THE ROLE OF GENETIC AND ENVIRONMENTAL FACTORS IN THE ETIOLOGY OF OROFACIAL CLEFTS IN THE ETHIOPIAN POPULATION

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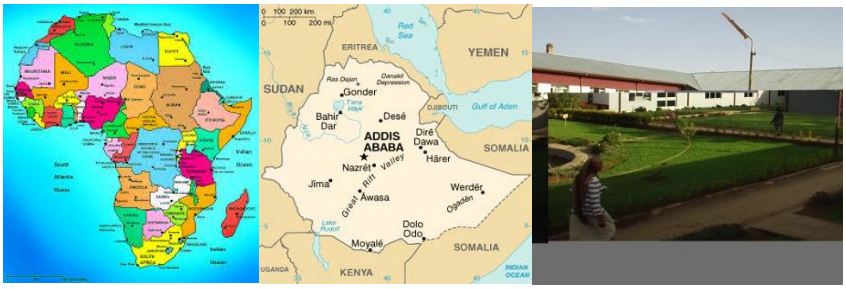

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THE ROLE OF GENETIC AND ENVIRONMENTAL FACTORS IN THE ETIOLOGY OF OROFACIAL CLEFTS IN THE ETHIOPIAN POPULATION

A Dissertation submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy (Ph.D.) in Public Health

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Dedication

To my father and memory of my late mother for the immense sacrifice that you paid to strengthen the path for me today.

I also dedicate this thesis to my wife Dr Shewalem Negash and children: Tsion Mekonen, Euael Mekonen, Natnael Mekonen and Elshaday Deme who constantly supported and encouraged me during the challenges of this work and life and also for accepting period of absences and late evenings from work. I am really grateful for having you in my life.

List of original papers

This Dissertation is based on the following four original research papers that will be referred by their respective Roman numerals (I-V) in the text.

I. Mekonen Eshete, Azeez Butali, Wakgari Deressa et al. Descriptive Epidemiology of Orofacial Clefts in Ethiopia. **Journal of Craniofacial Surgery 2017; Vol. 28:334-337**

II. L.J.J.Gowans*, W.L.Adeyemo*, Mekonen Eshete*, et al. Association Studies and Direct DNA Sequencing Implicate Genetic Susceptibility Loci in the Etiology of Nonsyndromic Orofacial Clefts in Sub-Saharan African Populations–**Journal of Dental Research 2016. Vol. 95(II) 1245-1256.**

III. Mekonen Eshete, Wakgari Deressa, Oladugba Bola et al. **Oral Health Related Quality of Life of Children Born with Orofacial Clefts in Ethiopia and their Parents.** Manuscript **The Cleft Palate-Craniofacial Journal 2018 January**

IV. Mekonen Eshete, Wakgari Deressa, Azeez Butali, Fikre abate et al. The role of environmental factors in the etiology of orofacial clefts in the Ethiopian Population-**Manuscript**

Acronyms/abbreviations

AAU	Addis Ababa University
BMP4	Bone Morphogenic Protein 4
CHS	College of Health Sciences
CI	Confidence Interval
CL(P)	Cleft lip with or without cleft palate
COHIP	Child oral health impact profile
CPO	Cleft Palate only
dbGaP	Database of Genotypes and Phenotypes
DNA	Deoxyribonucleic acid
FBAT	Family Based Association test
FGFR1	Fibroblast Growth Factor Receptor 1
FGFR2	Fibroblast Growth Factor Receptor 2
FOXE1	Forkhead box proteinE1
GWAS	Genome-wide association studies
HIV	Human immunodeficiency virus
IMF	International Monetary Funds
IRB	Internal review board
IRF6	Interferon regulatory factor 6
MSX1	Muscle specific homeobox 1
MTHFR	Methylenetetrahydrofolate Reductase
NIDCR	National Institute of Dental and Craniofacial Research
NIH	National Institutes of health
NSCL/P	Non-syndromic cleft lip and or palate
NTD	eural tube defect
OFC	Orofacial Clefts
OR	Odd Ratio
PAX7	Paired box 7
PCR	Polymerase chain reaction
SCL/P	Syndromic cleft lip and or palate
SHH	Sonic hedgehog

Taq1	Thermos aquaticus
TDT	Transmission Disequilibrium Test
TGFα	Transforming Growth Factor Alpha with Factor Alpha
UIN	unique identifying number PAX7
UN	United Nations
UNDP	United Nations developmental programme
UIN	Unique identifying number
VWS	Van der Woude syndrome
WHO	World Health Organization
Y12HMC	Yekatit 12 Hospital Medical College

Glossary

Cleft Lip - is an opening in the upper lip that can extend into the base of the nostril present in a baby at birth, it can also occur in the lower lip.

Cleft Palate - is an opening in the roof of the mouth present in a baby at birth.

Candidate Gene-A candidate gene is a gene, located in a chromosome region suspected of being involved in the expression of a trait such as a disease.

Genetics - is the study of genes.

A Gene - is the basic physical and functional unit of heredity, it contains biological information transmitted from parents to offspring.

DNA (Deoxyribonucleic acid)- Is the hereditary material in humans and almost all other organisms. The information in DNA is stored as a code made up of four chemical bases: Adenine (A), Guanine (G), Cytosine(C), and Thymine (T)

Genetic code- is the language in the DNA that carries the instruction required for making protein products.

Mutation- is a Change /variation in nucleotide base pairs which might lead to variable phenotypes (traits and diseases).

Frameshift mutation –deletion or insertion in a DNA sequence –shifts the way the sequence is read

Nonsense mutation –nonsense codons UAG, UAA, UGA-a point mutation that introduces a premature stop codon

Splice site mutation –inserts, deletes or changes several nucleotides in a specific site

Missense mutation –a single nucleotide change results in a codon that codes for a different amino acid

GWAS- An analysis of allelic association for genes throughout a genome

Congenital Birth defect – is a defect present at birth on any part of the body different from what we know as normal.

Table of Contents

List of Tables

List of Figures

Abstract

Background: Orofacial clefts (OFCs) are the commonest craniofacial birth defects with a worldwide birth prevalence of 1/700 live birth. It varies from 1/2500 to 1/500 births depending on the geographic origin, racial and ethnic backgrounds, and socioeconomic status. The incidence of this anomaly in Ethiopia is not known and the etiology has never been studied. We investigated the contribution of previously reported candidate genes and chromosomal loci to the risk of non-syndromic orofacial clefts (NSOFCs) in the Ethiopian population, which is important for improving the management and prevention. It is also of paramount importance in parental counseling and intervention. We investigated the role of environmental factors in the occurrence of NSOFCs in the Ethiopian population. The quality of life of children affected and their parents was also assessed.

Objectives: The main objective of this study was to investigate the role of genetic and environmental factors in the etiology of NSOFCs in the Ethiopian Population

Methods: We assessed the epidemiology, etiology and quality of life of those affected and the perception of their parents. The epidemiology of OFCs was assessed using the Ethiopian 2007 sensus retrived from the Federal Ministry of Health (EFMH) and the Smile Train (ST) databases. The data was collected from June 2007 to December 2013. ST is a charity organization, which supports cleft care in Ethiopia.

We used data collected from November 2012 to January 2016 on maternal and child demographic data, maternal illness, medication, lifestyle and exposures. Saliva samples collected from the participants (Cases, Case mothers, controls and Control Mothers) for analysis using Oragne saliva collection kits and sponges. The collected saliva samples were sent to University of Iowa, USA for DNA processing. We collaborated with similar projects in Ghana and Nigeria to perform the genetics part of this investigation. We selected &genotyped 48 single-nucleotide polymorphisms (SNPs) on 701 non-syndromic cleft lip and or palate (NSCL/P)&163 non-syndromic cleft palate only (NSCPO) cases, 1070 unaffected relatives &1078 unrelated controls. The association of these SNPs with NSOFCs in Asian and European population was confirmed through Genome-wide

association (GWAS)& candidate gene (CG) studies. We conducted association analyses for each population using the cleft-type cohorts described above, and a meta-analysis of the three subpopulations

The role of environmental factors in the etiology of NSOFCS was assessed using the data collected on maternal demographic data, maternal illness, maternal medication use, lifestyle and exposure before and during pregnancy. We interviewed 359 mothers of children born with NSOFCs and 401 mothers of children born without any congeneital anomaly.

We assessed the oral health related quality of life (OHRQoL) of children born with NSOFCS &the perception of their parents using the child oral health impact profile (COHIP) questionnaire. In this study 41 children born with NSOFCs and treated from December 2008 to December 2016 and equal number of parents participated.

Results: We determined the incidence/prevalence of OFCs in Ethiopia using the ST database, which was collected from June 2007-December 2013. During this time 18,073 patients with cleft lip and or palate (CL/P) were operated, out of the total operated patients with OFCs 8,232 are under seven years old. The total number of live births during this period was 18,811,316. This gives an incidence of 44/100,000 live births of orofacial clefts in Ethiopia. The prevalence was estimated using the total number of Cleft patients operated from June 2007 to December 2013 (N=18,073) as a numerator and the total number of population (N= 88,703,914) in 2013 as a denominator. It is estimated to be 20/100,000 populations.

We confiremed that SNPS, which were found to be associated withn the occurrence of NSOFCs in European population, were found to be associated with the occurrence of NSOFCs in our study populations. In the Ethiopian subpopulation PAX7 (rs742071, P = 0.005574,OR=1.329 and 95%CI 1.087-1.626), IRF6 (rs642961, P =0.01508;OR= 1.442; 95% CI 1.072-1.94), DYSF (rs2303596, P = 0.00231;OR= 0.6854; 95%CI 0.5371-0.8747), 8q24 (rs987525, P =0.000782; OR= 1.413; 95%CI 1.154-1.73), were found to be Nominally associated with the occurrence of NSCL/P. SNPS in NTN1 (rs8081823, P

= 0.03251; OR=0.4905, 95% CI 0.216-1.114) were also found to be nominally associated with NSCPO in the Ethiopian population.

The role of maternal environmental factors and diseases in the occurrence of NSOFCs was assessed and revealed that mothers who lived outside Addis Ababa during their pregnancy time had a higher risk of delivering a child with NSOFCs and mothers who gave history of threatened abortion and Bronchial Asthma were having a higher risk of delivering a child with NSOFCs. Mothers who had exposure to diagnostic X-Ray were also at higher risk of having a child with NSOFCs

No significant differences were found for overall and subscales COHIP scores between the patients and their parents. The maximum overall score parents obtained on the COHIP was 186 and the patients was 190. The mean overall score of the patients and parents was 155. This indicates good OHRQoL of children born with NSOFCs.

Conclusion and recommendations: The prevalence 20/100,000 populations found in this study are lower than the previous studies done in many parts of Africa including the study done in Addis Ababa. Loci, which contributed to the occurrence of NSOFCs in European and Asian population, were found to contribute to the occurrence of NSOFCs in sub-Saharan populations (Ethiopia, Ghana and Nigeria). We found out that the affected children who received multidisciplinary cleft care had good OHRQoL and the responses of the affected children and their parents did not differ.

We recommend community based prospective study to find out the true incidence of OFCs. We also recommend conducting a prospective case control study to better understand the contribution of environmental factors and the gene environment interaction in the occurrence of birth defects in general and OFCs in particular. Finally, we recommend that the child oral health impact profile (COHIP) questionnaire should be modified to fit the cultural beliefs of various populations and society around the world.

Key words: Orofacial clefts, genetic factors, Gene environment interaction

1. Introduction

1.1. Background of the study

Orofacial clefts (OFCs) are the most common craniofacial birth defects and one of the most common congenital abnormalities in humans, with a worldwide prevalence of approximately 1/700 births(1). The prevalence of these anomalies in Ethiopia is unknown and the etiology is not studied. The incidence of these anomalies in Addis Ababa is 1.49/1000 live births(2). OFCs can be described as part of a syndrome where it is called syndromic and non-syndromic or isolated when it occurs without other malformations or syndromes. The syndromic forms present with other congenital anomalies.

There are few studies, which investigated the cause of orofacial clefts in Africa. In Ethiopia, there is no study that investigated the cause of this anomaly prior to this study. Birth defects in general and orofacial clefts in particular are not in the priority list of the health care system for many African countries including Ethiopia. There is no birth defect registry system in Ethiopia and many other developing countries. This led to the non-existence of relevant information on the magnitude of birth defects, etiology and their contribution for morbidity and mortality on those affected. This study investigated the etiology of NSOFCs in the Ethiopian population using the available infrastructure and in collaboration with other universities (University of Iowa, USA, university of Lagos, Nigeria, university of Dundy, UK and Kwame Nkrumah University of Science and Technology, Ghana).

The existing literatures on the epidemiology of NSOFCs in Africa, the genetic role of cleft occurrence and the role of interaction between gene and environment in the occurrence of this anomaly was reviewed. Literatures on the quality of life of the children affected with OFCs and the perception of their parents were also reviewed. Saliva samples from the cases and controls including their parents were collected and investigated at the University of Iowa, USA Butali's Genetics laboratory to find out the role of genetic factors in the occurrence of OFCs in our study population. We collaborated with similar projects in Ghana and Nigeria in order to get adequate sample for the genetics part of this study.

1.1.1. Development of the face

Proper development of the face requires coordination of a complex series of events and includes cell growth, migration, differentiation, and apoptosis(3).The formation of facial

structures begins with the appearance of pharyngeal arches (Figure 1). Pharyngeal arches are columns of mesenchymal tissue found in the neck of developing embryos derived from neural crest cells. The neural crest cells migrate to form the five facial primordia: the frontonasal prominence, the paired mandibular and maxillary prominences, which surround the primitive oral cavity. Around the fifth week of fetal development the face begins to take shape starting with the nasal placodes that will become the nasal pits after invagination. In the sixth week, the nasal placodes of the frontonasal prominence invaginate to form the nasal pits and the lateral and medial nasal processes (figure 2 A, B). At this time the medial nasal processes fuse at the midline to form the intermaxillary process (Figure 2 C, D). Next, the nasolacrimal groove and duct develop in the seventh week. The frontonasal prominence forms the stomodeum from above like a primordial lip. When the mandibular prominences merge, they will form the beginnings of lower lip, chin and mandible. The nose is the result of a fusion of five separate prominences: the frontal prominence forms the bridge of the nose; the two medial nasal prominences form the crest, tip and central portion of the lip, or intermaxillary segment; and the lateral nasal prominences form the sides. By the 10th week, the intermaxillary process forms the philtrum. (Figure 2 E).

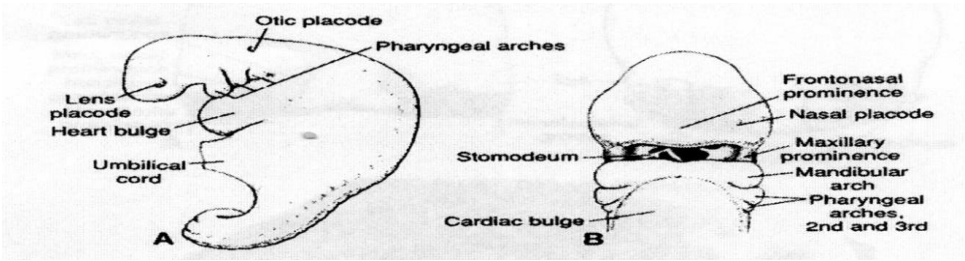


Figure 1: (A) Lateral view of an embryo at the end of the 4th week showing the position of the pharyngeal arches and (B). Frontal view of a four-and-a-half-week embryo (Adapted from Moore and Persaud, 2003).

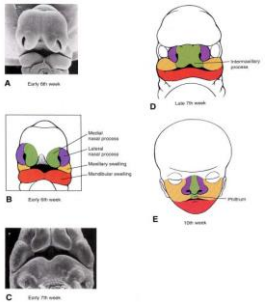


Figure 2: Facial development at different weeks of gestation A, B, C, D and E. (Photos courtesy of Dr. Arnold Tamarin.)

1.1.2. Formation of the primary and secondary palates

The palate is formed from two primordia and it can be classified as the primary and secondary palate. At around the sixth week of development the primary palate begins to take shape, arising from the medial nasal process, this wedge-shaped mass will eventually extend to form the floor of the nasal cavity. Around the end of the seventh and beginning of the eighth week of development the secondary palate begins to develop from the two lateral palatine processes. The Palatine processes grow vertically on either side of the tongue (Figure 3A, B). The medial walls of the maxillary processes produce a pair of thin medial extensions, called the palatal processes (shelves). Initially these grow predominantly vertically, downward and parallel to the lateral surfaces of the tongue. By the beginning of the eighth week the tongue begins to contract and move out of the way (Figure 3 C) and the lower jaw grow and drops downward and forward. By the end of the eighth week, the palatal processes rotate rapidly upward to a horizontal position and fuse with each other and with the primary palate (Figure 3 D) the fused palatal processes form the secondary palate - together with the primary palate they form the definitive palate (4).

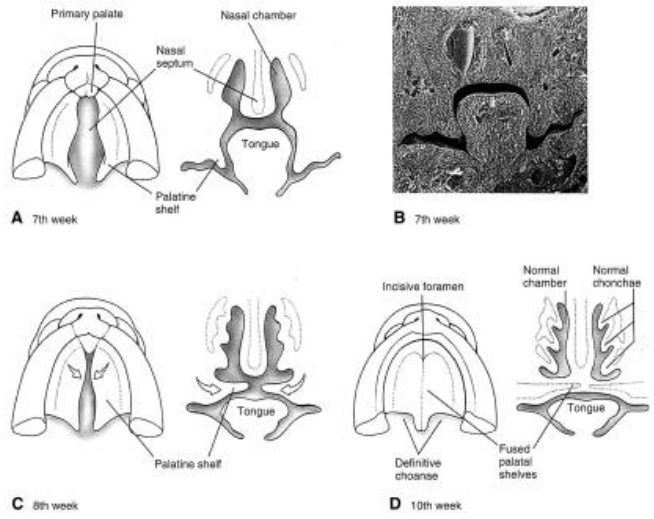


Figure 3**:**Formation of the secondary palate and nasal septum at different stages of embryologic development A, B, C, D. (B, Photo courtesy of Dr. Arnold Tamarin.)

Failure in growth or fusion of these processes results in orofacial clefting involving the upper lip, alveolus, and/or primary and secondary palate. OFCs affect the structure of the face and oral cavity. Phenotypically they are divided into three categories: those that affect the lip only, the lip and the palate and those that affect the palate only. Each could be unilateral or bilateral, complete or incomplete (figure 4).

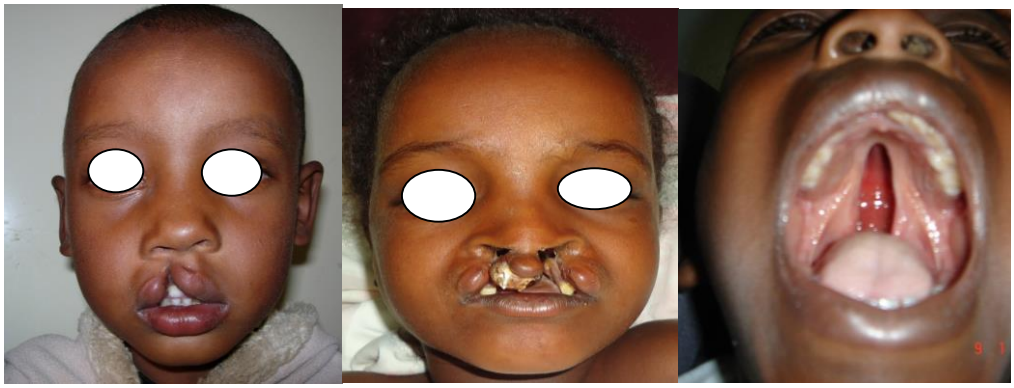


Figure 4 **Cleft phenotypes**

1.1.3 Ethiopia: The country

Ethiopia is the second most populous country in sub-Saharan Africa next to Nigeria with a population of 101,610,070 and the thirteenth populous country in the world(5). The population of Ethiopia is 1.35% of the total world population and 19.4% of the total population is urban. The International Monetary Fund (IMF) ranks Ethiopia as among the five fastest growing economies in the world. In 2013/14 the economy grew for its 11th consecutive year posting 10.3% growth (6).Although the industrial sector is said to be at its infant stage in Ethiopia, the few that are in and around the urban centers especially Addis Ababa, the capital city, are releasing untreated effluent/discharge into the stream and other water bodies. For example, the textile, tannery and iron tool industries are polluting the environment of the Akaki river systems and underground water in south- east of Addis Ababa. There is lack of sufficient system and regulation for the management of hazardous wastes, chemicals and radioactive substances. In addition, lack of environmental awareness concerning the linkage between environment and development in general, weak participation of the people and community-based organizations in environmental management activities are some of the environmental challenges Ethiopia is facing nowadays(7). There is no study, which assessed the role of environmental factors on the birth of a child with congenital anomalies in Ethiopia.

1.1.4 Establishment of Ethiopian orofacial clefts research infrastructure

Holistic cleft care in Ethiopia was started in 2003 in collaboration with Haukeland University Hospital Bergen cleft team, Addis Ababa Health Bureau and the Ethiopian Federal Ministry of Health. Norad financially supported this collaboration. It was started at two public institutions; Yekatit 12 Hospital Medical College and ALERT Hospital with the

aim of promoting holistic cleft care, teaching and research. The cleft care team at Yekatit 12 Hospital Medical College includes plastic surgeons, speech therapists, ear nose and throat specialists, social worker, orthodontists, oral hygienist and data encoder. Using the cleft care center at Yekatit 12 Hospital Medical College and in collaboration with Nigerian craniofacial research infrastructure, Dundy University Kwame Nkrumah University of Science and Technology, Ghana and Iowa University a research infrastructure for OFCs was established. Smile Train and Transforming Faces support the surgical treatment and rehabilitation of all Ethiopian cleft victims at the Yekatit 12 Hospital Medical College and other hospitals. The Addis Ababa University College of Health Sciences IRB and the National Research and Ethics Review committee (NRERC) approved the study project.

1.2. Statement of the problem

Orofacial clefts are the most common craniofacial birth defects in humans and are a substantial personal and societal burden. The treatment of clefts includes multiple craniofacial and dental surgeries, as well as speech, hearing and psychosocial therapies with an estimated lifetime cost associated with treatment of $200,000(8). The genetics of these anomalies are only partially understood but are of great importance in counseling of affected families(9). It has long been assumed that both genetic and environmental factors play important roles in the etiopathology of clefts, and this is supported by the varying incidence of clefting with ethnicity, geographic location, and socioeconomic status (10, 11). Reviewed studies on the incidence and birth prevalence of OFCs in Africa suggest that the prevalence is low (12) however the majority of the studies were small sample size and hospital based with a high tendency for under ascertainment of cases. The reason for under ascertainment could be that a significant proportion of births take place at home where there is no recording. This factor may have contributed to lower estimates from some countries. It is true that estimates can give an indication of rate, but they cannot be comparable with those where full ascertainment was achieved.

1.3. Rationale of the study

Studies on incidence, prevalence and etiology of birth defects in general and OFCs in particular are very limited in Ethiopia. There are only two published reports about cleft lip and or palate (CL/P). The first is the study among surgical patients less than 14 years of age admitted to the Ethio-Swedish children’s hospital in Addis Ababa between1984 and

1988(13). In this study, among 2,281 surgical patients treated 183 (8%) were cleft cases. The second is the incidence study conducted in health institutions in Addis Ababa, which reported an incidence of 1.49/1000 live births (2). This lack of information in fact has contributed to the poor cleft care in the country. This study contributed to feel the existing knowledge gap.

Understanding the effects of OFCs and other craniofacial conditions on long-term health outcomes is important for quantifying the health burden and improving service delivery and health care policies for affected populations and families. It is also important to understand the impact of OFCs on these outcomes for identifying unmet needs and developing public policies to reduce the burden of OFCs at the individual, family and societal levels(8).

As stated above OFCS are genetically heterogeneous therefore the long-term goal of this study was to investigate the contribution of previously reported candidate genes and chromosomal loci to the risk of OFCs in the Ethiopian population. This will improve our understanding of these anomalies that is important for improving the management and prevention. It is also of paramount importance in parental counseling and intervention. The knowledge we gained from this study about the environmental factors that could modify the risk of having a child with cleft lip and or palate and identifying the risk gene will enable us to provide a better counseling and establish a system for prevention. It will be also possible to predict more accurately the risk of having a cleft child in a family. The counseling, which will be provided for a family who already has a child with cleft lip and palate, will be more accurate. It is inevitable that we move to the era of personalized medicine and the knowledge of genetics and environmental influences will be very useful in providing focused clinical care and producing public health recommendations on risk assessment in reproductive health particularly regarding cleft lip and palate.

2. Literature review

In this part of the dissertation the available literature related to orofacial clefts were reviewed. The review is organized, in line with the study specific objectives.

2.1.Prevalence/Incidence of orofacial clefts

Orofacial Clefts are the most common craniofacial birth defects. The prevalence of these anomalies varies from 1/2500 -1/500 births depending on the geographic origin, racial and ethnic backgrounds, and socioeconomic status (1). S. K. Das et al stated that Asians have the highest risk (14/10,000 births) followed by whites (10/10,000 births) and African Americans (4/10,000 births)(14). The reported incidence of cleft lip and palate was greater than for cleft lip alone or for isolated cleft palate. In most reports, males outnumbered females in both cleft lip and cleft lip and palate, while isolated cleft palate was predominant in females. The lack of birth defect registry system in the vast majority of Africa led to the scarcity of information on birth defects in general and clefts in particular in this part of the world. The burden of congenital anomalies can only be addressed by establishing a birth defect registry system.

We **(2)**conducted a facility based cross sectional study in Addis Ababa city administration from September 2004 to February 28, 2007. The total number of live births in the study institutions during the study period was 42,986. Of those babies, 64 had some form of cleft anomalies, which makes an incidence of 1.49 per 1000 live births or 1 in 672 live births. A. Odhiambo et al 2012(15) did a prospective institution based descriptive cross-sectional study to find out the incidence of clinically manifest craniofacial anomalies (CFA) at birth. The study was done at the two largest government delivery centres in Nairobi, Kenya from November 2006 to March 2007. The study population included all mothers who delivered and their babies. They included all births at 20 weeks or more of gestation and/or at least 500 g birth weight. During the study period 7989 new births were registered: 4264 (53.4%) male, 3721 (46.6%) female and 4 (0.05%) with ambiguous external genitalia. 146 CFAs occurred (1.8%) of the total births. There were 7623 live births of these 1.3% had CFAs. Rachael Fels Elliott Et al 2008(16) did a retrospective chart review of live births at a university teaching hospital between 2001 and 2005 in Zambia. A total of 55,108 live births were documented between January 1, 2001, and December 31, 2005. The number of identified newborns with orofacial clefts was used to estimate the birth prevalence of Orofacial Clefts. Thirteen cases of orofacial clefts were identified from the total live births of 55108, this gave

a birth prevalence of 0.24/1000 live births.

Suleiman et al 2005(17) collected and analyzed the records of 15890 new-borns' delivered in the period from 1997 to 2000 at Omdurman Maternity Hospital, Al-Rahibat Maternity Hospital and Khartoum North General Hospital. Among the 15890 newborns thirteen cases of cleft lip and palate were recorded, this made a prevalence of 0.82/1000 live births. Dreise et al 2011(18) did a prospective study at seven hospitals and health institutions with maternity care in and around Kampala Over the course of 1 year (February 1, 2008 to January 31, 2009). All live births were examined for orofacial cleft anomaly. The birth of a baby with a cleft was communicated to the research coordinator by maternity staff, and the babies were assessed within 24 hours of delivery. The live births at each center were collected monthly and used as denominator. The total number of live births during the study period (February 1, 2008 to January 31, 2009) was 26,186. Of these nineteen babies were born with orofacial clefts this gave an incidence of 0.73/1000 live births. Kesande et al(19) did a study in Uganda, their study had two designs: The first design was a retrospective review of medical records of mothers who delivered live babies between January 2005 and December 2010 in Kisoro Hospital and St. Francis Hospital, Mutolere in Kisoro District, Uganda. The second design was a key informant design. They have interviewed 20 mothers who delivered children with orofacial clefts and 24 selected medical staffs that were working at the two study hospitals. They analyzed data using descriptive statistics. During the study period January 2005-December 2010 25,985 mothers delivered live babies in Kisoro Hospital (n = 13,199) and St. Francis Hospital, Mutolere (n = 12,786) with 20 babies having Orofacial clefts. The incidence of Orofacial clefts was calculated using the total number of live births as a denominator and it gave an incidence of 0.77/1,000 live births. Khrouf et al (20) performed a prospective study at the Wassila Bourgiba Hospital in Tunisia. The hospital was a referral and teaching hospital. They examined 10,000 newborns during the first 24 hours of age between October 4, 1983 and July 16,1984 for possible congenital anomalies. They recorded fifteen clefts of all types; among other congenital anomalies this makes an incidence of cleft lip and palate 1.5/1000 births.

Butali et al(21) did a retrospective review of the smile train database. They pooled the data of operated patients from September 2006 to June 2011at the seven of the largest Smile Train treatment centers in the six geopolitical zones in Nigeria. They identified a total of 2197 cases of clefts during the study period. The number of estimated birth for the six states (seven centers), which include Lagos (1.9million live births) and Kano (2 million live births)

two states with the highest population and number of live births during the study period in the six geopolitical zones in Nigeria was 4.6 million. The prevalence rate was calculated using the estimated birth as a denominator and it makes 0.5 per 1000 births. Msamati et al(22) reviewed delivery and nursery records of Obstetrics and Gynecology and Pediatrics department at a referral Hospital in Malawi between January 1998 and December 1999. They have investigated 25562 births, of which 39(0.2%) eight boys and 31 girls had some sort of congenital anomaly. Clefts of all types comprised the largest group, 43.6%, which made an incidence of 0.67/1000 births. Ogle et al 1993(23) did a retrospective hospital based study and reviewed the records of 56,637 live births at Mama Yemo Hospital, Zaire over a 20-month period. The prevalence of OFCs reported in this study was 0.46/1000 live births. Morrison et al (24) studied the incidence of cleft lip and palate in Western Cape, South Africa using data from the two cleft palate units at Tygerberg and Red Cross War Memorial Children's Hospitals, supplemented by data on cases seen by plastic surgeons in private practice. The study period was between January 1983 and January 1984. During this time a total of 52 orofacial cleft caes reported. The report included all phenotypes of cleft under one year of age (3 blacks, 43 coloureds and 6 whites). The birth figures of the 1982 census were used for denominators because the census figures for the corresponding period of the survey were not available. They found a high incidence among the coloureds (1.40/1000 births). The incidence among whites was 0.59/1000 births and among blacks it was 0.32/1000 births.

Yehoshua Shapira et al (25)investigated the prevalence, distribution and characteristic features of various types of non-syndromic clefts among Israeli Jews and Arabs. Their investigation included 976,578 live born infants, of these 684 presented with unilateral or bilateral clefts, with a prevalence of 7.00/10,000 live births; 479 were Jews and 205 were Arabs. The prevalence was higher among Arabs compared to Jews (11.12 and 6.22 per 10,000 live births in Arabs and Jews, respectively, $P < 0.00001$). Males had higher cleft rates than females (7.69/10,000 and 6.17/10,000 live births, respectively, $P = 0.05$). Soltani MK et al (26) assessed the incidence of cleft lip and palate and factors associated with them in the hospital births of Iran's Kurdistan province in 2010. Their study reported an incidence rate of 1.09 /1000 live births. Gregg TA et al (27) retrospectively analyzed the prevalence of cleft lip and/or palate (CL/P) in Northern Ireland (NI) for the 20-year period 1981 to 2000, and found a prevalence of 1.47 per 1000 live births, or 1:682. This was consistent with the findings reported by other U.K. studies. Rodrigues K et al (28) investigated the prevalence

of OFCs in live newborns from 1998 to 2002 in Brazilian state capitals and reported a mean prevalence of 0.36/1,000 live births. Per Tanka SA.et al the average prevalence of cleft lip with or without cleft palate were 7.75 per 10,000 live births in the United States and 7.94 per 10,000 live births internationally(29). The above-mentioned study found the highest rate in Japan (19.05/1000) and the lowest in South Africa 3.13 per 10,000 live births.

2.2 . The role of Genetic disorder in the occurrence of Orofacial Clefts

The etiology of non-syndromic orofacial clefts (NSOFCs) is not yet clearly understood. The works done so far at different countries and regions brought different results concerning the cause of NSOFCs. They are said to be multi factorial in origin. Genetic predisposition and various environmental factors can contribute to the occurrence of non-syndromic orofacial clefts specially if they act at the relevant time of embryologic development. The contribution of environmental factors is high in genetically predisposed patients(30). These include alcohol consumption(31), maternal illness and smoking (32). Several drugs have been implicated to cause OFCs in animal studies but only phenytoin has been reported to be a cause in humans. Folic acid may have a protective effect. In the case- control study Butali et al(33) showed that there is a statistically significant reduction in the occurrence of OFCs with maternal folic acid use.

2.2.1 The role of candidate genes in Orofacial Clefts

The genetic cause of NSOFCs can be investigated by studying the candidate genes responsible for syndromes associated with NSOFCs. Ardinger et al(34) first tested this approach using a case-contrl study design. The role of IRF6 and msh homeobox 1 (MXS1) in NSOFCs is identified through this approach(35).

2.2.2 Interferon regulatory factor 6 (IRF6)

Common birth defects such as neural tube defects, congenital heart disease, and cleft lip and palate can occur in both syndromic (which include structural abnormalities, developmental delay, or dysmorphic features), and non-syndromic forms(36). Genes, environment and their interaction play a role in the occurrence of non-syndromic forms of cleft lip and or palate. Theresa M. Zucchero, B.S et al (37) examined large number of affected patients and families to search for a specific genetic factor, which contributes to the development of cleft lip and or palate. They evaluated for a specific candidate gene based on its involvement in an

autosomal dominant form of cleft lip and palate (van der Woude'S syndrome) and identified the gene that encodes interferon regulatory factor 6(IRF6) as a candidate gene. They carried out transmission-disequilibrium testing (TDT) for valine 2741 (V274I) in 8003 individual subjects in 1968 families derived from 10 populations with ancestry in Asia, Europe, and South America. They found that 12% of the genetic contribution to cleft lip and or cleft palate is associated with variation of IRF6. This variation at IRF6 tripled the risk of recurrence in families that had already one affected child. Zucchero et al concluded that deoxyribonucleic acid **(**DNA)-sequence variants associated with IRF6 are major contributors to cleft lip and or palate.

Cleft lip with or without cleft palate (CL/P) and isolated cleft palate (CPO) are developmentally and genetically different however Van der Woude syndrome (VWS) is a single-gene disorder that covers both clefting phenotypes. Shinji Kondo et Al (38)confirmed this by a genotype analysis of families of VWS, which showed that affected individuals shared the 18 base pair (bp) deletion found in the proband in spite of their phenotype. They observed similar results in the other families and concluded that a single mutation in IRF6 can cause both types of clefts. Butali et al (39) confirmed the presence of IRF6-related VWS and Popliteal pterygium syndrome (PPS) in sub-Saharan Africa. Adrianna Mostowska et al(40) investigated the contribution of previously reported candidate genes and chromosomal loci to the risk of CL/P in Polish population. They performed analysis of 18 polymorphisms of forkhead box 1 (FOXE1), IRF6, MSX1, paired box 9 (PAX9), TBX10, fibroblast growth factor 10 (FGF10), fibroblast growth factor receptor 1 (FGFR1), transforming growth factor alpha (TGFa), transforming growth factor beta 3 (TGFb3), Small Ubiquitin-Like Modifier 1(SUMO1), and the chromosomal region 8q24 in a group of 175 patients with CL/P and a properly matched control group. They found significant association for the IRF6 rs642961 variant and the 8q24 region's rs987525 with CL/P. Chakravati et al proved that the risk of having a baby with cleft increases from 3-5% to 9% in parents that have a child with both copies of the susceptible IRF6 alleles (41).

2.2.3 MSH Homeobox 1 (MXS1)

Tongokobpetch et a (42) performed mutation analysis covering all the coding regions of the MSX1 gene for 100 Thai patients with nonsyndromic CL/P to find out if MSX1mutations contribute to the occurrence of nonsyndromic CL/P. In contrast to previous reports they found the P147Q variant in 8 out of 100 Thai controls. They could not detect association

between the variant and CL/P in their study population. This suggests that P147Q is not pathogenic. They found MSX1 mutations in 2% of cases of CL/P and they suggested that finding MSX1 mutation should be considered for genetic counseling. Andrew C. Lidral, et al(43) screened candidate genes for clefting, including transforming growth factor alpha (TGFA), B-Cell Leukemia/lymphoma 3 (BCL3), Distal-Less Homeobox 2 (DLX2), MSX1, and transforming growth factor beta 3 (TGFB3), for linkage-disequilibrium (LD) with either CL/P or CPO in a predominantly Caucasian population, using case-control and nuclear-family–based approaches. They could not confirm previously reported LD for TGFA with both CL/P and CPO, except in CL/P patients with a positive family history. They also could not find LD between BCL3 and either CL/P or CPO, in contrast to previous studies. They found Significant LD between CL/P and MSX1 and TGFB3 and between CPO and MSX1. This suggests that these genes are involved in the pathogenesis of clefting.

2.2.4 The role of MSX1 in the etiology of clefts in sub-Saharan Africa

Butali et al in 2011(44) investigated the role of candidate genes in the etiology of orofacial clefts in the Nigerian population. In this study a total of 118 cases and 166 controls included. Deoxyribonucleic acid **(**DNA) was isolated from the case's and control's saliva, genotyped for association study and direct sequencing was done on the cleft candidate genes: MSX1, IRF6, FOXE1, FGFR1, FGFR2, Bone Morphogenetic Protein 4(BMP4), MAFB, ATP Binding Cassette Subfamily A Member 4 (ABCA4), PAX7, Ventral Anterior Homeobox 1 (VAX1), and the chromosome 8q24 region. They have observed a missense mutation A34G in MSX1 in nine cases and four hap map controls. In the cases a deviation from Hardy-Weinberg principle was observed (p=00002). The conclusion they made was mutation in MSX1 A34G variant has a role in the etiology of CL/P in the investigated Nigerian children born with clefts.

2.2.5 Transforming Growth Factor Alpha (TGFA)

Ardinger et al(34) identified a major - locus model by comparing the frequencies of 12 restriction fragment length polymorphisms (RFLPS) at five loci-epidermal growth factor, transforming growth factor–a, epidermal growth factor receptor, glucocorticoid receptor and estrogen receptor in 80 subjects with NSCL/P and 102 controls. They observed a significant association between two RFLPS at transforming growth factor a (TGFA) locus and the occurrence of clefting. From this one can conclude that either the TGFA gene itself or DNA

sequences in or adjacent region contribute to the development of a portion of cases of CL/P in humans.

2.2.6 PAX7 and VAX1

Recent GWAS identified significant genetic associations for several genes with NSCL/P. Butali et al (45) investigated two of these GWAS signals; they investigated the role of common and rare variants in PAX7 and VAX1 genes. Their study replicated previous GWAS findings for markers in VAX1 in the Asian population and identified rare variants in PAX7 and VAX1 that may contribute to the etiology of NSCL/P.

2.2.7 Fibroblast growth factor receptor (FGFR)

Riley et al (46) identified thirty-seven point mutations in the exons or at the intron–exon junctions of the fibroblast growth factors (FGF) and fibroblast growth factor receptor (FGFR) genes, in a sequencing of the coding regions of the FGFs and FGFR genes in NSCL/P patients. In their finding nine of the mutations were either missense or nonsense, accounting for about 5–6% of the cases examined.

2.2.8 MAFB and FOXE1 genes

MAFB is a gene that encodes transcription factor MafB which also known as musculoaponeurotic fibrosarcoma oncogene homolog B (V-mafB). MAFB is a new gene that may be involved in susceptibility to cleft lip with or without cleft palate (CL/P). Mi N et al investigated the role of MAFB gene in NSCL/P, (47) and identified three single nucleotide polymorphisms in MAFB (rs13041247, rs6065259, and rs11696257). They examined the association of identified SNPS with NSCL/P in 344 patients and 324 healthy controls in northern Chinese Han population. They found that rs6065259 was the most important single nucleotide polymorphism in MAFB ($p = 0.0027$), followed by rs13041247; however, no association was found between rs11696257 and NSCLP. This study provided further evidence about the role of MAFB variations in the development of NSCLP in this northern Chinese Han population.

Mutations in FOXE1gene cause Bamforth-Lazarus syndrome and are associated with congenital hypothyroidism and cleft palate with thyroid dysgenesis(48). Linkage analysis studies with additional fine mapping and replication showed that the region surrounding FOXE1 has association in the pathogenesis of cleft lip and palate with genome-wide levels

of significance. Lina M. Moreno et al 2009(49) reported that FOX1 gene plays a significant role in the etiology of NSCL/P.

2.3 The role of environmental factors in the etiology of orofacial clefts

Maternal environmental exposures like exposures to medications, alcohol, or other exogenous factors that have adverse effects on the developing embryo or fetus are estimated to be the cause of congenital anomalies in around 10% of the cases(50). Warkaney J et al(51) recognized the environmental component of clefting in 1943; they associated nutritional deficiencies with cleft palate in animal study. Murray JC(30) suggested that nutritional or toxic environmental exposures may contribute directly to as much as one-third of cleft cases, and etiologies will be most identifiable in indigent populations. Teratogens that cause cleft are rare exposures to Phenytoin, valproic acid, thalidomide and environmental exposures such as maternal cigarette or alcohol use and more recently herbicides such as dioxin and altitude(52-54). In a South American study altitude was particularly noticed as a risk factor(54). The role of environmental factors is supported by other epidemiological studies done in different part of the world (55-57) and it can be categorized as follows:

2.3.1 Maternal medication and disease

In a retrospective study from eight health maintenance organizations, researchers estimated that approximately 59% of pregnant women were prescribed a medication other than a vitamin or mineral supplement at some time during pregnancy(58). Use of over-the-counter medications during pregnancy may be even higher, and many women take a dietary or herbal supplement other than multivitamins or folic acid while pregnant(59, 60). A study done at Bahirdar Ethiopia reported that 36% of pregnant women who reported history of illness during their pregnancy self-medicated, among these 68.7% took modern medicines and 21.1% took traditional medicine (61). The effects of these medications on the fetus are unpredictable and not well studied in Africans. A study done in Hungary by Julia Metneki, et al(62) found out that mothers who contracted influenza, common cold, orofacial herpes, and gastroenteritis during pregnancy have an increased risk of giving birth to a child with CL/P. The risk of having a child with isolated cleft Palate only was high in mothers who contracted influenza, sinusitis, and bronchitis. Among chronic maternal diseases, epilepsy and angina pectoris showed a higher prevalence in the mothers of children born with

NSOFCs. Zhang and Cai, (63) in study called association of the common cold in the first trimester of pregnancy with birth defects reported the possible association of common cold in the mother and orofacial clefts in the offspring. James et al (64) during hearing screening performed on 126 HIV-exposed and 121 HIV unexposed newborns identified the occurrence of cleft palate in three newborns exposed in-utero to human immunodeficiency virus (HIV) and highly active antiretroviral therapy (HAART). Jean Pierre W. Munsie et al(65)in a case control study observed a statistically significant association between maternal bronchodilator use during the periconceptional period and the risk of cleft lip only (CLO). It is unclear whether the increased odds ratios observed in this study are due to the bronchodilators, the severity of asthma, or both, or to chance alone.

