Parental Age and Severity of Non-Syndromic Orofacial Clefts: Relationship With De-Novo Mutations

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ABSTRACT

Background: This study investigates the relationship between paternal and maternal age, and the severity of orofacial clefts and the presence of de novo mutations in children.

Methods: This was a retrospective study of individuals who were diagnosed with non-syndromic cleft lip and/or palate (CL/P) and their unaffected parents, from 2012 to 2019. We obtained data from the AfriCRAN project database for Nigerians with non-syndromic orofacial clefts. These individuals were recruited at the Oral and Maxillofacial Surgery Clinic, Lagos University Teaching Hospital, Lagos.

Results: There was no statistically significant association between type of CL ± P and parental age in young fathers (p = 0.93). When older fathers were considered, the percentage of complete (more severe) CL ± P cases increased, especially when they were married to older mothers, and this was statistically significant (p = 0.036). In older fathers, the risk of CL ± P in their offspring was increased (OR: 2.66, CI: 1.04-6.80), and there was also an increased risk of developing right-sided CL ± P (OR: 1.61, CI: 1.0-2.59). There was a reduced risk of isolated clefts of the soft palate in younger fathers (OR: 0.36, CI: 0.07–1.71), but the risk increased when considering complete types (more severe) of isolated clefts of the hard and soft palates (OR: 1.63, CI: 0.7–1.7). There was an increase in de novo mutation in children as the difference between paternal and maternal age increased.

Conclusion: The study showed that a higher risk of CL ± P and de novo mutations in children is associated with increased parental age.

INTRODUCTION

Birth defects are reported to contribute significantly to infant morbidity and mortality globally[1]. Orofacial clefts (OFC) are among the most common craniofacial birth anomalies, with a prevalence of 1:700 live births[2]. OFCs can be syndromic or non-syndromic, with non-syndromic accounting for 70% of all OFCs. The phenotypic presentation of OFC differs and ranges from cleft lip (CL), cleft lip and palate (CLP), and cleft palate only (CPO), though other phenotypic expressions are noted but with a lower prevalence rate. The etiology of OFC is considered multifactorial, with polygenic, environmental, epigenetic, and interaction between genetics and environmental

factors playing a role[3]. Environmental factors implicated in the etiology of OFC include smoking, alcohol, metabolic syndromes such as diabetes mellitus and maternal obesity, as well as parental age.

Parental age has been proposed as a possible risk factor for OFC[4]. Previous studies conducted on the association between parental age and the incidence of birth defects have yielded inconsistent results.[5,6,7] It is generally reported that advanced age may predispose chromosomes to irreversible changes and genetic alterations leading to de novo mutations. In a study by Sartorelli et al.[8] the frequency of numerical and structural chromosomal aberrations (acentric fragments and complex radial figures) was significantly greater in the chromosomes of older donors when compared with those of the younger group. As such, many autosomal dominant diseases are associated with increasing paternal age.[9] Crouzon syndrome, Apert syndrome, and Pfeiffer syndrome are all autosomal dominant craniosynostosis disorders that can be caused by mutations in the FGFR2 gene occurring in a normal father's germ line. All the FGFR2 mutations were associated with increased paternal age and were molecularly proven to be of paternal origin.[10] A Danish population-based study of 1,920 OFC-affected births from 1,489,014 live births also concluded that paternal age is associated with CLP, independent of maternal age11. It is worthy to note that the foetal congenital anomalies attributed to advanced paternal age are low in absolute terms. This is despite a possible relationship that is not causal in effect.[9]

There are studies suggesting that maternal age and parity might play an important role in the development of certain isolated birth defects.[12] Kim et al.[13] reported that the risk of trisomy 21, trisomy 18, triple X syndrome, and all aneuploidies showed a significant increase related to older maternal age. For Down Syndrome, the risk of maternal age did not change when controlling for paternal age. On the other hand, paternal age effects changed from a very large risk to a small, sparing risk when controlling for maternal age.

There is no clear consensus on the effect of parental age regarding the risk of orofacial clefts, though many studies have reported associations between advanced maternal or paternal age and the risk of orofacial clefts. A study by Bille et al.[4], using the population-based Danish Facial Cleft Database, reported that the influence of maternal and paternal ages on the risk of cleft lip and/or palate (CL ± P) increases with the advancing age of one of the parents and that the influence vanishes if the other parent is young. In contrast, the risk of having a child with a cleft palate is influenced only by the father's age, not the mother's age. In a study of Brazilians with OFC, Martelli et al.[14] reported an association between maternal age and increased risk for CLP, while paternal age risk is not significant.

In addition to the fact that the association between parental age and the risk of orofacial clefts has been inconsistent, there is sparse literature on the influence of parental age on the severity of orofacial clefts. The severity of the cleft is usually determined by the extent of tissue involved. For example, an incomplete unilateral cleft lip is less severe compared to a complete unilateral cleft lip, while a complete unilateral cleft lip is less severe than a complete bilateral cleft lip.

This study, therefore, aims to investigate the relationship between paternal age and maternal age and the severity of orofacial clefts. In addition, we plan to evaluate the effect of parental age on the occurrence of de novo mutations in children.

MATERIAL AND METHODS

Study design: This was a retrospective study in which eligible individuals were