2.3.2 Maternal nutrition

Inadequate maternal nutrition during pregnancy has been suspected as a cause of orofacial clefts in humans since the early 1900s. Maternal nutritional deficiencies because of increased needs, inadequate intake, decreased absorption, disturbances in embryonic transfer, or underlying genetic aberrations in the mother or embryo or both, may significantly affect the nutritional status of the embryo. It may also affect gene expression and other developmental events in specific embryonic tissues. According to Murray JC (30) nutritional or toxic environmental exposures may contribute directly to as much as one-third of cleft cases, and etiologies will be most identifiable in indigent populations. Ingrid P. C. Krapels et al(66) found out that the dietary intake of energy, all macronutrients, vitamins, and minerals were lower in mothers of children born with OFCs than in controls. Christensen et al(67) noted that no change in frequency of cleft occurrence if the socioeconomic status does not change by a geographic move alone.

2.3.2.1 Folic acid

The human body needs folate to synthesize DNA, repair DNA, and methylate DNA. It also acts as a cofactor in certain biological reactions(68). Deficiencies of dietary folic acid can lead to abnormalities in the mother: anemia and peripheral neuropathy and the fetus: congenital abnormalities. Dietary supplementation with folic acid around the time of conception has been known to reduce the risk of neural tube defects (NTDs)(69). Folic acid is also thought to reduce the risk of preterm birth and congenital heart diseases. It is very important for a pregnant women to use folic acid and other vitamins throughout pregnancy, and it is preferable to start when pregnancy is planned(70). Ce´cile Chevrier, et al (71)in a

case control study design in France found significant difference in dietary folate intake between the mothers of OFC patients and controls mothers. The control mothers had significantly higher average dietary Folate intake (288 µg/day) than case mothers (264 µg/day P=0.03).

2.3.2.2 Vitamins B12 and B6

Vitamin B12 is a coenzyme, which plays a very important role in the process of converting homocysteine into methionine. Deficiency of vitamin B12 traps body folate in the 5-methylform, this trapping leads to increased homocysteine levels and decreased methylation of DNA, which is important in the regulation of gene activity. An inadequate amount of methionine caused by lack of vitamin B12 decreases the availability of S-Adenosylmethionine (SAMe). It is required for methylation reactions, which are also essential for myelin maintenance and thus neural function. A case control study in the Philippines evaluated the association between the risk for CL/P and maternal vitamin B6 status(72). The study concluded that inadequate vitamin B6 status was associated with an increased risk for CL/P. Vitamin B6 also plays a key role in the metabolism of folate and homocysteine.

2.3.3 Maternal life style, occupation and other environmental factors

The etiology of OFCs is multiple, both genetic and environmental factors such as lifestyles of both parents can contribute(73). Some of the ethnic and geographic variations in the epidemiology of cleft lip and or palate can be explained by the parental factors and genetic background. The identification and avoidance of harmful parental life style like smoking, drinking alcohol etc, before and during first trimester of pregnancy may contribute in the prevention of orofacial cleft occurrence. In N Taghavi et al (74)and Kraples et al (75)study low socioeconomic status and low maternal education are found to contribute for a birth of a child with orofacial clefts. The above-mentioned studies considered that low socioeconomic status could be a marker of parental health and lifestyle. They speculated that individuals with low education tend to smoke more and have less healthy diets and nutrients. The lifestyle factors, either alone or in combination with occupational activities and genetic background, could contribute to the occurrence of orofacial clefts(74). Tania A. Desrosiers et al 2012 (76) observed a positive association between maternal occupational exposure to chlorinated solvents during the periconceptional period and the prevalence of neural tube defects (NTDs) in offspring. Teratogens that could cause cleft are rare exposures to

phenytoin, valproic acid, thalidomide and environmental exposures such as maternal cigarette or alcohol use and more recently herbicides such as dioxin and altitude(52-54).

2.4 The role of Gene Environment Interaction in Orofacial Clefts

Studies to find out the role of gene and environment interaction in the occurrence of OFCs are going on. Romitti et al(77) examined allelic variants for transforming growth factor alpha (TGFA), transforming growth factor beta 3 (TGFB3), and MSX1, and their interactions with maternal cigarette smoking and alcohol consumption during pregnancy as risk factor for CL/P and CPO. Their finding shows that the occurrence of cleft palate only is significantly elevated in mothers who smoke 10 cigarettes /day and it is most elevated among infants with allelic variants at the TGFB3 or MSX1 sites. They have also compared the consumption of alcohol and MSX1 variants. If a mother consumes 4 drinks /month the risk of having a child with CL/P was significantly elevated and for those infants with allelic variants at the MSX1 site, the risk was most elevated. Their findings suggest that the development of CL/P and CPO may be influenced independently by maternal exposures but more significantly by interaction of such exposures and specific allelic variants. Iris A. L. M. van Rooij et al (78) performed a case-control triad study in the Netherlands between 1998 and 2000 and found that MTHFR C677T and MTHFR A1298Cpolymorphisms increase the risk of delivering a child with CL/P for mothers with a low periconceptional folate intake. Therefore, it suggests that it is possible to overcome the effect of the reduced enzyme activity, because of the MTHFR polymorphisms, by increasing maternal folate intake by supplement use and/or in the diet.

Frosst et al.(79)Concluded that MTHFR C677T and MTHFR A1298C polymorphisms are not independent risk factors for cleft lip and palate, but the low periconception folate intake increases the risk of CL/P in the offspring, and this risk is even more pronounced in mothers carrying the MTHFR 677TT or MTHFR 1298CCgenotype. Estandia-Ortega et al (80) in a case control study evaluated the association of NSCL/P with the MTHFR C677T and A1298C polymorphisms and assessed variant-variant and gene environment (maternal folic acid intake) interactions. They considered preconceptional, periconceptional and postconceptional folic acid use and collected DNA samples from blood and buccal swabs. The C677T and A1298C polymorphisms in the MTHFR gene were genotyped. The number of control mothers who took folic acid supplement during the preconceptional, periconceptional, and/or postconceptional periods is greater than the number of case mothers

73.3% versus 47.3%. This study showed that mothers who consumed folic acid supplement during the preconceptional, periconceptional, or postconceptional period were at significantly lower risk of delivering a child with NSCL/P than mothers who did not use folic acid ($P < 0.0001$). The CT and TT genotypes of the MTHFR C677T polymorphism were more frequent in controls than in patients; this showed that children with these two genotypes are at lower risk of NSCL/P than CC homozygotes. Analysis of the MTHFR A1298C variant showed that individuals with the AC and CC genotypes were at no greater risk of NSCL/P than were subjects with the AA genotype ($P > 0.5$). This study showed no variant–variant or gene–environment interaction effect between the MTHFR C677T and A1298C polymorphisms, and maternal folic acid intake during any period (pre-, peri- or postconceptional, each $P > 0.05$), regarding the risk of NSCL/P.

Butali ET, 2013(33) in an individual participant data (IPD) pooled-analysis examined the role of interaction between the MTHFR C667T polymorphism and folic acid in the etiology OFCs. They selected studies that reported the role of interaction between maternal folic acid use and MTHFR C677T genotypes for the IPD pooled-analysis. Their analysis showed that the risk of delivering a child with CL/P is significantly reduced with maternal folic acid use ($p=0.008$). Studies done by van Rooij et al. 2003(78) and Chevrier et al. 2007(71) reported that the risk of having a child with CL/P was found to be reduced in mothers carrying the MTHFR genotypes CT and TT, who gave a history of high dietary folate intake or folic acid supplements.

Asghar Ebadifar et al (81)in a case control study in Tehran showed that children carrying the 677TT variant of the *MTHFR* gene might have an increased risk of CL/P. This risk was higher in mothers who did not use folic acid and this supports the hypothesis which said that folic acid may play a role in the etiology of CL/P. Jugessur et al. (82) performed a case-parent triad study and examined the role of MTHFR variants C677T and A1298C, and their haplotypes, in the etiology of CL/P and CPO. Their finding showed that among CL/P cases, the child's genotype at C677T or A1298C did not influence the risk. However, children of mothers carrying the C677T variant allele had a lower risk of CL/P. For CPO, children carrying the C677T variant allele had about a twofold increased risk, whereas the mother's genotypes did not contribute to the risk.

2.5 Quality of life of patients with orofacial clefts and the perception of their parents

Quality of life (QoL) can be defined as an individual's perception about his or her own position in life from a cultural perspective based on the prevailing system of values, considering objectives, expectations, patterns and concerns(83). Oral health–related quality of life (OHRQoL) is part of HRQoL, which particularly measures the impact of oral disease on the child's physical and social functioning. It is possible to retrieve reliable information from children and adolescents affected with orofacial clefts regarding their own OHRQoL using appropriate questionnaire techniques. (84-86). Jared A. Ward, et al 2013 (87), evaluated the impact of OFCs on the OHRQoL of affected children using the child oral health impact profile (COHIP) questionnaire. They found that the quality of life scores in children with OFCs are significantly lower than the control groups for overall OHRQoL, functional well-being, the interaction of age group and social-emotional well-being. P- value .012, .001, and .048 respectively. They did not find statistically significant differences between the responses of children with orofacial clefts and their caregivers'.

Annemieke Bos, et al (88), assessed the oral health related quality of life (OHRQoL) of Dutch cleft lip, cleft lip and palate patients using the COHIP questionnaire, which consists of two parts one for parents and one for children. The participants were asked to complete these questionnaires. The perception of Patients and parents differed significantly on three of the five subscales ('oral symptoms,' 'emotional well-being,' and 'school' subscales). They found a significant difference in functional wellbeing' subscale between the different cleft types specially the cleft lip without alveolar cleft group had the highest scores. The patients with clefts aged 12 years and older scored significantly lower on the emotional wellbeing and oral symptoms subscales when compared with their younger peers.

Rosany Larissa Brito de Oliveira et al (89)assessed the health-related quality of life (HRQoL) of Brazilian patients with cleft lip and/or palate (CL/P) using the 36 items Short-Form Health Survey (SF-36), which were used in Portuguese, during outpatient visits. In this study the HRQoL scores were similar among the groups. In the vitality domain patients with CL/P scored better than the control and the family. For the emotional role domain, the controls scored higher than the CLP patients and the families. In this study, it was found that age had no effect on the HRQoL of patients with CL/P. This study concluded that orofacial clefts affect the quality of life of the victims and it requires early attention by a multi and interprofessional team. Ana Paula Corrˆea de Queiroz Herkrath, et al (90)in a systematic

literature review and meta-analysis which included 23 articles (seven case control and 16 cross-sectional) found out that twelve reported CL/P had a negative influence at least on one dimension of quality of life. The scores of QoL were mainly influenced by the cleft type, age and gender.

2.6 Conceptual framework

After reviewing several literatures on orofacial clefts we developed the conceptual framework shown in figure 5. It helped us to further improve and develop the study instruments and also assist in the process of analysis. The reviewed literatures indicated that nonsyndromic orofacial clefts are multifactorial in origin. It was shown that environmental factors which includes maternal socio demographic and economic status, maternal life style and exposure and maternal medication use before and during first trimester of pregnancy and genetics factors contribute alone or in combination to the occurrence of NSOFCs (91). Previous researches, which were done at different parts of the world proposed the contribution of many factors, like infections, contact with chemicals/smoking during first trimester of pregnancy and consanguinity(92-94). The role of geographic and ethnic variations in influencing the etiology and prevalence of orofacial clefts has been shown(95).The conceptual framework we developed was also used to show the relationship between history of cleft in the family, the presence of gene locus and the occurrence of OFCs as an out come variable. It was also used to show relationship between the occurrence of OFCs as an out come variable and maternal environmental exposure like diagnostic x-ray, maternal life style like smoking and maternal medication and vitamin use before and during pregnancy as an independent variable. The association between the quality of life (QoL) and NSOFCS was also shown.

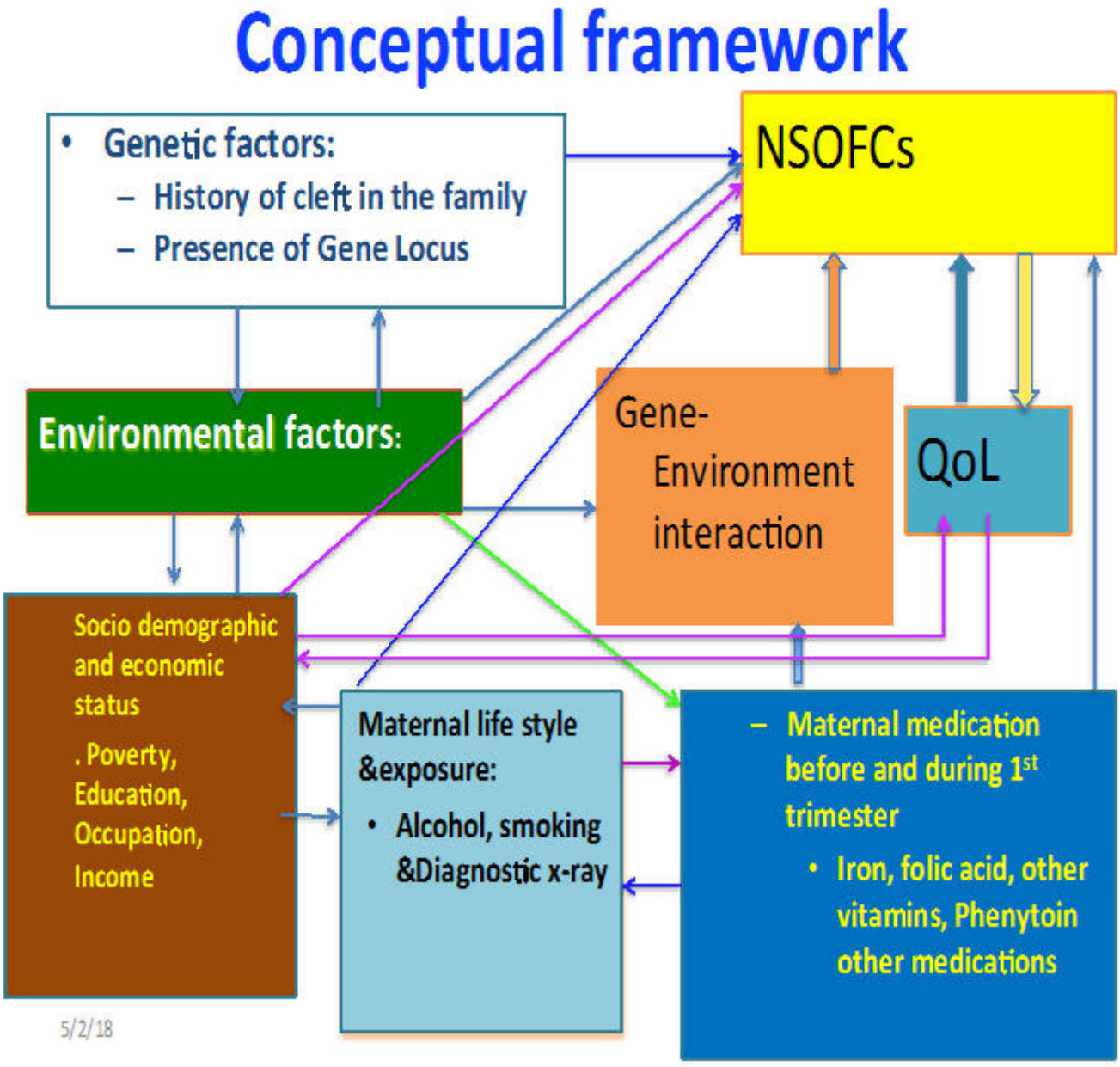


Figure 5: causes and associated factors of cleft lip and palate in the Ethiopian population

3. Research questions

1. What is the incidence and prevalence of orofacial clefts in Ethiopia?
2. Are genetic variants reported in European GWAS and candidate gene studies associated with non-syndromic orofacial clefts in Subsaharn population (Ethiopian, Ghana, Nigeria)?
3. What is the role of environmental factors in the occurrence of orofacial clefts in the Ethiopian population?
4. Does the Oral Health Related Quality of Life (OHRQoL) of Ethiopian children born with NSOFCs and the perception of their parents' differs?

4. Aim of the Dissertation

4.1 General objective

The overall aim of this study is to investigate the genetic and environmental causes of orofacial clefts in the Ethiopian population

4.2 Specific objectives

- To determine the prevalence and incidence of orofacial clefts in Ethiopia (Paper I).
- To taste the contribution of common and rare genetic variants reported in European GWAS and candidate genes studies in the occurrence of NSOFCs in population of sub-Saharan Africa (Ethiopia, Ghana and Nigeria (Paper II).
- To assess the role of environmental factors in the occurrence of orofacial clefts in the Ethiopian Population (Paper III).
- To assess the oral health related quality of life (OHRQoL) of children born with orofacial clefts in Ethiopia and their parents (Paper IV).

5. Materials and methods

5.1 Study area and setting

The main study site was Yekatit 12 Hospital Medical College reconstructive surgery unit in Addis Ababa. Yekatit 12 Hospital is one of the oldest public hospitals, which provides general and tertiary level service for all the disciplines. The cleft center at this hospital is the only center in Ethiopia that provides holistic care for patients with OFCs. The main services provided by the hospital include primary and secondary surgeries, speech therapy, orthodontics care, basic dental care, oral hygiene, ear nose and throat care, pediatrics care and psychological support for the parents for all cleft lip and palate patients coming from all over the country. The annual number of cleft lip and palate patients that were treated and reported to Smile Train during the study period was above 140 patients. In addition, all Ethiopian hospitals that provide cleft surgery on mission basis in collaboration with charity organizations were the site of this study. These are private and public general hospitals, which have the capacity to provide safe surgical care to patients with different diseases, which require surgical treatment. Their ability to provide safe cleft care was assessed by the collaborating charity organization which supports free cleft surgical care. The hospitals conduct cleft surgical campaigns 3-4 times per year and whenever a surgical

campaign is planned it was announced using local medias and brochures also distributed. It was also announced at religious places. This way maximum effort was done to call patients to these partner hospitals for surgical treatment. We also collaborated with the Ghanaian and Nigerian craniofacial anomalies project to get adequate samples to investigate the role of genetic factors in the occurrence of OFCs.

5.2 Study design

We employed a hospital based retrospective descriptive cross-sectional study design and assessed the incidence and prevalence of OFCs in Ethiopia. The data collected from June 2007 to December 2013 on OFCs from the study institutions were used as a numerator to assess the prevalence and the denominator was the total number of Ethiopian population in 2013. To assess the incidence we used the number of cleft patients born during the study period as a numerator and the total number of life births during the study period as denominator. We used unmatched case control study design to taste the contribution of common and rare genetic variants reported in European GWAS and candidate genes studies in the occurrence of NSOFCs in population of sub-Saharan Africa (Ethiopia, Ghana and Nigeria). The cases were all Ethiopian, Ghanaian and Nigerian patients with NSOFCs presented to the study institutions and who consented/assented to participate in this study. The controls were also from the same study institutions presented for treatment other than congenital anomaly of any type. After ensuring the absence of congenital anomalies they were asked to consent/assent for this study and those who agreed included. We used unmatched case control study to taste the contribution of environmental factors: maternal demographic data, vitamins and other medications use, maternal illness, maternal exposure and lifestile to the occurrence of NSOFCs in the Ethiopian population. We assessed the oral health related quality of life of children born with orofacial clefts in Ethiopia and their parents using cross-sectional study design.

5.3 Source and study population

5.3.1 To determine the prevalence and incidence of orofacial clefts in Ethiopia (Paper I).

The source population for this part of the study was all Ethiopian patients born with OFCs and lived in Ethiopia during the study period (June 2007- December 2013). The study population for this study was all patients born with OFCs and received single surgical

treatment during the study period at the mentioned study institutions. We assessed the incidence of OFCs in Ethiopia using the total number of children born with OFCs during the study period (June 2007- December 2013) and who received single surgical treatment at the 31 hospitals distributed throughout the country as a numerator and the total number of life births during this same period as denominator. The total number of patients operated during the study period was used as a numerator to assess the prevalence and the total number of Ethiopian population in 2013 as a denominator.

5.3.2 The source population to identify common and rare genetic variants in candidate genes reported in GWAS associated with non-syndromic orofacial clefts (Paper II)

All Ethiopian, Ghanaian and Nigerian children born with OFCs and presented for treatment to the study institutions and who agreed to take part in this study were recruited, but only children and adolecents born with non-syndromic orofacial clefts (NSOFCs) and their families were selected to determine the role of common and rare genetic variants in the occurrence of NSOFCs in population of (Ethiopia, Ghana and Nigeria) which were implicated in the etiology of NSOFCs in European and Asian population. The controls were Ethiopian, Ghanaian and Nigerian children born without any congenital anomaly who presented to the respected study institutions for any treatment other than congenital anomalies. Pediatricians, maxillofacial surgeons and plastic surgeons examined them to ensure that they do not have any congenital anomaly. The collection of cases was conducted regularly; every time a patient who was born with orofacial cleft came to seek treatment to the main cleft care providing center in Ethiopia (Yekatit 12 Hospital Medical College cleft unit) was assessed for possible inclusion in the study. All patients with NSOFCs and whose parents were willing included in the study. The collection of controls was also conducted on regular basis. The research assistants, collaborators (pediatrician, plastic and reconstructive surgeons) and the principal investigator all are working at this same hospital in the Ethiopian case. Recruitment of controls was conducted at the pediatrician's out patient clinic every week. The research assistants identified the controls whose parents were willing and the principal investigator or collaborators (pediatrician, plastic and reconstructive surgeons) examined the recruited controls to exclude the presence of any congenital anomaly and those who did not have identifiable congenital anomaly included in the study. The same procedure was followed in Ghana and Nigeria.

5.3.3 Source population to assess the role of environmental factors in relation to the occurrence of orofacial clefts (Paper III).

The source population of this study was all mothers of children born with OFCs who brought their children for treatment to Yekatit 12 Hospital Medical College reconstructive surgery unit, but only those mothers who delivered a child with NSOFCs were recruited for this study. The controls were all mothers who brought their children to the same institutions as the cases for treatment other than congenital anomaly of any type, but only mothers who agreed included.

5.3.4 The source population to assess the *oral health related quality of life of children born w*ith OFCs in Ethiopia and the perception of their parents (Paper IV)

The source populations to assess the quality of life of patients born with NSOFCs were all Ethiopian patients born with NSOFCs and presented for treatment to Yekatit 12 Hospital Medical College and Orthodontics unit of Dental School AAU but only those patients who received multididciplinary cleft care provided at the two institutions were included for this study.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

All patients with NSOFCs presented to the study institutions and their families who agreed to participate were included in this study. The controls were also children and parents of controls who where presented for treatment to the same institutions but with out any congenital anomaly.

5.5 Sample size determination

5.5.1 Sample size determination for objective one:

To determine the prevalence and incidence of OFC in Ethiopia we used single population proportion formula. The study was done at 31 hospitals, which are distributed throughout the country. We have calculated sample size required to estimate the incidence and prevalence of OFC in each hospital and then added all the samples to get a nationally representative sample size.

The following table shows the sample size required for each hospital

	Objective	Sample size calculation formula	Confidence level	Power	Proportion (from other studies)	Non response rate	Sample size
1	Prevalence of OFC	Single population proportion formula	95%	80%	Not known in Ethiopia- so assumed 50% to get maximum sample size	10%	422
2	Incidence of OFC	Single population proportion formula	95%	80%	Not known in Ethiopia- so assumed 50% to get maximum sample size	10%	422

As this is a sample size for one hospital, for 31 hospitals the sample size is 422 *31= 13,082

5.5.2 Sample size determination for objective two

We assessed the contribution of genetic factors in the occurrence of NSOFCs in the Ethiopian population using a case control study design. We calculated sample size using sample size calculation for unmatched case control study. The review of the records of the cleft unit at Yekatit 12 Hospital Medical College revealed that, two to three new cleft cases are reported each week. Thus, on average, 140 cleft patients report to the cleft unit yearly (medical records of cleft unit at Yekatit 12 Hospital Medical College 2015). We recruited about 132 case families annually for a period of two years December 2014-December 2016. In all, a minimum of 264 families with NSOFCs was targeted. An online sample size calculator at http://www.surveysystem.com/sscalc.htm was used to calculate the sample size and it was calculated at a confidence level of 95% and a confidence interval of 5.

The sample size was justified for the case-control analyses using Epi tools http://epitools.ausvet.com.au/conent.php. According to Mossey et al (Mossey and Modell, 2012) the prevalence of OFCs in Africans is 0.54 per 1,000 live births. The average odds ratio (OR) for the genotyped SNPs was 2. Considering these observations as well as a confidence level of 0.95 and a power of 0.80, Epitools indicated that the average sample size for the NSOFCs cases only should be 134 whereas the sample size for both control and cases should be 268. Epitools suggested a total sample size of 265 for cases and 357 for controls, giving a total sample size of 622 participants used for this study has at least 80% power to detect associations at an assumed OR of 2 in the Ethiopian population.

We selected and genotyped 48 SNPS based on European GWAS and candidate gene studies. All together 1244 Ethiopian samples were genotyped at these markers. These samples came from 265 NSOFCs, 357 controls and 622 case and control parents. In addition 2626 samples from Ghana and Nigeria that included NSOFC cases, controls and parents were also included to the genotyping cohort to increase the sample size. All together 3,585 sub-Saharan African samples were genotyped: 872 NSOFC cases, 1635 unaffected relatives and 1078 unrelated controls. The 872 NSOFC cases comprised 163 NSCP cases, 340 NSCL cases, 361 NSCLP cases and 8 "un-typed" cases. "Un-typed" refers to samples from case samples that failed quality control checks, which were carried out after genotyping and were therefore not included in the final statistical analyses

5.5.3 Sample Size determination for objective three

To assess the contribution of environmental factors in the etiology of nonsyndromic orofacial clefts we have used sample size calculation for unmatched case control study.
The table below shows the sample size required for this objective

	Exposure	Sample size calculation formula	Confidence level	Power	Case to control ratio	Percent of controls exposed	Odds ratio	Sample size for controls (including 10% non-response)	Sample size for cases (including 10% non-response)
1	Maternal smoking	Sample size for unmatched case control study	95%	80%	1:1	10%	2	338	338

Epitools suggested a total sample size of 359 for cases and 401 for controls, giving a total sample size of 760 participants used for this study has at least 80% power to detect associations at an assumed OR of 2 in the Ethiopian population.

5.6 Sampling procedure

The sampling procedure we followed for the incidence/prevalence part of the study was a census type and we took all the available records from Smile Train database and used to

assess the incidence and prevalence of OFCs in Ethiopia. We collected samples to assess the role of genetics and environmental factors from cases, controls and their mothers at the Yekatit 12 Hospital Medical College. This unit is the only unit, which provides multidisciplinary cleft care for the whole country so we receive patients from all parts of the country. When a child who was born with OFC anomaly presented to the cleft unit of Yekatit 12 Hospital Medical College, the cleft care coordinator recorded the Child's details on the cleft care registry database. Since the research assistants are working at this same unit they were involved in the process immediately. If this was a new born visiting the unit for the first time the unit's social worker provided psychosocial support for the parents and demonstrated on how to feed the neonate. They were informed about the multidisciplinary cleft care provided for free to all patients born with OFCs with the support of a charity organization. They were also informed that it is not a requirement to participate in the study project to get the care. For those who can read and write the participant information sheet, which was prepared in Amharic language was given and also explained to them. For those who cannot read it was explained. The principal investigator and collaborators (Pediatrician, plastic and reconstructive surgeons) examined the patients carefully to excluded additional congenital anomalies. The parents of the patients who did not have any additional birth defect other than NSOFC were asked to participate in the study project and those who agreed included. The controls were recruited from the same hospital pediatrics department out patient clinic and neonatology unit. The research assistants visited the mentioned places once per week and looked for a participant who did not have congenital anomaly of any type and presented to seek treatment for other health problems. The principal investigator and collaborators examined the controls for the presence of congenital anomalies. Those who did not have any congenital anomaly and who are willing to participate included. To assess the oral health related quality of life of patients we recruited all patients who received multidisciplinary cleft care at the Yekatit 12 Hospital Medical College cleft unit and dental department of school of medicine AAU.

5.7 Survey instruments

Research assistants and collaborators recruited. The research assistants were assistant speech therapists with nursing background that have an experience working with children born with OFCs. The collaborators were plastic and reconstructive surgeons, pediatrics surgeon and pediatrician with special interest in cleft lip and palate from the Ethiopian side and

maxillofacial surgeons and nurses from the Ghanaian and Nigerian side. After the recruitment training was organized (at Yekatit 12 Hospital Medical College, the main study center) on how to collect information using the questionnaire, which is adopted from the NigeriaCRAN study for this study. The same procedure was followed in Ghana and Nigeria. Information about giving a unique identifier number (UIN) for each family is provided. The importance of asking all the questions and recording the responses of the mother was emphasized. They were shown how to collect saliva samples from the parents and from the children who can spit using the Oragene collection kits and from the children who cannot spit using oragene sponges. Before the training was concluded they were given opportunities to ask questions and appropriate answers were given.

5.7.1 Survey questionnaire

We adopted and used the NigeriaCRAN study questionnaire for this study. It was translated in to Amharic language, which is spoken, by most of our patients. Professional translator made the translation. The principal investigator and research assistants approached the parents of the index child and invited to take part in the study. Environmental exposure data information obtained from the mothers using a structured questionnaire. The details obtained from each family were date of birth for each member, place of birth, current and previous contact addresses (to elicit information on environmental exposures to chemicals), contact telephone numbers, and nine main environmental exposure variables such as maternal age at delivery, gestational age; educational level; dietary folic acid intake, vitamin supplementation, maternal illness, maternal medication use, maternal tobacco use, and alcohol consumption during the periconceptional period, medical and obstetric history, previous reproductive history and dietary history. In addition, details on the birth weight, multiple births, cleft details (sidedness and involving the lip, alveolus and palate) and other anomalies, were obtained.

We also used the translated child oral health impact profile (COHIP) questionnaire, which consists of children and parent inquiries to evaluate the oral health related quality of life (OH-RQoL) of Ethiopian children born with OFCs and who received holistic cleft care, and the perception of their parents. The COHIP was resulted from an international research conducted in 2001. The aim of this research was to develop a measure for oral health-related quality of life in children, ages 8 to 15 that could be applied cross-culturally (Broder and Wilson-Genderson, 2007). NIH supported this research project (project nr. 1 r21 de13721-

01). Researchers representing nine countries (New Zealand, USA, France, UK, Canada, South Africa, China, Brazil, Netherlands) gathered for the initial adaptation of an existing oral health-related quality of life questionnaire. The resulting questionnaire was then translated (if necessary), and developed further in each country. The researchers met several times to evaluate their results (Broder et al., 2002). In the Netherlands, this process resulted in the Dutch version of the COHIP (Bakker et al., 2004).

We translated and used the Dutch version of the COHIP. The translation was to Amharic language, which is spoken by most of our patients and their families. Two professional translators made the translation independently. Both the translators' mother tongue was Amharic language. One of the translators had an experience in translating medical records like medical certificates. The translation was checked by another professional translator for accuracy, the principal investigator and two other plastic and reconstructive surgeons who have experience working with cleft patients and also involved in cleft and related researches. It was articulated differently for parents and victims, for the parents for example it was articulated like this: In the past three months does your child have pain in his teeth? And for the victim the same question was articulated like this: in the past three months have you had pain in your teeth?

The instrument was validated and its internal consistencies of the overall scale and for all the subscales responses from both the parents and patients were excellent with Cronbach's alpha: .985, .951, .906, .971, .829, .953 for parents, and .979, .933, .961, .948, .678, .979 for children. The questions for the children born with OFCs and their parents consisted of the 38 items of COHIP, which were divided into: Oral symptoms and emotional well being (contained 10 items each), functional well being (eight items), school (four items), and peer interaction (six items). The items were answered on a 5-point likert scale (1=Very often and 5=Never, with the additional response option of 0=I don't know). Poor oral health-related quality of life was indicated by low response. The general health of the child was assessed by one more additional question, which was added to both the parent and the child questionnaire. It had the following response categories: 1= Bad, 5=great and 0 =I do not know. The patients and the parents completed the measures in separate rooms with the principal investigator and research assistant orienting both the children and parents to the questionnaire and assisted them whenever they had difficulty. A high response showed good oral health-related quality of life.

5.7.2 Codes

A unique identifier number (UIN) was assigned for every family (case and control) who agreed to take part in this study. The number begins with ET to indicate that the sample comes from Ethiopia followed by the family number. For example, the first family recruited is coded as ET001. This way confidentiality was maintained during the study and the participants' details remained anonymous. It means the laboratory at Iowa where DNA was extracted and analysis done did not know the identity of the participant. These codes were used to link the information the participant provided and the DNA analysis and stored in the database where only authorized personnel can access. The same procedure was followed both in the Nigerian and Ghanaian cases and control samples.

5.7.3 Saliva Sample collection

After the consent form was signed, the mothers were interviewed using the study questionnaire and saliva sample collected. Saliva samples were obtained from the cases, controls, mothers of cases and controls and when available from the fathers, labeled with the unique identifying number (UIN) for each member of the family and sent to Iowa University, USA for DNA extraction and analysis. The details of the cleft lip and or palate patients and the controls participating in this study were registered prospectively and record kept at the Yekatit 12 Hospital Medical College cleft unit library. The following records were entered on line into the Iowa University Redcap database: the unique identifying number (UIN), date of birth, the type of sample collected from the child and the mother, questionnaire and the date the sample collected. The principal investigator and the collaborators at Iowa University can access the redcap data.

5.7.3.1 Collection of saliva from the parents and children who can spit

The Mothers and children who can spit were asked to rinse their mouth with drinking water, and after 5 minutes given the DNA self-collection orange tubes and they spat into the tube. We made sure the tube is empty has a white lid cap containing oragne liquid sealed with a plastic film and labeled with the right ID before they spat into it. (Figure7). They were asked to spit into the tube until it reaches the fill line as it is shown on the figure below. Then the lid was closed by firmly pushing the lid until a loud click was heard. The liquid in the lid was released into the tube to mix the saliva. After the lid is closed tightly the saliva was mixed with the orange fluid by shaking.

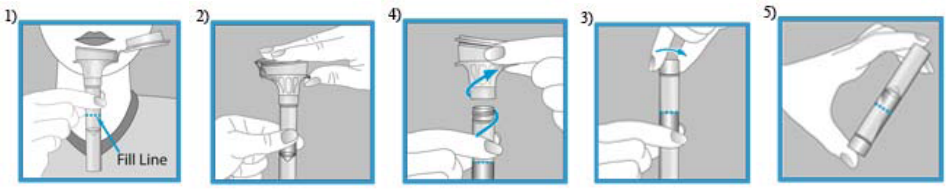


Figure 6:**Devices for saliva collection and steps in collection from parents and children who can spit**

5.7.3.2 Collection of saliva from infants and young children who cannot spit

A saliva sample from infants and children was collected using saliva sponges, a scissor and oragene self-collection kit. The collection was done at the operation room before the beginning of surgery and at the speech therapy unit by the principal investigator and research assistants. The sponges have a long handle with a narrow end attached to the sponge and come in packs of five. The sponges were placed into the child's cheek pouch along the gums and inner cheeks to soak up as much saliva as possible (Figure 7 A). We used 10 sponges (two packs) for each child to increase the yield. Each saturated sponge was inserted in V-notch of funnel and saliva wringed out against the inner wall of the V-notch or cut at the narrow end of the handle and inserted in to the tube (Fig 7 B). Saliva will flow into the tube. The lid was closed tightly by firmly pushing down until we heard a loud click (Fig 7 C). The funnel was unscrewed and the tube was closed tightly using the small cap, which is found in the collection kit (Fig 7 D). We shook the capped tube for 5 seconds. The procedure is done 30 minutes before or after the child is fed for those from whom saliva was collected at the speech therapy unit.

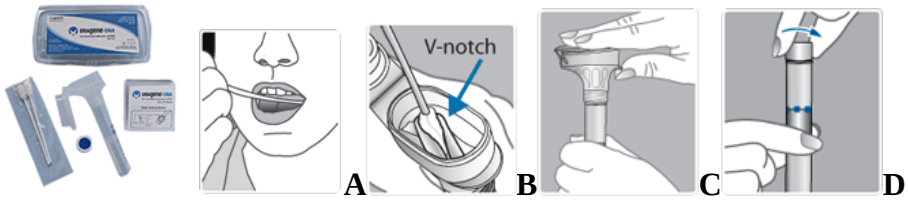


Figure 7:Saliva **collecting sponges and steps A,B, C, D for children who can not spit**

The collected saliva samples were accurately coded. All had UIN on the container and on the tubes and kept at room temperature at the allocated room in the speech therapy unit until transfer to Iowa, USA.

5.7.3.3 Transfer of saliva samples

The collected samples were accurately labeled with the unique identifier number both on the tubes and on the container. The national research and ethics review committee wrote a letter to the customs authority and we were permitted to ship the saliva samples. (Annex 2). The principal investigator provided a letter, which states that the specimen is not infective (annex 3) and samples shipped via FedEx to Iowa University Butali's laboratory for DNA extraction and analysis. This was the procedure followed every time sample was shipped.

5.7.3.4 Sponge samples

All fluid from the saliva container is transferred into a 15ml conical tube. A barrel of a 3-5 ml syringe was placed into the same 15 ml conical tube and the sponges were transferred into the syringe barrel using forceps. The 15ml conical tube containing the syringe barrel and sponges were spanned at 1000 rotation per minute (RPM) for 10 minutes and the sample was made ready for proceeding along with the regular saliva samples.

5.7.3.5 Saliva samples

The saliva sample was transferred into a 15-ml conical tube that is already labeled with 1-20 the sample name was verified from the vial it corresponded to the number on the processing sheet. Samples were incubated in a 50°C water bath for one hour and Oragene Purifier (40 ul /1 ml sample) was added to each tube and mixed by vortexing for a few seconds. The sample incubated on ice for 10 minutes and centrifuged at room temperature for 10 minutes at a minimum of 3500 g's. Another set of conical tubes was labeled and the clear supernatant was carefully transferred to a new 15 ml tube with the same number on it. Example: 1 to 1, 2 to 2 etc. The pellet was discarded. An equal volume of 100% EtOH was added and mixed by inverting 10 times. Strands of DNA formed. The strands of DNA were kept at room temperature for 10 minutes and centrifuged at room temperature for 10 minutes at a minimum of 3500 g's. The supernatant was carefully removed without disturbing the DNA pellet. The tubes turned upside down on a Teri wipe and all the ethanol drained from the pellet. The DNA pellet was rehydrated with 1 ml of elution buffer for saliva samples and 750ul for cheek swab samples. The bottom of the tube was moved to loosen the pellet and left on the bench for overnight at room temperature. The DNA was transferred into a 1.5 ml eppendorf tube. The DNA was spinned down at room temperature for 15min. @ 14000 g's and any remaining turbid material was removed. The DNA sample, which was labeled with

processing ID and divided evenly, was transferred into 2.0 ml screw cap freezer tubes and made ready for analysis (Figure 8).

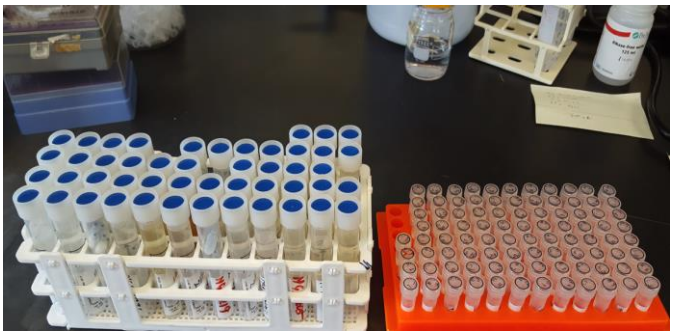


Figure 8:**Saliva samples and DNA samples**

5.7.4 Sample analysis

5.7.4.1 DNA Dilution

It is important to dilute the DNA before any type of analysis. The dilution is made depending on the amount of volume required for a specific analysis. For genotyping and sequencing, the amount of DNA concentration required was 20ng/ml. All the samples were made to have the same concentration using the formula below: C1V1=C2V2. Where C 1 was the measured concentration on the tube, V1 volume of DNA, C2 is the desired concentration and V2 is the desired final volume.

5.7.4.2 DNA quality

The DNA quality was checked using 1% agarose gel. 2-3ul of each sample was transferred to a PCR plate and 2ul of loading dye rich in glycerol was added and run in a 1% agarose gel at 200V for 30 min and picture taken (figure 9).

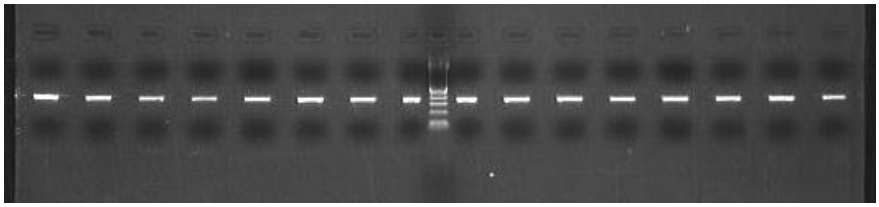


Figure 9: **Picture of DNA sample on agarose gel.**

5.7.4.3 Polymerase chain reaction (PCR)

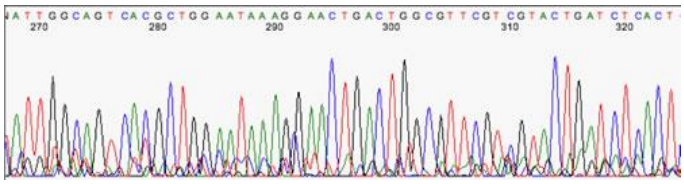
A large quantity of DNA was produced using PCR and made ready for analysis. PCR amplifies a particular region of a DNA strand. The DNA strand to be amplified is added to a solution, which contains buffer. The solution promotes Taq usage and deoxynucleotide

triphosphates (dNTP's). These were incorporated into the newly synthesized DNA strands during the extension step of PCR. Taq polymerase facilitates strand synthesis and oligonucleotide primers anneal to the template strand of DNA allowing the Taq Polymerase to synthesize a strand complementary to the template strand. These solutions were combined in a conical tube and mixed well. The PCR machine was made ready. Reagents were removed from freezer boxes and allowed to defrost and vortexed well. Plate was made ready and labeled with appropriate information. 1 ul of DNA was added to each well of the plate using the multichannel pipeter. Master Mix was made by combining the appropriate amounts of each reagent and kept in a tube labeled master mix. The master mix was mixed well by pipetting up and down with the p200 set at 200ul. The master mix was distributed to the plate with a motorized pipetman. The plate was sealed with the plastic PCR covers; plate sealer was used around all the edges. The plate was put in the prepared machine. The program was edited and the gradient needed selected and the PCR reactions run. This process involves three steps. The first step is denaturation, which separates the double strands and it is accomplished in 30 seconds at a temperature of 94°c. The second step is annealing, during this step the primers bind to DNA. It happens in 30 seconds at a temperature of 68°c. The final step is extension during this step a new DNA strand is synthesized extending from each primer along the template. This also happens in 30 seconds at a temperature of 72°c.

5.7.4.4 DNA sequencing

Polymerase chain reaction performed as described above, and the DNA sample amplified. The quality of the amplified DNA product was reviewed using the alpha-imager software and the amplified product of DNA with good quality was sent to the functional biosciences Inc. laboratory in Wisconsin for sequencing. The exact order of nucleotides within a DNA molecule is determined by DNA sequencing. DNA sequencing includes any method or technology that is used to determine the order of the four bases –adenine (A), guanine (G), cytosine (C) and thymine (T) in a strand of DNA. A program called CONSED was used for viewing, editing, and finishing DNA sequence assembly. It was originally developed for sequence assemblies created with phrap, which is a program widely, used for DNA assembly. The sequencing chromatogram was down loaded and examined for its quality. We saw evenly spaced peaks, each with only one color. Peak heights varied 3-fold, which is normal (Figure 10).

figure 10 DNA Chromatogram



5.7.4.5 DNA Genotyping

Genotyping of case and control samples was performed using Taqman technology and family based association was investigated. A sample with a volume of 30 ul on a plate with a concentration of 5ng/µl DNA was made. The volume of DNA needed to make 60 ml dilution was calculated using this formula: C1V1= C2V2. Where C1 is the new DNA concentration =5ng/ml, V1 is the volume of the new DNA required =60µl, C2 is the previous DNA concentration = 5ng/ml and V2 is the desired final volume. V2=C1V1/C2, V2= 2*60/20. Thus V2=6ul.The multi-channel pipette was used to distribute 6ul of 20ng/µl into each well of a new 96-PCR plate. To make a 2ng/ml dilution 54ml of DDH20 was added. This was done for all the case and control family samples.

The Hydra II machine and the control mate software, the 96 well plates for proband cases, proband controls, case mothers and control mothers were used to distribute into a 384 well plate. The samples of proband cases were dispensed into the first well of the 384 well plates, proband controls into second well, case mothers into 3rd and control mothers into the fourth well. Targeted genotyping was carried out to examine the significance of candidate genes like MSXI, BMP4, FOXE1 and GWAS (IRF6, MAFB, VAX1, ARHGAB29, PAX7) significant genes for orofacial clefts in the study population using 13 markers. The Taqman PCR reaction for all the markers at initial phase was 95 ^{o}C for 10 mins, denaturation phase 92 ^{o}C for 15 seconds over 50 cycles, and anneal/extension phase 60^{o}c for 1 min and a hold of 4^{o}c. The number of cycles was increased to 70 for some plates. The plates were read using the 7900 machine after they have been through the PCR step, which was controlled by SDS 2.2 software. The door of the machine was opened and the plate was loaded in its holder after checking the orientation of A1, which was in the left upper corner. The machine read allelic discrimination, and allelic end point in a plot diagram with the FAM dye on the x-axis and Vic dye on y-axis. For each sample in an allelic discrimination assay, unique pair of fluorescent dye detectors was used, for example, two Taqman® MGB (minor groove binder) probes that target SNP site(96, 97). One fluorescent dye detector is a perfect match to the

wild type (allele 1) and the other fluorescent dye detector is a perfect match to the mutation (allele 2).

The allelic discrimination assay classified unknown samples as:

• Homozygotes (samples having only allele 1 or allele 2)

• Heterozygotes (samples having both allele 1 and allele 2)

The allelic discrimination assay measured the change in fluorescence of the dyes associated with the probes. This allowed for calls to be made on samples that are either heterozygous or homozygous for the vic and fam in each marker. All the files with samples and markers were imported into Progeny to check for Mendelian errors and discrepancies in each file. The document was saved in an Ethiopian folder name.

5.8 Study variables

5.8.1 Dependent variable

The birth of a child with NSOFC

5.8.2 Independent variables

Environmental exposure variables: dietary folic acid intake, vitamin supplementation, maternal illness, maternal medication use, maternal tobacco use, and alcohol consumption during the periconceptional period, Exposure to diagnostic X-ray medical and obstetric history, previous reproductive history, family history of OFCs and dietary history

5.9 Operational Definitions

- Child Oral Health Impact Profile (COHIP) - is a 38 question survey designed to measure self-reported Oral Health Related Quality of Life (OHRQoL) in school age children ages 8-15 years
- Oral Health Related Quality of Life (OHRQoL)- is the impact of oral health on quality of life
- Prevalence - The total number of Ethiopian patients with OFCs who received single surgical treatment and registered at the Smile Train data base from June 2007 to December 2013 as numerator and the total number of Ethiopian population in 2013 as denominator multiplied by 1000.
- Incidence -The total number of children born with OFCS during the study period (June 2007-December 2013) as numerator and the total number of life births during

the study period as denominator. Data of live births during the study period and total number of population were projected from the 2007 Ethiopian census.

- Orofacial Cleft (OFC)- the second commonest congenital anomaly in humans and the most common congenital anomaly in the head and neck region which affects the face and oral cavity. It can be syndromic (SOFC) when it is associated with other birth defects and non syndromic (NSOFC) when it occurs with out any identifiable birth defect
- Candidate Gene-A candidate gene is a gene, located in a chromosome region suspected of being involved in the expression of a trait such as a disease.
- GWAS- An analysis of allelic association for genes throughout a genome
- Environmental Factors –Factors to what the case or control are exposed
- Congenital Birth defect – is a defect present at birth on any part of the body different from what we know as normal**.**

5.10 Data management

5.10.1 Data quality control

Research assistants and collaborators were recruited. They were professionals working with patients born with orofacial clefts. The research assistants were assistant speech therapists with nursing background that have an experience working with children born with OFCs. The collaborators were plastic and reconstructive surgeons, Pediatrics surgeon and pediatrician with special interest in craniofacial anomalies (cleft lip and palate). After the recruitment of research assistants and collaborators we organized and conducted intensive training at Yekatit 12 hospital medical college for research assistants and collaborators. The questionnaire, which was, adopted from the Nigerian craniofacial (NigeriaCRAN) anomalies study, was translated into Amharic language and used to collect data on the environmental exposure of the mothers. The training included introducing this questionnaire to the research assistants and collaborators. The same procedure was followed in Ghana and Nigeria. Information about giving a unique identifier number (UIN) for each family is provided. The importance of asking all the questions and recording the responses of the mother was emphasized. They were shown how to collect saliva samples from the parents and from the children who can spit using the Oragene collection kits and from the children who cannot spit using Oragene sponges. Before the training was concluded they were given opportunities to ask questions and appropriate answers were given.

The collected data was interned to a REDCap (Research Electric Data Capture) which is is a data management tool that provides a secure multi user web based interface for storing study information and also registered on a hard copy, this way the reliability of the collected information was assured. The collected saliva samples were, stored and transported to Iowa University, USA in line per the international guidelines. Analysis of the saliva samples (DNA processing, sequencing and genotyping) was conducted at the university of Iowa genetic laboratory, which is one of the best genetic laboratories in the world. The principal investigator regularly supervised the research assistants and collaborators. The same procedure was followed in Ghanaian and Nigerian samples and data collection.

5.10.2 Data entry

Data entry was carried out by an experienced data entry clerks at all study sites with close supervision of the principal investigator. The principal investigator conducted data cleaning. The data were entered using XL spread sheet. Before analysis the data were cleaned, prepared and exported to SPSS version 20 for further analysis.

5.11 Data analysis

The dataset analyses to find the incidence/prevalence of OFCs in Ethiopia was based on all Ethiopian cleft patients surgically treated at the 31 hospitals distributed through out the country with the support of Smile Train from June 2007 to decemeber 2013. The denominator to assess the prevalence was the number of Ethiopian population in 2013 projected from the 2007 Ethiopian census. We used as numerator all Ethiopian cleft lip and or palate patients operated at the mentioned hospitals during the study period. The incidence was calculated using the number of cleft patients born and operated during the study period as numerator and the total number of live births during the study period as denominator. Both, incidence and prevalence are based on the information at hand. If someone was not able to attend one of the hospitals during the study period, he/she is not included in these counts and therefore, the incidence and prevalence might be underestimated. It also did not include abortions and stillbirths. The statistical analyses included cross-tabulations and tests for proportions. We presented in tables how many people are in each category (bilateral CLP (BCLP), unilateral CLP (UCLP), bilateral cleft lip only (BCLO), and unilateral cleft lip only (UCLO). The unilateral CLP and UCLO were further classified into right and left. We also

determined if the proportion of children in one category was the same with the proportion in another category.

We conducted case- control analyses to determine associations in each subpopulation and meta-analyses of the 3 subpopulations. Meta analysis was conducted to account for population stratification due to variation in allele frequencies. For this test, we used $P < 0.05$ to denote nominal association. There might be a nominal association with $p<0.05$ when there is no actual association because of multiple testing, to account for this a Bonferroni correction was done. It is used to determine a threshold (true) association for formal significance of $P = 0.000354$. The 141 tests comprised 47 SNPs that passed HWE X 3 cleft sub phenotypes X 1 racial group x 1 test. Of the 48 SNPs, only 1 failed HWE ($P < 0.05$). Additional analyses to determine over transmission of the rare alleles were conducted with the transmission disequilibrium test (TDT) and through the family-based association for disease traits (DFAM).

In order to assess the role of environmental factors in the occurrence of orofacial clefts in the Ethiopian population we collected data using standardized questionnaire. The collected data was entered into excel worksheet cleaned then transferred to SPSS version 20 for analysis. Descriptive summaries such as frequencies, percentages and proportions were determined and presented in tables. To identify variables which contribute to the occourence of NSOFCs first bivariate binary logistic regression analyses were carried out and candidate variables for multivariable model at p-value <0.05 were determined. We then identified the significant predictors of NSOFC occurrence by enterning variables that were associated with the occurrence of NSOFCs in the bivariate models at P-value<0.05 in the multivariate logistic regression model.

We performed statistical analysis using SPSS 20 for data entry and producing analysis in order to assess the oral health related quality of life of children born with NSOFCs and the perception of their parents. Internal consistencies of the overall scale and for all the subscales responses from both the parents and patients were examined by defining Cronbach's alpha. We summed up the responses of all items of each subscale and determined the subscale scores of each subscale and summed up the subscale scores to determine the overall OH-RQoL Score. Both the parents and patients answered all the questions. Comparing their overall and subscale scores using independent sample t-test determined the similarity between parents and patients. We calculated Pearson correlations

coefficients and intraclass correlation coefficients (ICCs) between subscales of parents and patients.

5.12 Ethical consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) College of Health Sciences Addis Ababa University, from the National Research Ethics Review Committee (NRERC) cand written permission from Addis Ababa Regional Health Bureau (AARHB), copy of letters of clearance were annexed.

5.12.1 Consent and voluntary participation

The purpose of the research, how it is going to be conducted, the benefits, confidentiality, risks, the right to withdraw and the incentive was verbally communicated and also given in written. Participants were informed that they have the right to participate or not in this study and their decision wouldn't affect the care and services they receive in any part of the health system. They have been informed that they have the right to withdraw from participation at any point during their participation if they want to withdraw. Participants were encouraged to ask any concerns or question they may have before agreeing to participate in the study. Data collectors also ensured that participants fully understand about the study before getting consent. The participants were given the information sheet and consent forms, to sign if they agree. The Research assistants collected the completed consent forms prior to data collection.

5.12.2 Privacy and confidenciality

All information collected in this study was given code numbers and was not linked to any participants' name. All data collected was kept in a locked shelf and password protected electronic version to prevent access from unauthorized person. All DNA samples were coded and any name and personal identifier was removed from the sample prior to transportation to Iowa Univesity USA genetic laboaratory for analysis. This way confidentiality was secured. All participants were interviewed in a separate room to secure privacy.

5.12.3 Benefit and harm

The participants were informed that they are not going to get special benefit from participating in this study but other people might benefit from this study by learning some of the causes of OFCs. This would help devise strategies to prevent and predict these conditions in the future, as well as give a better understanding of typical development.

During saliva collection from participants who cannot spit there might be a risk of infection but using sterile technique minimized this and this was communicated to the parents. The participants were informed that their saliva samples will be sent to Iowa University, USA for DNA extraction and analysis, but the identifiers such as name, date of birth, address, etc will be stripped from the samples. This was communicated to the parents.

The purpose of the study and the right of the patient to say no to participate in this study without any affect to their treatment is explained throughly. The same procedure was followed in Ghana and Nigeria. The IRB College of Health Sciences Addis Ababa University, approved the consent form during the ethical approval process meeting No: 053/2013 and protocol number: 003/10/Surg. and the NRERC approved the consent form during the ethical approval process, meeting No: 3.10/790/06, date 19/10/06 EC. The summary of study design and participants was shown in Table 1.

Table 1: Summary of study designs and participants

Paper	Title (or problem addressed)	Study design	Participants and sample size	Data collection period
I	Prevalence/Incidence of ORFCs in Ethiopia	Hospital based Descriptive study	18,073 Patients with OFCs who received single surgical treatment at 31 hospitals distributed throughout the country, out of the total operated patients with OFCs 8,232 are under seven years old. The total number of live births during this period was used as denominator to find the incidence. The 2007 Ethiopian census was used as denominator to find the prevalence of OFCs	We retrieved data of operated patients at the mentioned hospitals from Smile Train data base from June 2007 –December 2013
II	The role of common and rare genetic variants reported in European GWAS and candidate genes studies in the occurrence of NSOFCs in population of sub-Saharan Africa (Ethiopia, Ghana and Nigeria	A hospital based case - control analysis and meta-analysis.	A total of 3585 samples (872 NSOFCS cases, 1,635 unaffected relatives and 1,078 unrelated controls)	We collected data of OFC patients, their families, controls and their families from December 2014-December 2016
III	The Role of environmental factors in the Occurrence of NSOFCs in the Ethiopian population	Retrospective hospital based case control study design	A total of 359 case mothers and 401 control mothers participated in this study	Environmental factors: Maternal exposure and life style, maternal demographic data, maternal vitamin and medication use, maternal illness of NSOFC patients and control mothers collected from December November 2012 to January 2016.
IV	Oral health related quality of life of Ethiopian children born with OFCs and fully rehabilitated and the Perception of their Parents	Hospital based descriptive study design	In this study 41 patients with NSOFCs and their parents participated	The data of patients treated and followed from 2008-2016.

6. Main Findings

6.1 Paper I: Descriptive epidemiology of OFCs in Ethiopia

The prevalence and incidence of orofacial clefts in Ethiopia was estimated using the smile Train database and the 2007 Ethiopian census. The number of patients born with OFC patients and received single surgical treatment during the study period (June 2007-December 2013) was18073. During this time 8,232 patients were born and received single surgical treatment. Incidence was calculated using this as numerator and the total number of live births during the study period, which was 18,811,316 (projected from 2007 sensus) as denominator and gave 44/100,000 live births. The prevalence was estimated using the total number of clefts operated during the study period (N=18,073) as a numerator and the total number of population (N= 88,703,914) in 2013 as a denominator. It is estimated to be 20/100,000 populations. Most of the individuals examined had unilateral cleft lip only (UCLO). Cleft palate only was the least finding in this study. Table 2.

Table 2 Laterality distribution of the cleft types by gender

	BCLP	UCLP	CPO	BCLO	UCLO	Total
Female	344	1185	248	764	3895	6436
Male	902	2201	293	1214	6958	11568
Total	1246	3386	541	1978	10853	18004

BCLP- bilateral cleft lip and palate, BCLO- bilateral cleft lip only, CPO- cleft palate only, UCLO- unilateral cleft lip only, UCLP- unilateral cleft lip and palate.

The differences in proportion were tested and showed that the proportion of bilateral cleft lip and palate is smaller than the proportion of unilateral, right and left cleft lip and palate (all p-values <0.0001). It also shows that the proportion of left cleft lip and palate is smaller than the proportion of right cleft lip and palate (p value 0.00367). The proportion of bilateral cleft lip only is smaller than the proportion of right, left and unilateral cleft lip only (all p-values <0.0001). It was also showed that the proportion of left cleft lip only is bigger than the proportion of right cleft lip only (p-value <0.0001). Table 3

Table 3 Tests for diffeence in proportions

Test	Percent of first category	Confidence Interval	Exact p-value*
BCLP vs UCLP	26.90	(25.63, 28.20)	<0.0001
BCLP vs LCLP	43.66	(41.83, 45.50)	<0.0001
BCLP vs RCLP	41.20	(39.44, 42.98)	<0.0001
LCLP vs RCLP	47.49	(45.80, 49.19)	0.00367
BCLO vs UCLO	15.42	(14.79, 16.05)	<0.0001
BCLO vs LCLO	20.21	(19.41, 21.02)	<0.0001
BCLO vs RCLO	39.40	(38.05, 40.77)	<0.0001
LCLO vs RCLO	71.97	(71.12, 72.81)	<0.0001

*Significance probability (p-value) associated with the test of the null hypothesis that equal proportions (50%) of subjects were found in the two cleft subcategories specified, assessed by the exact binomial test.

6.2 Paper II: Association of loci implicated in the occurrence of NSOFCS in Asian and European population in Sub-Saharan African population (ETHIOPIA, Ghana, Nigeria)

For this part of the study we collaborated with the Nigerian and Ghanaian craniofacial anomalies study projects and recruited 3,585 participants. The participants were 872 cases of NSOFCs, 1635unaffecrted relatives and 1078 controls. The case probands were composed of 423 males and 441 females, whereas unrelated controls were made up of 441 males and 637 females. The cleft subphentypes were 163 non-syndromic cleft palates only, 340 non-syndromic cleft lips only 361 non nsyndromic cleft lip and palate and 8 untyped. Table 4. Some of the parental samples failed data cleaning and were dropped from statistical analyses. These families were labeled as singletons and were informative in the case-control arm of our study. In families where two individuals were affected we collected tetrads and pentads. The dyads and trios refer to case-mother-maternal grandmother trios, case-mother-sibling trios, as well as case-siblings trios and dyads. In the case control analysis case probands and unrelated controls were included.

Table 4 Subphenotypes and Sample Types of Study Cohort

Cleft subphenotype of participants	Samples per Population,			
	Ethiopia	Ghana	Nigeria	Total
NSCL	101	162	77	340
NSCLP	143	144	74	361
NSCPO	21	102	40	163
Unrelated controls	357	408	313	1078
	Case Parent trios			
NSCL	2	52	20	74
NSCLP	3	48	26	77
NSCPO	1	34	7	42
	Case Parent dyads			
NSCL	84	77	51	212
NSCLP	134	76	47	257
NSCPO	20	53	32	105
	Other trios			
NSCL	0	18	0	18
NSCLP	0	14	0	14
NSCPO	0	11	0	11
	Other dyads			
NSCL	0	8	0	
NSCLP	0	3	0	
NSCPO	0	3	0	
	Singltons			
NSCL	13	5	6	24
NSCLP	8	1	1	10
NSCPO	0	2	1	3
	Tetrads			
NSCLP	0	2	0	2
	Pentads			
NSCLP	0	1		1

NSCL, nonsyndromic cleft lip; NSCL/P, nonsyndromic cleft lip with or without cleft palate; NSCLP, nonsyndromic cleft lip and palate; NSCPO, nonsyndromic cleft palate only.

We conducted case- control analyses to determine associations in each subpopulation and meta-analyses of the 3 subpopulations. Meta analysis was conducted to account for population stratification due to variation in allele frequencies in each population. For this test, we used $P < 0.05$ to denote nominal association. There might be a nominal association with $p<0.05$ when there is no actual association because of multiple testing; to account for this a Bonferroni correction was done. It is used to determine a threshold (true) association for formal significance of $P = 0.000354$. The 141 tests comprised 47 SNPs that passed HWE X 3 cleft sub phenotypes X 1 racial group x 1 test. Of the 48 SNPs, only 1 failed HWE ($P < 0.05$.

In the Ethiopian subpopulation PAX7 (rs742071, P = 0.005574,OR=1.329 and 95%CI 1.087-1.626), IRF6 (rs642961, P =0.01508;OR= 1.442; 95% CI 1.072-1.94), DYSF (rs2303596, P = 0.00231;OR= 0.6854; 95%CI 0.5371-0.8747), 8q24 (rs987525, P =0.000782; OR= 1.413; 95%CI 1.154-1.73), were found to be Nominally associated with the occurrence of NSCL/P Table 5. In addition MAFB (rs13041247, P=0.04303; OR= 0.7994; 95%CI 0.6434-0.9932 and rs11696257, P=0.03628;OR=0.7929, 95%CI, 0.6379-0.9855) were found to be nominally associated with NSCL/P (Table 5).

SNPS in ABCA4 (rs481931 and rs4147811 were found to be nominally associated with the occurrence of NSCPO in the Ethiopian population. SNPS in NTN1 (rs8081823, P = 0.03251; OR=0.4905, 95% CI 0.216-1.114) were also found to be nominally associated with NSCPO Table 6. We performed subphenotype analysis of the Ethiopian NSCL/P cohort and found that the PAX7, DYSF, MSX1, SPRY2 (rs9574565, p=0.00705) & MAFB signals were mainly found to be associated with non-syndromic cleft lip only, whereas the IRF6 (rs642961, p=0.00911) and 8q24 (rs987525, p=0.00107) signals were stronger for NSCLP. In the 7 genes, we sequenced rare and novel variants were observed but de novo occurrences were not demonstrated. All VAX1 variants were occurred in controls but none of the ARHGAP29 variants occurred in controls.

Table 5. Analysis of NSCL/P\ case control Cohorts from Ethiopia

SNP	**Probable Gene Loci**	A1	F_A	F_U	A2	OR	L95	U95	P
rs1801131	**MTHFR**	1	0.3429	0.3211	2	1.103	0.892	1.364	0.3655
rs1801133	**MTHFR**	2	0.1102	0.1132	1	0.9706	0.7052	1.336	0.8548
rs766325	**PAX7**	2	0.2327	0.2517	1	0.9012	0.7117	1.141	0.3876
rs742071	**PAX7**	2	0.5369	0.4659	1	1.329	1.087	1.626	0.005574
rs560426	**ABCA4**	2	0.4429	0.4444	1	0.9936	0.8119	1.216	0.9502
rs481931	**ABCA4**	2	0.102	0.1071	1	0.9478	0.6815	1.318	0.75
rs4147811	**ABCA4**	2	0.102	0.1083	1	0.9352	0.6727	1.3	0.6904
rs138751793	**ARHGAP29**	2	0.002041	0.003476	1	0.5862	0.07041	4.881	0.6173
rs6677101	**SLC25A24**	2	0.3422	0.3677	1	0.8944	0.7242	1.105	0.3001
rs861020	**IRF6**	1	0.2714	0.2358	2	1.207	0.9612	1.517	0.105
rs34743335	**IRF6**	2	0.0551	0.06917	1	0.7848	0.5101	1.207	0.2691
rs642961	**IRF6**	1	0.1429	0.1036	2	1.442	1.072	1.94	0.01508
rs7590268	**THADA**	1	0.2408	0.2569	2	0.9174	0.7264	1.159	0.4691
rs4332945	**DYSF**	1	0.3245	0.3426	2	0.9216	0.7443	1.141	0.4542
rs2303596	**DYSF**	2	0.2723	0.2041	1	0.6854	0.5371	0.8747	0.002306
rs227782	**DYSF**	1	0.3469	0.3876	2	0.8394	0.6806	1.035	0.1015
rs115200552	**MSX1**	1	0.01434	0.009849	2	1.463	0.6032	3.548	0.3972
rs12532	**MSX1**	2	0.3673	0.354	1	1.06	0.8602	1.305	0.5862
rs2674394	**Gene desert**	1	0.1918	0.1999	2	0.9502	0.7371	1.225	0.6933
rs651333	**TULP4**	1	0.3878	0.4038	2	0.935	0.7613	1.148	0.5217
rs6558002	**EPHX2**	1	0.4592	0.479	2	0.9234	0.755	1.129	0.438
rs987525	**8q24**	1	0.5594	0.4733	2	1.413	1.154	1.73	0.0007822
rs894673	**FOXE1**	1	0.3258	0.3432	2	0.925	0.747	1.145	0.4747
rs3758249	**FOXE1**	2	0.3272	0.3428	1	0.9322	0.7526	1.155	0.5199

Table 5
continuation

rs7078160	**VAX1**	1	0.1996	0.1855	2	1.095	0.8499	1.411	0.4822
rs4752028	**VAX1**	1	0.4082	0.4113	2	0.9873	0.805	1.211	0.9024
rs10785430	**ADAMTS20**	2	0.3094	0.3043	1	1.024	0.824	1.274	0.8281
rs9574565	**SPRY2**	2	0.4177	0.4219	1	0.9828	0.8014	1.205	0.868
rs8001641	**SPRY2**	1	0.3143	0.2851	2	1.149	0.9245	1.428	0.2103
rs17563	**BMP4**	2	0.3327	0.3353	1	0.9883	0.7987	1.223	0.9137
rs1258763	**GREM1**	1	0.3857	0.4222	2	0.8594	0.6997	1.055	0.1482
rs8049367	**ADCY9**	1	0.3327	0.3343	2	0.9926	0.8022	1.228	0.9456
rs16260	**CDH1**	1	0.1516	0.1385	2	1.112	0.8378	1.475	0.4634
rs11642413	**CDH1**	1	0.4796	0.4576	2	1.092	0.8934	1.335	0.3891
rs1546124	**CRISPLD2**	2	0.4037	0.4234	1	0.9218	0.7513	1.131	0.4351
rs4783099	**CRISPLD2**	2	0.3898	0.3939	1	0.9828	0.8	1.207	0.8685
rs8069536	**NTN1**	2	0.2265	0.2203	1	1.036	0.8149	1.318	0.7714
rs8081823	**NTN1**	1	0.2735	0.3031	2	0.8653	0.6919	1.082	0.2046
rs17760296	**NOG1**	1	0.1796	0.1937	2	0.911	0.7026	1.181	0.4818
rs227731	**NOG1**	1	0.4592	0.4786	2	0.9251	0.7565	1.131	0.4483
rs7224837	**AXIN2**	2	0.05102	0.04424	1	1.162	0.7307	1.846	0.5261
rs3923086	**AXIN2**	2	0.2245	0.2076	1	1.105	0.8676	1.408	0.418
rs17820943	**MAFB**	2	0.2878	0.3333	1	0.808	0.6486	1.007	0.05701
rs13041247	**MAFB**	1	0.365	0.349	2	0.7994	0.6434	0.9932	0.04303
rs11696257	**MAFB**	2	0.3487	0.298	1	0.7929	0.6379	0.9855	0.03628

Table 6 Analysys of the NSCPO in the Ethiopian Case control cohorts

SNP	**Probable Gene Loci**	A1	F_A	F_U	A2	OR	L95-U95	P
rs1801131	**MTHFR**	1	0.2857	0.3198	2	0.8506	0.4312-1.678	0.6403
rs1801133	**MTHFR**	2	0.09524	0.1055	1	0.8928	0.314-2.539	0.8316
rs766325	**PAX7**	2	0.2381	0.2496	1	0.9394	0.4568-1.932	0.865
rs742071	**PAX7**	2	0.4048	0.4552	1	0.814	0.4354-1.522	0.5183
rs560426	**ABCA4**	2	0.5238	0.4521	1	1.333	0.7207-2.467	0.3579
rs138751793	**ARHGAP29**	2	0.02381	0.003811	1	6.376	0.7284-55.81	0.05476
rs6677101	**SLC25A24**	2	0.381	0.3552	1	1.117	0.5933-2.104	0.7313
rs861020	**IRF6**	1	0.3095	0.2309	2	1.493	0.7664-2.908	0.236
rs34743335	**IRF6**	2	0.04762	0.0743	1	0.6229	0.1483-2.617	0.5142
rs642961	**IRF6**	1	0.1667	0.1065	2	1.677	0.7312-3.847	0.2173
rs7590268	**THADA**	1	0.2857	0.2595	2	1.142	0.5779-2.255	0.7029
rs4332945	**DYSF**	1	0.3571	0.3547	2	1.011	0.5322-1.919	0.9744
rs2303596	**DYSF**	2	0.1667	0.2819	1	0.5094	0.2243-1.157	0.1009
rs227782	**DYSF**	1	0.2381	0.3933	2	0.4821	0.235-0.9891	0.04222
rs115200552	**MSX1**	1	0.02381	0.009132	2	2.646	0.3361-20.84	0.3366
rs12532	**MSX1**	2	0.2857	0.3514	1	0.7384	0.3745-1.456	0.3796
rs2674394	**Gene desert**	1	0.2143	0.1921	2	1.147	0.542-2.428	0.7194
rs651333	**TULP4**	1	0.381	0.4085	2	0.8909	0.4734-1.677	0.7203
rs6558002	**EPHX2**	1	0.4286	0.4885	2	0.7854	0.4222-1.461	0.4445
rs987525	**8q24**	1	0.5476	0.4459	2	1.504	0.8115-2.789	0.192
rs894673	**FOXE1**	1	0.4286	0.3569	2	1.351	0.7259-2.516	0.3407

Table 6 continuation								
rs3758249	**FOXE1**	2	0.4286	0.3565	1	1.354	0.7272-2.52	0.3377
rs7078160	**VAX1**	1	0.119	0.1761	2	0.6322	0.2458-1.626	0.3373
rs4752028	**VAX1**	1	0.3571	0.4084	2	0.8048	0.4241-1.527	0.5057
rs10785430	**ADAMTS20**	2	0.2857	0.3031	1	0.9199	0.4662-1.815	0.8097
rs9574565	**SPRY2**	2	0.35	0.4224	1	0.7362	0.3809-1.423	0.3607
rs8001641	**SPRY2**	1	0.1905	0.2824	2	0.5978	0.2742-1.303	0.1911
rs17563	**BMP4**	2	0.3095	0.3326	1	0.8996	0.463-1.748	0.7549
rs1258763	**GREM1**	1	0.4762	0.4297	2	1.207	0.6522-2.233	0.549
rs8049367	**ADCY9**	1	0.2619	0.327	2	0.7305	0.3636-1.467	0.3756
rs16260	**CDH1**	1	0.1429	0.1434	2	0.9955	0.4137-2.395	0.992
rs11642413	**CDH1**	1	0.4762	0.461	2	1.063	0.5745-1.966	0.846
rs1546124	**CRISPLD2**	2	0.3571	0.4298	1	0.7371	0.3885-1.399	0.349
rs4783099	**CRISPLD2**	2	0.2857	0.3902	1	0.6252	0.3172-1.232	0.1713
rs8069536	**NTN1**	2	0.1429	0.209	1	0.6306	0.263-1.512	0.2974
rs8081823	**NTN1**	1	0.2896	0.1667	2	0.4905	0.216-1.114	0.03251
rs17760296	**NOG1**	1	0.2381	0.1969	2	1.274	0.6184-2.626	0.5102
rs227731	**NOG1**	1	0.4762	0.4817	2	0.978	0.5287-1.809	0.9436
rs3923086	**AXIN2**	2	0.2381	0.2026	1	1.23	0.597-2.534	0.574
rs17820943	**MAFB**	2	0.2619	0.3425	1	0.6812	0.3392-1.368	0.2777
rs13041247	**MAFB**	1	0.3095	0.3586	2	0.8019	0.4129-1.558	0.5138
rs11696257	**MAFB**	2	0.3095	0.3581	1	0.8034	0.4136-1.561	0.5175

6.3 Paper III. The role of environmental factors in the etiology of orofacial clefts in the Ethiopian population

We assessed the role of environmental factors in the occurrence of NSOFCs in the Ethiopian population using a case control study design. We have included 760 participants in this study: 359 mothers of children born with NSOFCs and 401 Mothers of children born with out any identifiable birth defect. The demographic data of the participants like mother's age at the time of subject's delivery, educational level, religion, location during pregnanacy etc was presented in Table 7. Most of the mothers in this study were in 21-26 years age group (35.9% case mothers and 40.29% control mothers) followed by 27-32 years age group (26.5% case mothers and 24.2% control mothers). The majority of the mothers in this study lived in Oromia region 112(31.2%) during their pregnanacy followed by Addis Ababa 82(22.8%). Mothers who lived in other regions during their pregnanacy had a higher risk of delivering a child with OFCs than those mothers who lived in Addis Ababa (reference category) Table 7. NSOFC is obsereved more in children who's birth weight was not known p-value 0.010; COR=4.321; 95% CI 1.411-13.235. This study showed that the occurrence of OFCs is more common in Muslim families p-value 0.000, COR 2.284; 95% CI 1.624-3.213. Table 7.

Table 7 The role of maternal demographic data in the occurence of NSOFCs

Variables	Case (N(%))	Control (N(%))	Odds ratio	95%CI	P-value
Gender					
Female	148(41.2%)	186 (46.4%)	1.00		
Male	211 (58.8%)	215 (53.6%)	0.811	0.608-1.081	0.153
Birth weight					
VLBW	5(1.4%)	10(1.4%)	1.00		
LBW	31(8.6%)	48(8.6%)	1.292	0.403-4.139	0.667
NBW	185 (51.5%)	242(60.3%)	1.529	0.514-4.549	0.445
Macrosomia	17(4.7%)	45(11.2%)	0.756	0.225-2.533	0.650
Unknown	121(33.%)	56(14.0%)	4.321	1.411-13.235	0.010
Mothers' age at subjects birth in years					
15-20	76 (21.2%)	81(20.2%)	1.00		
21-26	129 (35.9%)	164 (40.9%)	0.838	0.568-1.237	0.374
27-32	95(26.5%)	97(24.2%)	1.004	0.685-1.592	0.842
33-38	44 (12.3%)	44(11.0%)	1.066	0.632-1.796	0.811
≥39	15 (4.2%)	15 (3.7%)	1.066	0.488-2.328	0.873
Mothers' education level					
Illiterate	151(42.1%)	125 (31.2%)	1.00		.
Primary	107 (29.8%)	129 (32.2%)	.687	0.484-0.973	0.035
Secondary	64 (17.8%)	95 (23.7%)	.558	0.375-0.828	0.004
Tertiary	37 (10.3%)	52 (13.0%)	.589	0.363-0.955	0.032
Mothers' Religion					
Christian	236(65.7%)	331 (82.5%)	1.000		
Muslim	114 (31.8%)	70 (17.5%)	2.284	1.624-3.213	0.000
Others	9 (2.5%)	0(0.0%)	2265771919	0.000-	0.999
Mothers' Birth Place					
Addis Ababa	26 (7.2%)	107 (26.7%)	.1.00		
Oromia	126 (35.1%)	106 (26.4%)	4.892	2.966-8.068	0.000
Amhara	95(26.5%)	77 (19.2%)	5.077	3.008-8.570	0.000
SNNPR	85 (23.7%)	105 (26.2%)	3.332	1.990-5.577	0.000
Others	27(7.5%)	6(1.5%)	18.519	6.930-49.489	0.000
Mothers' Location during pregnancy					
Addis Ababa	82(22.8%)	268(66.8%)	1.00		
Oromia	112(31.2%)	61(15.2%)	6.001	4..030-8.935	0.000
Amhara	70(19.5%)	15(3.7%)	15.252	8.287-28.02	0.000
SNNPR	65(18.1%)	53(13.2%)	4.008	2.584-6.218	0.000
Others	30(8.4%)	4(1.1%)	24.521	8.389-71.619	0.000
Number of pevious births					
≤ 2 Children	276(76.9%)	350(8.3%)	1.00		
3-4 chldren	55(15.3%)	43(10.7%)	1.622	1.056-2.491	0.027
>4 children	28(7.8%)	8(2.0%)	4.438	1.991-9.892	0.000

SNNPR-Southern Nations Nationalities Peoples Republic

We assesd the role of maternal smoking, alcohol consumption and exposure to diagnostic x-ray before and during the first week of pregnanacy and found out that exposure to diagnostic x-ray was a risk factor p-value 0.011; COR= 3.145; 95% CI 1.306-7.573. Table 8.

Table 8 The role of Maternal exposure(Diagnostic x-ray, Smoking and alchol

	Case mothers	Control mothers	OR	95% CI	P-value
Diagnostic X-Ray during and before pregnancy (three months)					
No	**340(94.7%)**	**394(98.3%)**	**1.00**		
Yes	**19(5.3%)**	**7(1.7%)**	**3.145**	**1.306-7.573**	**0.011**
Smoking during and before pregnancy (three months)					
No	334(93.0%)	378(94.3%)	1.00		
Yes	25(7.0%)	23(5.7%)	1.230	0.685-2.208	0.488
Alcohol use before an during pregnancy conception (three months)					
No	346(96.4 %)	394(98.3%)	1.00		
Yes	13(3.6%)	7(1.7%)	2.115	0.834-5.361	0.115

We also assessed the role of maternal illness and maternal medication use to the occurrence of NSOFCs in the offspring and found out that mothers who suffer from bronchial asthma, vomiting during first trimester of pregnanacy and mothers who were admitted for treatend abortion were at a higher risk of delivering a child with NSOFCs with p-value 0.002; COR 3.729; 95% CI 1.205-11.543; p-value 0,031,COR 0.676; 95% CI 0.474-0.965; and p-value 001, COR 0.367, 95% CI 0.203-0.661 respectively. Maternal folic acid, iron and other medication use and the occurrence of NSOFCs was assessed but no significant association was found.

Binary logistic regration analysis showed that residential area, socioeconomic sataus (indirectly shown by maternal education, number of previous births and a number of home delivery), maternal exposure to diagnostic x-ray, and maternal illness (Bronchial asthma, vomiting and threatened abortion) were found to be associated with the occurrence of NSOFCs. Tables: 7 and 8. Some variables like exposure to diagnostic x-ray, maternal education, maternal birthplace, and maternal illness like vomiting were significantly associated with the occurrence of NSOFCs. However the association was not persistent in the second model when it was adjusted for other variables Table 9.

Table 9 The effect of variables on the occurence of NSOFCs

Variable	COR	Adjusted OR	P-Value
Childrens' Birth weight			
VLBW	**1**	**1**	
LBW	**1.292 (0.403-4.139)**	**0.962(0.483-7.962)**	**0.346**
NBW	**1.529 (0.514-4.349)**	**2.265(0.616-8.332)**	**0.218**
Macrosomia	**0.756 (0.225-2.533)**	**0.974(0.234-4.006)**	**0.972**
Unknown	**4.321 (1.411-13.235)**	**2.985(0.786-11.336)**	**0.108**
Mothers location during pregnancy			
Addis Ababa	**1**	**1**	
Oromia	**6.001 (4.030-8.935)**	**4.165(2.379-7.292)**	**0.000**
Amhara	**15.212 (8.287-28.072)**	**11.543(5.284-25.214)**	**0.000**
SNNPR	**4.008 (2.584-6.218)**	**4.530(2.344-8.755)**	**0.000**
Others	**24.512 (8.389-71.619)**	**7.432(1.551-35.613)**	**0.012**
Mothers birth place			
Addis Ababa	**1**	**1**	
Oromia	**4.892 (2.966-8.068)**	**1.843(0.951-3.573)**	**0.070**
Amhara	**5.077 (3.008-8.570)**	**1.750(0.883-3.467)**	**0.109**
SNNPR	**3.332 (1.990-5.577)**	**1.305(0.649-2.626)**	**0.455**
Others	**18.519 (6.930-49.489)**	**3.292(0.702-15.424)**	**0.131**
Mothers religion			
Christian	**1**	**1**	
Muslim	**2.284 (1.624-3.213)**	**2.130(1.379-3.290)**	**0.001**
Others	**2265771919(0.000**	**465990145.8(1.379-3.290)**	**0.999**
Mathernal Education			
Illiterate	**1**	**1**	**.**
Primary	**1.698 (1.047-2.754)**	**1.399(0.892-2.163)**	**0.146**
Secondary	**1.166 (0.712-1.909)**	**1.510(0.881-2.589)**	**0.134**
Tertiary	**0.947 (0.559-1.604)**	**1.540(0.824-2.880)**	**0.176**
Number of previous births			
≤2 Children	**1**	**1**	
3-4 Children	**1.622 (.056-2.491)**	**0.909((0.533-1.552)**	**0.728**
>4 Children	**4.478 (1.991-9.892)**	**1.974(0.789-4.942)**	**0.148**

Table 9 continued			
Diagnostic X-ray before conception and during first trimester			
No	**1**		
Yes	**3.145 (1.306-7.573)**	**2.369(0.872-6.434)**	**0.091**
Vomiting			
No	**1**		
Yes	0.676 (0.474-0.965)	**0.770(0.498-1.192)**	**0.242**
Threatened abortion			
No	**1**		
Yes	0.367 (0.203-0.661)	**0.5.716(2.879-11.347)**	**0.000**
Bronchial Asthma			
No	**1**		
Yes	3.729 (1.205-11.543)	**4.159(1.075-16.097)**	**0.039**

NBW- normal birth weight; LBW- low birth weight, VLB- very low birth weight

6.4 Paper IV: Oral health related quality of life of children born with OFCs in Ethiopia and their parents

In this study 41 children and adolescents born with NSOFCs and treated by a team of Professionals at our unit and their parents participated. There were 21 (51.21%) males and 20 (48.78%) females. The mean age of the patients' was 12.37 years (SD = 2.5), with more adolescents (60.97%) than children (39.02%). The majority of the parents were mothers (70.73%). The parents' age ranged between 27 and 53 years and 74% were under the age 40 years. The phenotype of the cleft patients included in this study were as follows: 24 (58.5%) children born with unilateral cleft lip and palate (UCLP), nine (22.0%) with bilateral cleft lip and palate (BCLP), three (7.3%) with unilateral cleft lip only (CLO), two (4.9%) with bilateral cleft lip only (BCLO), and three (7.3%) with cleft palate only (CPO). The Internal consistencies using cronbach's alpha of the overall scale (0.958 for parents and 0.979 for children) and for the majority of the subscales responses were excellent ranging from: 0.829 to 0.971 for parents, and 0.961, to 0.979 for children. The one subscale with a lower internal consistency of 0.678 was for children's school, which appears to be due to the small number of items in this subscale. There is no cronbach's alpha for "General Health" because it contains only one item Table 10.

Table 10. Internal consistency of the chlid oral health impact profile (COHIP) and its subscales for parents and children

	Cronbach Alpha	
	Parent	Children
Overall	.985	.979
Subscales		
Oral symptoms	.951	.933
Functional well-being	.906	.961
Emotional well-being	.971	.948
School	.829	.678
Peer interaction	.953	.979

Note that: There is no Cronbach's alpha for "General Health" because it contains only one item

Parents and patients COHIP scores appear in Table 11. The minimum overall score the parents obtained on the COHIP was 67 and the maximum was 186. The minimum score patients obtained was 78 and the maximum was 190. The mean overall score of both the patients and parents was 155. There are minor differences between patients and parents on subscales, but no significant differences were shown between patients and parents on overall scores.

Table 11 Mean Subscale &Overall Scale for both Parents and Patients, t, P- values

	Parents				Patients					
Subscale	Mean	SD	Min	Max	Mean	SD	Min	Max	t	P
Oral Symptoms (10 Items)	39.90	7.47	19.00	49.00	39.14	5.81	25.00	50.00	0.512	0.610
Functional Wellbeing (8Items)	33.58	6.95	12.00	40.00	35.60	6.23	14.00	40.00	1.388	0.781
Emotional Wellbeing (10 Items)	40.00	10.11	14.00	50.00	39.88	9.22	17.00	50.00	0.057	0.980
School Environmen t (4 items)	16.61	4.37	4.00	20.00	16.93	4.61	2.00	20.00	0-.320	0.938
Peer Interaction (6 Items)	26.39	5.29	10.00	30.00	24.60	5.88	6.00	30.00	1.441	0.488
COHIP Overall (38 Items)	155.51	30.79	67.00	186.00	155.56	26.20	78	190.00	0-.008	0.386

Intraclass correlation coefficients between the parents and the patients were calculated to show their agreement across subscales and significant correlation was found with $p < 0.05$ (Table 12). The correlation coefficient for the emotional well-being was found to be high followed by oral symptoms and functional wellbeing subscales. The correlation on school environment and general health was found to be relatively low.

Table 12. Intraclass Correlation Coefficients (ICCs) Between Parents and Children on COHIP Subscale and Overall Score

	ICC	95% Confidence Interval	
		Lower Bound	Upper Bound
Overall	.982*	.976	.987
Subscales			
Oral symptoms	.941*	.920	.958
Functional well-being	.930*	.905	.951
Emotional well-being	.961*	.947	.972
School environment	.769*	.676	.841
Peer interaction	.916*	.884	.941
General Health	.807*	.701	.876

***** Correlation is significant with $p < .05$

Pearson's correlation coefficients between subscale scores, overall and general health in the parent and patient group showed significant correlations between the subscales, overall, and general health.

Table 13: Summary of the major findings of the different studies of the dessertation

Paper	Objective	Major findings
I	To investigate the prevalence and incidence of OFCs in Ethiopia	From June 2007-December2013 8,232 patients were born and received single surgical treatment. Incidence was calculated using this as numerator and the total number of live births during the study period, which was 18,811,316 (projected from 2007 sensus) as denominator and gave 44/100,000 live births. The prevalence was estimated using the total number of OFC pateints operated from June 2007-December 2013 (N=18,073) as a numerator and the total number of population (N= 88,703,914) in 2013 as a denominator. It is estimated to be 20/100,000 populations.
II	To identify the role genetic variants reported in candidate gene studies &GWAS in the occurrence of NSOFCs in European population in sub-saharan African countries (Ethiopian, Ghanaian and Nigerian)	The case control analysis revieled that PAX7 (rs742071, P = 0.005574,OR=1.329 and 95%CI 1.087-1.626), IRF6 (rs642961, P =0.01508;OR= 1.442; 95% CI 1.072-1.94), DYSF (rs2303596, P = 0.00231;OR= 0.6854; 95%CI 0.5371-0.8747), 8q24 (rs987525, P =0.000782; OR= 1.413;95%CI 1.154-1.73), were found to be associated with the occurrence of NSCL/P .In addition MAFB (rs13041247, P=0.04303;m OR= 0.7994; 95%CI 0.6434-0.9932 and rs11696257, P=0.03628;OR=0.7929, 95%CI, 0.6379-0.9855) were found to be nominally associated with NSCPO. In the Ethiopian subpopulation. SNPS in ABCA4 (rs481931 and rs4147811 were found to be associated with the occurrence of NSCPO all with P= 0.03, and NTNI (rs8081823, P = 0.03; were also found to be associated with NSCPO In the Ethiopian population. PAX7 (rs742071, p=0.00557, IRF6 (rs642961,p=0.02), DYSF (rs2303596, p= 0.00231, 8q24(rs987525,p=0.000782 and MAFB (rs13041247 and rs11696257, all with p= 0.04 were nominally associated with NSCL/P.
III	To assess the role of environmental factors in relation to the occurrence of orofacial clefts in the Ethiopian population	Mothers who were admitted for treatend abortion were at a higher risk of deliverioing a child with NSOFCs p-value =0.000; AOR= 5.716; 95% CI =2.879-11.347. We observed significant increase in the occurrence of OFCs in mothers who gave history of Bronchial Asthma p-value 0.039; AOR= 4.159; 95%CI= 1.075-16.097.
IV	To evaluate the OHRQoL of Ethiopian children born with NSOFCs and the perception of their parents.	This study found that the parents and children's responses were similar when evaluating the child OH-RQoL using an Amharic translation of the COHIP that had strong internal consistency.

7. Discussion

The discussion was presented in the same sequence as the results were presented above.

7.1 The prevalence/incidence of OFCs in Ethiopia. (Paper I).

This study revealed an incidence of 44/100,000 live births and prevalence of 20/100,000 populations. The incidence rate reported in this study is not comparable with the report from Addis Ababa, Ethiopia by Eshete el al in 2011(2). The incidence report in our previous study was 1.49/1000 live births much higher than the current study. The reason for this could be that the previous study was a prospective study, which included all live births during a specific period of time, while this one was a retrospective review of patients'charts, which is prone to bias. The other reason could be the rate of unattended delivery is less in the capital city when it is compared with the country at large. Attended delivery to some extent means structured registration and also could minimize the death of infants born with congenital anomalies.

The prevalence reported in this study is lower than the reports from different African countries. It is less than the Nigerian prevalence reported by Butali et al in 2014(21). Both these studies had a similar design and data source. It came from the Smile Train database of Ethiopia and Nigeria respectively. A study done in Uganda by Kesande et al(19)reported a prevalence of 0.77/1000 live births. It was a hospital based retrospective analysis of births at two Ugandan hospitals. The prevalence rate report in African American by Gundlach KK et al (98) is also higher than our study.

The majority of cleft lip and or palate cases in this study were males (55.6%). This is the same finding with the studies done in Uganda by Kesande et al (19) and in Tanzania by Manyama et al(99). Martelli Junior et al(100)reported similar findings in Brazilian population. In their study males were 54.4% and females were 45.6%. As stated above all types of clefts including isolated cleft palate were more common in males in this study. In our previous study which was conducted in Addis Ababa, Ethiopia (2) cleft lip alone and isolated cleft palate were more common in females, this is in consistence with the existing accepted pattern of cleft occurrence. Cleft lip and palate was more common in males, in contradiction to the current study. The reason for the higher number of all types of clefts in males could be explained by the fact that this study captured those cleft patients presented to the study institutions and received single surgical treatment and in the African concept males

have priorities for many things including medical care.

The patterns of clefts found in this study are similar to the studies done in Tanzania by Manyama et al (99). The commonest type of cleft was isolated cleft lip (70%) followed by cleft lip and palate (26%). Isolated cleft palate consisted of only 3%. In Manyama et al study isolated cleft lip constituted 49.2% of all cleft deformities, while clefts of both lip and palate and isolated cleft palate constituted 39.2% and 11.7% of cleft deformities respectively. As it was mentioned earlier our study captured only patients who presented for surgery at the study institutions and the surgeons were of different level qualification. The number of plastic and reconstructive surgeons is few and obviously general surgeons conducted the large majority of the surgeries. It is obvious that they choose the simplest type of cases in order to avoid complications, in addition the hospitals where the surgery was conducted were not equipped for such type of delicate surgeries and we think that this could be the reason for the higher number of isolated cleft lip only which is the simplest among the cleft cases. The situation in Tanzania concerning plastic and reconstructive surgery is worse than our country and this could be the reason for similarities in findings. Isolated cleft palate was the list in our study; this is similar to the study done by Manyama et al(101) in Tanzania and other African countries. We speculate that this could be due to lack of proper examination of a neonate at delivery and large number of unattended deliveries.

Recent unpublished survey, which was conducted in Addis Ababa health institutions, found out that there is no established birth defect registry system in the capital city of Ethiopia. We think it is the same in other parts of the country. This is the main reason for the non-existence of relevant data on the incidence and prevalence of birth defects including orofacial clefts in Ethiopia.

7.2 Association studies and direct DNA sequencing implicate genetic susceptibility loci in the etiology of NSOFCs in Sub-Saharan African Populations (Ethiopia, Ghana, Nigeria) (Paper II).

This study demonstrated nominal associations between some loci and the occurrence of non-syndromic cleft lip and or palate (NSC/P) in cohorts from Ethiopia, Ghana and Nigeria.Transmission disequilibrium test (TDT) and family based association test for diseases trait (DFAM) analysis in the same cohort demonstrated threshold association between some loci and NSC/P. The contribution of these loci in the occurrence of NSCPO was also observed. The strongest nominal association was shown between 8q24 locus and NSC/P in a case control Meta analysis.

Our study showed reduced susceptibility in minor C allele rs987525 while the major A allele is high risk in the same cohort of study population. Even though there is difference in minor alleles our study's result is the same with studies done by Grant et al (102); Mangold et al (103); Beaty et al (104) and Ludwig et al (105) which showed that the A allele of rs987525 is a risk allele for NSCL/P in Europeans. Our study and the studies mentioned above suggest that the A allele of rs987525 and the actual risk variant is (or variants are) in linkage disequilibrium. The findings in our study confirm the findings by Beaty et al (106) and Murray et al (107) which suggested that the varied ethnic association of the rs987525 allele mainly depends on its MAF in different populations.

In the TDT analysis we demonstrated that MTHFR (Methylenetetrahydrofolate Reductase) is significantly associated with NSCL among our study populations and it was the C minor allele of the A1298C (rs1801131) SNP that presents a reduced risk, this suggests that A is the risk allele. In Asians it was the C677T (rs1801133) SNP of the MTHFR that has been mainly associated with reduced risk for nonsyndromic cleft lip and or palate (108); (109) and Pan et al (110). AXIN2 was found to play a role in the occurrence of NSOFCs in multiple populations but not in Africans. Among Asians the rs3923086, was found to be associated with the occurrence of NSOFCS (111). The association between the occurrence of non-syndromic cleft lip and or palate and AXIN2 was confirmed by other studies ((112).Our DFAM analysis showed that rs3923086 (AXIN2) is associated with NSCLP among sub-Saharan African populations (Ethiopia, Nigeria and Ghana). It was also shown that DYSF candidate gene was also associated with NSC/P among Africans. The association between rs560426 of ABCA4 and NSCLP was demonstrated in both TDT and DFAM analyses.

Our case control meta-analysis showed nominal association of PAX7 (rs742071) with NSCL/P. The subpopulation analysis suggested that this signal originated mainly from the Ethiopian and Nigerian cohorts that revealed some level of heterogeneity, but TDT and DFAM sub phenotype analyses showed that rs742071 revealed over-transmission in NSCL cases in all the three populations. Our case control meta-analysis found out that VAX1 (rs7078160) was nominally associated with NSCL/P and the subpopulation analysis suggested that Nigeria and Ghana drive this signal. This study could not detect a formal association between some GWASs and candidate gene loci and NSCL/P, the reason for this could be: these loci may not play a role in the etiology of NSCL/P in Africans or the genotyped SNPs may not be the tag SNPs for Africans. Lack of statistical power due to sample size and low MAF of the genotyped SNPs in Africans could also be possible reasons.

In the genes sequenced we found many missense mutations and one frameshift mutation. We have not observed any de novo occurrence for any of these variants because some parental samples were not available. The novel variants we observed were also shown in clinically unaffected parents and controls. The contribution of rare variants in the occurrence of NSOFCs has been observed in ARHGAP29(113), PAX7 and VAX1(45), (113) BMP4 (114), FOXE1 (49), MAFB(39), and MSX1(115).

7.3 The role of environmental factors in the etiology of NSOFCs in the Ethiopian Population (Paper III)

The role of environmental factors in the occurrence of orofacial clefts was known since 1943 when Warkany et al(116) related nutritional deficiencies with the occurrence of cleft palate in animal studies. Teratogens like exposure to phenytoin; valproic acid and thalidomide can cause clefts. In addition common environmental exposures such as maternal alcohol and cigarette use (117) before and during first trimester of pregnancy can be the cause of clefts. In this case control study which included 359 mothers of children born with non-syndromic orofacial clefts and 401 mothers of children born with out any congenital anomaly we evaluated the role of exposures like smoking, alcohol consumption and exposure to diagnostic x-ray before conception and during pregnancy.

We showed that mothers who had exposure to diagnostic x-ray before conception and during first trimester had a higher risk of delivering a child with non-syndromic orofacial clefts COR= 3.145**; 95% CI = 1.306-7.573;** p-value 0.011. Similar to our study Mohammad Zandi et al (118) found significant association between maternal exposure to diagnostic x-ray during pregnancy and the occurrence of orofacial clefts in the offspring. Sutapa Bandyopadhyay Neogi et al(119) in an Indian study found significant association between the occurrence of orofacial clefts and diagnostic x-ray exposure in the first three month of pregnancy.

In our study maternal smoking was not found to be a risk factor for delivering a child with NSOFCs. This is in contradiction to other researches done in different part of the world. Kallen (120) did a case-control analysis in Sweden and found significant association between maternal smoking and non-syndromic cleft lip and or palate in the offspring (OR = 1.64, 95% CI = [1.33 to 2.02]) and CP (OR = 1.42, 95% CI = [1.06 to 1.90]). He examined a total of 1,834 orofacial cleft cases using the Swedish registry. In a meta-analysis using 11 published studies Wyszynski *et al (117)* found significant association between maternal smoking and non-syndromic orofacial clefts OR 1.29 (95% CI = [1.18, 1.42]). The role of passive smoking in the etiology of orofacial clefts was evaluated (121) and was found to be significantly associated similar to active smoking. An increased risk of non-syndromic cleft lip and or palate in the offspring of smoking mothers in the Danish and Iowan case control studies was observed by Min Shi et al (122). Asghar Ebadifar et al (123) found significant association between maternal smoking and increased risk for oral clefts (OR = 14.7, 95% CI = [5.4-75.4], *P*= 0.001).

This study also evaluated the relationship between maternal vomiting and threatened abortion during first trimester of pregnancy and the occurrence of NSOFCS and found significant association with OR= 0.676; 95% CI= 0.474-0.965; p-value =0.031; OR=0.367; 95% CI =0.203-0.661; p-value= 0.001 respectively. We also showed the role of low socioeconomic status, of the mothers that was indirectly indicated by the high proportion of an attended delivery, multiple births and low maternal education, in the occurrence of orofacial clefts OR=4.321; 95% CI= 1.411-13.235; p-value 0.010; OR=4.438; 95% CI=1.991-9.892; p-value= 0.000; respectively. This is similar to Kraples et al.(124) and N Taghavi et al (125) studies. Kraples et al. speculated that low socioeconomic status can be a marker of parental health and life style there fore should be considered as a risk factor. Warkany et al associated nutritional deficiencies with cleft palate in animal studies (116). Education plays a role in changing the life of individuals. The chance of getting healthy diets and nutrients for uneducated individuals is less. Maternal healthy diets and nutrients are very important for the normal development of a fetus. Nutritional deficiency in mothers before conception and during early pregnancy could lead to failure of cell growth, differentiation, migration and fusion. This alone or in combination with other factors could cause orofacial clefts.

We assessed the impact of using folic acid, vitamins and other medications on the occurrence of NSOFCs but found no association, this could be because very few mothers reported that they took vitamins and other medications.

7.4 Oral health related quality of life of children born with orofacial clefts in Ethiopia and their parents (paper IV)

The main objective of this study was to evaluate the oral health-related quality of life of children born with NSOFCs and their parents with the use of an Amharic translation of the COHIP. The study included those patients with non-syndromic OFCs that received multidisciplinary cleft care and their parents. The findings in this study indicated good oral health-related quality of life, which was shown by the high overall score obtained by parents and patients. (Geels et al. 2008) (126) Reported similar findings in Rotterdam, Amsterdam. (Munz et al. 2011) (127) also found similar positive OH-RQoL for young patients with cleft lip and palate who completed treatment using the Michigan Oral Health-Related Quality of Life Scale (MOH-RQoL). (Wilson-Genderson et al. 2007) (128) assessed the similarity of

the responses of children born with orofacial clefts and their caregivers using the COHIP; however, they found low to modest rates of similarity between child and caregiver responses for the sample overall. In our study, the proportion of the mean scores to the maximum scores were the same as those reported by (Bos et al. 2011) (129) in their Dutch sample. Their sample also had similar overall mean scores; however, there were significant differences between patients and parents on the emotional well being, oral symptoms and school subscales.

Our study indicated that it is possible to use an Amharic translation of COHIP scores to assess the oral health-related quality of life of children affected with orofacial clefts and their parents; however, some of the questions need to be expressed differently based on culture/language. For instance, "Felt that you were attractive (good looking) because of your teeth, mouth or face". This question might not be appealing for our culture, because patients feel shy to respond to this question. Geels et al. 2008) (126) also emphasized the importance of formulating these questions when administering COHIP in children born with OFCs.

8. Validity and generalizability

Validity can be defined as the degree that measuring procedure reflects, captures, or assesses the specific concept that a research is trying to measure. It usually applies to both the method and design of the research. If the procedure followed in the collection of data is valid the findings of the research truly represent the fact that one is claiming to measure. There are two types of validity internal and external. Internal validity can be affected by not controlling some of the major variables or any problems with the research instrument. (Powell, & Connaway, 2010.

If the findings of a research can be generalized to a larger group or in other contexts the research is referred as externally valid. The findings of such type of a research can be applied in the context outside the one the research took place. Internal and external validity are important in analyzing the usefulness, appropriateness, and meaningfulness of the research study.

8.1 Internal Validity

Internal validity refers to how well an experiment is done, especially whether it avoids confounding. It shows how much degree of the findings is correct for the particular group of people at particular settings. The less chance for confounding in a study, the higher its internal validity is. Internal validity can be affected by not controlling some of the major variables or any problems with the research instrument. This dissertation took all the possible measures to avoid the role of chance, bias and confounding at all stages.

Information collection bias: To insure that the information collected was accurate research assistances recruited and trained. The principal investigator regularly supervised them. The questionnaire used for NigeriaCRAN study was translated into local language. The research assistants and principal investigator were speakers' of two local languages. Information on environmental factors was obtained using a protocol which was designed per the WHO guide lines. Saliva samples collected using standardized saliva kits and saliva sponges stored and transported to Iowa University, USA in line according to international guidelines. Saliva sample analysis (DNA processing, sequencing and genotyping) was conducted at the university of Iowa genetic laboratory, which is one of the best genetic laboratories in the world.

Confounding: The association of independent and dependent variables was assessed using multivariate logistic regression models. This way confounding was controlled. We used different statistical procedures.

8.2 External Validity and generalizability

The samples were collected from different ethnic groups of Ethiopian and collaborated with the Ghanaian and Nigerian study projects. Therefore, the finding in this study could be representative to many Ethiopian ethnic groups and could provide relevant information to the sub-Saharan African population regarding etiology of orofacial clefts. The quality of life of patients affected with orofacial clefts and the perception of their parents was assessed using the child oral health impact profile questionnaire. The Internal consistencies of the overall scale and for all the subscales responses from both the parents and patients were excellent with Cronbach's alpha: .985, .951, .906, .971, .829, .953 for parents, and .979, .933, .961, .948, .678, .979 for children.

The fact that we used standardized questionnaire to collect data and collecting samples using standardized tools and inline with the international rules and regulations and also the inclusion of samples from different African population will make it valid both nationally and internationally. The findings in this study can be generalized to all Ethiopians and sub-Saharan Africa.

9. Strengths and limitations of the study

Strengths: This study investigated the three aspects of orofacial clefts (epidemiology, etiology and quality of life of patients affected with OFCs) for the first time in Ethiopia. We included all the 31 hospitals, which were providing cleft surgical treatment in collaboration with Smile Train and other charity organizations on mission basis. The hospitals did maximum effort to call patients to the treatment centers during the surgical missions. These hospitals are distributed through out the country. All the operated patients data was enterred into the Smile Train database and we used this data to assess the prevalence and incidence of this anomaly.

The main study site was the only cleft care unit which provides multidisciplinary cleft care for patients coming from all over the country. This gave us the chance to include nearly all-ethnic groups in our study samples. We collected DNA samples using Oragene saliva collection kits and sponges, which are, standardized tools for this purpose. The collected saliva samples were transported according to the international rules and regulations. DNA extraction and analysis was done at the best genetics laboratory in the world. This makes our findings nationally and internationally valid.

The other strength of this study is that we were able to translate and use the Child Oral Health Impact Profile COHIP) questionnaire which is a valide international tool to assess the quality of life of children affected withn OFCS and the perception of their parents. We also translated the NigriaCran questionnaire, which is a valide tool to collect data on materanal environmrntal factors.

Limitations: The prevalence study was limited by using data only from the hospitals and may not be representative of the true estimate of the prevalence. A population-based study is preferred but there is lack of resources both human and capital to undertake such an exercise at this moment. However, the data provides a baseline data on the prevalence and will serve

as reference for future population based studies. Another limitation is that, environmental exposure data is collected retrospectively and subjectively leaving room for recall biases. It was not also collected at a standardized time, which is 24 months after the periconception period of the index pregnancy (124). Considering the situation and available research infrastructure, we are limited to these approaches. The number of patients included in the quality of life of children affected with OFCS was only 41; this has limited the types of appropriate analyses that could be completed with the data. In addition, the sample included only a small proportion of less than 3% of the total number of patients who received surgical treatment at our unit. We therefore cannot generalize these results to other cleft populations in Ethiopia. Another limitation could be that the sample of children born with OFCs and their parents may have not fully expressed their feelings and experience, with the possibility of social desirability in their responses.

10. Conclusions

- The findings in this research indicated that: The incidence and prevalence of orofacial clefts in Ethiopia is low, but this can not be representative because this is a retrospective hospital based study which included only patients who received single surgical treatment
- We demonstrated that SNPs in: PAX7, IRF6, DYSF, 8q24 and MAFB (rs13041247 and rs11696257, were found to be nominally associated with NSCL/P in the Ethiopian Ghanaian and Nigerian population. It was also shown that SNPs in ABCA4 (rs481931 and rs4147811, and NTN1 (rs8081823), were found to be nominally associated with NSCPO in the three subpopulations. Sub phenotype analyses of the Ethiopian NSCL/P cohort showed that the PAX7, DYSF, MSX1, SPRY2 (rs9574565, and MAFB signals were mainly associated with NSCLO, whereas the IRF6 (rs642961, and 8q24 (rs987525 signals were stronger for NSCLP.
- Exposure to diagnostic X-ray had a higher risk of delivering a child with OFCs. The role of socioeconomic status of the mother in the occurrence of OFCS was shown indirectly: mothers who were illiterate had a higher risk of delivering a child with OFCS, it was also shown that maternal illness like Bronchial asthma and treatend abortion contributed to the occurrence of NSOFCs.
- We assessed the similarity of the responses of children with orofacial clefts and their parents regarding children's oral health related quality of life (OHRQoL) using the

Child Oral Health Impact Profile (COHIP) questionnaire. We found high rates of similarity between children and Parents responses for the sample overall and for the subscales.

10. Recommendations

For policy makers

During our study we found out that birth defect registry is not in the health care system of Ethiopia, therefore we strongly recommend establishing a birth defect registry system. We found out that the rate of unattended delivery is high. Unattended delivery is associated with adverse birth out comes and unrecorded biths/birth defects. The policy makers should establish a system where skilled health care professionals should attend all births. The number of patients who received multididciplinary cleft care was very few; some of the reasons for this were lack of awareness, distance and shortage of centers, which provide multidisciplinary cleft care. It is important to establish more centers, which provide multidisciplinary cleft care in different regions. It is important to teach families of children born with OFCs and the community at large about the importance of comprehensive multidisciplinary cleft care. We also recommend to involve the primary health-care providers in cleft care so that they will appreciate the need for team cleft care.

For service providers

This study found out that a number of mothers were exposed to diagnostic x-ray investigation, which is a known environmental hazard. We recommend collecting proper reproductive history before ordering an x-ray to mothers at the reproductive age. We also recommend that all investigations and medications should be properly selected for mothers at the reproductive age. This study indicated that very few mothers took folic acid and other vitamins before and during pregnancy. The benefit of folic acid in preventing the occurrence of craniofacial anomalies including OFCs is a proven fact. We recommend that health providers should prescribe folic acid and other vitamins to pregnant women during their early pregnancy time and when possible even at the time of planning pregnancy.

Recommendation for researchers:

It is very crucial to conduct community-based studies to find out the true incidence/ prevalence of OFCs. This will provide relevant information to the health planners of the country to include birth defect registry in the health care system. To understand the

contribution of environmental factors and the gene environment interaction in the occurrence of birth defects in general and orofacial clefts in particular it is important to conduct a prospective case control study. It is also very crucial to perform a genome wide association studies (GWASs) and whole genome sequencing for every ethnicity as far as complex traits are concerned. Finally, we recommend that the child oral health impact profile (COHIP) questionnaire should be modified to fit the cultural belifs of various populations and society around the world.

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14. Appendices

14.1 Participant information sheet English and Amharic

14.1.1 English participant information sheet

TITLES OF THE PROJECT:

The role of genetic and environmental factors in the etiology of orofacial clefts in the Ethiopian population

Principal investigator: Dr Mekonen Eshete Abebe

Supervisors: Dr Wakgari Deressa, Dr Azeez Butali and Prof Peter Mossey

Coordinating Office: Addis Ababa University, College of Health Sciences, School of Public Health

Purpose(s) of research:

1. The purpose of this research is to research into the causes and possible methods of prevention of cleft lip and cleft palate.
2. To assess the quality of life of children born with OFCs that were fully rehabilitated and the perception of their parents

As the parent of a participating child and as a participant we would like your permission to do the following things:

1. Collect information on forms and by means of a simple interview on aspects of family history, medical background, diet, vitamin supplements, occupational exposures and lifestyle around the time of conception and early pregnancy. This interview should take approximately 20 minutes.
2. We also wish to perform certain *laboratory tests* to look at genes, which we suspect might be involved in cleft lip and palate. The samples we require are saliva and we will collect at the time of operation or at the clinic.
3. Collect information to understand the quality of life of your child and your perception using the child oral health impact profile questionnaire

Your sample, information and data will be stripped of identifiers (such as name, date of birth, address, etc) and placed in a central storage place (repository). Your information may be placed in the central information place such as the database of genotypes and phenotypes (what the disease looks like) also known as dbGaP or other national repository at United States of America, National Institute for Health National Institute for Health (NIH). Other

qualified researchers who obtain proper permission may gain access to your sample and /or data for use in approved research studies that may or may not be related to the present study. Sample and data used through such repositories will be monitored and usage approved by the repositories' administrators.

Samples may be used for reading out all the genetic information (sequencing) and /or studies that show differences in the genetic information across the entire human genome (genome wide studies). The tests we might want to use to study your biological sample may not even exist at this time. Therefore, we are asking for your permission to store your biological sample so that we can study it in the future. In total we expect to recruit 300 participants from this center into this study per year.

Expected duration of research and of participant(s)' involvement:

In total, we expect you to be involved in this research throughout the duration of the study. Effort will be made to ensure that you do not spend more than 1 hour at each clinic visit. We may recontact you after your visit to obtain further information and samples for the study.

The Study will be done at Yekatit 12 Hospital medical College all cleft lip and palate patients born in Addis Ababa health institutions home deliveries and all Ethiopian cleft patients born anywhere in the country and presented to our study health institution will be included in the case parent study. In Addition, all cleft patients managed at any hospital in collaboration with Smile Train in the country on outreach basis will be included in the study. If you agree to participate in this study you will be asked when you attend the hospital clinic, to complete a questionnaire on diet and lifestyle factors in a personal interview. You will also be asked if you would be willing if saliva sample is collected from you and your child. If it is convenient for the child's father to be present at the hospital and he is willing then a saliva sample would also be requested at this time. All children born with cleft lip and palate including those who are not included in our study will receive comprehensive cleft care free of charge.

Confidentiality: All information collected in this study will be given code numbers. However, names will remain in the case notes and medical records. The code numbers assigned to you for the purpose of this study cannot be linked to you in anyway and your name or any identifier will not be used in any publication or reports from this study. As part of our responsibility to conduct this research properly, officials from NHREC and NIDCR / NIH from the United States may have access to these records.

Benefit: You will not benefit from being in this study. However, we hope that, in the future, other people might benefit from this study by learning some of the causes of cleft lip and cleft palate. This would help devise strategies to prevent and predict these conditions in the future, as well as give a better understanding of typical development.

Risk: Risks For saliva sample collection in children that cannot spit, risk of infection may present and will be minimized by the use of routine sterile sampling procedures i.e. use of saliva sponges to soak up saliva in the buccal and sublingual area

Costs to the participants, if any, of joining the research: Your participation in this research will not cost you anything.

Inducement, incentive and compensation: You will be compensated to assist with the cost of transport to and from the research site but you will not be paid any fees for participating in this research.

Results Dissemination:

After the completion of the study, findings will be disseminated to all pertinent local, regional, national and international stakeholders and beneficiaries. The final document of study will be distributed to all regions health bureau, to the faculty of health Sciences School of public health, school of Medicine and To Iowa University. The finding is also planned to be disseminated to national stakeholders through a conference. The annual national conference of Ethiopian Public Health Association, Ethiopian Medical Association and the surgical society of Ethiopia will be considered as an opportunity. All possible efforts will be made to reach out to the international scientific community through publishing the research in international journals.

Freedom to withdraw: If you want to participate in this study, you have full right to with draw from the study any time you wish. Please note that some of the information that has been obtained about you before you chose to withdraw may have been modified or used in reports and publications. These cannot be removed anymore. However the researchers promise to make good faith effort to comply with your wishes as much as is practicable.

Person to Contact: The participant has the right to ask information that is not clear about the research context and content before and or during the research work. You can contact the principal investigator and his supervisor. Moreover this research-undergone ethical reviewed and approved by Addis Ababa University College of Health Sciences IRB. The main task of this board is to make sure that the ethical principles is adhered or not and the research participants are protected from harm.

If you want more information and check about this project you can contact the following people

Addis Ababa University College of Health Sciences IRB Secretary Office Tel. 0115512876

Principal Investigator name and address: Mekonen Eshete Tel: 251 911 254170

Supervisor name and address: Dr Wakgari Deressa, School of Public Health, Collegfe of Health Science, Addis Ababa University; Mobile: 251 911 483714

Informed Consent Form

Title of the Project: THE ROLE OF GENETIC AND ENVIRONMENTAL FACTORS IN THE ETIOLOGY OF OROFACIAL CLEFTS IN THE ETHIOPIAN POPULATION

I have been well aware of that this research undertaking is a postgraduate degree partial fulfillment of research dissertation, which is fully supported and coordinated by AAU School of Public Health, and the designate principal investigator is **Dr Mekonen Eshete**. I have been fully informed in the language I understand about the research project objectives that are to understand the environmental health conditions and associated health threats in refugee.

I have been informed that all the information I shall provide to the interviewer will be kept confidential. I understood that the research has no any risk and no composition. I also knew that I have the right to withhold information, skip questions to answer or to withdraw from the study any time. I have acquainted no body will impose me to explain the reason of withdrawal. It is also enlighten there would have no effect at all in my health benefit or other administrative effect that I get from the refuge.

I have assured that the right to ask information that is not clear about the research before and or during the research work and to contact

Addis Ababa University College of Health Sciences IRB Secretary Office Tel. 0115512876

Principal Investigator Name: Dr Mekonen Eshete Tel: +251 911254170

Supervisers Name: Dr Wakgari Deressa Tel +251 911483714

Address: Addis Ababa University college of Health Sciences School of Public Health

I have read this form, or it has been read to me in the language I comprehend and understood the condition stated above, therefore, I am willing and confirm my participation by signing the consent.

Name of the participant _____

Agreed to participate in the study: Yes /No (mark one of them for verbal consent)

Signature _____ (if written consent)

Name of witness signature _____ (Data collector, supervisor, any third person)

Signature _____ Date _____

Name(s) and affiliation(s) of researcher(s) of applicant(s): This study is being conducted by: Dr. Mekonen Eshete

Ass prof Surgery FoHS, AAU

Sponsor(s) of research: United States of America National Institute of Health, National Institute of Dental and Craniofacial Research.

Purpose(s) of research: The purpose of this research is to *Research into the causes and* possible methods of prevention of cleft lip and cleft palate.

Procedure of the research

This is a case control study. This means that we must identify children or adults born with Orofacial Clefts in a number of Ethiopian families. We wish to approach the mothers of cases and controls to obtain some details about medical history, diet and lifestyle factors. We also need to take family history details and obtain a saliva sample from mother, child and Father if possible. Father sample is optional.

As the parent of a child with a cleft lip or palate we would like your permission to do the following things:

Statement about sharing of benefits among researchers and whether this includes or exclude research participants: If this research leads to scientific innovations, the University of Addis Ababa and University of Iowa shall jointly own it.

Any apparent or potential conflict of interest:

We are not aware of any other information that may cause the researchers not to do their work with fear or favor.

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including about risks and benefits, to make an informed decision.

DATE: _____ SIGNATURE: _____

NAME: _____

Statement of person giving consent:

I have read the description of the research or have had it translated into language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of

the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

DATE: _____ SIGNATURE: _____

NAME: _____

WITNESS' SIGNATURE (if applicable): _____

WITNESS' NAME (if applicable): _____

Contact Address _____

14.1.2 Amharic Participant information sheet

ዘረመልና እካባቢ በተፈጥሮ ከከንፈር እና ላንቃ ከፍተት ጋር ለመወለድ በኢትዮጵያዊያን ላይ ያላቸው አስተዋጾ

ከቡራን ወላጆች

ዋና የጥናቱ ተመራማሪ፡- ዶ/ርመኮንን እሸቴ

ጥናቱ የሚከሄደው በየከቲት 12 ሆስፒታል ሜዲካል ኮሌጅ እና ከላይ ለተጠቀሰው ችግር ህክምና በሚሰጥበት በማንኛውም የኢትዮጲያ ሆስፒታል ውስጥ ነው፡፡በጥናቱ ውስጥ ተሳታፊ የሚሆኑት በማንኛውም የጤና ድርጅትና በቤትም ከዚህ ችግር ጋር የተወለዱና ችግሩ የሌለባቸው እድሜያቸው ከአስር አመት እና ከእስር አመት በታች የሆኑ ኢትዮጲያውያን ናቸው፡፡

የጥናቱ አላማዎች

አጠቃላይ አላማ

ዘረመልና እካባቢ በተፈጥሮ ከከንፈር እና ላንቃ ከፍተት ጋር ለመወለድ ኢትዮጵያዊያን ላይ ያላቸው አስተዋጾ ማወቅ

በዚህ ጥናት ውስጥ ለመሳተፍ ከፈቀዱ ወደ ሆስፒታሎቹ ለሕክምና ልጆዎን ይዘው ሲመጡ በግላዊ የኑሮ ዘይቤዎቻችሁና ስለአመጋጋባቸሁ ቃለ መጠየቅ ይደረግላቸሁዋል፡፡

ከዚህ በተጨማሪ የምራቅ ናሙና እርሶና ልጅዎ ለመስጠት ፈቃደኛ ስለመሆናችሁ ይጠየቃሉ

ፈቃዳኛ ከሆኑ የምራቅ ናሙና ከእርሶና ከልጅዎ ይወሳዳል፡፡

የሕፃኑ አባት ከሕፃኑ ጋር በሆስፒታሉ ከተገኘ እርሱም የምራቅ ናሙና ለመስጠት ፈቃዳኛ ከሆነ ከእሱም ይወሰዳል፡፡

በተፈጥሮ ከከንፈር እና ላንቃ ከፍተት ጋር የተወለዱት ሁሉም ሕፃናት በጥናታችን ውስጥ ያልተካተቱትም ቢሆኑ ነፃ የሕክምና እንክብካቤን እንዲያገኙ ይደረጋል፡፡

ለወላጆች የቀረበ የተሳትፎ የጥሪ ደብዳቤ

ዘረመልና እካባቢ በተፈጥሮ ከከንፈር እና ላንቃ ከፍተት ጋር ለመወለድ ኢትዮጵያዊያን ላይ ያላቸው አስተዋጾ

ከቡራን ወላጆች

ዋና የጥናቱ ተመራማሪ፡- ዶ/ርመኮንን እሸቴ

ጥናቱ የሚከሄደው በየከቲት 12 ሆስፒታል ሜዲካል ኮሌጅ እና ከላይ ለተጠቀሰው ችግር ህክምና በሚሰጥበት በማንኛውም የኢትዮጲያ ሆስፒታል ውስጥ ነው፡፡በጥናቱ ውስጥ ተሳታፊ የሚሆኑት በማንኛውም የጤና ድርጅትና በቤትም ከዚህ ችግር ጋር የተወለዱና ችግሩ የሌለባቸው እድሜያቸው ከአስር አመት እና ከእስር አመት በታች የሆኑ ኢትዮጲያውያን ናቸው፡፡

የጥናቱ አላማዎች

አጠቃላይ አላማ

ዘረመልና እካባቢ በተፈጥሮ ከከንፈር እና ላንቃ ከፍተት ጋር ለመወለድ ኢትዮጵያዊያን ላይ ያላቸው አስተዋጾ ማወቅ

በዚህ ጥናት ውስጥ ለመሳተፍ ከፈቀዱ ወደ ሆስፒታሎቹ ለሕክምና ልጆዎን ይዘው ሲመጡ በግላዊ የኑሮ ዘይቤዎቻችሁና ስለአመጋጋባቸሁ ቃለ መጠየቅ ይደረግላቸሁዋል፡፡

ከዚህ በተጨማሪ የምራቅ ናሙና እርሶና ልጅዎ ለመስጠት ፈቃደኛ ስለመሆናችሁ ይጠየቃሉ

ፈቃዳኛ ከሆኑ የምራቅ ናሙና ከእርሶና ከልጅዎ ይወሳዳል፡፡

የሕፃኑ አባት ከሕፃኑ ጋር በሆስፒታሉ ከተገኘ እርሱም የምራቅ ናሙና ለመስጠት ፈቃዳኛ ከሆነ ከእሱም ይወሰዳል፡፡

በተፈጥሮ ከከንፈር እና ላንቃ ክፍተት ጋር የተወለዱት ሁሉም ሕፃናት በጥናታችን ውስጥ ያልተካተቱትም ቢሆኑ ነፃ የሕክምና እንክብካቤን እንዲያገኙ ይደረጋል፡፡

<u>ለወላጆች የቀረበ የተሳትፎ የጥሪ ደብዳቤ</u>

የመረጀ ሰጪነት ፈቃድ / ስምምነት/
ውድ ወላጅ: በከንፈር እና/ወይም በላንቃ መሰንጠቅ ክስተት እና በስነ-ምግብ፣ በኑሮ ዘይቤ፣ በአንዳንድ ዘረመሎች መካከል ሊኖሩ የሚችሉ ግንኙነቶችን ለማየት እንድንችል ፈቃደኛ ከሆኑ በጥናቱ ውስጥ እንዲሳተፉ እንጠይቅዎታለን፡፡

የዚህ ምርምር ፕሮጀክት ተሳታፊ በምርምር ሂደቱ ውስጥ የሚጠየቀው አስፈላጊ መረጃዎችን እና የምራቅ ናሙና ከመስጠት ባለፈ ምንም ዓይነት ተጨማሪ ነገር አይጠየቅም፡፡ ተጥናቱ አከናዋኞች የሚሰበሰቡት መረጃዎች በሙሉ በምስጢራዊነት ይጠበቃሉ፣ የሚያገለግሉትም ለምርምር ዓላማዎች ብቻ ይሆናል፡፡ የሚሰበሰበው የምራቅ ናሙና ወደ አይዋ ዩኤስ አሜሪካ ይላካል፣ ከዚያም ከምራቁ ውስጥ ዲኤንኤ የሚባለው ነጥረ-ነገር እንዲወጣ ተደርጎ የሚከተሉት ጉዳዮች ይከናወናሉ፡-

1. ናሙናዎቹን የሚመለከቱ መረጃዎች እና የውህቡን ምንነት የሚያሳቁ መለያዎች (ስም፣ የትውልድ ቀን፣ አድራሻ፣ ወ.ዘ.ተ. የመሳሰሉት እንዲነሱ ተደርገው ማከመቻ ስፍራ እንዲቀመጡ ይደረጋሉ፡፡ ናሙናዎች በኮምፒተዩር የዘረመል ዓይነቶች (ጂኖታይፕስ) እና የውጫዊ ገጽታ ዓይነቶች መረጃ ቋት (ዳታቤዝ) (ዲቢጂኤፒ) ውስጥ ወይም በዩኤስ አሜሪካ ብሔራዊ የጤና ኢንስቲትዩት በሚገኝ ሌላ ማከማቻ እና አግባብነት ባላቸው ሌሎች የአሜሪካ ላቦራቶሪዎች ሊቀመጡ ይችላሉ፡፡ ተገቢውን ፈቃድ ያገኙ ሌሎች ብቁ ተመራማሪዎች በውልደት በጭንቅላት እና በፊት ላይ ከሚደረሱ እንከኖች ጋር ተያያዥ ለሆኑ ወይም ላልሆኑ የተፈቀዱ የምርምር ጥናቶች አገልግሎት ላይ ለማዋል ናሙናዎቹን እና/ወይም ውህቡን ሊያገኙዋቸው ይችላሉ፡፡ በእንደነዚህ ያሉት ማከማቻዎች በኩል ጥቅም ላይ እንዲውሉ በሚደረግበት ጊዜ በማከማቻ ስፍራዎቹ አስተዳዳሪዎች ቁጥጥር እየተደረገባቸው እና አጠቃቀሙም ከእርሱ በሚገኝ ፈቃድ ይሆናል፡፡
2. ናሙናዎች ቅደም-ተከተል ለመስጠት (ሁሉንም የዘረመል መረጃዎች ለማንበብ) እና/ወይም ሙሉ ጂኖሚን ለሚያካትት ጥናት ጥቅም ላይ ሊውል ይችላል፡፡ የስነህይወታዊ ናሙናዎን ለማጥናት አሁን ለመጠቀም የምንፈልጋቸው ምርመራዎች በዚህን ጊዜ የሌሉ እንኳን ሊሆኑ ይችላሉ፡፡ በመሆኑም፣ ናሙናዎን ለወደፊቱ ለማጥናት እንድንችል ናሙናውን አስቀምጠን እንድናቆየው የእርስዎን ፈቃድ እንጠይቃለን፡፡

በዚህ ምርምር ውስጥ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ በመሆኑ ምንም አይነት ምክንያት ማቅረብ ሳይስፈልግዎ በማንኛውም ጊዜ ከጥናቱ የመውጣት መብትዎ የተጠበቀ ነው፤ ከጥናቱ መውጣትዎ እርስዎ ወይም ልጅዎ በሚያገኘው ሕክምናም ላይ ምንም ዓይነት ተጽእኖ እንደማይኖረው እንዲያውቁት ይሁን፡፡ከዚህ ፕሮጀክት ከወጡ፣ ናሙናዎችዎ እና ውህብዎ ለማንኛውም አዲስ ምርምር ጥቅም ላይ አይውልም፡፡ ሆኖም ግን፣ ለምርምር ጥናት ጥቅም ላይ እየዋሉ የሚገኙ ናሙናዎችን መልሶ ለመውሰድ አይቻልም፡፡ በጥናቱ ውስጥ ለመካተት የሚፈልጉ ከሆነ፣ በፈቃደኝነት መግለጫ ቅጹ ላይ ይፈርሙ፡፡

የፈቃደኝነ ትመግለጫ ቅጽ

የተመርማሪው (ዎቹ) እናየአመልካቹ (ቾቹ) ስም (ሞች) እና ዝምድናቸው፡-ይህ ጥናት እየተከናወነ የሚገኘው፡- በዶ/ር መኮንን እሸቴ፣የቀዶ ጥገና ሕክምና ረዳት ፕሮፌሰር፣አአዩ

የምርምሩ ስፖንሰር (ሮች)፡- የዩኤስ አሜሪካ ብሔራዊ ጤና ተቋም፣ የጥርስ፣ የጭንቅላት እና የፊት ምርምር ብሔራዊ ተቋም፡፡

የዚህ ምርምር ዓላማ የከንፈር መሰንጠቅ እና የላንቃ መሰንጠቅ ምክንያቶች ላይ እና ከስተቶቹን ለመከላከል በሚያስችሉ ዘዴዎች ላይ ምርምር ለማድረግ ነው፡፡

የምርምሩሥነ-የምርምሩ ሥነ-ሥርዓት፣ ከእያንዳንዱ ተሳታፊ የሚጠበቅ እና በምርምሩ ውስጥ ተሳታፊ ሰዎች ጠቅላላ ብዛት ግምት፡-

ይህ ጥናት የክስተት (ኬዝ) ቁጥጥር ነው፡፡ ይህም ማለት፣ በበርካታ ኢትዮጵያዊያን ቤተሰቦች ውስጥ ከአፍ እና ፊት መሰንጠቅ ጋር የሚወለዱ ሕፃናት እና አዋቂዎችን የግድ ለይተን ማወቅ ይኖርብናል ማለት ነው፡፡ ከህመም ታሪክ፣ ከስነ-ምግብ እና ከኑሮ ዘይቤ ጋር ተያያዥነት ያላቸውን አንዳንድ ዝርዝሮች ለማግኘት የተነጂዎቹን እናቶች እና መቆጣጠሪያዎችን ለመቅረብ እንሻለን፡፡ ከዚህ በተጨማሪ የቤተሰብ ታሪክ ዝርዝሮችን እና ከእናት፣ ከልጅ እና የሚቻል ከሆነ ከአባት የምራቅ ናሙና መውሰድ ያስፈልገናል፡፡ ከአባት የሚገኘው ናሙና ሊወሰድም፣ ሊቀርም ይችላል፡፡

የከንፈር ወይም የላንቃ መሰንጠቅ ያለበት ልጅ ወላጅ እንደመሆንዎ መጠን፣ የሚከተሉትን ነገሮች እንድናከናውን የእርስዎን ፈቃድ ለመጠየቅ እንፈልጋለን፡-

1. የቤተሰብ፣ የጤና ስር-መሰረት፣ የስነ-ምግብ፣ የተጨማሪ ቫይታሚኖች፣ በስራ ላይ ተጋላጭነት በሚፈጥሩ እና በእርግዝና ጊዜ አካባቢ እና በእርግዝና የመጀመሪያ ወቅት ገፅታዎችን የሚያሳዩ መረጃዎችን በቅጾቹ ላይ እና ቀለል ባለ ቃለ-መጠይቅ አማካኝነት መሰብሰብ፡፡ ይህ ቃለ-መጠይቅ ከ20 ደቂቃ ገደማ በላይ አይፈጅም፡፡
2. በከንፈር እና በላንቃ መሰንጠቅ ጋር ግንኙነት ሊኖራቸው ይችላል ብለን በምንጠራጠራቸው ዘረመሎች ላይም አንዳንድ የላቦራቶሪ ምርመራዎችን እናደርጋለን፡፡ የምንፈልጋቸው ናሙናዎች ምራቅ ሲሆኑ፣ የምንሰበስባቸውም በኦፕሬሽን ጊዜ ወይም በክሊኒክ ይሆናል፡፡

3. ናሙናዎ፣ መረጃዎ፣ ውህቦች፣ ማንነትን የሚያሳዩ አመላካቾች (እንደ ስም፣ የትውልድ ቀን፣ አድራሻ፣ ወዘተ) እንዲወገዱ ተደርገው በማዕከላዊ ማከማቻ ስፍራ እንዲቀመጡ ይደረጋል፡፡ መረጃዎ በማዕከላዊ የመረጃ ማስቀመጫ ቦታ፣ ማለትም እንደ ዳታቤዝ፣ የዘረመል አይነቶች (ጂኖታይፕስ) እና የገጽታ አይነቶች (በሽታው ምን እንደሚመስል) ዲቢጂኤፒ ተብሎ የሚጠራ ወይም አሜሪካ ውስጥ በሚገኝ በሌላ ብሔራዊ ማከማቻ ስፍራ፣ የጤና ብሔራዊ ተቋም (ኤን አይ ኤች)፡፡ ሌሎች ብቁ የሆኑ፣ ተገቢውን ፈቃድ ያገኙ ተመራማሪዎች ናሙናዎን እና/ወይም ውህብዎን ለተፈቀዱ የምርምር ጥናቶች ለመጠቀም ይችላሉ፣ ጥናቶቹን ከአሁኑ ጥናት ጋር ተዛማጅነት ያላቸው ወይም የሌላቸው ሊሆኑ ይችላሉ፡፡ በእነዚህ አይነቶቹ ማከማቻዎች በኩል ጥቅም ላይ የሚውሉት ናሙና እና ውህብ ክትትል የሚደረግባቸው እና አጠቃቀማቸውም በማከማቻዎቹ አስተዳዳሪዎች ፍቃድ የሚሰጥባቸው ይሆናሉ፡፡

ናሙናዎቹ ሁሉንም የዘረመል መረጃ (በቅደም ተከተል የተቀመጡ) እና/ወይም ጥናቶች፣ በሰው ልጅ ሙሉ ጂኖም (ጂኖምን የሚያጠቃልሉ ጥናቶች) ለማንበብ ጥቅም ላይ ሊውሉ የሚችሉ ናቸው፡፡ የስነ ሕይወት (ባዮሎጂካል) ናሙናውን ለማጥናት ልንጠቀምባቸው የምንፈልጋቸው ምርመራዎች በአሁኑ ጊዜ የሌሎ እንኳን ሊሆኑ ይችላሉ፡፡ በመሆኑም፣ ስነ ሕይወታዎ ናሙናዎን ለወደፊት ጥናታችን ለመጠቀም እንድንችል እንድናስቀምጠው ፍቃድዎን እንጠይቃለን፡፡ በድምሩ ከዚህ ማዕከል በየአመቱ 300 ሰዎችን በመመልመል በዚህ ጥናት ውስጥ እንደምናካትት እንጠብቃለን፡፡

የሚጠበቀው የምርምሩ ቆይታ እና የተሳታፊ (ዎች) ተሳትፎ፤

በጠቅላላው፣ እርስዎ በዚህ የምርምር ጥናት ሙሉ የቆይታ ጊዜ በተሳትፎ እንደሚቀጥሉ እንጠብቃለን፡፡ በእያንዳንድ የክልኒክ ምርመራ የቆይታ ጊዜዎ ከ1 ሰዓት በላይ እንዳይሆን ጥረት ይደጋል፡፡ ለጥናቱ የሚሆኑ ተጨማሪ መረጃዎችን እና ናሙናዎችን ለመውሰድ ከምርመራዎ በኋላ በድጋሚ ልናገኝዎት እንችላለን፡፡

ስጋት(ቶች)፡-

ምራቃቸውን ለመትፋት ከማይችሉ ሕፃናት የምራቅ ናሙና በሚወሰድበት ጊዜ የኢንፌክሽን ስጋት ሊኖር ይችላል፡፡ መደበኛ ከጀርም ነፃ የሆኑ የናሙና አወሳሰድ ስነ ስርዓቶችን፣ ማለትም ምራቅን ከአፍ ውስጥ እና ከምላስ ስር ለመውሰድ የምራቅ መምጠጫ ስፖንጅን በመጠቀም፣ የኢንፌክሽን ስጋቱ እንዲቀንስ ይደረጋል፡፡

ወደ ምርምሩ ለመቀላቀል በተሳታፊዎች ላይ የሚያስከትለው ወጪ/ካለ/፡-

በዚህ ምርምር መሣተፍዎ ምንም አይነት ወጪ አያስከትልብዎትም፡፡

ጥቅም (ሞች)፡-

በዚህ ጥናት ውስጥ በመሣተፍዎ ተጠቃሚ አይሆኑም፡፡ ሆኖም ግን ለወደፊቱ ሌሎች ሰዎች በዚህ ጥናት ውስጥ ከሚያገኙዋቸው አንዳንድ የከንፈር መሰንጠቅ እና የላንቃ መሰንጠቅ ምክንያቶች ትምህርት በመውሰድ ተጠቃሚ ሊሆኑ እንደሚችሉ ተስፋ እናደርጋለን፡፡ ይህ ሁኔታ እነዚህን ክስተቶች ለመከላከል የሚያስችሉ ስትራቴጂዎችን (ስልቶችን) ለመንደፍ እና ለወደፊቱ ክስተቶቹን ለመተንበይ እንዲሁም አይነተኛ እድገትን በተሻለ ሁኔታ ለመገንዘብ ሊረዳ ይችላል፡፡

ሚስጥራዊነት፡-

በዚህ ጥናት የሚሰበሰቡ ሁሉም መረጃዎች ኮድ ቁጥሮች ይሰጧቸዋል፤ ሆኖም ግን ስሞች በክስተት (ኬዝ) ማስታወሻዎች እና የሕክምና መዛግብት ውስጥ ይቆያሉ፡፡ ለዚህ ጥናት አላማ ለእርስዎ የሚሰጡት የኮድ ቁጥሮች በማንኛውም መንገድ ከእርስዎ ጋር ተያያዥነት ያላቸው ሊሆኑ አይችሉም፣ እንዲሁም ስምዎ ወይም ማንኛውም እርስዎን ለመለየት የሚያስችሉ ጠቋሚዎች በዚህ ጥናት ማንኛውም ሕትመት ወይም ሪፖርቶች ውስጥ ጥቅም ላይ አይውሉም፡፡ ይህንን ምርምር በተገቢው አኳኋን ለማከናወን እንደ የኃላፊነታችን አካል በመሆኑ ከአሜሪካ ኤንኤችአርኢሲ እና ኤንአይዲሲአይ/ኤንአይኤች የሚመጡ ባለስልጣናት እነዚህን መዛግብት ሊመለከቱ ይችላሉ፡፡

ፍቃደኝነት፡-

በዚህ ምርምር ላይ የእርስዎ ተሳታፊነት ሙሉ በሙሉ በፍቃደኝነትዎ ላይ የተመሰረተ ነው፡፡

ያለመሳተፍ አማራጮች፡-

ላለመሳተፍ ከመረጡ፣ በምርምሩ ያለመሳተፍዎ እርስዎ ወይም ልጅዎ በዚህ ሆስፒታል ውስጥ በሚያገኙት ሕክምና ላይ በምንም አይነት መልኩ ተፅእኖ አይኖረውም፡፡

ማበረታቻ (ዎች)፡- ወደ ምርምር ቦታው ለመምጣትና ለመመለስ የሚያወጡትን የትራንስፖርት ወጪ ለመተባበር ማካካሻ ይሰጥዎታል፣ ነገር ግን በዚህ ምርምር ውስጥ ለሚያደርጉት ተሳትፎ የሚፈጸም ምንም ዓይነት ክፍያ አይኖርም፡፡

ተሳታፊዎች ከምርመሩ ለመውጣት የሚደርሱበት ውሳኔ እና ተሳትፎን የማቋረጥ ሥርዓት፡- በማንኛውም ጊዜ ከምርምሩ ራስዎን የማግለልም ምርጫም ሊኖርዎት ይችላል፡፡ እባክዎን፣ ለመውጣት ከመምረጥዎ በፊት እርስዎን በተመለከተ የተወሰዱ አንዳንድ መረጃዎች ለውጥ ተደርጎባቸው ሊሆን እንደሚችል ወይም በሪፖርቶች እና በህትመቶች ውስጥ ጥቅም ላይ ውለው ሊሆን እንደሚችል ልብ ይበሉ፡፡ እነዚህ ከአሁን በኋላ እንዲወጡ ለማድረግ አይቻልም፡፡ ሆኖም ግን፣ ተመራማሪዎቹ እስከሚቻለው ድረስ እንደፍላጎትዎ ለመፈጸም በቅን ልቦና ላይ የተመሰረተ ጥረት ያደርጋሉ፡፡

በተመራማሪዎች መካከል ጥቅሞችን መጋራትን በተመለከተ፣ እና ይህም ተሳታፊዎችን የሚያካትት ወይም የማያካትት ስለመሆኑ የተሰጠ መግለጫ፡- እነዚህ ምርምሮች ወደ ሳይንሳዊ ፈጠራዎች ካመራ፣ አዲስ አበባ ዩኒቨርስቲ እና አይዋ ዩኒቨርስቲ የጋራ ባለቤቶች ይሆናሉ፡፡

ማንኛውም በግልጽ የሚታይ ወይም ሊከሰት የሚችል የጥቅም ግጭት፡-

ተመራማሪዎቹ ስራቸውን በፍርሃት ወይም በውለታ እንዲሰሩ ሊያደርጋቸው ስለሚችል ምክንያት የምናውቀው ምንም አይነት ሌላ መረጃ የለም።

በእውቀት ላይ የተመሰረተ ፍቃደኝነትን በሚቀበለው ሰው የተሰጠ መግለጫ፡-

ተሳታፊው በእውቀት ላይ የተመሠረተ ውሣኔ ላይ እንዲደርሱ ለማድረግ ይህንን ምርምር ለ_____

_____ ሙሉ ማብራሪያ፣ በቂ መረጃ (ከመረጃ በኋላ ሊኖሩ የሚችሉ ስጋካቶች እና ጥቅሞችን ጨምሮ) ሰጥቻቸዋለሁ።

ቀን: _____ ፊርማ: _____

ስም: _____

ፍቃደኝነትን በሚገልፀው ሰው የተሰጠ መግለጫ:-

የምርምሩ ማብራሪያ አንብቤያለሁ ወይም እኔ በምረዳው ቋንቋ ተተርጉሞልኛል። ከዚህ በተጨማሪም እኔን ሊያረካኝ በሚችል ደረጃ ከዶ/ሩ ጋር ተነጋግረንበታል። ተሳትፎዬ በፈቃደኝነት ላይ የተመሰረተ መሆኑን እረዳለሁ። በምርምሩ ላይ ለመሳተፍ ፍላጎት ያለኝ ስለመሆኑ ፍርድ ለመስጠት በሚያስችለን ሁኔታ የምርምር ጥናቱን አላማዎች፣ የሚጠቀማቸውን ዘዴዎች፣ ሊኖሩ የሚችሉ ስጋቶች እና ጥቅሞችን በበቂ ሁኔታ አውቄያለሁ። በማንኛውም ጊዜ በነፃነት ተሳትፎዬን ለማቆም እንደምችልም እገነዘባለሁ። እኔ ዘንድ እንዲቀመጡ የዚህን የፍቃደኝነት መግለጫ ቅጽ እና ተጨማሪ የመረጃ ቅጽን ተቀብያለሁ።

ቀን: _____ ፊርማ: _____

ስም: _____

የምስክር ፊርማ (ተፈፃሚ ከሆነ): _____

የምስክር ስም (ተፈፃሚ ከሆነ): _____

14.2 Adopted Questionnaire from the NigeriaCRAN study to find out the risk factors in the occurrence of OFCs in the Ethiopian population (Please indicate the response with this sign ✓) (English and Amaharic)

14.2.1 English questionnaire

Family Id	
Hospital Name	
Case or control	Case
	Control
Please indicate who has consented and provided a sample for this research	Child only
	Mother and child
	Father, mother and child
Sample Information	
Child Sample Type	Saliva
	Cheek swab
	Not sampled
Date child sample obtained	
Mother sample type	Saliva
	Cheek swab
	Not sampled
Date mother sample obtained	
Father sample type	Saliva
	Cheek swab
	Not sampled
Date father sample obtained	
Family history of clefting or craniofacial Anomalies	Yes
	No
	Unknown
Child Demographics data	
Name	
Father's name	
Grandfather's name	
Mother's Demographics data	
Name	
Father's name	
Grandfather's name	
Address	Region
	Zone
	Woreda
	Kebele or farmers association, Tel.
Father's Demographics data	
Name	
Father's name	
Grandfather's name	
Address	Region

	Zone
	Woreda
	Kebele or farmers association Tel:
Child data	
Birth weight (KG)	
Gestational age (Weeks)	
Multiple birth	No
	Yes, liked sex
	Yes, Unlinked
	Unknown
Consanguinity of Parents	Yes
	No
	Unknown
If yes, do you know the relationship?	Yes
	NO
Please describe the relationship of the parents.	
Multiple Malformed Infant (MMI)	Yes
	No
	Unknown
Limb Abnormality	Yes
	No
	Unknown
Please describe limb abnormality	
Craniofacial Anomaly (besides cleft lip and/or cleft palate	Yes
	No
	Unknown
Please describe craniofacial anomaly	
Cardiovascular Anomaly	Yes
	No
	Unknown
Please describe cardiovascular anomaly	
Other Anomalies	Yes
	No
	Unknown
Please describe other anomalies	
Cleft and Craniofacial Details	
Cleft type	Cleft lip
	Cleft palate
	Cleft Alveolus
	Submucous cleft
	None
	Unknown
Cleft lip details	Right
	Left
	Right and Left
	Unknown
Type of cleft lip	Incomplete
	Complete

	Unknown
Cleft palate details	Hard palate
	Soft palate
	Both hard and soft palate
Alveolar cleft details	Right
	Left
	Right and Left (bilateral)
	Unknown
Type of alveolar cleft	Incomplete
	Complete
	Unknown
Prenatal diagnosis of cleft	Yes
	No
	Unknown
Simonarts bands	Right
	Left
	Unknown
Pierre robin	Yes
	No
	Unknown
Pierre Robin: Additional features	Respiratory disease
	Micrognatia
	Glossoptosis
	Other (please indicate all that apply)
Maternal Data	
Cleft or craniofacial anomaly	Yes
	No
	Unknown
Religion	Christian
	Muslim
	Other
Age at subject's birth	
Education	Illiterate
	Primary
	Secondary
	Tertiary
Mother's birth location	
Mother's Location during pregnancy	
Consanguinity of Parents	Yes
	No
	Unknown
If yes, Do you know the relationship	Yes
	No
Please describe the relationship of the consanguineous partners and how they are related to the mother of this family	
Previous birth information	
Number of previous births	
Date of Birth of Previous Child 1	

Gender of Previous Child 1	Male
	Female
	Unknown
Is the father of this child the same as the father of the subject child?	Yes
	No
	Unknown
Date of birth of previous child 2	
Gender of previous child 2	Male
	Female
	Unknown
Is the father of this child the same as the father of the subject child?	
Can continue based on the number of children	
If you answered that any of your previously born children had a different father than the subject child, please describe how many different partners the mother had and how each of them are related to each of the children described above and the subject child	
Malformations in ANY Previous Live Births	Cleft
	Other craniofacial anomaly
	Cardiovascular anomaly
	Limb anomaly
	Multiple malformations
	None (please indicate all that apply)
Maternal Illnesses	
Diabetes	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
	Select all that apply
Hypertension	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
	Select all that apply
Bronchial Asthma	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Other chronic diseases	Before conception
	During first trimester
	During second trimester
	During second trimester
	No history
	Unknown

Vomiting	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Vaginal bleeding	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
History of threatened Abortion	Yes
	No history
	Unknown
Other Maternal Illnesses: List any other maternal illnesses not already described above and include whether they occurred before conception or during 1st, 2nd, or 3rd trimesters	
Maternal medications	
Folic acid	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
	Select all that apply
Iron	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Vitamins	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
How many other medications (besides folic acid, iron, and vitamins) have been taken?	
Other medication 1	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Other medication 1 name	
Other medication 2	Before conception

	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Other medication 2 name	
Other Medication 3	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Other medication 3 name	
Other medication 4, 5 etc	
Maternal Life Style and Exposure	
Cigarette smoking	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Cigarette amount	
Alcohol (including locally made)	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Alcohol amount	
Diagnostic x-ray	Before conception
	During first trimester
	During second trimester
	During third trimester
	No history
	Unknown
Diagnostic X-ray number of times	

14.2.2 የአማርኛ ቃለ መጠይቅ (Please indicate the response with this sign ✓)

የቤተሰብ መለያ	
የተመዘገበበት ቀን	
የጤና ድርጅቱ ስም	
ጉዳዩ (ኬዝ) ወይም ቁጥጥር	ኬዝ (ጉዳይ)
	ቁጥጥር
የምልመላ አቋም፡- እባክዎን ለዚህ የምርምር ፕሮጀክት ፈቃደኝነቱን የሰጠውን እና ናሙናውን ያቀረበውን አካል ይግለፁ	ሕፃኑ ብቻ
	እናት እና ሕፃን
	አባት እና ሕፃን
	ሕፃኑ፣አባት፣እና እናት
	ምንም
የናሙና መረጃ	
የሕፃኑ ናሙና አይነት	የምራቅ
	ከጉንጭ ውስጥ የተወሰደ እርጥበት
	ናሙና የለውም
	(የሚመለከቱትን በሙሉ ይምረጧቸው)
የሕፃኑ ናሙና የተወሰደበት ቀን	
የእናት ናሙና አይነት	ምራቅ
	ከጉንጭ ውስጥ የተወሰደ እርጥበት
	ናሙና የለም
	(የሚመለከቱትን በሙሉ ይምረጧቸው)
የእናት ናሙና የተወሰደበት ቀን	
ማንኛውም የከንፈር /ላንቃ ክፍተት ወይም የራስ ቅል እና የፊት እንከኖች የቤተሰብ ታሪክ አለን?	አዎ
	የለም
	አይታወቅም
እባክዎን የከንፈር/የላንቃ ክፍተት ወይም የጭንቅላት እና የፊት እንከኖች የነበረውን ሰው ማንነት እንዲሁም የክፍተቱን አይነት ወይም የጭንቅላት እና የፊት እንከኑን አይነት ይግለፁ	
የሕፃኑ የሰነ ሕዝብ (ዲሞግራፊክ) መረጃዎች	
ስም	
የአባት ስም	
የአያት ስም	
ፆታ	ሴት
	ወንድ
	አይታወቅም
የትውልድ ቀን	
አድራሻ	
የእናት የሰነ ሕዝብ (ዲሞግራፊክ) መረጃዎች	
ስም	
የአባት ስም	
የአያት ስም	
የትውልድ ቀን	

አድራሻ	
የአባት የስነ ሕዝብ (ዲሞግራፊክ) መረጃዎች	
ስም	
የአባት ስም	
የአያት ስም	
የትውልድ ቀን	
አድራሻ	
ሕፃኑ ሲወለድ የነበረው ክብደት (ኪ.ግ.)	
የእርግዝና እድሜው መጠን (ሳምንታት)	
የተወለዱ መንትያዎች	የሉም
	አዎ
	አዎ ፣ ተመሳሳይ ዖታ ያላቸው
	አዎ፣ የተለያዩ ዖታ ያላቸው
	አዎ፣አይ,ታወቅም
	አይ,ታወቅም
በወላጆቹ መካከል የስጋ ዝምድና አለን?	አዎ
	የለም
	አይ,ታወቅም
መልስዎ ‹አዎ› ከሆነ፣ዝምድናቸውን ያውቁታልን?	አዎ
	አላውቅም
እባክዎን በሕፃኑ ወላጆች መካከል ያለውን የስጋ ዝምድና ይግለፁ	
በርካታ የአፈጣጠር እንከኖች የነበሩበት ጨቅላ ሕፃን ነበርን?	አዎ
	የለም
	አይ,ታወቅም
የእጅ /የእግር የጤና እንከን	አዎ
	የለም
	አይ,ታወቅም
እባክዎን የእጅ /የእግር ጤና እንከኑን ይግለፁት	
የራስ ቅል እና የፊት እንከን (ከከንፈር እና /ወይም ከላንቃ ክፍተት በተጨማሪ)	አዎ
	የለም
	አይ,ታወቅም
እባክዎን የጭንቅላት እና የፊት እንከኑን ይግለፁት	
የልብ እና የደም ስር እንከን	አዎ
	የለም
	አይ,ታወቅም
እባክዎን የልብ እና የደም ስር እንከኑን ይግለፁት	
ሌሎች እንከኖች	አዎ
	የለም
	አይ,ታወቅም
እባክዎን ሌሎቹን እንከኖች ይግለጿቸው	
የከንፈር ና የላንቃ ክፍተት እና የራስ ቅል እና የፊት እንከን ዝርዝሮች	
የክፍተት አይነት	የከንፈር መሰንጠቅ
	የላንቃ መሰንጠቅ
	የአልቪዮራል መሰንጠቅ (alveolar cleft)
	የለም
	አይ,ታወቅም
የከንፈር ክፍተት ዝርዝሮች	በቀኝ በኩል
	በግራ በኩል
	ቀኝ እና ግራ (በሁለቱም በኩል)

	አይ,ታወቅም
የከንፈር ክፍተት አይነት	ሙሉ በሙሉ
	ሙሉ በሙሉ ያልሆነ
	አይ,ታወቅም
የላንቃ ክፍተት ዝርዝሮች	የፊት ለፊት ላንቃ (ጠንካራው)
	የኋላ ላንቃ (ለስላሳው)
	ሁለቱም (ጠንካራው እና ለስላሳው)
የአልቪዮራል ክፍተት ዝርዝሮች (alveolar cleft)	በቀኝ በኩል
	በግራ በኩል
	ቀኝ እና ግራ (በሁለቱም በኩል)
	አይ,ታወቅም
የአልቪዮራል ክፍተት አይነት (Type of alveolar cleft)	ሙሉ በሙሉ
	ሙሉ በሙሉ ያልሆነ
	አይ,ታወቅም
ቅድመ ወሊድ በምርመራ የታወቀ ክፍተት	አዎ
	የለም
	አይ,ታወቅም
ሲሞናርትስ ባንድ (Simonarts band)	በቀኝ በኩል
	በግራ በኩል
	ቀኝ እና ግራ (በሁለቱምበኩል)
	አይ,ታወቅም
ፒዬሬሮቢን (Pire robin sequence)	አዎ
	የለም
	አይ,ታወቅም
ፒዬሬሮቢን፡- ተጨማሪ ገፅታዎች	የትንፋሽ አካላት በሽታ
	ጤናማ ያልሆነ የመንጋጋዎች መጠን ፣በተለይ የታችኛው መንጋጋ
	የምላስ ወደ ላንቃ አቅጣጫ መውረድ
	ሌሎች
	የለም
	(የሚመለከቱትን በሙሉ ይምረጡ)
ሲንድሮሚክ (የተቀናጁ የጤና እንከኖች) - ልዩ ሲንድሮሙ የማይታወቅ እንኳን ከሆነ ፣ነገር ግን ሕመምተኛው ሲንድሮሚክ ከሆነ፣ ‹አዎ› የሚለውን ይምረጡት	አዎ
	የለም
	አይታወቅም
ሲንድሮሚክ የምርመራ ውጤት (ከታወቀ) የሲንድሮሚክ ዝርዝር መግለጫ ያልተገለፁትን ገፅታዎች በሙሉ ያብራሩ	
ሲንድሮሚክ መሆኑ በምርመራ ካልታወቀ ፣እባክዎን ታካሚው ሲንድሮም እንዳለበት ያመላክቱ	
የእናት መረጃዎች	
የከንፈር ና የላንቃ ክፍተት እና የራስ ቅል እና የፊት እንከን ዝርዝሮች	አዎ
	የለም
	አይ,ታወቅም
እባክዎን እናት በዚህ የጤና ችግር የተጠቃች ከሆነች ክፍተትቱን ወይም የጭንቅላት እና የፊት እንከኑን ይግለፁት	
ሐይማኖት	
ሕመምተኛው በተወለደበት ጊዜ የእናት እድሜ	

የትምህርት ደረጃ	ያልተማሩ
	አንደኛ ደረጃ
	ሁለተኛ ደረጃ
	ከፍተኛ ትምህርት የተማሩ
የእናት የትውልድ ቦታ	
በእርግዝናቸው ወቅት የነበሩበት ቦታ	
የወላጆች የስጋ ዝምድና	አዎ
	የለም
	አይታወቅም
መልስዎ‹አዎ› ከሆነ፣ዝምድናቸውንያውቃሉን?	አዎ
	የለም
የስጋ ዝምድና ያላቸው ወላጆችን የስጋ ዝምድና አይነት እና ከዚህ ቤተሰብ እናት ጋር ምን አይነት ዝምድና እንዳላቸው ይግለፁ	
የቀድሞ ወሊድ መረጃ	
ከዚህ ቀደም በሕይወት የተወለዱ ሕፃናት ብዛት	
የመጀመሪያው ልጅ የተወለደበት ቀን	(የማይታወቅ ከሆነ፣ክፍት ይተውት)
የመጀመሪያው ልጅ ፆታ	ሴት
	ወንድ
	አይታወቅም
ይህ ልጅ እና ሕመምተኛው ልጅ ከአንድ አባት የተወለዱ ናቸውን?	አዎ
	አይደሉም
	አይታወቅም
ሁለተኛው ልጅ የተወለደበት ቀን	
የሁለተኛው ልጅ ፆታ	ሴት
	ወንድ
	አይታወቅም
ይህ ልጅ እና ሕመምተኛው ልጅ ከአንድ አባት የተወለዱ ናቸውን?	አዎ
	አይደሉም
	አይታወቅም
ሶስተኛው ልጅ የተወለደበት ቀን	
የሶስተኛው ልጅ ፆታ	ሴት
	ወንድ
	አይታወቅም
ይህ ልጅ እና ሕመምተኛው ልጅ ከአንድ አባት የተወለዱ ናቸውን?	አዎ
	አይደሉም
	አይታወቅም
የእናት ሕመሞች	
የስኳር ሕመም	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ከፍተኛ የደም ግፊት	ከእርግዝና በፊት

	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
አስም	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ትውከት	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የውርጃ ስጋት	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
በብልት በኩል የደም መፍሰስ	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ለረጅም ጊዜ የዘለቀ በሽታ (Other Chronic illness)	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ሌሎች የእናት ሕመሞች-ከላይ ያልተገለፁ ማናቸውም የእናት ሕመሞች ካሉ ይዘርዝሯቸው ፣ሕመሞቹም ከእርግዝና በፊት ወይም በመጀመሪያዎቹ፣በሁለኛዎቹ ወይም በሶስተኛዎቹ ሶስት የእርግዝና ወራቶች የተከሰቱ መሆናቸውን ጭምር ያካቱ	
እናት የወሰዷቸው መድሃኒቶች	
ፎሊክ አሲድ (Folic acid)	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የብረት መአድን (Iron)	ከእርግዝና በፊት

	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ቫይታሚኖች (Other vitamins)	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
ከፎሊክ አሲድ ፣ከብረት መአድን እና ቫይታሚኖች ውጪ ቁጥራቸው ስንት የሆነ ሌሎች መድሃኒቶች ተወስደዋል	
ሌላ መድሃኒት 1	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የሌላ መድሃኒት 1 ስም	
ሌላ መድሃኒት 2	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የሌላ መድሃኒት 2 ስም	
ሌላ መድሃኒት 3	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የሌላ መድሃኒት 3 ስም	
የእናት የሕይወት ዘይቤ እና ተጋላጭነት	
ትምባሆ (Cigarette)	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የትምባሆ መጠን (amount of smoking)	
አልኮሆል (Alcohol including locally made)	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ

	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
የአልኮሆሉ መጠን	
በሽታን ለማወቅ የተደረገ የራጅ ምርመራ	ከእርግዝና በፊት
	በመጀመሪያዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሁለተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	በሶስተኛዎቹ ሶስት የእርግዝና ወራት ውስጥ
	ታሪክ የለም
	አይታወቅም
	(የሚመለከታቸውን በሙሉ ይምረጡ)
በሽታን ለማወቅ የተደረጉት የራጅ ምርመራዎች ብዛት	

14.3. Oral health related quality of life of Children born with NSOFCs and their parents (English and Amaharic version)

14.3.1 English version parents questionnaire

Instruction: Dear Parents thank you for agreeing to participate in this study. The main purpose of this study is to understand how children who were born with orofacial clefts and received multidisciplinary cleft care feel about themselves and if they have any functional problem.

Instructions: Please read each statement carefully and choose the answer that best describes your child in the past three months regarding his/her teeth mouth and face. Please be as honest as possible, we want to know how he/she really feel.

Example: In the past 3 months how often has your child had bleeding gums? If your child had bleeding gums circle the appropriate response if your child did not have gum-bleeding circle never

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Patient details (filled by researcher)

Patient ID_____

DOB of child_____

Gender_____

Ethnicity_____

Diagnosis_____

Date_____

(Circle one of the following)

Intervention Provided 1) none 2) surgery 3) other if , other please specify

Filled by participant

Please circle one response on each question

Oral symptoms (10 items)

In the past three months how often has your child?

1. Had pain in his/her teeth/toothache

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

2. Been breathing through his/her mouth or snoring

1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

3. Had discolored teeth or spots on his/her teeth

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

4. Had crooked teeth or spaces between his/her teeth

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

5. Had sores or sore spots in or around his/her mouth

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

6. Had bad breath

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

7. Had bleeding gums

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

8. Had food sticking in or between his/her teeth

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

9. Had pain or sensitivity in his/her teeth with hot or cold things

   1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

10. Had dry mouth or lips

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Functional well-being (8 items)

11. Had trouble biting off or chewing foods such as apple, carrot or firm meat

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

12. Had difficulty eating foods he/she would like to eat because of his/her teeth, mouth or face

1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

13. Had trouble keeping teeth clean

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

14. Had trouble sleeping because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

15. Had difficulty saying certain words because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

16. Been unable to eat foods he/she would like to eat b/c of his her tooth, mouth, face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

17. Had people difficulty understanding what he/she was saying because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

18. Had difficulty keeping his/her teeth clean because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Emotional well-being (10 items)

19. Been unhappy or sad because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

20. Been confident because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

21. Felt worried or anxious because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

22. Felt shy or withdrawn because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

23. Felt unattractive because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

24. Been angry because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

25. Felt that he/she look different because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

26. Felt that he/she was attractive (good looking) because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

27. Been worried about what other people think about his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

28. Been upset of uncomfortable with being asked questions about his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

School (4 items)

29. Missed school for any reason because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

30. Had difficulty paying attention in school because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

31. Not wanted to speak/read out loud in class because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

32. Not wanted to go to school because of his/her teeth, mouth or face

    Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Peer interaction (6 items)

33. Avoided smiling or laughing with other children because of his/her teeth, mouth or face?

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

34. Been teased, bullied or called names by other children because his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

35. Felt left out by peers because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

36. Been asked questions because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

37. Not wanted to meet new people because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

38. Been fighting or arguing with other children or family members because of his/her teeth, mouth or face

    1) Very often 2) Fairly often) 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

General health

39. In general, would you say his/her health is: (circle one)

    1) Bad 2) fair 3) good 4) very good 5) excellent 6) I don't know

14.3.2 Oral health related quality of life of children born with NSOFCs and their parents in Ethiopia. English questionnaire for children and adolescents

Patient details (filled by researcher)

Patient ID_____

DOB of child_____

Gender_____

Ethnicity_____

Diagnosis_____

Date_____

(Circle one of the following)

Intervention Provided 1) none 2) surgery 3) other if , other please specify

Oral symptoms (10 items)

In the past three months, how often have you?

1. Had pain in your teeth/toothache
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
2. Been breathing through your mouth or snoring
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
3. Had discolored teeth or spots on your teeth
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
4. Had crooked teeth or spaces between your teeth
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
5. Had sores or sore spots in or around your mouth
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
6. Had bad breath
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
7. Had bleeding gums
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
8. Had food sticking in or between your teeth
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
9. Had pain or sensitivity in your teeth with hot or cold things
   1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know
10. Had dry mouth or lips
    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Functional well-being (8 items)

11. Had trouble biting off or chewing foods such as apple, carrot or firm meat

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

12. Had difficulty eating foods you would like to eat because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

13. Had no trouble keeping teeth clean

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

14. Had trouble sleeping because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

15. Had difficulty saying certain words because of your teeth, mouth or face

    1) Fairly often 2) Very often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

16. Been able to eat foods you like to eat

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

17. Had people have difficulty understanding what you were saying because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

18. Had difficulty keeping your teeth clean because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Emotional well-being (10 items)

19. Been unhappy or sad because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

20. Been confident because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

21. Felt worried or anxious because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

22. Felt shy or withdrawn because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

23. Felt unattractive because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

24. Been angry because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

25. Felt that you look different because of your teeth, mouth or face

    1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

26. Felt that you were attractive (good looking) because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

27. Been worried about what other people think about your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

28. Been upset of uncomfortable with being asked questions about your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

School (4 items)

29. Missed school for any reason because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

30. Had difficulty paying attention in school because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

31. Not wanted to speak/read out loud in class because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

32. Not wanted to go to school because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

Peer interaction (6 items)

33. Avoided smiling or laughing with other children because of your teeth, mouth or face?

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

34. Been teased, bullied or called names by other children because your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

35 Felt left out by peers because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

36. Been asked questions because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don't know

37. Not wanted to meet new people because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don’t know

38. Been fighting or arguing with other children or family members because of your teeth, mouth or face

1) Very often 2) Fairly often 3) Occasionally 4) Hardly ever 5) Never 6) I don’t know

General health

39. In general, would you say your health is: (circle one)

1) Bad 2) Fair 3) Good 4) Very good 5) Excellent 6) I dont know

14.3.3 Amharic Questionnaire for parents

ከከንፈር እና ላንቃ ክፍተት ጋር **ከሚወለዱ ልጆች የሕይወት ጥራት ጋር በተያያዘ የአፍ ጤና የወላጆች ቃለመጠይቅ**

የታካሚ ዝርዝሮች (በተመራማሪ የሚሞሉ)

የታካሚው መለያ፡- ----------

የልጁ የትውልድ ቀን፡- ----------

ፆታ፡- ----------

ብሔረሰብ፡- ----------

የምርመራ ውጤት/ግኝት፡- ----------

ቀን፡- ----------

(ከሚከተሉት መካከል በአንዱ ላይ አክብቡ)

የተሰጠ እርዳታ፡-

1. ምንም የለም
2. ቀዶ ሕክምና
3. ሌላ ካለ፣እባክዎን ይግለፁት

በተሳታፊው የሚሞላ

እባክዎን ለእያንዳንዱ ጥያቄ በአንድ መልስ ላይ ያክብቡ

አፍ ላይ የሚታዩ ጠቋሚ ምልክቶች (10 ነገሮች) (አንዱ ላይ ያክብቡ)

1. ልጅዎ የጥርሶች ሕመም ኣጋጥሞታልን /ሚታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/በጭራሽ 6/ አላውቅም

2. ልጅዎ በአፉ /ፏይ ተነፍሳልን/ ትተነፍሳለችን ወይም ያንኮራፋልን /ፋለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

3. ልጅዎ የጥርስ ቀለም መበላሸት ወይም በጥርሶቹ /ቿ ላይ ጠብታ ነጥብ አለበትን/ባትን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

4. ልጅዎ የጥርሶች መደራረብ ወይም በጥርሶች መካከል ክፍተት አለበትን /ባትን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/በጭራሽ 6/ አላውቅም

5. ልጅዎ በአፉ /ፏ ውስጥ ወይም በአፉ /ፏ ዙሪያ ቁስሎች ወይም ነጠብጣብ ቁስሎች አለበትን/ባትን?

1/በጣምብዙጊዜ 2/ በመጠኑብዙጊዜ 3/ አንዳንድጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

6. ልጅዎ መጥፎ የአፍ ጠረን አለበትን/ባትን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

7. ልጅዎ የድድ መድማት ችግር አለበትን/ባትን?

1/በጣም ብዙጊዜ 2/ በመጠኑብዙጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

8. ልጅዎ በጥርሶች ውስጥ ወይም መካከል የምግብ መግባት ችግር ያጋጥመዋልን/ያጋጥሚታልን?

1/በጣምብዙጊዜ 2/ በመጠኑብዙጊዜ 3/ አንዳንድጊዜ 4/ እጅግአልፎአልፎ 5/ በጭራሽ 6/ አላውቅም

9. ልጅዎ በሙቅ ወይም በቀዝቃዛ ነገሮች የጥርስ ሕመም ወይም የሚነዝር ስሜት ይሰማዋልን/ታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

10. ልጅዎ የአፍ ወይም የከንፈሮች መድረቅ ችግር አለበትን /ባትን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

የከንውን ደህንነት (በአንዱ ላይ አክብቡ)

11. ልጅዎ እንደፖም፣ካሮት ወይም ጠጣር ስጋን የመሳሰሉ ምግቦችን ጨምሮ የመግመጥ ወይም የማኘክ ችግር አለበትን/ባትን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

12. ልጅዎ በጥርሶቹ /ቿ፣በአፉ/ፏ ወይም በፊቱ/ ቷ ላይ ባለው ችግር ምክንያት ለመብላት የሚፈልጋቸውን /የምትፈልጋቸውን ምግቦች መብላት ያስቸግረዋልን/ ራታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

13. ልጅዎ ጥርሶቹን /ቿን ማፅዳት ያቅተዋልን/ታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

14. ልጅዎ በጥርሶቹ /ቿ፣ በአፉ /ፏ ወይም ፊቱ/ቷ ላይ ባለው ችግር ምክንያት መተኛት ያስቸግረዋልን/ ታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

15. ልጅዎ በጥርሶቹ /ቿ፣ በአፉ/ፏ ወይም በፊቱ /ቷ (ላይ) ባለው ችግር ምክንያት አንዳንድ ቃላቶች ያስቸግሩታልን /ታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

16. ልጅዎ ለመብላት የሚፈልጋቸውን /የምትፈልጋቸውን ምግብ ለመብላት ይችላልን /ላለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

17. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ላይ ባለው ችግር ምክንያት የሚናገረውን /የምትናገረውን ነገር ሌሎች ሰዎች ለመረዳት ያስቸግራቸዋልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

18. ልጅዎ በጥርሶቹ/ቿ፣ በአፉ/ፏ ወይም በፊቱ/ቷ ላይ ባለው ችግር ምክንያት የጥርሶቹን/ቿን ንፅህና መጠበቅ ያስቸግረዋልን /ያስቸግራታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

ከንግግር ጋር በተያያዘ በስሜትህ ደህንነት ላይ ያጋጠመህ ክስተቶች (አንዱን ያክብቡ)

19. ልጅዎ በንግግሩ/ሯ አኳኋን ምክንያት ደስተኛ ያለመሆን ወይም የማዘን ሁኔታ ገጥሞታልን /ገጥሟታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

20. ልጅዎ በንግግሩ/ሯ አኳኋን ምክንያት በራስ መተማመን ይሰማዋልን /ታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

21. ልጅዎ በአነጋገሩ/ሯ አካኋን ጭንቀት ይሰማዋልን/ታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

22. ልጅዎ በአነጋገሩ አካኋን በአፍረት መሸማቀቅ፣ራስን ማግለል ያጋጥመዋልን /ታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

23. ልጅዎ በአነጋገሩ አካኋን የመልከ ጥፉነት ስሜት ኣጋጥሞታልን /ኣጋጥሟታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

24. ልጅዎ በአነጋገሩ አካኋን የመበሳጨት ስሜት ይከሰት በታል/ባታል?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

25. ልጅዎ በአነጋገሩ አካኋን ከሌሎች ሰዎች የተለየ /የች ሰው እንደሆነ /ነች ይሰማዋልን/ታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

26. ልጅዎ በአነጋገሩ/ሯ አካኋን ማራኪ እንደነበረ /ረች ተሰምቶት /ቷት ያውቃልን /ታውቃለችን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

27. ልጅዎ በአነጋገሩ አካኋን ሌሎች ሰዎች ምን ሊሉ እንደሚችሉ አስጨንቆት /ቋት ያውቃልን/ታውቃለችን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

28. ልጅዎ የአነጋገር አካኋኑን /ኗን በተመለከተ ጥያቄዎችን ሲጠየቅ /ስትጠየቅ የመበሳጨት ወይም የምኞት ማጣት ስሜትን ፈጥሮበታልን /ባታልን?

1/ በጭራሽ 2/ እጅግ አልፎ አልፎ 3/ አንዳንድ ጊዜ 4/ በመጠኑ ብዙ ጊዜ 5/ በጣም ብዙ ጊዜ 6/ አላውቅም

ትምህርት ቤት (4 ነገሮች) (አንዱ ላይ ያክብቡ)

29. ልጅዎ ከጥርሶቹ /ቿ፣ከአፉ/ፏ ወይም ከፊቱ/ቷ ጋር በተያያዘ በማንኛውም ምክንያት ከት/ቤት ቀርቷልን/ታለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

30. ልጅዎ ከጥርሱ/ሷ፣ከአፉ/ፏ ወይም ከፊቱ/ቷ ጋር በተያያዘት/ቤት ውስጥ ትኩረቱን /ቷን የማሰባበብ ችግር ገጥሞታልን/ሚታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

31. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያት ክፍል ውስጥ ድምፁን/ጿን ከፍ አድርጐ /ጋ የማንበብ ፍላጐት እንዳያድርበት/ባት ሆኗልን/ለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

32. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያትት/ቤት ያለመሄድ ፍላጐት እንዲያድርበት /ባት ሆኗልን/ሆናለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

ከአቻህ ጋር የሚደረግ መስተጋብር (6 ነገሮች) (አንዱ ላይ ያክብቡ)

33. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያት ፈገግ ያለማለት ወይም ከሌሎች ልጆች ጋር አብሮ/ራ አለመሳቅ ችግር ፈጥሮበታልን/ባታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

34. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያት ሌሎች ልጆች ያበሽቁታልን /ቋታልን ወይም ሌሎች ስሞችን ይሰጡታል/ጧታል ወይ?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

35. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያትበአቻዎቹ/ቿ እንደተገለለ/ለች ተሰምቶታልን/ቷታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

36. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያት ጥያቄዎችን ተጠይቷልን/ለችን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

37. ልጅዎ በጥርሶቹ/ቿ ፣በአፉ/ፏ ወይም በፊቱ/ቷ ምክንያት ከአዳዲስ ሰዎች ጋር ለመገናኘት አለመፈለግ ያድርበታልን/ባታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

38. ልጅዎ ከጥርሶቹ/ቿ ፣ከአፉ/ፏ ወይም ከፊቱ/ቷ ጋር በተያያዘ ከሌሎች ልጆች ወይም ከቤተሰብ አባላት ጋር የመጣላት ወይም የመጨቃጨቅ ሁኔታ ያጋጥመዋልን /ማታልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

39. በአጠቃላይ ጤና ጤንነትህን እንዴት ትገልፀዋለህ? (አንዱ ላይ ያክብቡ)

1/ መጥፎ 2/ ደህና 3/ ጥሩ 4/ በጣም ጥሩ 5/ እጅግ በጣም ጥሩ 6/ አላውቅም

14.3.4 Amharic Questionnaire for children and adolescents

ከከንፈር እና ላንቃ ክፍተት ጋር ከሚወለዱ ልጆች የሕይወት ጥራት ጋር በተያያዘ የአፍ ጤና

ለሕፃናት ቃለ መጠይቅ

የታካሚ ዝርዝሮች (በተመራማሪ የሚሞሉ)

የታካሚው መለያ፡- ----------

የልጁ የትውልድ ቀን፡- ----------

ፆታ፡- ----------

ብሔረሰብ፡- ----------

የምርመራ ውጤት/ግኝት፡- ----------

ቀን፡-

ጉብኝት (ከሚከተሉት መካከል በአንዱ ላይ አክብቡ)

1. ከንግግር ሕክምና በፊት
2. ከንግግር ሕክምና በኋላ ከ3 ወር ባነሰ ጊዜ ውስጥ
3. ከንግግር ሕክምና በፂላ ከ3 ወር በበለጠ ጊዜ ውስጥ

አፍ ላይ የሚታዩ ጠቋሚ ምልክቶች (10 ነገሮች) (አንዱ ላይ ያክብቡ)

1. የጥርሶች ሕመም/የጥርስ ሕመም አጋጥሞሃል?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
2. በአፍ ተንፍስሃል ወይም አንኮራፍተሃል?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
3. የጥርስ ቀለም መበላሸት ወይም በጥርሶችህ ላይ ጠብታ ነጥብ ኖሮብሃል?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
4. የጥርሶች መደራረብ ወይም በጥርሶች መካከል ክፍተት ነበረብህን?
   1/በጣምብዙጊዜ 2/ በመጠኑብዙጊዜ 3/ አንዳንድጊዜ 4/ እጅግአልፎአልፎ 5/ በጭራሽ 6/ አላውቅም
5. በአፍህ ውስጥ ወይም በአፍህ ዙሪያ ቁስሎች ወይም ነጠብጣብ ቁስሎች ነበሩብህን?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
6. መጥፎ የአፍ ጠረን ነበረብህን?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
7. የድድ መድማት ነበረብህን?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
8. በጥርሶች ውስጥ ወይም መካከል የምግብ መግባት አጋጥሞሃልን?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም
9. በሙቅ ወይም በቀዝቃዛ ነገሮች የጥርስ ሕመም ወይም የሚነዝር ስሜት ያጋጥምሃልን?
   1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

10. የአፍ ወይም የከንፈሮች መድረቅ ያጋጥምሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

    የከንውን ደህንነት (በአንዱ ላይ አክብቡ)

11. እንደፖም፣ ካሮት ወይም ጠጣር ስጋን የመሳሰሉ ምግቦችን በመግመጥ ወይም ማኘክ ያስቸግርሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

12. በጥርሶችህ ፣በአፍህ ወይም በፊትህ ላይ ባለው ችግር ምክንያት ለመብላት የምትፈልጋቸውን ምግቦች መብላት አስቸግሮሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

13. ጥርሶችህን ማፅዳት አስቸግሮሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

14. በጥርሶችህ፣በአፍህ ወይም ፊትህ ላይ ባለው ችግር ምክንያት መተኛት አስቸግሮሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

15. በጥርሶችህ፣በአፍህ ወይም በፊት ህላ ይባለው ችግር ምክንያት አንዳንድ ቃላቶችን አስቸግሮሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

16. ለመብላት የምትፈልጋቸውን ምግብ ለመብላት ችለህ ነበርን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

17. በጥርሶችህ፣በአፍህ ወይም በፊትህ ላይ ባለው ችግር ምክንያት የምትናገረው ነገር ሰዎች ለመረዳት ችግር ያጋጥማቸዋልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

18. በጥርሶችህ፣በአፍህ ወይም በፊትህ ላይ ባለው ችግር ምክንያት የጥርሶችህን ንፅህና መጠበቅ አስቸግሮሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

    ከንግግር ጋር በተያያዘ በስሜትህ ደህንነት ላይ ያጋጠሙህ ክስተቶች (አንዱን ያክብቡ)

19. በንግግርህ አኳኋን ምክንያት ደስተኛ ያለመሆን ወይም የማዘን ሁኔታ ኣጋጥሞሃል?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

20. በንግግርህ አኳኋን ምክንያት በራስ መተማመን ይሰማሃል ወይ?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

21. በአነጋገርህ አኳኋን ጭንቀት ይሰማሃል?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

22. በአነጋገርህ አኳኋን በአፍረት ማሸማቀቅ ራስን ማግለል ያጋጥመሃል?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

23. በአነጋገርህ አኳኋን የመልከ ጥፉነት ስሜት ኣጋጥሞሃልን?

    1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

24. በአነጋገርህ አኳኋንየመበሳጨት ስሜት ኣጋጥሞሃል?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

25. በአነጋገርህ አካኋን ከሌሎች ሰዎች የተለየህ ሰው እንደሆንክ ተሰምቶሃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

26. በአነጋገርህ አካኋን ማራኪ እንደነበርክ ተሰምቶህ ያውቃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

27. በአነጋገርህ አካኋን ሌሎች ሰዎች ምን ሊሉ እንደሚችሉ አስጨንቆሃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

28. የአነጋገር አካኋንህን በተመለከተ ጥያቄዎችን ስትጠየቅ የመበሳጨት ወይም የምኞት ማጣት ስሜትን ፈጥሮባሃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

ትምህርት ቤት (4 ነገሮች) (አንዱ ላይ ያክብቡ)

29. ከጥርሶችህ፣ከአፍህ ወይም ከፊትህ ጋር በተያያዘ በማንኛውም ምክንያት ከት/ቤት ቀርተሃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

30. ከጥርሶችህ ፣ከአፍህ ወይም ከፊትህ ጋር በተያያዘ ት/ቤት ውስጥ ትኩረትህን የማሰባበብ ችግር ገጥሞሃልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

31. በጥርሶችህ፣በአፍህ ወይም በፊትህ ምክንያት ክፍል ውስጥ ድምፅህን ከፍ አድርገህ የማንበብ ፍላጐት እንዳያድርብህ ሆኗልን?

1/በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/በጭራሽ 6/ አላውቅም

32. በጥርሶችህ፣ በአፍህ ወይም በፊትህ ምክንያትት/ቤት ያለመሄድ ፍላጐት እንዲያድርብህ ሆኗልን?

1/በጣም ብዙ ጊዜ 2/በመጠኑ ብዙ ጊዜ 3/አንዳንድጊ ዜ 4/እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

ከአቻህ ጋር የሚደረግ መስተጋብር (6 ነገሮች) (አንዱ ላይ ያክብቡ)

33. በጥርሶችህ፣በአፍህ ወይም በፊትህ ምክንያት ፈገግሳትል ወይም ከሌሎች ልጆች ጋር አብረህ ሳትስቅ ቀርተሃልን?

1/ በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

34. በጥርሶችህ፣በአፍህ ወይም በፊትህ ምክንያት ሌሎች ልጆች ያበሽቁሃል ወይም ሌሎች ስሞችን ይሰጡሃልወይ?

1/ በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

35. በጥርሶችህ፣በአፍህ ወይም በፊትህ ምክንያት በአቻዎችህ እንደተገለልክ ተሰምቶሃልን?

1/ በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

36. በጥርሶችህ፣ በአፍህ ወይም በፊትህ ምክንያት ጥያቄዎችን ተጠይቀሃልን?

1/ በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

37. በጥርሶችህ፣በአፍህ ወይም በፊትህ ምክንያት ከአዲስ ሰዎች ጋር ለመገናኘት ሳትፈልግ ቀርተሃልን?

1/ በጣም ብዙ ጊዜ 2/ በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

38. ከጥርሶችህ፣ከአፍህ ወይም ከፊትህ ጋር በተያያዘ ከሌሎች ልጆች ወይም ከቤተሰብ አባላት ጋር የመጣላት ወይም የመጨቃጨቅ ሁኔታ አጋጥሞሃልን?

1/ በጣም ብዙ ጊዜ 2/በመጠኑ ብዙ ጊዜ 3/ አንዳንድ ጊዜ 4/ እጅግ አልፎ አልፎ 5/ በጭራሽ 6/ አላውቅም

አጠቃላይ ጤና

39. በአጠቃላይ ጤንነትህን እንዴት ትገልፀዋለህ? (አንዱ ላይ ያክብቡ)

1/ መጥፎ 2/ ደህና 3/ ጥሩ 4/ በጣም ጥሩ 5/ እጅግ በጣምጥሩ 6/ አላውቅም

14.4 Published original articles and manuscripts

14.4.1 Published articles

I. Descriptive Epidemiology of Orofacial Clefts in Ethiopia.

II. Association Studies and Direct DNA Sequencing Implicate Genetic Susceptibility Loci in the Etiology of Nonsyndromic Orofacial Clefts in Sub-Saharan African Populations

III. Oral Health Related Quality of life of Children born with orofacial clefts in Ethiopia and their parents.

14.4.2 Manuscript

I. The role of environmental factors in the etiology of orofacial clefts in the Ethiopian Population.

Descriptive Epidemiology of Orofacial Clefts in Ethiopia

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Background: The prevalence of birth defects including orofacial clefts (OFC) in Ethiopia is not known and there is no established birth defects registration system.

Objectives: To investigate the prevalence and incidence of OFC in Ethiopia.

Design: Retrospective hospital-based descriptive study.

Methods: The authors obtained data from the Smile Train database on Ethiopian patients with OFC who underwent surgical treatment from June 2007 to December 2013 at 31 hospitals distributed throughout the country. Data related to live births in Ethiopia during the mentioned period were obtained from the Federal Ministry of Health database for estimates of the incidence and prevalence rates.

Results: The total number of life births during the study period was 18,811,316. During this same period, 18,073 cleft patients approximately ranging from 1 to 75 years old were examined and treated at the hospitals mentioned earlier. The incidence rate estimated from the total number of affected children during the study period (N = 8232) is 0.44/1000 live births. The prevalence rate is 0.20/1000 and this was estimated using the number of total population in 2013 (N = 88,703,914). There is a significant difference in frequency between bilateral clefts of the lip and/or palate (CLP) (26.9%) versus unilateral CLP (73.1%) ($P < 0.0001$). There is also a significant difference in frequency between bilateral cleft lips only (15.4%) versus unilateral cleft lip only (84.6%), $P < 0001$.

Conclusion: It is obvious that the findings in this study cannot be representative of the true picture but provides a previously unavailable national estimate of incidence and prevalence of OFC in Ethiopia. It can also be used as comparison for future community-based studies.

Key Words: Cleft lip, cleft lip and palate, cleft palate, epidemiology, Ethiopia

(*J Craniofac Surg* 2017;28: 334–337)

Ethiopia is the second most populous country in Africa with a population of more than 96 million people. The average growth rate is about 2.89%, with 50.3% females and 49.7% males. Only 19% of pregnant women have 4+ visits during their pregnancy and only 10% of the deliveries were attended by skilled healthcare professionals.[1] This means that about 90% are seen by either unskilled persons or traditional birth attendants with the risk of adverse birth outcomes and unrecorded births/birth defects. Clefts of the lip and/or palate (CLP) are the most common craniofacial birth defects with a worldwide birth prevalence of approximately 1/700.[2] It varies from 1/2500 to 1/500 births depending on the geographic origin, racial and ethnic backgrounds, and socioeconomic status.[3,4] Das et al[5] stated that Asians have the highest risk (14/10,000 births) followed by Whites (10/10,000 births) and African Americans (4/10,000 births). There has been varying reports and rates from Africa. Khan[6] reported a birth prevalence of 1.65/1000 births in Kenya. Odhiambo et al[7] in a descriptive cross-sectional study at the Kenyatta National Hospital and Pumwani Maternity Hospital done from November 2006 to March 2007 found an incidence of preauricular tags and CLP 1.5/1000 births.[7] Suleiman et al[8] reported a prevalence of 0.9/1000 live births of orofacial clefts (OFC) among a group of Sudanese hospital newborns in Khartoum. Kesande et al[9] in a retrospective analysis of births at 2 Ugandan hospitals found a prevalence of 0.77/1000 live births.[9] The reported rates of OFC in Nigeria are low. Ireqbulem[10] reported a prevalence of 0.3/1000 in the Eastern part of Nigeria. Butali et al[11] reported a countrywide prevalence of 0.5/1000. The incidence and prevalence of these anomalies in Ethiopia are not known and there are only 2 published reports about these anomalies. The first is the study among surgical patients <14 years of age admitted to the Ethio-Swedish children's hospital in Addis Ababa from 1984 to 1988. Among 2281 surgical patients treated, 183 (8%) were cleft patients.[12] The second is a study conducted at Addis Ababa health institutions by Eshete et al[13] that reported an incidence of 1.49/1000 live births. OFC represent significant public health problems because their treatment requires comprehensive surgical, orthodontic, speech, and psychological management. As Christensen et al[14] noted in 2004, in spite of these comprehensive management efforts, patients with OFC can experience lifelong psychosocial effects from the malformation. They also noted that the incidence of mental health problems is higher in individuals born with OFC. These complications are more severe in the developing world where medical care is limited. In the majority

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TABLE 1. Distribution Into the Various Cleft Types by Gender

	BCLP	BCLO	CPO	LCLO	LCLP	N/A	RCLO	RCLP	Total
Female	344	764	248	2809	559	24	1086	626	6460
Male	902	1214	293	5002	1049	45	1956	1152	11,613
Total	1246	1978	541	7811	1608	69	3042	1778	18,073

BCLO, bilateral cleft lip only; BCLP, bilateral cleft lip and palate; CPO, cleft palate only; LCLO, left cleft lip only; LCLP, left cleft lip and palate; N/A, cleft type unknown; RCLO, right cleft lip only; RCLP, right cleft lip and palate.

of the patients, affected individuals receive only a single surgical treatment.

In Ethiopia, there is only 1 center, which provides multidisciplinary cleft care in the entire country. This center was established in 2003 in collaboration with the Cleft Lip and Palate Team in Bergen, Norway supported by Norwegian Agency for Development Cooperation (NORAD) and later strengthened by Smile Train (American-based charity organization, which organizes and supports free cleft surgical treatment in Ethiopia and other countries) and Transforming Faces (a Canadian-based charity organization which supports holistic cleft care in Ethiopia and other countries). There are also other hospitals, which provide surgical treatment to cleft patients in collaboration with Smile Train. Patient population to these hospitals is a clear mix of urban and rural. The publicity regarding the care of individuals with clefts in Ethiopia is optimal and widespread to all areas at the moment. Our center and other hospitals, which provide cleft surgical treatment, are involved in surgical missions to rural areas to ensure that no cleft patient is left untreated.

METHODS

After obtaining ethical clearance from Institutional Review Board College of Health Sciences, Addis Ababa University Ethiopia Approval number 3.10/027/2015, and permission from Smile Train. We retrieved data from Smile Train database. This included data of all cleft patients who received single surgical treatment with the support of Smile Train at 31 hospitals. The hospitals are distributed throughout the country. Children and adults identified with clefts and provided with surgical repair are included in the database. All individuals with undiagnosed or unoperated cleft were not included. The dataset analyses are based on all Ethiopian cleft patients surgically treated at the above-mentioned institutions from June 2007 to December 2013 (N = 18,073). The cleft types were classified as bilateral CLP (BCLP), unilateral CLP (UCLP) further broken down into right and left CLP, bilateral cleft lip only (BCLO), and unilateral cleft lip only (UCLO)—again broken down into right and left CLO and cleft palate only (CPO). This classification does not include the syndromic and atypical clefts. The cleft types were also divided into bilateral and unilateral in order to examine the cleft laterality. This was done by merging the left/right categories into a unilateral category. That is, instead of having left CLP and right CLP, we have now UCLP. Frequency tables were constructed for the overall sample and stratified by gender. Exact binomial tests for differences in proportions were used for the whole population, and for each gender to assess whether there was a significant difference in the proportion of BCLP, left/right CLP, and UCLP. An analogous procedure was followed for the patients with CLO. Information about immediate and distant relatives with clefts was also collected and frequency tables were constructed based on this information.

TABLE 2. Laterality Distribution of the Cleft Types by Gender

	BCLP	UCLP	CPO	BCLO	UCLO	Total
Female	344	1185	248	764	3895	6436
Male	902	2201	293	1214	6958	11,568
Total	1246	3386	541	1978	10,853	18,004

BCLO, bilateral cleft lip only; BCLP, bilateral cleft lip and palate; CPO, cleft palate only; UCLO, unilateral cleft lip only; UCLP, unilateral cleft lip and palate.

TABLE 3. Tests for Difference in Proportions

Test	Percent of First Category	Confidence Interval	Exact P^*
BCLP vs UCLP	26.90	(25.63, 28.20)	<0.0001
BCLP vs LCLP	43.66	(41.83, 45.50)	<0.0001
BCLP vs RCLP	41.20	(39.44, 42.98)	<0.0001
LCLP vs RCLP	47.49	(45.80, 49.19)	0.00367
BCLO vs UCLO	15.42	(14.79, 16.05)	<0.0001
BCLO vs LCLO	20.21	(19.41, 21.02)	<0.0001
BCLO vs RCLO	39.40	(38.05, 40.77)	<0.0001
LCLO vs RCLO	71.97	(71.12, 72.81)	<0.0001

BCLO, bilateral cleft lip only; BCLP, bilateral cleft lip and palate; LCLO, left cleft lip only; LCLP, left cleft lip and palate; RCLO, right cleft lip only; RCLP, right cleft lip and palate; UCLO, unilateral cleft lip only; UCLP, unilateral cleft lip and palate.

*Significance probability (*P*-value) associated with the test of the null hypothesis that equal proportions (50%) of subjects were found in the 2 cleft subcategories specified, assessed by the exact binomial test.

RESULTS

During the study period, 18,073 patients with CLP were operated. Out of the total operated cleft patients, 8232 are under 7 years old. In these 7 years (the study period), the total number of live births was 18,811,316. This gives an incidence of 0.44/1000 live births of OFC in Ethiopia, although this is likely to be an underestimate. We also estimated the prevalence to be 0.20/1000 using the total number of clefts (N = 18,073) and number of total population in 2013 (N = 88,703,914). Individuals with no diagnosis, no cleft but prior unspecified surgeries are under the N/A category. The syndromic and atypical clefts are not included in the tables. Table 1 presents the distributions of these categories by gender based on the individuals who presented for surgery. We noted that these do not include termination of pregnancy, stillbirths, neonatal deaths with clefts, and untreated patients or misdiagnosed patients. Overall, most of the individuals examined had left CLO. The category with the least individuals examined was CPO. When stratified by gender, these patterns remained the same.

The distributions of the cleft types by laterality and by gender are presented in Table 2. Most of the individuals had UCLO, and the category with the least individuals examined is CPO.

Tests for differences in proportions were performed to see if there was a significant difference in the proportion of BCLP, left/right CLP, and UCLP. An analogous procedure was followed for the CLO patients. These assessments were done for all individuals (Table 3).

Table 3 shows that the proportion of BCLP is smaller than the proportion of UCLP, right CLP, and left CLP (all $P < 0.0001$). It also shows that the proportion of left CLP is smaller than the proportion of right CLP ($P = 0.00367$). Likewise, the proportion of BCLO is smaller than the proportion of right CLO, left CLO, and UCLO (all $P < 0.0001$). However, the proportion of left CLO is bigger than the proportion of right CLO ($P < 0.0001$).

An analogous procedure was done but now stratifying by gender. The results were similar to the ones without the stratification. The only exception happens when we compare the proportion

TABLE 4. Tests for Difference in Proportions by Gender

Test	Female		Male	
	Percent of First Category (95% CI)	Exact P^*	Percent of First Category (95% CI)	Exact P^*
BCLP vs UCLP	22.50 (20.43, 24.68)	<0.0001	29.07 (27.48, 30.70)	<0.0001
BCLP vs LCLP	38.10 (34.92, 41.35)	<0.0001	46.23 (44.00, 48.47)	0.00094
BCLP vs RCLP	35.46 (32.45, 38.57)	<0.0001	43.91 (41.75, 46.09)	<0.0001
LCLP vs RCLP	47.17 (44.30, 50.06)	0.05516	47.66 (45.56, 49.77)	0.02967
BCLO vs UCLO	16.40 (15.35, 17.49)	<0.0001	14.86 (14.09, 15.65)	<0.0001
BCLO vs LCLO	21.38 (20.05, 22.76)	<0.0001	19.53 (18.55, 20.54)	<0.0001
BCLO vs RCLO	41.30 (39.04, 43.58)	<0.0001	38.30 (36.60, 40.01)	<0.0001
LCLO vs RCLO	72.12 (70.68, 73.52)	<0.0001	71.89 (70.82, 72.94)	<0.0001

BCLO, bilateral cleft lip only; BCLP, bilateral cleft lip and palate; CI, confidence interval; LCLO, left cleft lip only; LCLP, left cleft lip and palate; RCLO, right cleft lip only; RCLP, right cleft lip and palate; UCLO, unilateral cleft lip only; UCLP, unilateral cleft lip and palate.

*Significance probability (*P*-value) associated with the test of the null hypothesis that equal proportions (50%) of subjects were found in the 2 cleft subcategories specified, assessed by the exact binomial test.

of left CLP versus right CLP among females. In this patient, the difference is not significant at the $\alpha = 0.05$ level (Table 4).

Information about immediate and distant relatives with clefts was also collected. The frequencies and percent are shown in Table 5. The majority of the individuals reported that they did not have an immediate relative with cleft or a distant relative with cleft. Less than 2% of them had immediate or distant relative with cleft, and <1% reported that they did not know if they had either of those.

DISCUSSION

There is no relevant information about OFC in Ethiopia. The multidisciplinary cleft care, which was started in 2003 in collaboration with the Cleft Lip and Palate Team in Bergen supported by NORAD and strengthened by Transforming Faces and Smile Train created an opportunity for teaching and research. This research was done based on the database of Smile Train, which is the largest and most representative database available at the moment. It revealed an incidence of 0.44/1000 live births and prevalence of 0.20/1000. The distribution of the cleft types which is done for all operated cleft patients (18,073) during the study period is: CLO = 12,831, CLP = 4632, and CPO = 541. The number of isolated cleft palate in this study (3%) is low similar to other African studies.[11,15,16] It is also more common in males similar to the study done by Conway et al.[16]

The incidence and prevalence rates reported in this study are less than what has been reported in Addis Ababa, Ethiopia by Eshete et al.[13] They are lower than the Nigerian prevalence reported by Butali et al[11] and the prevalence report in African American by Gundlach and Maus.[17] However, these rates are similar in the sense that they are lower than other population and consistent with what has been reported for clefts in Africa. Kesande et al[9] in a retrospective analysis of births at 2 Ugandan hospitals found a prevalence of 0.77/1000 live births. This also is higher than our study. In our current study, most of the patients with OFC (55.6%) were males. This is similar to the Ugandan study[9] and the study conducted in Tanzania by Manyama et al.[18] The study done by Martelli-Junior et al[19] in a Brazilian population reported similar findings. They found 54.5% males and 45.6% females. In the previous Ethiopian study done by Eshete et al[13] cleft lip alone and isolated cleft palate were more common in females, whereas CLP were more common in males, in contradiction to the current study. In the current study, all types of clefts including isolated cleft palate are more common in males than in females. This can be explained by the fact that this study captured only those patients who came to get surgical treatment, and the previous one captured all hospital deliveries at specified institutions. This might also be the reflection of the attitude of the community to give priority for males for everything including treatment. Isolated cleft lip constituted the most common type of cleft (70%), CLP (26%), and isolated cleft palate (3%). This is the same finding with a study done in Tanzania by Manyama et al.[18] In their study, isolated cleft lip constituted 49.2% of all cleft deformities, while clefts of both lip and palate and isolated cleft palate constituted 39.2% and 11.7% of cleft deformities, respectively. In our study, isolated cleft palate is low as it is in Manyama et al[18] study and other studies in Africa. One of the reasons for this could be lack of proper examination of the neonate before discharge from the delivery ward and unattended deliveries. Congenital anomalies like isolated cleft palate are not evident to everybody including parents and physicians unless a proper physical examination is done. It is very common to find patients with an isolated cleft palate who do not exactly know the pathology they have until adulthood at our set-ups. We think it is not different in other institutions in developing world. The other reason could be the higher mortality rate in these patients because of difficulties in feeding neonates and infants in the absence of supportive feeding devices.[10,20] This raises several concerns that can be addressed by surveillance, community participation, and education.

There is no established system of birth defect registry including OFC in Ethiopia (recent unpublished review). We think this has contributed to the nonexistence of relevant information on the incidence of congenital anomalies including OFC. The main reason for planning and conducting this research is to obtain relevant information on the incidence and prevalence of OFC. We retrieved data from Smile Train database. During the past 7 years, more than 18,073 patients were operated at different hospitals. Of the total

TABLE 5. Relatives With a Diagnosis of Cleft

Test	Immediate Relative With Clefts		Distant Relative With Clefts	
	Frequency	%	Frequency	%
Yes	351	1.95	272	1.51
No	17,609	97.81	17,688	98.24
Do not know	44	0.24	44	0.24

operated patients, 8232 were born and received surgical cleft repair during the study period. These data contain the information of all the patients operated during this period. It is limited by the use of data only from the hospitals and may not be representative of the true estimate of the prevalence. A population-based study is preferred but there is lack of resources human and capital to undertake such an exercise at this moment. However, the current study provides a baseline data on the prevalence and incidence that will serve as reference for future population-based studies.

LIMITATION

This study is hospital-based and captures only patients who presented for surgical treatment. This is bias and may not give a true reflection of the prevalence and incidence of clefting. However, it gives a baseline upon which future population studies can be conducted. Our rates are smaller than other African countries but the methodology reported in these other studies are different. Most of them reported single center data for the prevalence estimate, which is largely an under-ascertainment. This observation was reported by Butali and Mossey. Our approach is similar to other standardized studies in Africa conducted in Nigeria[11] and South Africa.[21] Furthermore, our rates are comparable to rates reported by Kromberg and Jenkins, 1982 in South Africa and Butali et al[11] in Nigeria.

CONCLUSION AND RECOMMENDATION

The incidence rate (0.44/1000 live births) and prevalence rate (0.20/1000 population) found in this study are lower than previously reported in Ethiopia and other African countries. The reason for this lower rate could be that in the numerator we included only individuals who presented for surgery through the Smile Train outreach, considering the available surgical set-up. This finding could not be representative therefore we highly recommend establishing a system of birth defect registry to know the burden of birth defects including CLP.

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Association Studies and Direct DNA Sequencing Implicate Genetic Susceptibility Loci in the Etiology of Nonsyndromic Orofacial Clefts in Sub-Saharan African Populations

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Abstract

Orofacial clefts (OFCs) are congenital dysmorphologies of the human face and oral cavity, with a global incidence of 1 per 700 live births. These anomalies exhibit a multifactorial pattern of inheritance, with genetic and environmental factors both playing crucial roles. Many loci have been implicated in the etiology of nonsyndromic cleft lip with or without cleft palate (NSCL/P) in populations of Asian and European ancestries, through genome-wide association studies and candidate gene studies. However, few populations of African descent have been studied to date. Here, the authors show evidence of an association of some loci with NSCL/P and nonsyndromic cleft palate only (NSCPO) in cohorts from Africa (Ghana, Ethiopia, and Nigeria). The authors genotyped 48 single-nucleotide polymorphisms that were selected from previous genome-wide association studies and candidate gene studies. These markers were successfully genotyped on 701 NSCL/P and 163 NSCPO cases, 1,070 unaffected relatives, and 1,078 unrelated controls. The authors also directly sequenced 7 genes in 184 nonsyndromic OFC (NSOFC) cases and 96 controls from Ghana. Population-specific associations were observed in the case-control analyses of the subpopulations, with West African subpopulations (Ghana and Nigeria) showing a similar pattern of associations. In meta-analyses of the case-control cohort, *PAX7* (rs742071, $P = 5.10 \times 10^{-3}$), 8q24 (rs987525, $P = 1.22 \times 10^{-3}$), and *VAX1* (rs7078160, $P = 0.04$) were nominally associated with NSCL/P, and *MSX1* (rs115200552, $P = 0.01$), *TULP4* (rs651333, $P = 0.04$), *CRISPLD2* (rs4783099, $P = 0.02$), and *NOG1* (rs17760296, $P = 0.04$) were nominally associated with NSCPO. Moreover, 7 loci exhibited evidence of threshold overtransmission in NSOFC cases through the transmission disequilibrium test and through analyses of the family-based association for disease traits. Through DNA sequencing, the authors also identified 2 novel, rare, potentially pathogenic variants (p.Asn323Asp and p.Lys426IlefsTer6) in *ARHGAP29*. In conclusion, the authors have shown evidence for the association of many loci with NSCL/P and NSCPO. To the best of this knowledge, this study is the first to demonstrate any of these association signals in any African population.

Keywords: genetic heterogeneity, rare variants, genome-wide association studies (GWAS), candidate genes, craniofacial genetics, population genetics

Introduction

Human orofacial clefts (OFCs) are congenital malformations of the face and oral cavity due to dysregulation of embryologic processes. The global incidence of OFCs is 1 per 700 live births. However, race, ethnicity, geographic locations, environmental factors, and socioeconomic status influence the incidence of OFCs (Gorlin et al. 2001). The highest incidence occurs in Asians, followed by populations of European ancestry, whereas African populations have the lowest incidence (Mossey and Modell 2012). Although there are no national prevalence data for Ghana and Ethiopia, an estimate of 0.5 per 1,000 has been observed for Nigeria (Butali, Adeyemo, et al. 2014). These observations presuppose that the relative contributions of individual susceptibility genes may vary across different human populations. OFCs may be syndromic or nonsyndromic, with the syndromic forms presenting with other congenital anomalies. The etiology of the more common nonsyndromic OFCs (NSOFCs) is complex, exhibiting multifactorial pattern of inheritance. NSOFCs are classified into nonsyndromic cleft lip with or without cleft palate (NSCL/P) and nonsyndromic cleft palate only (NSCPO), and these 2

groups have a heterogeneous genetic architecture. NSCL/P comprises nonsyndromic cleft lip only (NSCL) and nonsyndromic cleft lip and palate (NSCLP; Dixon et al. 2011).

To date, 6 genome-wide association studies (GWASs) and a meta-analysis have been published for NSOFCs, with these signals demonstrating an association with NSCL/P but not NSCPO. In a GWAS involving Europeans, an association was observed between a locus in Chr8q.24 and NSCL/P (Birnbaum et al. 2009). The 8q.24 signal was subsequently replicated in another GWAS of NSCL/P in Europeans from the United States (Grant et al. 2009). A third GWAS that involved cohorts of European ancestries also revealed that 2 additional loci, 17q22 (*NOG1*) and 10q25 (*VAX1*), were associated with NSCL/P. Other loci yielded a suggestive association with NSCL/P: 15q13.3 (*GREM1*), 13q31.1 (*SPRY2*), and 2p21 (*THADA*; Mangold et al. 2010). Employing trios of Asian and European ancestries, a GWAS implicated 20q12 (*MAFB*) and 1p22.1 (*ABCA4*) in the etiology of NSCL/P, with 17p13 (*NTN1*) showing a suggestive association. Stratified analyses based on ancestries by the same GWAS showed that some signals were ancestry specific: trios of European ancestry gave the strongest association for 8q.24, whereas those of Asian ancestry were strongly associated with *MAFB, ABCA4*, and *IRF6* (Beaty et al. 2010). A meta-analysis revealed additional NSCL/P susceptibility loci: *THADA, SPRY2*, 15q22.2 (*TPM1*), and 1p36 (*PAX7*; Ludwig et al. 2012). Recently, a GWAS involving Asians implicated 16p13.3 (*ADCY9*; Sun et al. 2015) in the etiology of NSCL/P, whereas a GWAS involving dogs and a Guatemalan population gave a suggestive association for *ADAMTS20* (Wolf et al. 2015).

In the pre- and post-GWAS era, candidate gene and replication studies have been instrumental in identifying cleft susceptibility loci. Pathogenic variants in *IRF6* were shown to cause van der Woude syndrome and popliteal pterygium syndrome (Kondo et al. 2002). Subsequently, a missense variant in *IRF6* (rs2235371) demonstrated overtransmission in NSCL/P cases of European ancestry (Zucchero et al. 2004). Another *IRF6* locus, rs642961, has been shown to be associated with NSCL/P but not NSCPO (Rahimov et al. 2008). Corollary to these observations, some studies (Birnbaum et al. 2009; Kerameddin et al. 2015) have confirmed a role of *IRF6* as a NSCL/P risk locus in populations of Asian and European ancestries. Other candidate genes implicated in the etiology of NSCL/P included *MSX1* (Rafighdoost et al. 2013), *BMP4* (Suzuki et al. 2009), *FOXE1* (Moreno et al. 2009), *AXIN2* (Letra et al. 2012), *CRISPLD2* (Chiquet et al. 2007), *NOG1*, and *FGFR2* (Leslie et al. 2015).

Among Africans, genetic studies on OFCs are limited. A study involving a Nigerian cohort implicated *MSX1*, but not other loci, in the etiology of NSCL/P (Butali et al. 2011). Other studies that recruited Kenyans (Weatherley-White et al. 2011) and Congolese (Figueiredo et al. 2014) could not replicate the association for cleft susceptibility loci among Africans, probably due to the small sample size and population heterogeneity. Moreover, sequencing of GWAS loci in cohorts from Ethiopia and Nigeria reported some rare, potentially causative variants (Butali, Mossey, et al. 2014). Conducting genetic and genomics studies with a cleft cohort from Africa may identify novel and population-specific signals. However, it is also important for us to investigate the role of identified signals and biologically relevant genes from existing European and Asian studies in the African population. The present study aimed to replicate the association between reported GWASs and candidate gene loci in our NSCL/P cohort. We also tested the hypothesis that NSCL/P loci may contribute to NSCPO susceptibility in Africans. Finally, we screened for rare, potentially pathogenic variants in 7 candidate genes at risk loci usually associated with NSCL/P.

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Subjects and Methods

We recruited 3,585 participants from Ghana, Ethiopia, and Nigeria (Table 1; Appendix Methods). All sample and data collection at various study sites were approved by the local institutional review boards: College of Health Sciences, KNUST (Ghana; CHRPE/AP/217/13); College of Medicine, University of Lagos (Nigeria; ADM/DCST/HREC/APP/1374); and College of Health Sciences, Addis Ababa University (Ethiopia; 3.10/027/2015). Before sample and data collection, written informed consent was obtained from each participating family. DNA processing is shown in the Appendix Methods.

Single-Nucleotide Polymorphism Selection

We selected single-nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥5% in the African population for genotyping; these were previously reported in peer review journals or identified in animal studies and during our resequencing studies. These include SNPs that are associated with NSCL/P in candidate genes studies and GWASs in European and Asian populations (Appendix Table 1).

SNP Genotyping

We genotyped 48 SNPs (Appendix Table 1) on a total of 3,585 samples—872 NSOFC cases (163 NSCPO, 340 NSCL, 361 NSCLP, and 8 "untyped"), 1,635 unaffected relatives, and 1,078 unrelated controls—with the 192.24 Fluidigm SNP genotyping protocol (Appendix Methods). The "untyped" samples (from probands) and other samples, however, failed quality control checks and were not included in the final statistical analyses (Table 1).

Statistical Analyses for Association Studies

During quality control checks, we resolved Mendelian errors in case-parent triads and dropped from the final analyses samples that were not successfully genotyped on at least 95% of the 48 genotyped SNPs. We computed Hardy-Weinberg equilibrium (HWE) through PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/). We then conducted 1) case-control analyses to determine associations in each subpopulation and 2) meta-analyses of the 3 subpopulations based on Table 1. For this test, we used $P < 0.05$ to denote nominal association and a Bonferroni correction of 141 tests to ascertain a threshold for formal significance of $P = 3.54 \times 10^{-4}$. The 141 tests comprised 47 SNPs that passed HWE × 3 cleft subphenotypes × 1 racial group × 1 test. Of the 48 SNPs, only 1 failed HWE ($P < 0.05$). Additional analyses to determine overtransmission of the rare alleles were conducted with the transmission disequilibrium test (TDT) and through the family-based association for disease traits (DFAM). The TDT used only the case-parent triad information (Table 1), while the DFAM allowed us to combine triad and dyad data. For these tests, the significant *P* value was 0.05. Parent-of-origin effects and gene-gene interactions (epistasis) were also calculated. The probands in the case-control arm of the study (Table 1) are the same probands in the family-based studies.

Table 1. Subphenotypes, Sex, and Sample Types of Study Cohort That Passed Quality Control Checks and Were Included in Statistical Analyses.

Cleft Subphenotype of Probands	Samples per Population, *n*			Total
	Ghana	Ethiopia	Nigeria	
Case-control cohort				
NSCL	162	101	77	340
NSCLP	144	143	74	361
NSCPO	102	21	40	163
Unrelated controls	408	357	313	1078
Case-parent trios				
NSCL	52	2	20	74
NSCLP	48	3	26	77
NSCPO	34	1	7	42
Case-parent dyads				
NSCL	77	84	51	212
NSCLP	76	134	47	257
NSCPO	53	20	32	105
Other trios				
NSCL	18	0	0	18
NSCLP	14	0	0	14
NSCPO	11	0	0	11
Other dyads				
NSCL	8	0	0	8
NSCLP	3	0	0	3
NSCPO	3	0	0	3
Singletons				
NSCL	5	13	6	24
NSCLP	1	8	1	10
NSCPO	2	0	1	3
Tetrads				
NSCLP	2	0	0	2
Pentads				
NSCLP	1	0	0	1

Case probands consisted of 423 males and 441 females, whereas unrelated controls were made up of 441 males and 637 females. The probands in the case-control arm of the study are the same probands in the family-based studies. In some of the designated singletons, parental samples failed data cleaning and were dropped from statistical analyses—hence, the designation of such families as *singletons*. Singletons were informative in the case-control arm of our study but not the family-based studies. Tetrads and pentads were collected from families where 2 individuals were affected with clefts. "Other trios and dyads" largely refers to case-mother-maternal grandmother trios, case-mother-sibling trios, as well as case-siblings trios and dyads. Case-parent trios, tetrads, and pentads were employed in the transmission disequilibrium test, whereas all sample types, except singletons and unrelated controls, were used for analyses of the family-based association for disease traits. Only case probands and unrelated controls were included in the case-control analyses.
NSCL, nonsyndromic cleft lip; NSCL/P, nonsyndromic cleft lip with or without cleft palate; NSCLP, nonsyndromic cleft lip and palate; NSCPO, nonsyndromic cleft palate only.

DNA Sequencing

We directly sequenced *VAX1, PAX7, ARHGAP29, MSX1, FOXE1, BMP4*, and *MAFB* in 184 NSOFC cases (131 NSCL/P and 53 NSCPO) from Ghana using Sanger Sequencing (Appendix Methods; Butali, Mossey, et al. 2014). We also performed segregation

analyses on observed potentially pathogenic missense, frameshift, and splice site variants by sequencing available parental samples. We further sequenced 96 unrelated Ghanaian controls to ascertain whether the novel variants that we encountered in NSOFC cases also occurred in controls.

Results

Association Analyses

In meta-analyses of the case-control cohorts from the 3 subpopulations, we successfully demonstrated nominal association of *PAX7* (rs742071, $P = 5.10 \times 10^{-3}$), 8q24 (rs987525, $P = 1.22 \times 10^{-3}$), as well as *VAX1* (rs7078160, $P = 0.04$) with NSCL/P; in addition, *MSX1* (rs115200552, $P = 0.01$), *TULP4* (rs651333, $P = 0.04$), *CRISPLD2* (rs4783099, $P = 0.02$), and *NOG1* (rs17760296, $P = 0.04$) were nominally associated with NSCPO (Table 2), with the direction of effect being the same as reported by earlier studies. Among Ethiopians (Appendix Table 2), *PAX7* (rs742071, $P = 5.57 \times 10^{-3}$), *IRF6* (rs642961, $P = 0.02$), *DYSF* (rs2303596, $P = 2.31 \times 10^{-3}$), 8q24 (rs987525, $P = 7.82 \times 10^{-4}$), and *MAFB* (rs13041247 and rs11696257, all with $P = 0.04$) were nominally associated with NSCL/P; *ABCA4* (rs481931 and rs4147811, all with $P = 0.03$) and *NTN1* (rs8081823, $P = 0.03$) were nominally associated with NSCPO. Moreover, subphenotype analyses of the Ethiopian NSCL/P cohort showed that the *PAX7, DYSF, MSX1, SPRY2* (rs9574565, $P = 7.05 \times 10^{-3}$) and *MAFB* signals were particularly stronger for NSCL, whereas the *IRF6* (rs642961, $P = 9.11 \times 10^{-3}$) and 8q24 (rs987525, $P = 1.07 \times 10^{-3}$) signals were stronger for NSCLP (Appendix Table 2). Among Ghanaians (Appendix Table 3), *ABCA4* (rs560426, $P = 0.03$) and *VAX1* (rs7078160, $P = 0.03$) were nominally associated with NSCL/P with subphenotype analyses of the NSCL/P cohort showing that the *ABCA4* locus was strongly associated with NSCLP. *ABCA4* (rs4147811, $P = 7.48 \times 10^{-3}$) and *CRISPLD2* (rs4783099, $P = 0.04$) were nominally associated with NSCL/P and NSCPO, respectively, among Nigerians (Appendix Table 4). Subphenotype analyses of the Nigerian NSCL/P (Appendix Table 4) showed that *PAX7* (rs742071, $P = 0.02$) and *ARHGAP29* (rs138751793, $P = 0.04$) signals were stronger for NSCL, whereas another SNP at the *ABCA4* locus (rs481931, $P = 2.87 \times 10^{-3}$) was strongly associated with NSCLP. However, none of these case-control associations passed Bonferroni correction.

For the TDT and DFAM (Tables 3 and 4) for all 3 subpopulations, 7 loci demonstrated formal significance with NSOFCs at $P \leq 0.05$. Formal significance for the TDT and DFAM was evaluated at $P \leq 0.05$ because these are secondary analyses compared with case-control analyses and are not true independent tests. All family-based studies suggested that the minor allele of *ABCA4* (rs560426) was overtransmitted in NSCLP cases among Africans. *PAX7* (rs742071) also consistently showed evidence of overtransmission in NSCL cases in the TDT and DFAM. *MSX1* (rs115200552) and *AXIN2* (rs3923086) also demonstrated strong overtransmission in NSCLP cases in DFAM analyses, whereas *MTHFR* (rs1801131) and *DYSF* exhibited overtransmission in NSCL cases in TDT and DFAM analyses, respectively. Only an SNP of *VAX1* demonstrated overtransmission in NSCPO cases.

Parent-of-Origin Effects

Parent-of-origin effects were not observed for almost all SNPs, except rs16260 of *CDH1*. For rs16260, a trend toward association ($P = 0.0764$) was observed for all clefts. The rs16260 SNP exhibited a maternal imprinting or maternal overtransmission effect.

Gene-Gene Interactions

In gene-gene (G × G) or epistatic interactions, 3 SNPs exhibited evidence of epistasis with other SNPs. Each of these epistatic interactions yielded $P = 0.02$. A SNP for *ABCA4*, rs560426, interacted with *Chr6*, rs2674394 (gene desert). Moreover, rs2303596 of *DYSF* interacted with rs3923086 of *AXIN2*. Finally, rs8069536 of *NTN1* interacted with rs17820943, rs13041247, and rs11696257, all of *MAFB*. However, none of these G × G interactions passed Bonferroni correction.

Direct DNA Sequencing of 7 Selected Genes

We observed several rare and/or novel variants in the 7 genes that we sequenced (Table 5, Appendix Table 5). "Rare variants," as used here, refer to either a novel variant or a variant whose MAF is ≤1%. Some of these variants were predicted to be potentially pathogenic by various bioinformatics tools, whereas others were depicted as benign. A de novo occurrence could not be demonstrated for any of these variants, because either the variant was present in at least 1 parent, or not both parents were available for segregation analysis. Last, some of the novel variants that we observed occurred in controls (e.g., all *VAX1* variants), whereas others were not observed in controls (e.g., all *ARHGAP29* variants).

Discussion

We have successfully demonstrated associations (both nominal in case-control analyses and threshold in the TDT and DFAM analyses) between some loci and NSCL/P in cohorts from Africa. We also tested the hypothesis that these loci contribute to NSCPO in Africans, and we observed some interesting associations. The 8q24 locus exhibited the strongest nominal significance with NSCL/P in case-control meta-analyses, with the trends suggesting that this locus may be relevant in all 3 subpopulations. The test of heterogeneity also largely suggested the absence of heterogeneity at this locus among the 3 African populations. We observed that among Africans, the associated minor C allele of rs987525 (http://browser.1000genomes.org) conferred reduced susceptibility, while the major A allele is the risk allele. Irrespective of these differences in minor alleles, our result is in harmony with earlier

Table 2. Meta-analyses of the Case-Control Cohorts from Ghana, Ethiopia, and Nigeria.

Part A: Meta-analyses of NSCL/P and NSCPO Case-Control Cohorts from All 3 Countries

				NSCL/P			NSCPO		
SNP	Probable Gene/Loci	Minor Alleles[a]	African MAF	*P*	OR	I	*P*	OR	I
rs1801131	*MTHFR*	C/A[b]	0.15	0.32	1.08	0.00	0.19	0.79	0.00
rs1801133	*MTHFR*	A/G[c]	0.09	0.49	1.08	18.19	0.44	0.83	0.00
rs766325	*PAX7*	G/A[b,d,e]	0.18	0.29	0.92	0.00	0.23	0.82	0.00
rs742071	*PAX7*	T/G[b]	0.39	**5.10E-03**[f]	**1.19**	**54.68**	0.76	0.96	0.00
rs560426	*ABCA4*	C/T[b,g]	0.49	0.10	0.90	6.15	0.16	1.18	0.00
rs481931	*ABCA4*	T/G[c]	0.10	0.40	1.09	11.13	0.49	0.85	0.00
rs4147811	*ABCA4*	T/C[c]	0.11	0.23	1.13	67.35	0.93	1.02	0.00
rs138751793	*ARHGAP29*	C/T[h]	0.02	0.24	1.32	0.00	0.47	1.34	27.90
rs6677101	*SLC25A24*	G/T[b,e,g]	0.33	0.80	0.98	12.11	0.87	1.02	53.89
rs861020	*IRF6*	A/G[b]	0.11	0.23	1.11	0.00	0.83	0.96	24.15
rs34743335	*IRF6*	T/A	0.02	0.59	0.90	0.00	0.84	0.89	38.34
rs642961	*IRF6*	A/G[b]	0.09	0.32	1.11	68.47	0.57	0.88	44.17
rs7590268	*THADA*	G/T[b]	0.20	0.74	0.98	0.00	0.38	0.87	0.00
rs4332945	*DYSF*	T/G[b,e,g]	0.16	0.94	0.99	0.00	0.97	1.01	0.00
rs2303596	*DYSF*	T/C[c,d,e]	0.22	0.20	0.91	75.32	0.57	1.09	73.54
rs227782	*DYSF*	A/G[b,g]	0.42	0.33	1.06	0.00	0.35	1.12	61.90
rs115200552	*MSX1*	C/G[h]	0.02	0.38	1.16	28.63	**0.01**[f]	**1.81**	**0.00**
rs12532	*MSX1*	G/A[c,e]	0.44	0.49	0.96	0.00	0.37	0.90	0.43
rs2674394	Gene desert	A/C[b]	0.17	0.62	1.04	0.00	0.68	1.07	0.00
rs651333	*TULP4*	C/T[b,d,g]	0.34	0.97	1.00	0.00	**0.04**[f]	**1.29**	**0.00**
rs6558002	*EPHX2*	C/T[b,g]	0.24	0.39	1.06	0.00	0.87	1.02	0.00
rs987525	8q24	A/C[b,g]	0.38	**1.22E-03**[f]	**0.81**	**40.55**	0.22	0.86	0.00
rs894673	*FOXE1*	A/T[c]	0.33	0.42	0.95	0.00	0.93	1.01	0.00
rs3758249	*FOXE1*	T/C[c]	0.33	0.56	0.96	0.00	0.90	1.02	0.00
rs7078160	*VAX1*	A/G[b]	0.25	**0.04**[f]	**1.16**	**0.00**	0.88	1.02	0.00
rs4752028	*VAX1*	C/T[b,g]	0.45	0.51	0.96	0.00	0.80	0.97	0.00
rs10785430	*ADAMTS20*	G/A[b]	0.32	0.90	0.99	0.00	0.49	1.09	0.00
rs9574565	*SPRY2*	T/C[c,g]	0.35	0.75	1.02	0.00	0.45	1.10	0.00
rs8001641	*SPRY2*	G/A[c,d,e,g]	0.10	0.35	1.08	0.00	0.37	0.85	0.00
rs17563	*BMP4*	T/C[b,d,e,g]	0.18	0.95	0.99	0.00	0.77	1.04	0.00
rs1258763	*GREM1*	C/T[c,d,e,g]	0.49	0.11	1.11	0.00	0.50	0.92	0.00
rs8049367	*ADCY9*	C/T[c,d,e]	0.30	0.20	1.09	0.00	0.10	0.81	0.00
rs16260	*CDH1*	A/C[b]	0.13	0.59	1.05	0.00	0.39	0.85	0.00
rs11642413	*CDH1*	G/A[b,e,g]	0.28	0.83	1.02	0.00	0.21	0.83	0.00
rs1546124	*CRISPLD2*	G/C[b,e]	0.25	0.60	0.96	0.00	0.89	0.98	0.00
rs4783099	*CRISPLD2*	T/C[b]	0.33	0.59	1.04	0.00	**0.02**[f]	**0.74**	**0.00**
rs8069536	*NTN1*	T/G[b]	0.32	0.13	1.11	0.97	0.88	0.98	0.00
rs8081823	*NTN1*	A/G[c]	0.24	0.08	0.88	0.00	0.63	0.94	32.54
rs17760296	*NOG1*	G/T[b]	0.02	0.92	0.99	0.00	**0.04**[f]	**1.74**	**0.00**
rs227731	*NOG1*	G/T[b,g]	0.22	0.86	0.99	0.00	0.26	1.17	0.00
rs7224837	*AXIN2*	G/A[b]	0.11	0.75	1.04	0.00	0.81	0.95	0.00
rs3923086	*AXIN2*	A/C[b,d,e,g]	0.02	0.25	1.15	0.00	NA	NA	NA
rs17820943	*MAFB*	T/C[c]	0.25	0.33	0.93	15.15	0.68	1.06	22.99
rs13041247	*MAFB*	C/T[c]	0.25	0.37	0.94	34.01	0.42	1.12	0.00
rs11696257	*MAFB*	T/C[c]	0.25	0.30	0.93	32.24	0.61	1.07	0.00

Part B: Meta-analyses of Subphenotypes of NSCL/P Cohorts from the 3 Countries

				NSCL			NSCLP		
rs1801131	*MTHFR*	C/A[b]	0.15	0.78	1.03	0.00	0.22	1.13	0.00
rs1801133	*MTHFR*	A/G[c]	0.09	0.71	1.06	8.24	0.30	0.30	0.00
rs766325	*PAX7*	G/A[b,d,e]	0.18	0.91	0.99	0.00	0.17	0.86	0.00
rs742071	*PAX7*	T/G[b]	0.39	**0.02**[f]	**1.23**	**68.74**	**0.03**[f]	**1.19**	**0.00**
rs560426	*ABCA4*	C/T[b]	0.49	0.73	1.03	0.00	**0.03**[f]	**1.20**	**10.33**
rs481931	*ABCA4*	T/G[c]	0.10	0.81	0.97	0.00	0.08	1.27	63.75
rs4147811	*ABCA4*	T/C[c]	0.11	0.50	1.10	65.82	0.15	1.21	15.35
rs138751793	*ARHGAP29*	C/T[h]	0.02	0.19	1.53	66.38	0.41	1.29	0.00
rs6677101	*SLC25A24*	G/T[b,e,g]	0.33	0.92	0.99	0.00	0.98	1.00	58.97
rs861020	*IRF6*	A/G[b]	0.11	0.18	1.17	17.72	0.57	1.07	0.00
rs34743335	*IRF6*	T/A	0.02	0.87	0.96	0.00	0.50	0.85	23.72
rs642961	*IRF6*	A/G[b]	0.09	0.96	0.99	15.60	0.15	1.21	62.97
rs7590268	*THADA*	G/T[b]	0.20	0.45	0.92	0.00	0.50	1.07	0.00

(continued)

Table 2. (continued)

Part B: Meta-analyses of Subphenotypes of NSCL/P Cohorts from the 3 Countries									
				NSCL			NSCLP		
SNP	Probable Gene/Loci	Minor Alleles[a]	African MAF	*P*	OR	I	*P*	OR	I
rs4332945	*DYSF*	T/G[b,e,g]	0.16	0.54	0.94	10.40	0.71	1.04	0.00
rs2303596	*DYSF*	T/C[c,d,e]	0.22	0.29	0.89	63.58	0.44	0.93	75.54
rs227782	*DYSF*	A/G[b,g]	0.42	0.85	0.98	0.00	0.13	1.14	0.00
rs115200552	*MSX1*	C/G[h]	0.02	0.18	1.37	61.30	0.68	1.10	0.00
rs12532	*MSX1*	G/A[c,e]	0.44	0.55	0.95	0.00	0.51	0.95	0.00
rs2674394	Gene desert	A/C[b]	0.17	0.06	1.22	0.00	0.42	0.91	0.00
rs651333	*TULP4*	C/T[b,d,g]	0.34	0.63	0.96	0.00	0.74	0.97	0.00
rs6558002	*EPHX2*	C/T[b,g]	0.24	0.82	1.02	0.00	0.11	0.11	0.00
rs987525	8q24	A/C[b,g]	0.38	**5.38E-03**[f]	**1.28**	**0.00**	**0.01**[f]	**0.80**	**54.21**
rs894673	*FOXE1*	A/T[c]	0.33	0.54	0.95	42.39	0.45	0.94	0.00
rs3758249	*FOXE1*	T/C[c]	0.33	0.53	0.94	46.73	0.68	0.96	0.00
rs7078160	*VAX1*	A/G[b]	0.25	**0.03**[f]	**1.23**	**0.00**	0.20	1.13	24.04
rs4752028	*VAX1*	C/T[b,g]	0.45	0.55	1.05	16.64	0.50	0.95	0.00
rs10785430	*ADAMTS20*	G/A[b]	0.32	0.88	1.01	41.30	0.86	0.98	3.00
rs9574565	*SPRY2*	T/C[c,g]	0.35	0.53	1.06	72.62	0.43	1.07	65.44
rs8001641	*SPRY2*	G/A[c,d,e,g]	0.10	0.99	1.00	0.00	0.26	1.13	0.00
rs17563	*BMP4*	A/G[b,d,e,g]	0.18	0.89	0.99	25.84	0.98	1.00	0.00
rs1258763	*GREM1*	C/T[c,d,e,g]	0.49	0.22	0.90	0.00	0.10	1.15	0.00
rs8049367	*ADCY9*	C/T[c,d,e]	0.30	0.36	1.09	10.19	0.35	1.08	0.00
rs16260	*CDH1*	A/C[b]	0.13	0.46	0.91	10.51	0.20	1.16	0.00
rs11642413	*CDH1*	G/A[b,e,g]	0.28	0.98	1.00	0.00	0.55	1.05	0.00
rs1546124	*CRISPLD2*	G/C[b,e]	0.25	0.26	0.90	0.00	0.88	1.01	0.00
rs4783099	*CRISPLD2*	T/C[b]	0.33	0.85	1.02	0.00	0.32	1.09	0.00
rs8069536	*NTN1*	T/G[b]	0.32	0.72	1.03	3.47	**0.04**[f]	**1.20**	**0.00**
rs8081823	*NTN1*	A/G[c]	0.24	0.55	0.95	0.00	0.05	0.83	0.00
rs17760296	*NOG1*	G/T[b]	0.02	0.83	1.04	5.85	0.85	0.97	0.00
rs227731	*NOG1*	G/T[b,g]	0.22	0.38	0.92	0.00	0.59	1.05	0.00
rs7224837	*AXIN2*	G/A[b]	0.11	0.61	1.08	0.00	0.81	1.04	0.00
rs3923086	*AXIN2*	A/C[b,d,e,g]	0.02	0.62	1.10	40.28	NA	NA	0.00
rs17820943	*MAFB*	T/C[c]	0.25	0.25	0.89	15.55	0.43	0.93	0.00
rs13041247	*MAFB*	C/T[c]	0.25	0.25	0.89	31.03	0.54	0.94	0.00
rs11696257	*MAFB*	T/C[c]	0.25	0.24	0.89	27.17	0.40	0.92	0.00

All *P* values reported are for the minor alleles. All initial studies were carried out in Asians and/or Caucasians but not Africans. Source of minor alleles and MAF: http://browser.1000genomes.org.

I, test of heterogeneity of which 0 to 40 represents no heterogeneity; MAF, minor allele frequency; NA, not applicable; NSCL, nonsyndromic cleft lip; NSCL/P, nonsyndromic cleft lip with or without cleft palate; NSCLP, nonsyndromic cleft lip and palate; NSCPO, nonsyndromic cleft palate only; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]The first allele is the minor allele in Europeans unless otherwise indicated. The first allele is also the minor allele in East Asians, South Asians, and Africans.

[b]Minor allele was the risk allele in initial study.

[c]Minor allele was protective in initial study.

[d]The first allele is the major allele, while the second allele is the minor allele in South Asians.

[e]The first allele is the major allele ,while the second allele is the minor allele in East Asians.

[f]Loci that reached nominal significance in meta-analyses (in bold).

[g]The first allele is the major allele, while the second allele is the minor allele in Africans.

[h]The first allele is the minor allele, and the variation exists only in Africans.

studies (Birnbaum et al. 2009; Grant et al. 2009; Mangold et al. 2010; Beaty et al. 2010; Ludwig et al. 2012) demonstrating that the A allele of rs987525 is a risk allele for NSCL/P in Europeans. These observations suggest that the actual risk variant is (or variants are) in linkage disequilibrium with the A allele of rs987525. Fine mapping of the African haplotype (which is smaller in the 8q24 region) will help identify the risk variant (or variants). Our observations corroborate those made elsewhere (Beaty et al. 2010; Murray et al. 2012) suggesting that the varied ethnic association of the rs987525 allele largely depends on its MAF in various populations. Current evidence suggests that although the 8q24 window is a gene desert, it harbors very remote *cis*-acting craniofacial enhancer elements that regulate the expression of oncogenic *MYC* in the developing face; perturbation of this regulatory network leads to craniofacial dysmorphologies, including sporadic CL/P, in mice (Uslu et al. 2014).

The C677T (rs1801133) SNP of *MTHFR* but not A1298C (rs1801131) has largely been associated with reduced risk for NSCL/P in Asians (Zhao et al. 2014; Martinelli et al. 2015; Pan

Table 3. Transmission Disequilibrium Test for Case-Parent Trios Only.

Part A: Transmission Disequilibrium Test Analyses for NSCL/P and NSCPO

		NSCL/P			NSCPO		
SNP	Probable Gene/Loci	T:NT	*P*	OR (95% CI)	T:NT	*P*	OR (95% CI)
rs1801131	*MTHFR*	27:34	0.37	0.79 (0.48 to 1.32)	10:9	0.82	1.11 (0.45 to 2.73)
rs1801133	*MTHFR*	22:23	0.88	0.96 (0.53 to 1.72)	6:8	0.59	0.75 (0.26 to 2.16)
rs766325	*PAX7*	43:52	0.36	0.83 (0.55 to 1.24)	11:11	1.00	1.00 (0.43 to 2.31)
rs742071	*PAX7*	82:75	0.58	1.09 (0.80 to 1.50)	16:11	0.34	1.46 (0.68 to 3.13)
rs560426	*ABCA4*	78:59	0.10	1.32 (0.94 to 1.85)	18:18	1.00	1.00 (0.52 to 1.92)
rs481931	*ABCA4*	28:25	0.68	1.12 (0.65 to 1.92)	3:8	0.13	0.38 (0.10 to 1.41)
rs4147811	*ABCA4*	26:25	0.89	1.04 (0.60 to 1.80)	5:10	0.20	0.50 (0.17 to 1.46)
rs138751793	*ARHGAP29*	5:7	0.56	0.71 (0.23 to 2.25)	1:2	0.56	0.50 (0.05 to 5.51)
rs6677101	*SLC25A24*	65:75	0.40	0.87 (0.62 to 1.21)	21:14	0.24	1.50 (0.76 to 2.95)
rs861020	*IRF6*	35:29	0.45	1.21 (0.74 to 1.97)	3:7	0.21	0.43 (0.11 to 1.66)
rs34743335	*IRF6*	4:2	0.41	2.00 (0.37 to 10.92)	0:0	NA	NA (NA)
rs642961	*IRF6*	29:29	1.00	1.00 (0.60 to 1.67)	2:7	0.10	0.29 (0.06 to 1.38)
rs7590268	*THADA*	49:48	0.92	1.02 (0.69 to 1.52)	8:8	1.00	1.00 (0.38 to 2.66)
rs4332945	*DYSF*	43:40	0.74	1.08 (0.70 to 1.65)	11:8	0.49	1.38 (0.55 to 3.42)
rs2303596	*DYSF*	45:57	0.23	0.79 (0.53 to 1.18)	12:8	0.37	1.50 (0.61 to 3.67)
rs227782	*DYSF*	73:65	0.50	1.12 (0.80 to 1.57)	20:13	0.22	1.54 (0.77 to 3.09)
rs115200552	*MSX1*	10:13	0.53	0.77 (0.34 to 1.75)	7:2	0.10	3.50 (0.72 to 16.85)
rs12532	*MSX1*	77:71	0.62	1.09 (0.79 to 1.50)	20:22	0.76	0.91 (0.50 to 1.67)
rs2674394	Gene desert	40:44	0.66	0.91 (0.59 to 1.40)	9:9	1.00	1.00 (0.40 to 2.52)
rs651333	*TULP4*	56:59	0.78	0.95 (0.66 to 1.37)	21:16	0.41	1.31 (0.68 to 2.52)
rs6558002	*EPHX2*	47:40	0.45	1.18 (0.77 to 1.79)	13:12	0.84	1.08 (0.49 to 2.37)
rs987525	8q24	71:59	0.29	1.20 (0.85 to 1.70)	19:20	0.87	0.95 (0.51 to 1.78)
rs894673	*FOXE1*	60:67	0.53	0.90 (0.63 to 1.29)	16:15	0.86	1.07 (0.53 to 2.16)
rs3758249	*FOXE1*	59:66	0.53	0.89 (0.63 to 1.27)	16:15	0.86	1.07 (0.53 to 2.16)
rs7078160	*VAX1*	60:44	0.12	1.36 (0.92 to 2.01)	18:10	0.13	1.80 (0.83 to 3.90)
rs4752028	*VAX1*	73:76	0.81	0.96 (0.70 to 1.32)	**27:13**	**0.03**[a]	**2.08 (1.07 to 4.03)**
rs10785430	*ADAMTS20*	61:59	0.86	1.03 (0.72 to 1.48)	15:11	0.43	1.36 (0.63 to 2.97)
rs9574565	*SPRY2*	69:55	0.21	1.26 (0.88 to 1.79)	18:17	0.87	1.06 (0.55 to 2.05)
rs8001641	*SPRY2*	22:22	1.00	1.00 (0.55 to 1.81)	9:6	0.44	1.50 (0.53 to 4.21)
rs17563	*BMP4*	44:44	1.00	1.00 (0.66 to 1.52)	10:15	0.32	0.67 (0.30 to 1.48)
rs1258763	*GREM1*	73:58	0.19	1.26 (0.89 to 1.78)	19:21	0.75	0.90 (0.49 to 1.68)
rs8049367	*ADCY9*	67:67	1.00	1.00 (0.71 to 1.40)	12:13	0.84	0.92 (0.42 to 2.02)
rs16260	*CDH1*	31:28	0.70	1.11 (0.66 to 1.85)	6:13	0.11	0.46 (0.18 to 1.21)
rs11642413	*CDH1*	62:49	0.22	1.27 (0.87 to 1.84)	14:11	0.55	1.27 (0.58 to 2.80)
rs1546124	*CRISPLD2*	53:44	0.36	1.21 (0.81 to 1.80)	9:14	0.30	0.64 (0.28 to 1.49)
rs4783099	*CRISPLD2*	75:64	0.35	1.17 (0.84 to 1.64)	15:21	0.32	0.71 (0.37 to 1.39)
rs8069536	*NTN1*	67:70	0.80	0.96 (0.68 to 1.34)	14:13	0.85	1.08 (0.51 to 2.29)
rs8081823	*NTN1*	58:56	0.85	1.04 (0.72 to 1.50)	14:15	0.85	0.93 (0.45 to 1.93)
rs17760296	*NOG1*	7:8	0.80	0.88 (0.32 to 2.41)	2:0	0.16	NA (NA)
rs227731	*NOG1*	47:49	0.84	0.96 (0.64 to 1.43)	20:11	0.11	1.82 (0.87 to 3.80)
rs7224837	*AXIN2*	19:27	0.24	0.70 (0.39 to 1.27)	1:6	0.06	0.17 (0.02 to 1.38)
rs3923086	*AXIN2*	2:3	0.65	0.67 (0.11 to 3.99)	1:0	0.32	NA (NA)
rs17820943	*MAFB*	49:42	0.46	1.17 (0.77 to 1.76)	15:12	0.56	1.25 (0.59 to 2.67)
rs13041247	*MAFB*	49:43	0.53	1.14 (0.76 to 1.72)	15:12	0.56	1.25 (0.59 to 2.67)
rs11696257	*MAFB*	48:43	0.60	1.12 (0.74 to 1.69)	14:12	0.69	1.17 (0.54 to 2.52)

Part B: Transmission Disequilibrium Test Subphenotype Analyses for NSCL/P

		NSCL			NSCLP		
rs1801131	*MTHFR*	**9:20**	**0.04**[a]	**0.45 (0.20 to 0.99)**	18:14	0.48	1.29 (0.64 to 2.59)
rs1801133	*MTHFR*	7:8	0.80	0.88 (0.31 to 2.41)	15:15	1.00	1.00 (0.49 to 2.05)
rs766325	*PAX7*	18:24	0.35	0.75 (0.41 to 1.38)	25:28	0.68	0.89 (0.52 to 1.53)
rs742071	*PAX7*	**50:30**	**0.03**[a]	**1.67 (1.06 to 2.62)**	32:45	0.14	0.71 (0.45 to 1.12)
rs560426	*ABCA4*	32:35	0.71	0.91 (0.57 to 1.48)	**46:24**	**8.55E-03**[a]	**1.92 (1.17 to 3.14)**
rs481931	*ABCA4*	10:13	0.53	0.77 (0.34 to 1.75)	18:12	0.27	1.50 (0.72 to 3.14)
rs4147811	*ABCA4*	8:10	0.64	0.80 (0.32 to 2.03)	18:15	0.60	1.20 (0.60 to 2.38)
rs138751793	*ARHGAP29*	1:2	0.56	0.50 (0.05 to 5.51)	4:5	0.74	0.80 (0.21 to 2.98)
rs6677101	*SLC25A24*	26:41	0.07	0.63 (0.39 to 1.04)	39:34	0.56	1.15 (0.72 to 1.82)
rs861020	*IRF6*	20:14	0.30	1.43 (0.72 to 2.83)	15:15	1.00	1.00 (0.49 to 2.05)
rs34743335	*IRF6*	2:1	0.56	2.00 (0.18 to 22.06)	2:1	0.56	2.00 (0.18 to 22.06)
rs642961	*IRF6*	16:15	0.86	1.07 (0.53 to 2.16)	13:14	0.85	0.93 (0.44 to 1.98)

(continued)

Table 3. (continued)

Part B: Transmission Disequilibrium Test Subphenotype Analyses for NSCL/P							
		NSCL			NSCLP		
SNP	Probable Gene/Loci	T:NT	*P*	OR (95% CI)	T:NT	*P*	OR (95% CI)
rs7590268	*THADA*	21:32	0.13	0.66 (0.38 to 1.14)	28:16	0.07	1.75 (0.95 to 3.23)
rs4332945	*DYSF*	21:17	0.52	1.24 (0.65 to 2.34)	22:23	0.88	0.96 (0.53 to 1.72)
rs2303596	*DYSF*	18:22	0.53	0.82 (0.44 to 1.53)	27:35	0.31	0.77 (0.47 to 1.27)
rs227782	*DYSF*	33:28	0.52	1.18 (0.71 to 1.95)	40:37	0.73	1.08 (0.69 to 1.69)
rs115200552	*MSX1*	6:3	0.32	2.00 (0.50 to 8.00)	4:10	0.11	0.40 (0.13 to 1.28)
rs12532	*MSX1*	39:32	0.41	1.22 (0.76 to 1.95)	38:39	0.91	0.97 (0.62 to 1.52)
rs2674394	Gene desert	21:17	0.52	1.24 (0.65 to 2.34)	19:27	0.24	0.70 (0.39 to 1.27)
rs651333	*TULP4*	26:26	1.00	1.00 (0.58 to 1.72)	30:33	0.71	0.91 (0.55 to 1.49)
rs6558002	*EPHX2*	15:18	0.60	0.83 (0.42 to 1.65)	32:22	0.17	1.46 (0.85 to 2.50)
rs987525	8q24	35:28	0.38	1.25 (0.76 to 2.06)	36:31	0.54	1.16 (0.72 to 1.88)
rs894673	*FOXE1*	27:31	0.60	0.87 (0.52 to 1.46)	33:36	0.72	0.92 (0.57 to 1.47)
rs3758249	*FOXE1*	27:31	0.60	0.87 (0.52 to 1.46)	32:35	0.71	0.91 (0.57 to 1.48)
rs7078160	*VAX1*	37:23	0.07	1.61 (0.96 to 2.71)	23:21	0.76	1.10 (0.61 to 1.98)
rs4752028	*VAX1*	32:38	0.47	0.84 (0.53 to 1.35)	41:38	0.74	1.08 (0.69 to 1.68)
rs10785430	*ADAMTS20*	25:28	0.68	0.89 (0.52 to 1.53)	36:31	0.54	1.16 (0.72 to 1.88)
rs9574565	*SPRY2*	35:29	0.45	1.21 (0.74 to 1.97)	34:26	0.30	1.31 (0.78 to 2.18)
rs8001641	*SPRY2*	12:12	1.00	1.00 (0.45 to 2.27)	10:10	1.00	1.00 (0.42 to 2.40)
rs17563	*BMP4*	22:16	0.33	1.38 (0.72 to 2.62)	22:28	0.40	0.79 (0.45 to 1.37)
rs1258763	*GREM1*	31:27	0.60	1.15 (0.69 to 1.92)	42:31	0.20	1.36 (0.85 to 2.16)
rs8049367	*ADCY9*	25:28	0.68	0.89 (0.52 to 1.53)	42:39	0.74	1.08 (0.70 to 1.67)
rs16260	*CDH1*	12:14	0.69	0.86 (0.40 to 1.85)	19:14	0.38	1.36 (0.68 to 2.71)
rs11642413	*CDH1*	25:22	0.66	1.14 (0.64 to 2.02)	37:27	0.21	1.37 (0.83 to 2.25)
rs1546124	*CRISPLD2*	25:22	0.66	1.14 (0.61 to 2.02)	28:22	0.40	1.27 (0.73 to 2.23)
rs4783099	*CRISPLD2*	39:35	0.64	1.11 (0.71 to 1.76)	36:29	0.39	1.24 (0.76 to 2.02)
rs8069536	*NTN1*	32:35	0.71	0.91 (0.57 to 1.48)	35:35	1.00	1.00 (0.63 to 1.60)
rs8081823	*NTN1*	30:20	0.16	1.50 (0.85 to 2.64)	28:36	0.32	0.78 (0.47 to 1.27)
rs17760296	*NOG1*	5:2	0.26	2.50 (0.49 to 12.89)	2:6	0.16	0.33 (0.07 to 1.65)
rs227731	*NOG1*	22:26	0.56	0.85 (0.48 to 1.49)	25:23	0.77	1.09 (0.62 to 1.92)
rs7224837	*AXIN2*	10:9	0.82	1.11 (0.45 to 2.73)	9:18	0.08	0.50 (0.22 to 1.11)
rs3923086	*AXIN2*	1:2	0.56	0.50 (0.05 to 5.51)	1:1	1.00	1.00 (0.06 to 15.99)
rs17820943	*MAFB*	18:22	0.53	0.82 (0.44 to 1.53)	31:20	0.12	1.55 (0.88 to 2.72)
rs13041247	*MAFB*	18:22	0.53	0.82 (0.44 to 1.53)	31:21	0.17	1.48 (0.85 to 2.57)
rs11696257	*MAFB*	18:22	0.53	0.82 (0.44 to 1.53)	30:21	0.21	1.43 (0.82 to 2.50)

95% CI, 95% confidence interval; NA, not applicable; NSCL, nonsyndromic cleft lip; NSCL/P, nonsyndromic cleft lip with or without cleft palate; NSCLP, nonsyndromic cleft lip and palate; NSCPO, nonsyndromic cleft palate only; NT, not transmitted; OR, odds ratio; SNP, single nucleotide polymorphism; T, transmitted.

[a]Loci that demonstrated overtransmission at threshold significance of $P \leq 0.05$ (in bold).

et al. 2015) and, to some extent, in European-derived populations (Estandia-Ortega et al. 2014; de Aguiar et al. 2015), though not all studies (Sozen et al. 2009) replicated the association. Interestingly, we have demonstrated in TDT analyses that *MTHFR* is significantly associated with NSCL among Africans and that it is the C minor allele of the A1298C (rs1801131) SNP that confers a reduced risk, suggesting that A is the risk allele. *AXIN2* has been implicated in the etiology of NSOFCs in multiple populations, except Africans, with rs3923086 demonstrating an association with NSCLP among Asians (Letra et al. 2012). Other studies (Mostowska et al. 2012; de Araujo et al. 2015) have replicated the association between *AXIN2* and NSCL/P. Here, we have demonstrated that rs3923086 (*AXIN2*) is also associated with NSCLP among Africans in DFAM analyses. Other candidate genes (e.g., *DYSF*) also showed evidence of association with NSOFCs among Africans, buttressing the relevance of this approach in etiologic "gene hunting."

Other SNPs, other than the already-reported ones, may be responsible for the associations between certain loci and NSOFCs in some ethnicities. Through direct DNA sequencing of the *MSX1* gene, we observed overtransmission of the minor allele of rs115200552 in NSOFC cases. Subsequent genotyping of this SNP in 3,585 individuals showed that this SNP was associated with NSCPO ($P = 0.01$) in case-control meta-analyses, although family-based studies suggest that this marker may be a risk allele for NSCLP. Earlier studies involving Africans from Nigeria implicated *MSX1* in the etiology of NSCL/P (Butali et al. 2011).

We could not detect a formal association between some GWASs and candidate gene loci and NSCL/P, presupposing that 1) these loci may not play a role in the etiology of NSCL/P in Africans or 2) the genotyped SNPs may not be the tag SNPs for Africans. Lack of statistical power due to sample size and low MAF of the genotyped SNPs in Africans could also be possible reasons. For example, rs2235371—an SNP of *IRF6*

Table 4. Family-Based Association for Disease Traits for Cases and Relatives.

		P Values			
SNP	Probable Gene/Loci	NSCL/P	NSCL	NSCLP	NSCPO
rs1801131	*MTHFR*	0.70	0.68	0.24	0.67
rs1801133	*MTHFR*	0.82	0.51	0.59	0.29
rs766325	*PAX7*	0.61	0.71	0.74	0.24
rs742071	*PAX7*	0.32	**0.02**[a]	0.29	0.96
rs560426	*ABCA4*	**2.59E-02**[a]	0.72	**4.75E-03**[a]	0.80
rs481931	*ABCA4*	0.15	0.55	0.16	0.61
rs4147811	*ABCA4*	0.29	0.44	0.48	0.51
rs138751793	*ARHGAP29*	0.38	0.66	0.43	0.40
rs6677101	*SLC25A24*	1.00	0.80	0.64	0.24
rs861020	*IRF6*	0.43	0.23	0.98	0.35
rs34743335	*IRF6*	0.32	0.52	0.47	0.61
rs642961	*IRF6*	0.83	0.99	0.98	0.15
rs11119388	*SYT14*	0.83	0.85	0.92	0.91
rs7590268	*THADA*	0.85	0.30	0.18	0.77
rs4332945	*DYSF*	**0.04**[a]	**0.02**[a]	0.60	0.62
rs2303596	*DYSF*	0.81	0.84	0.53	0.60
rs227782	*DYSF*	0.36	0.48	0.55	0.47
rs115200552	*MSX1*	0.89	0.13	**3.50E-02**[a]	0.08
rs12532	*MSX1*	0.67	0.96	0.30	0.43
rs2674394	Gene desert	0.59	0.11	0.58	0.51
rs651333	*TULP4*	0.92	0.90	0.63	0.20
rs6558002	*EPHX2*	0.38	0.77	0.27	0.52
rs987525	8q24	0.80	0.50	0.52	0.99
rs894673	*FOXE1*	0.69	0.88	0.46	0.55
rs3758249	*FOXE1*	0.69	0.86	0.46	0.55
rs7078160	*VAX1*	0.21	0.18	0.77	0.28
rs4752028	*VAX1*	0.88	0.44	0.30	0.06
rs10785430	*ADAMTS20*	0.84	0.86	0.62	0.66
rs9574565	*SPRY2*	0.07	0.16	0.28	0.22
rs8001641	*SPRY2*	0.32	0.19	0.88	0.64
rs375489721	*MIR17HG*	NA	NA	NA	NA
rs185831554	*MIR17HG*	0.32	0.32	NA	NA
rs17563	*BMP4*	0.66	0.15	0.80	0.70
rs1258763	*GREM1*	0.14	1.00	0.06	0.98
rs8049367	*ADCY9*	0.23	0.24	0.56	0.18
rs16260	*CDH1*	0.59	0.59	0.36	0.46
rs11642413	*CDH1*	0.33	0.81	0.08	0.88
rs1546124	*CRISPLD2*	0.30	0.53	0.45	0.15
rs4783099	*CRISPLD2*	0.17	0.14	0.89	0.37
rs8069536	*NTN1*	0.58	0.47	0.87	0.23
rs8081823	*NTN1*	0.97	0.30	0.19	0.89
rs17760296	*NOG1*	0.63	0.25	0.97	0.63
rs227731	*NOG1*	0.24	0.41	0.43	0.09
rs7224837	*AXIN2*	0.20	0.75	0.12	0.35
rs3923086	*AXIN2*	0.89	0.70	**2.88E-03**[a]	0.85
rs17820943	*MAFB*	0.31	0.88	0.14	0.65
rs13041247	*MAFB*	0.37	0.83	0.21	0.63
rs11696257	*MAFB*	0.46	0.89	0.26	0.77

NA, not applicable; NSCL, nonsyndromic cleft lip; NSCL/P, nonsyndromic cleft lip with or without cleft palate; NSCLP, nonsyndromic cleft lip and palate; NSCPO, nonsyndromic cleft palate only; SNP, single-nucleotide polymorphism.
[a]Loci that demonstrated overtransmission at threshold significance (in bold).

that is in high-linkage disequilibrium and the same locus as rs642961 and that has been associated with NSCL/P among mostly Asians (Sun et al. 2015) and in some Europeans (Zucchero et al. 2004)—does not exist in the African population (http://browser.1000genomes.org/index.html). It is also possible that even when no associations are detected between reported loci and NSOFCs, potentially pathogenic variants may be observed in NSOFC cases. Therefore, GWASs and whole genome sequencing of NSOFC cases from Africa are required to detect more risk loci.

Subphenotype and subpopulation analyses (even among the same racial group) may be crucial in detecting an association

Table 5. Novel, Rare, and Potentially Etiologic Variants Observed in Sequenced Genes.

Part A: Variants Observed in Cases and Some Parents but Not in Controls				
HGVS	HGVp	Total No. of Cases with Variant	Subphenotype of Cases with Variant	Segregation Analyses
			ARHGAP29	
c.341-30T>A	NA	1	NSCL	NA
c.511-107T>C	NA	2	NSCLP and NSCPO	NA
c.967A>G	p.Asn323Asp	1	NSCL	Absent in father
c.1277delAinsTA	p.Lys426IlefsTer6	1	NSCLP	Absent in mother
c.1281+4A>G	NA	1	NSCLP	Observed in clinically unaffected mother
			PAX7	
c.1227G>A	p.Leu409Leu	1	NSCL	NA

Part B: Bioinformatics-Predicted Effects of Potentially Pathogenic Variants				
HGVS	Polyphen-2	SIFT	Human Splice Finder	RegulomeDB
			ARHGAP29	
c.341-30T>A	NA	NA	Alteration of ESS site	NA
c.511-107T>C	NA	NA	Alteration of ESS site and creation of new ESE site	NA
c.967A>G	Benign	Deleterious	NA	NA
c.1277delAinsTA	NA	NA	NA	NA
c.1281+4A>G	NA	NA	Alteration of wildtype donor site	NA
			PAX7	
c.1227G>A	Benign	Tolerated	Alteration of an ESE site	NA

All analyses were based on genome assembly number GRCh37/hg19, 2009 (http://genome.ucsc.edu).
ESE, exonic splicing enhancer; ESS, exonic splicing silencer; NA, not applicable; NSCLP, nonsyndromic cleft lip and palate; NSCL, nonsyndromic cleft lip only; NSCPO, nonsyndromic cleft palate only.

between certain loci and NSOFCs. In both TDT and DFAM analyses, we observed that rs560426 of *ABCA4* was associated with NSCLP but not the other OFC subphenotypes. Case-control analyses further suggested that the *ABCA4* locus may be crucial in NSOFC etiology in all 3 African populations. *PAX7* (rs742071) exhibited nominal association with NSCL/P in case-control meta-analyses, with subpopulation analyses suggesting that this signal originated mainly from the Ethiopian and Nigerian cohorts that exhibited some level of heterogeneity. However, TDT and DFAM subphenotype analyses demonstrated that rs742071 exhibited overtransmission in NSCL cases in all 3 populations. In case-control meta-analyses, *VAX1* (rs7078160) was nominally associated with NSCL/P, with subpopulation analyses suggesting the 2 West African countries (largely Ghana) drive this signal.

Rare variants, but not necessarily common variants, may account for the link between certain loci and NSOFCs. We observed many missense mutations and 1 frameshift mutation in sequenced genes. No de novo occurrence was observed for any of these variants due to the unavailability of some parental samples. Moreover, some of the novel variants were also observed in clinically unaffected parents and controls. We sequenced the novel variants in 96 controls from Ghana, and the likelihood of identifying these novel variants in more controls (i.e., >96) is possible. Nonetheless, these variants are absent in >1,000 individuals in the 1000 Genomes database (with >300 Africans), >61,000 individuals in the ExAC database, as well as 6,500 individuals in the EVS database. There is also the need to functionally validate the pathogenicity or otherwise of these variants in vivo. Rare variants in *ARHGAP29* (Leslie et al. 2012), *PAX7* and *VAX1* (Butali et al. 2013; Leslie et al. 2015), *BMP4* (Suzuki et al. 2009), *FOXE1* (Moreno et al. 2009), *MAFB* (Butali, Mossey, et al. 2014), and *MSX1* (Liang et al. 2012) have been observed in NSOFC cases.

The incidence of OFC in Africans is much lower than in Europeans and Asians (Mossey and Modell 2012; Butali, Adeyemo, et al. 2014), even though these populations may share the same or similar genetic susceptibility loci for OFCs, as observed in the present study. Although underascertainment due to a lack of birth defect registries in most African countries could be a contributing factor (Butali, Adeyemo, et al. 2014), the low incidence of OFCs among Africans may be real, as African-derived populations in the Caribbean have a low OFC incidence similar to that of their ancestral population (Mossey and Modell 2012). We therefore hypothesize the possible existence of genetic protective variants in the African genome, whose "rescue mission" reduces clefting. The identification and elucidation of such protective variants can be translated to European and Asian populations to bring about reduced OFC incidence and eventually prevention.

Conclusion

The present study has shown evidence of an association of certain loci with NSOFCs at both nominal and threshold significance. For instance, we have for the first time shown that the

8q.24 locus is a risk locus in Africans. Our study has thus corroborated an earlier suggestion that the 8q24 locus may be a risk locus for NSCL/P across major ethnicities, although the effect size is smaller in Asians due to a lower MAF. Subphenotype as well as subpopulation analyses and genotyping of other SNPs, other than those already reported for some loci, may be crucial in identifying NSOFC loci in various ethnicities and populations. We have also demonstrated the existence of rare variants, both novel and known, in NSOFC cases from Africa. In conclusion, we have for the first time demonstrated associations between the SNPs that we studied and NSOFC among Africans. Our study is crucial for understanding the genetic architecture of NSOFCs in Africans and further suggests the need to carry out GWASs and whole genome sequencing for every ethnicity as far as complex traits are concerned.

Author Contributions

L.J.J. Gowans, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; W.L. Adeyemo and M. Eshete, contributed to conception, design, and data acquisition, critically revised the manuscript; P.A. Mossey, contributed to conception, data acquisition, and analysis, critically revised the manuscript; T. Busch, contributed to design, data acquisition, and interpretation, critically revised the manuscript; B. Aregbesola, contributed to data acquisition and critically revised the manuscript; P. Donkor, contributed to design, data acquisition, and interpretation, critically revised the manuscript; F.K.N. Arthur, contributed to design, data acquisition, and analysis, critically revised the manuscript; S.A. Bello, contributed to data acquisition, critically revised the manuscript; A. Martinez, M. Li, and E. Augustine-Akpan, contributed to data acquisition and analysis, critically revised the manuscript; W. Deressa, contributed to data acquisition, critically revised the manuscript; P. Twumasi, contributed to design, critically revised the manuscript; J. Olutayo, M. Deribew, P. Agbenorku, A.A. Oti, R. Braimah, G. Plange-Rhule, M. Gesses, S. Obiri-Yeboah, G.O. Oseni, P.B. Olaitan, L. Abdur-Rahman, F. Abate, T. Hailu, P. Gravem, and M.O. Ogunlewe, contributed to data acquisition, critically revised the manuscript; C.J. Buxó, M.L. Marazita, and A.A. Adeyemo, contributed to data analysis and interpretation, critically revised the manuscript; J.C. Murray and A. Butali, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Oral Health–Related Quality of Life of Children Born With Orofacial Clefts in Ethiopia and Their Parents

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Abstract

Objective: To assess the oral health–related quality of life (OH-RQoL) using a translated standardized measure in an understudied population of Ethiopian children born with orofacial clefts (OFCs) and their parents.

Methods: Using a descriptive study design, we assessed the OH-RQoL of 41 patients with OFCs between the ages of 8 and 17 years and their parents. Participants received multidisciplinary cleft care from 2008 to 2016. They completed an Amharic translation of the Child Oral Health Impact Profile (COHIP).

Results: There was strong internal reliability with the translated COHIP for parents and patients. Parents' COHIP scores ranged from 67 to 186, and patients' scores were 78 to 190. The mean for patients and parents was 155, indicating good OH-RQoL.

Conclusion: The Amharic translation of the COHIP appears appropriate for use with families in Ethiopia. Both parents and patients reported OH-RQoL at similar levels as other international populations.

Keywords

cleft lip and palate, birth defect, oral health–related quality of life, perception, Ethiopia, Child Oral Health Impact Profile

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Introduction

Orofacial clefts (OFCs) are the most common congenital anomalies in the head and neck region and one of the most prevalent birth defects in human beings (Mossey and Modell, 2012). The incidence varies from 1 per 2500 to 1 per 500 births (Mossey et al., 2009, depending on ancestry, geographic and residential location, maternal age, prenatal exposures to teratogens, and socioeconomic status (Clark et al., 2003; Durning et al., 2007; Mossey et al., 2009).

The psychosocial and economic impact of birth defects, including OFCs, on those affected and on the society is enormous. Early in life, OFCs are associated with complications, such as feeding problems and recurrent ear infections, which can result in increased risks of morbidity and mortality. This is more common in developing countries where early systematic pediatric care may not be commonly accessible (Wehby et al., 2006). The burden of OFCs on the affected individuals, families, and the society can be improved by understanding the effects of OFCs on the well-being of affected individuals and families. It is also important to identify their health-care needs and make changes in health-care practices and public policies in order to improve the well-being of the victims.

Patients born with OFCs usually need multidisciplinary care over a long period of time. Care starts in infancy and continues into adult life depending on the availability of the services and the complexity of the associated cleft-related concerns, such as malocclusion, hearing and speech disorders, facial appearance, and psycho-emotional problems (Konan et al. 2015). The aim of the multidisciplinary cleft care is to fully rehabilitate the affected individuals, which includes facilitating normal speech, hearing, occlusion, facial appearance satisfaction, and self-confidence.

One outcome of the multidisciplinary cleft care provided can be evaluated by assessing the oral health–related quality of life (OH-RQoL). It is a subjective evaluation of the individual's oral health, functional well-being, emotional well-being, expectations and satisfaction with care, and sense of self (Geels et al., 2008).

In Ethiopia, there are 31 hospitals, which provide surgical treatment to patients with a cleft, but only Yekatit 12 Hospital Medical College provides multidisciplinary cleft care. As there is only 1 cleft unit serving all of Ethiopia, which is limited to seeing a small portion of patients within the institution, the vast majority of Ethiopian children born with OFCs do not receive multidisciplinary care. There is also lack of information on the quality of life, long-term health, and health-care use and costs of affected individuals and families in Ethiopia.

We evaluated the OH-RQoL of Ethiopian children born with OFCs who received multidisciplinary cleft care from December 2008 to December 2016 at Yekatit 12 Hospital Medical College cleft unit and the perception of their parents using an Amharic translation of the Child Oral Health Impact Profile (COHIP). Jokovic et al., (2002) evaluated a questionnaire used to measure the OH-RQoL of children and found it to be valid and reliable. They showed that oral and orofacial conditions have a substantial effect on functional and psychosocial well-being of the affected. It was also shown that children are able to give psychometrically acceptable accounts of that effect (Jokovic et al. 2002). During the study period, 1427 children and adults born with OFCs received surgical treatment at this unit, but only 41 patients with OFCs received multidisciplinary cleft care due to mainly lack of awareness and distance needed to travel to get the service.

Methods

Study Area and Setting

This study was conducted at Yekatit 12 Hospital Medical College cleft unit and the orthodontics unit of Dental Department of the School of Medicine at Addis Ababa University. These 2 institutions provide multidisciplinary cleft care in collaboration with charity organizations (Transforming Faces and Smile Train). The main services provided by these 2 institutions are primary and secondary surgeries, speech therapy, orthodontics care, basic dental care, oral hygiene, ear nose and throat care, pediatrics care, and psychosocial support for all cleft lip and palate patients coming from all over the country. The participants were 41 children who were born with OFCs and received the multidisciplinary cleft care provided at our unit.

Study Design

We performed a hospital-based descriptive study design and evaluated the OH-RQoL of Ethiopian patients with OFCs who received multidisciplinary care at the study institution. The treatment started by counseling their parents, followed by primary surgery, and when needed, secondary surgeries, and follow-up and treatment by a team of professionals working for the 2 units. Forty-one patients and their parents participated in this study and completed the COHIP, which consists of parallel enquiries for children and parents. The participants were asked to come to the speech therapy unit at any convenient time during the week and weekends. The data were collected from September 2016 to March 2017

Inclusion criteria. Patients were included if they consented to participate, had nonsyndromic cleft lip and/or palate, were aged between 8 and 17 years old, and had established multidisciplinary cleft care during the 8-year study period. Inclusion criteria also included having no surgeries completed in the previous 3 months or planned surgeries with 3 months of the study visit.

Exclusion criteria. Of the 123 patients assessed for possible inclusion, 3 families declined to participate and 79 patients were excluded. There were 20 patients excluded because they had secondary surgery planned within 3 months and 23 patients were excluded for having recently completed surgery (eg, fistula closure, lip revision, rhinoplasty) within the past 3 months.

Table 1. Mean Subscale and Overall Scale for Parents and Patients, *t*, *P* Values.

	Parents				Patients					
Subscale	Mean	SD	Min	Max	Mean	SD	Min	Max	*t*	*P*
Oral symptoms (10 Items)	39.90	7.47	19.00	49.00	39.14	5.81	25.00	50.00	0.512	.610
Functional well-being (8 items)	33.58	6.95	12.00	40.00	35.60	6.23	14.00	40.00	1.388	.781
Emotional well-being (10 items)	40.00	10.11	14.00	50.00	39.88	9.22	17.00	50.00	0.057	.980
School environment (4 items)	16.61	4.37	4.00	20.00	16.93	4.61	2.00	20.00	0-.320	.938
Peer interaction (6 items)	26.39	5.29	10.00	30.00	24.60	5.88	6.00	30.00	1.441	.488
COHIP overall (38 items)	155.51	30.79	67.00	186.00	155.56	26.20	78	190.00	0-.008	.386

There were 30 patients who had just begun multidisciplinary care and were not yet established with the team. Finally, 6 patients had syndromic OFC or Tessier clefts.

Data Collection

Patients and their parents who were found to be eligible for this study according to the inclusion criteria were invited to participate. We used the translated COHIP questionnaire, which consists of children and parent inquiries to evaluate the OH-RQoL. The translation was to Amharic language, which is spoken by most of our patients and their families. Two professional translators made the translation independently. Both the translators' mother tongue was Amharic language. One of the translators had an experience in translating medical records, such as medical certificates. The translation was checked by another professional translator for accuracy. In addition, the principle investigator and 2 other plastic and reconstructive surgeons who have experience working with cleft patients and are also involved in cleft research also reviewed the translated measure. The questions for the patients and their parents consisted of the 38 items of COHIP, which were divided into oral symptoms and emotional well-being (each contained 10 items), functional well-being (8 items), school (4 items), and peer interaction (6 items). The items were answered on a 5-point Likert scale (1 = very often and 5 = never, with the additional response option of 0 = I don't know). Poor OH-RQoL was indicated by a low response. The general health of the patients was assessed by one more additional question, which was added to both the parent and the child questionnaire. It had the following response categories: 1 = bad, 5 = great, and 0 = I do not know. The patients and the parents completed the measures in separate rooms with the principle investigator and research assistant orienting both the children and parents to the questionnaire and assisted them whenever they had difficulty. Ethical clearance was obtained from the institutional review board of College of Health Sciences, Addis Ababa University (10/027/2015). We also obtained informed consent from all parents and assent from the children who were above 12 years old.

Data Analysis

Internal consistencies of the overall scale and for all the subscale responses from both the parents and patients were examined by defining Cronbach α. The do not know (DK) responses frequency of each item of each subscale was analyzed. We summed the responses of all items of each subscale to determine the subscale scores and summed the subscale scores to determine the overall OH-RQoL score. Both the parents and patients answered all the questions. Comparing their overall and subscale scores using independent sample *t* test determined the similarity between parents and patients. We calculated Pearson correlation coefficients and intraclass correlation coefficients between subscales of parents and patients. We also examined the differences between boys and girls using independent *t* tests.

Results

In this study, 41 children and adolescents born with OFCs and treated by a team of professionals at our unit and their parents participated. There were 21 (51.21%) males and 20 (48.78%) females. The mean age of the patients was 12.37 years (standard deviation = 2.5), with more adolescents (60.97%) than children (39.02%). The majority of the parents were mothers (70.73%). The parents' age ranged between 27 and 53 years and 74% were under the age 40 years. The phenotype of the cleft patients included in this study were as follows: 24 (58.5%) children born with unilateral cleft lip and palate, 9 (22.0%) with bilateral cleft lip and palate, 3 (7.3%) with unilateral cleft lip only, 2 (4.9%) with bilateral cleft lip only, and 3 (7.3%) with cleft palate only. The analysis of the DK response revealed that both the parents and the patients gave the highest DK response for the emotional well-being.

The internal consistencies using Cronbach α of the overall scale (0.958 for parents and 0.979 for children) and for the majority of the subscales responses were excellent, ranging from 0.829 to 0.971 for parents, and 0.961 to 0.979 for children. The one subscale with a lower internal consistency of 0.678 was for children's school, which appears to be due to the small number of items in this subscale. There is no Cronbach α for "General Health" because it contains only 1 item.

Parents and patients' COHIP scores appear in Table 1. The minimum overall score the parents obtained on the COHIP was 67 and the maximum was 186. The minimum score patients obtained was 78 and the maximum was 190. The mean overall score of both the patients and parents was 155. There are minor differences between patients and parents on subscales, but no

Table 2. Intraclass Correlation Coefficients (ICCs) Between Parents and Children on COHIP Subscale and Overall Scores.

		95% Confidence Interval	
Overall & subscale	ICC	Lower Bound	Upper Bound
Overall	.982[a]	.976	.987
Subscales			
Oral symptoms	.941[a]	.920	.958
Functional well-being	.930[a]	.905	.951
Emotional well-being	.961[a]	.947	.972
School environment	.769[a]	.676	.841
Peer interaction	.916[a]	.884	.941
General health	.807[a]	.701	.876

Abbreviation: COHIP, Child Oral Health Impact Profile.
[a]correlation is significant with $p<.05$.

Table 3. Correlations Between Overall, Subscale Scores, and General Health for Parents (Below the Diagonal) and Patients (Above the Diagonal).[a]

	Correlations						
	S1	S2	S3	S4	S5	S6	Overall
S1	1	.693[b]	.908[b]	.698[b]	.859[b]	.799[b]	.913[a]
S2	.875[b]	1	.777[b]	.281[b]	.585[b]	.600[b]	.757[b]
S3	.938[b]	.830[b]	1	.654[b]	.915[b]	.743[b]	.953[b]
S4	.914[b]	.830[b]	.939[b]	1	.744[b]	.522[b]	.736[b]
S5	.770[b]	.936[b]	.700[b]	.767[b]	1	.649[b]	.893[b]
S6	.628[b]	.569[b]	.557[b]	.450[b]	.479[b]	1	.748[b]
Overall	.945[b]	.923[b]	.933[b]	.905[b]	.825[b]	.617[b]	1

[a]S1 = Oral symptoms, S2 = functional well-being, S3 = emotional well-being, S4 = school, S5 = peer interaction, and S6 = general health.
[b]Correlation is significant at the 0.01 level (2 tailed).

significant differences were shown between patients and parents on overall scores.

Intraclass correlation coefficients between the parents and the patients were calculated to show their agreement across subscales, and significant correlation was found with $P < .05$ (Table 2). The correlation coefficient for the emotional well-being was found to be high followed by oral symptoms and functional well-being subscales. The correlation on school environment and general health was found to be relatively low.

Pearson correlation coefficients between subscale scores, overall, and general health in the parent and patient group are shown in Table 3. There were significant correlations between the subscales, overall, and general health. Similarly, the results of the children showed significant correlations between the subscales.

Discussion

The main objective of this study was to evaluate the OH-RQoL of children born with OFCs and their parents with the use of an Amharic translation of the COHIP. The study included those patients with nonsyndromic OFCs who received multidisciplinary cleft care and their parents. The findings in this study indicated good OH-RQoL, which was shown by the high overall score parents and patients obtained. Geels et al. (2008) reported similar findings in Rotterdam, Amsterdam. Munz et al. (2011) also found similar positive OH-RQoL for young patients with cleft lip and palate who completed treatment using the Michigan Oral Health–Related Quality of Life Scale. Wilson-Genderson et al. (2007) assessed the similarity of the responses of children born with OFCs and their caregivers using the COHIP; however, they found low to modest rates of similarity between child and caregiver responses for the sample overall. This contrasts with our findings, which showed high similarity between child and caregiver responses. This might be due to cultural differences, which might have resulted in expectation differences. In our study, the proportion of the mean scores to the maximum scores were the same as those reported by Bos and Prahl (2011) in their Dutch sample. Their sample also had similar overall mean scores for patients and parents; however, there were significant differences between patients and parents found on the emotional well-being, oral symptoms, and school subscales.

Our study indicated that it is possible to use an Amharic translation COHIP scores to assess the OH-RQoL of children affected with OFCs and their parents; however, some of the questions need to be expressed differently based on culture/language. For instance, "Felt that you were attractive (good looking) because of your teeth, mouth or face". Geels et al. (2008) also emphasized the importance of formulating these questions when administering COHIP in children born with OFCs.

The number of patients and parents included in this study based on the inclusion criteria of having received team care is far fewer than the patients who received surgical treatment at our unit. The importance of rehabilitative care in improving OH-RQoL for patients born with a cleft is not well understood in the community or by families of patients with a cleft and their providers. Even though all the cleft care at our unit is provided free of charge with the support of charity organizations (Transforming Faces and Smile Train), we have not received many patients for full rehabilitation. The reason for this could be the lack of knowledge on the importance of holistic cleft care and the need for long-term follow-up. The distance needed to travel to get the service could also contribute to low utilization. Awoyale et al. (2016) also reported similar concerns in the quality of life of family caregivers of children with OFCs in Nigeria. This could be improved through teaching of families of patients with OFCs and the community at large about the importance of comprehensive multidisciplinary cleft care. It is also very important to involve the primary health-care providers so that they will appreciate the need for team cleft care.

Limitations of the Study

This study has several limitations. The number of patients included in this study was small and limited the types of appropriate analyses that could be completed with the data. In addition, the sample included only a small proportion of less than

3% of the total number of patients who received surgical treatment at our unit. We therefore cannot generalize these results to other cleft populations in Ethiopia. We did not complete measures with patients outside of team care and therefore cannot comment on the OH-RQoL for the majority of our patients. Another limitation could be that the sample of children born with OFCs and their parents may have not fully expressed their feelings and experience, with the possibility of social desirability in their responses.

Conclusion and Recommendation

This study found that the parents and children's responses were similar when evaluating the child OH-RQoL using an Amharic translation of the COHIP that had strong internal consistency. Although the OH-RQoL was high in this sample of patients who received multidisciplinary care, they represent less than 3% of patients with a cleft at our institution. We recommend that the cleft care at selected hospitals in Addis Ababa and other regions should be expanded to provide multidisciplinary cleft care.

Authors' Note

Mekonen Eshete contributed to the conception, design, data procurement, analysis, and interpretation. He drafted and analytically revised the manuscript. Drs Wakgari Deressa and A. Butali contributed to conception, design, data acquisition, and analysis. They also interpreted the data and critically revised the manuscript. Drs Fikre Abate, Taye Hailu, Abiye Hailu, Yohannes Demissie, Milliard Deribew, Mulualem Gessesse, Peter Mossey, and P.E Gravem critically revised the manuscript. Bizuwork Alemayhu contributed to data collection and critically revised the manuscript. All authors approved this work and they agree to be accountable for all aspects of the work.

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The Role of Environmental Factors in the Etiology of Non syndromic Orofacial Clefts in the Ethiopian Population

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Abstract

Background: Orofacial Clefts represent the most common congenital anomalies in the head and neck region and one of the commonest congenital anomalies in human beings. Multiple factors contribute to the occurrence of Non-Syndromic Orofacial Clefts (NSOFCs). The etiology of NSOFCs in the Ethiopian population has not been investigated before but understanding the causes of this common anomaly is very important to develop preventive strategies in the future.

Aims of the study: the purpose of this study was to assess the role of maternal demographic data, maternal illness, maternal medication, and maternal life style and exposure to the occurrence of NSOFCs in the Ethiopian population.

Methods: We used unmatched case control study design and evaluated the role of environmental factors to the occurrence of NSOFCs in the Ethiopian population. The participants (cases and controls) were recruited from the same institution (Yekatit 12 Hospital Medical College) and interviewed 760 mothers: 359 mothers of children born with NSOFCs and 401 mothers of children born with out any congenital anomalies. Univariate and multivariate logistic regression analyses were used to calculate relative risk by odds ratio (OR) and 95% confidence interval.

Results: OFC is observed more in children who's birth weight was not known (unattended delivery) p-value 0.010; COR=4.321; 95% CI=[1.411-13.235]. This indirectly indicates the role of socioeconomic status in the occurrence of NSOFCs. Mothers who gave history of bronchial asthma had a higher risk of delivering a child with NSOFCS p-value=0.022; COR= 3.729; 95% CI= [1.205-11.543]. This study showed that the occurrence of NSOFCs is more common in Muslim families p-value 0.000, OR= 2.284; 95% CI= [1.624-3.213].

Conclusion: The role of socioeconomic status was shown indirectly: those mothers who delivered at home had a higher risk of delivering a child with NSOFCs. We think that this study will contribute in designing primary prevention mechanisms, since identification of modifiable risk factors is the first step in this process.

Key words: Orofacial clefts, Environmental factors, etiology

Introduction

Orofacial Clefts are major congenital defects with a worldwide prevalence of 1/700 live births (1, 2). The prevalence ranges from 1 in 2500 to 1 in 500 (3) depending on geographic origin, racial and ethnic backgrounds as well as environmental exposures and socioeconomic status (SES). In general, Asian and Amerindian populations have the highest reported birth prevalence rates. The incidence of this anomaly in Africa varies from 0.44/1000 live births to 1.65/1000 births. The incidence of this anomaly in Ethiopia is 0.44/1000 live births(4) while in Addis Ababa the capital city of Ethiopia it is 1.49/1000 live births (5).

At the moment OFCs are not major cause of mortality in developed countries, but cause considerable morbidity. The effect on affected children goes beyond the noticeable defect of face and extends to repeated infections, social stigma, and mental impairment that affect the speech, hearing, and teeth formation(6).

OFCs can be described as part of a syndrome where it is called syndromic and Non-syndromic or isolated when it occurs without other malformations or syndromes. The syndromic forms present with other congenital anomalies. Non-syndromic clefts are said to be multi factorial in origin. Genetic predisposition and various environmental factors can contribute to the occurrence of Non-syndromic Orofacial clefts specially if they act at the relevant time of embryologic development. The contribution of environmental factors is high in genetically predisposed patients (1). These include alcohol consumption (7), maternal illness and smoking (8). Several drugs have been implicated to cause OFCs in animal studies but only phenytoin has been reported to be a cause in humans. The etiology of this common anomaly is not known in Ethiopia. Investigating the contribution of factors such as socioeconomic status and exposure to environmental factors to the occurrence of NSOFCs in different populations at different locations may contribute in the identification of new factors in NSOFCs. Therefore the aim of this study was to assess the role of socioeconomic and environmental factors to the occurrence of NSOFCs in the Ethiopian population.

Methods

Study area and setting

The study site was Yekatit 12 Hospital Medical College reconstructive surgery unit and pediatrics department of the same hospital in Addis Ababa, Ethiopia. Yekatit 12 Hospital is one of the oldest public hospitals, which provide general and tertiary level service for all the disciplines. The cleft center at this hospital is the only center in Ethiopia that provides multidisciplinary cleft care for patients born with orofacial clefts (OFCs). This center was established with the support of the Bergen Cleft Team, Norway sponsored by Norad in 2003. It is now providing the care in collaboration with charity organizations (Transforming Faces and Smile Train). All participants (cases and controls) were recruited from the same study institution.

Study design

We used unmatched case control study design to taste the contribution of maternal demographic data, maternal medication use, maternal illness, maternal life style and exposure to the occurrence of NSOFCs in the Ethiopian population. We interviewed 760 mothers, who were divided into 2 groups: Cases –324 mothers of a child born with non-syndromic cleft lip and or palate (NSCL/P), 35 mothers of a child born with non syndromic cleft palate only (NSCPO) and 401 control mothers (mothers of children born with out any congenital anomaly). The principal investigator, collaborators and research assistants interviewed the participants using the questionnaire adopted from the NigeriaCRAN study project. The data was collected from November 2012 to January 2016. We have excluded from the analysis mothers who gave history of clefts in the family (CL/P 16, CPO 3) and also excluded mothers who delivered a child with syndromic cleft lip and or palate all together 10, one of the mothers delivered a child with Van Der Woude syndrome and one with Pire robin sequence one Tessier 1, 13 and the rest is not known. The Van Der Woude had bilateral complete cleft lip and bilateral lower lip pits and was included in our previous publication.

Inclusion Criteria: Mothers of children born with NSOFCs who visited our unit and who agreed to participate included. The controls were also from the same hospital that brought their child for treatment other than congenital anomaly of any type

Exclusion Criteria: Mothers of children born with syndromic clefts, disability, and mothers who did not agree to participate excluded.

Data Collection

A protocol was designed per the WHO guidelines for obtaining minimal information on environmental factors. The questionnaire used for the NigeriaCRAN study was adapted and used for this study. The parents of the index child approached and invited to take part in the study. The details obtained from each family were, mothers' birth place and location during pregnancy, and main environmental exposure variables such as maternal age at delivery, gestational age; educational level, folic acid intake, vitamin supplementation, other medications use, maternal illness, maternal tobacco use, and alcohol consumption some months prior to conception and during the periconceptional period, medical and obstetric history, and dietary history. In addition, details on the birth weight and delivery status was obtained. The research protocol was reviewed and approved by IRB College of Health Sciences, Addis Ababa University meeting No: 042/2012; Protocol No: 00/10/Surg and by NRERC 3.10/715/04 EC after that yearly renewed. We also retrieved informed consent from the participants.

Data analysis and statistics

In order to assess the role of environmental factors in the occurrence of orofacial clefts in the Ethiopian population we collected data using standardized questionnaire. The collected data was entered into excel worksheet cleaned then transferred to SPSS version 20 for analysis. Descriptive summaries such as frequencies, percentages and proportions were determined and presented in tables. To identify variables which contribute to the occurrence of NSOFCs first bivariate binary logistic regression analyses was carried out and candidate variables for multivariable model at p-value <0.05 were determined. We then identified the significant predictors of NSOFC occurrence by entering variables that were associated with the occurrence of NSOFCs in the bivariate models at P-value<0.05 in the multivariate logistic regression model. First descriptive data was presented in tables and association between environmental factors and the occurrence of NSOFCs was determined by calculating odds ration and 95% CI.

Results

We assessed the role of environmental factors in the occurrence of NSOFCs in the Ethiopian population using a case control study design. We have included 760 participants in this study: 359 mothers of children born with NSOFCs and 401 Mothers of children born with out any identifiable birth defect. The demographic data of the participants like mother's age at subject's delivery, educational level, religion, location during pregnancy etc. was presented in Table 1. Most of the mothers in this study were in 21-26 years age group (35.9% case mothers and 40.29% control mothers) followed by 27-32 years age group (26.5% case mothers and 24.2% control mothers). The majority of the mothers in this study lived in Oromia region 112 (31.2%) during their pregnancy followed by Addis Ababa 82 (22.8%). Mothers who lived in other regions during their pregnancy had a higher risk of delivering a child with NSOFCs than those mothers who lived in Addis Ababa (reference category) Table 1. NSOFC is observed more in children who's birth weight was not known p-value 0.010; COR=4.321; 95% CI 1.411-13.235. This study showed that the occurrence of NSOFCs is more common in Muslim families p-value 0.000, COR 2.284; 695% CI 1.624-3.213. Table 1

Table 1 The role of maternal demographic data in the occurrence of NSOFCs

Variables	Case (N(%))	Control (N(%))	Odds ratio	95%CI	P-value
Gender					
Female	148(41.2%)	186 (46.4%)	1.00		
Male	211 (58.8%)	215 (53.6%)	0.811	0.608-1.081	0.153
Birth weight					
VLBW	5(1.4%)	10(1.4%)	1.00		
LBW	31(8.6%)	48(8.6%)	1.292	0.403-4.139	0.667
NBW	185 (51.5%)	242(60.3%)	1.529	0.514-4.549	0.445
Macrosomia	17(4.7%)	45(11.2%)	0.756	0.225-2.533	0.650
Unknown	121(33.%)	56(14.0%)	4.321	1.411-13.235	0.010
Mothers' age at subjects birth in years					
15-20	76 (21.2%)	81(20.2%)	1.00		
21-26	129 (35.9%)	164 (40.9%)	0.838	0.568-1.237	0.374
27-32	95(26.5%)	97(24.2%)	1.004	0.685-1.592	0.842
33-38	44 (12.3%)	44(11.0%)	1.066	0.632-1.796	0.811
≥39	15 (4.2%)	15 (3.7%)	1.066	0.488-2.328	0.873
Mothers' education level					
Illiterate	151(42.1%)	125 (31.2%)	1.00		.
Primary	107 (29.8%)	129 (32.2%)	.687	0.484-0.973	0.035
Secondary	64 (17.8%)	95 (23.7%)	.558	0.375-0.828	0.004
Tertiary	37 (10.3%)	52 (13.0%)	.589	0.363-0.955	0.032
Mothers' Religion					
Christian	236(65.7%)	331 (82.5%)	1.000		
Muslim	114 (31.8%)	70 (17.5%)	2.284	1.624-3.213	0.000
Others	9 (2.5%)	0(0.0%)	2265771919	0.000-	0.999
Mothers' Birth Place					
Addis Ababa	26 (7.2%)	107 (26.7%)	.1.00		
Oromia	126 (35.1%)	106 (26.4%)	4.892	2.966-8.068	0.000
Amhara	95(26.5%)	77 (19.2%)	5.077	3.008-8.570	0.000
SNNPR	85 (23.7%)	105 (26.2%)	3.332	1.990-5.577	0.000
Others	27(7.5%)	6(1.5%)	18.519	6.930-49.489	0.000
Mothers' Location during pregnancy					
Addis Ababa	82(22.8%)	268(66.8%)	1.00		
Oromia	112(31.2%)	61(15.2%)	6.001	4.030-8.935	0.000
Amhara	70(19.5%)	15(3.7%)	15.252	8.287-28.02	0.000
SNNPR	65(18.1%)	53(13.2%)	4.008	2.584-6.218	0.000
Others	30(8.4%)	4(1.1%)	24.521	8.389-71.619	0.000
Number of previous births					
≤ 2 Children	276(76.9%)	350(8.3%)	1.00		
3-4 children	55(15.3%)	43(10.7%)	1.622	1.056-2.491	0.027
>4 children	28(7.8%)	8(2.0%)	4.438	1.991-9.892	0.000

SNNPR-Southern Nations Nationalities Peoples Republic

We assessed the role of maternal smoking, alcohol consumption and exposure to diagnostic x-ray before and during the first trimester of pregnancy and found out that exposure to diagnostic x-ray was a risk factor. Table 2.

Table 2 The role of maternal exposure (Diagnostic X-Ray, Smoking and Alcohol use)

	Case	Control	OR	95% CI	P-value
Diagnostic X-Ray during and before pregnancy (three months)					
No	**340(94.7%)**	**394(98.3%)**	**1.00**		
Yes	**19(5.3%)**	**7(1.7%)**	**3.145**	**1.306-7.573**	**0.011**
Smoking during and before pregnancy (3 months)					
No	334(93.0%)	378(94.3%)	1.00		
Yes	25(7.0%)	23(5.7%)	1.230	0.685-2.208	0.488
Alcohol use before an during pregnancy conception (3 months)					
No	346(96.4 %)	394(98.3%)	1.00		
Yes	13(3.6%)	7(1.7%)	2.115	0.834-5.361	0.115

We also assessed the role of maternal illness and maternal medication use to the occurrence of NSOFCs in the offspring and found out that mothers who suffer from Bronchial Asthma, vomiting during first trimester of pregnancy and mothers who were admitted for threatened abortion were at a higher risk of delivering a child with NSOFCs with p-value 0.002; COR 3.729; 95% CI 1.205-11.543; p-value 0,031,COR 0.676; 95% CI 0.474-0.965; and p-value 001, COR 0.367, 95% CI 0.203-0.661 respectively table 3. Maternal folic acid, iron and other medication use and the occurrence of NSOFs was assessed but no significant association was found Table 4.

Table 3 The role of maternal illness in NSOFCs

Maternal illness					
Vomiting during first trimester					
	Case mothers	Control mothers	OR	95% CI	p-value
No	296(82.5%)	305(76.1%)	1.00		
Yes	63(17.5%)	96(23.9%)	0.676	0.474-0.965	0.031
Threatened abortion					
No	314(8.5%)	385(9.0%)	1.00		
Yes	45(12.5%)	16(4.0%)	.367	.203-.661	0.001
Diabetes					
No	353(98.3%)	397(99.0%	1.00		
Yes	6(1.7%)	4(1.0%)	1.607	0.472-6.027	0.421
Hypertensive Disease					
No	354(98.6%)	394(98.3%)	1.00		
Yes	5(1.4%)	7(1.7%)	0.795	0.250-2.527	0.697
Bronchial Asthma					
No	346(96.4%)	397(99.0%)	1.00		
Yes	13(3.6%)	4(1.0%)	3.729	1.205-11.543	0.022

Table 4 maternal medication uses and NSOFCs risk

	Case mothers	Control mothers	OR	95% CI	P-value
Folic acid use during 1st trimester					
No	**347(96.7%)**	**388(96.8%)**	**1.00**		
Yes	**12(3.3%)**	**13(3.2%)**	**1.032**	**0.465-2.292**	**0.938**
Iron during first trimester					
No	351(97.8%)	384(95.8%)	1.00		
Yes	8(2.2%)	17(4.2%)	0.515	0.219-1.208	0.127
Vitamin use					
No	355(98.9%)	389(97.0%)	1.00		
Yes	4(1.1%)	12(3.0%)	1.701	0.117-1.143	0.084
Other medications use					
No	315(87.7%)	368(91.8%)	1.00		
Yes	44(12.3%)	33(8.2%)	1.558	0.968-2.507	0.068

Binary logistic regression analysis showed that residential area, socioeconomic status indirectly shown by (maternal education, number of previous births and number of home delivery), maternal exposure to diagnostic X-ray, maternal illness (bronchial asthma, vomiting and threatened abortion) and religion contributed to the occurrence of NSOFCs. Tables: 1,2 and 3. Some variables like exposure to diagnostic x-ray, maternal education, maternal birthplace, and maternal illness like vomiting were significantly associated with the occurrence of NSOFCs. However the association was not persistent in the second model when it was adjusted for other variables Table 5.

Table 5 the effect of variables on the occurrence of NSOFCs

Variable	COR	Adjusted OR	P-Value
Childrens' Birth weight			
VLBW	**1**	**1**	
LBW	**1.292 (0.403-4.139)**	**0.962(0.483-7.962)**	**0.346**
NBW	**1.529 (0.514-4.349)**	**2.265(0.616-8.332)**	**0.218**
Macrosomia	**0.756 (0.225-2.533)**	**0.974(0.234-4.006)**	**0.972**
Unknown	**4.321 (1.411-13.235)**	**2.985(0.786-11.336)**	**0.108**
Mothers location during pregnancy			
Addis Ababa	**1**	**1**	
Oromia	**6.001 (4.030-8.935)**	**4.165(2.379-7.292)**	**0.000**
Amhara	**15.212 (8.287-28.072)**	**11.543(5.284-25.214)**	**0.000**
SNNPR	**4.008 (2.584-6.218)**	**4.530(2.344-8.755)**	**0.000**
Others	**24.512 (8.389-71.619)**	**7.432(1.551-35.613)**	**0.012**
Mothers birth place			
Addis Ababa	**1**	**1**	
Oromia	**4.892 (2.966-8.068)**	**1.843(0.951-3.573)**	**0.070**
Amhara	**5.077 (3.008-8.570)**	**1.750(0.883-3.467)**	**0.109**
SNNPR	**3.332 (1.990-5.577)**	**1.305(0.649-2.626)**	**0.455**
Others	**18.519 (6.930-49.489)**	**3.292(0.702-15.424)**	**0.131**
Mothers religion			
Christian	**1**	**1**	
Muslim	**2.284 (1.624-3.213)**	**2.130(1.379-3.290)**	**0.001**
Others	**2265771919(0.000**	**465990145.8(1.379-3.290)**	**0.999**
Maternal Education			
Illiterate	**1**	**1**	**.**
Primary	**1.698 (1.047-2.754)**	**1.399(0.892-2.163)**	**0.146**
Secondary	**1.166 (0.712-1.909)**	**1.510(0.881-2.589)**	**0.134**
Tertiary	**0.947 (0.559-1.604)**	**1.540(0.824-2.880)**	**0.176**
Number of previous births			
≤2 Children	**1**	**1**	
3-4 Children	**1.622 (.056-2.491)**	**0.909((0.533-1.552)**	**0.728**
>4 Children	**4.478 (1.991-9.892)**	**1.974(0.789-4.942)**	**0.148**

Table 5 continued			
Diagnostic X-ray before conception and during first trimester			
No	**1**		
Yes	**3.145 (1.306-7.573)**	**2.369(0.872-6.434)**	**0.091**
Vomiting			
No	**1**		
Yes	0.676 (0.474-0.965)	**0.770(0.498-1.192)**	**0.242**
Threatened abortion			
No	**1**		
Yes	0.367 (0.203-0.661)	**0.716(2.879-11.347)**	**0.000**
Bronchial Asthma			
No	**1**		
Yes	3.729 (1.205-11.543)	**4.159(1.075-16.097)**	**0.039**

NBW- normal birth weight; LBW- low birth weight, VLB- very low birth weight

There was no difference between the case mothers and control mothers with regard to the use of folic acid, iron and other medications before and during first trimester of pregnancy. Very few mothers of both groups reported to take folic acid and other vitamins before and during first trimester of pregnancy. The use of folic acid and iron was found to be more common during the second and third trimester of pregnancy in both groups.

Discussion

The role of environmental factors in the occurrence of orofacial clefts was known since 1943 when Warkany et al (9) related nutritional deficiencies with the occurrence of cleft palate in animal studies. Teratogens like exposure to phenytoin; valproic acid and thalidomide can cause clefts. In addition common environmental exposures such as maternal alcohol and cigarette use (10) before and during first trimester of pregnancy can be the cause of clefts. In this case control study which included 359 mothers of children born with non-syndromic orofacial clefts and 401 mothers of children born with out any congenital anomaly we evaluated the role of exposures like smoking, alcohol consumption and exposure to diagnostic x-ray before conception and during pregnancy.

We showed that mothers who had exposure to diagnostic x-ray before conception and during first trimester had a higher risk of delivering a child with non-syndromic orofacial

clefts COR= 3.145**; 95% CI = 1.306-7.573;** p-value 0.011. Similar to our study Mohammad Zandi et al (11) found significant association between maternal exposure to diagnostic x-ray during pregnancy and the occurrence of orofacial clefts in the offspring. Sutapa Bandyopadhyay Neogi et al (12) in an Indian study found significant association between the occurrence of orofacial clefts and diagnostic x-ray exposure in the first three months of pregnancy.

In our study maternal smoking was not found to be a risk factor for delivering a child with NSOFCs. This is in contradiction to other researchers done in different part of the world. Kallen (13) did a case-control analysis in Sweden and found significant association between maternal smoking and non-syndromic cleft lip and or palate in the offspring (OR = 1.64, 95% CI = [1.33 to 2.02]) and CP (OR = 1.42, 95% CI = [1.06 to 1.90]). He examined a total of 1,834 orofacial cleft cases using the Swedish registry. In a meta-analysis using 11 published studies Wyszynski *et al (10)* found significant association between maternal smoking and non-syndromic orofacial clefts OR 1.29 (95% CI = [1.18, 1.42]). The role of passive smoking in the etiology of orofacial clefts was evaluated (14) and was found to be significantly associated similar to active smoking. An increased risk of non-syndromic cleft lip and or palate in the offspring of smoking mothers in the Danish and Iowan case control studies was observed by Min Shi et al (15). Asghar Ebadifar et al (16) found significant association between maternal smoking and increased risk for oral clefts (OR = 14.7, 95% CI = [5.4-75.4], *P*= 0.001).

This study also evaluated the relationship between maternal vomiting and threatened abortion during first trimester of pregnancy and the occurrence of NSOFCS and found significant association with COR= 0.676; 95% CI= 0.474-0.965; p-value =0.031; COR=0.367; 95% CI =0.203-0.661; p-value= 0.001 respectively. We also showed the role of low socioeconomic status, of the mothers that was indirectly indicated by the high proportion of an attended delivery, multiple births and low maternal education, in the occurrence of orofacial clefts COR=4.321; 95% CI= 1.411-13.235; p-value 0.010; COR=4.438; 95% CI=1.991-9.892; p-value= 0.000; respectively. This is similar to Kraples et al.(17) and N Taghavi et al (18) studies. Kraples et al. speculated that low

socioeconomic status can be a marker of parental health and life style there fore should be considered as a risk factor. Warkany et al associated nutritional deficiencies with cleft palate in animal studies (9). Education plays a role in changing the life of individuals. The chance of getting healthy diets and nutrients for uneducated individuals is less. Maternal healthy diets and nutrients are very important for the normal development of a fetus. Nutritional deficiency in mothers before conception and during early pregnancy could lead to failure of cell growth, differentiation, migration and fusion. This alone or in combination with other factors could cause orofacial clefts.

We assessed the impact of using folic acid, vitamins and other medications on the occurrence of NSOFCs but found no association, this could be because very few mothers reported that they took vitamins and other medications.

Strength and limitations

The main study site for this study was Yekatit 12 Hospital Medical College Cleft Lip and Palate center, which is the only cleft center in the country, which provides holistic cleft care. Because of this we receive patients from all over the country. This and the large sample size enabled us to extend the results to the Ethiopian population. We also evaluated the contribution of the main exposure factors, which were evaluated in other studies. The main limitation of this study was that environmental exposure data was collected retrospectively leaving some recall bias.

Conclusion and Recommendations

In conclusion, this study for the first time demonstrated the role of some environmental factors in the etiology of NSOFCs in the Ethiopian population. We observed significant association between exposure to diagnostic X-ray and the occurrence of NSOFCs. A statistically significant association between low educations and high risk of NSOFCs was observed. We also observed that very few case and control mothers took folic acid and other vitamins before and during first trimester of pregnancy.

Preventative public health measures can decrease some of the congenital anomalies. We recommend preventative health care measures, which include improving the diet of women throughout their reproductive years, ensuring an adequate dietary intake of

vitamins and minerals, and particularly folic acid, through daily oral supplements or fortification of staple foods such as wheat or maize flours

Any exposure of pregnant women to medications or medical radiation e.g. imaging rays should be justified, based on careful health risk–benefit analysis. The education of health care staff and others involved in promoting prevention of congenital anomalies should be strengthened.

Contributions of the authors

Mekonen Eshete contributed to the conception, design, data procurement, analysis, and interpretation. He drafted and analytically revised the manuscript. Drs: Wakgari Deressa and A. Butali contributed to conception, design, data acquisition and analysis. They also interpreted the data and critically revised the manuscript. Drs: Fikre Abate, Taye Hailu, Abiye Hailu, Yohannes Demissie, Shiferaw Degu, Milliard Deribew, Peter Mossey and P.E Gravem critically revised the manuscript. All authors approved this work and they agree to be accountable for all aspects of the work.

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15 Letter of decleration (Dissertation work)

I, the undersigned, declared that this is my original work, has never been presented in this or any other University, and that all the resources and materials used for the dissertation, have been fully acknowledged

Name: Mekonen Eshete Abebe

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Date: May 3, 2018

Place: Addis Ababa

Date of submission

This dissertation has been submitted for examination with my approval as a University Supervisor

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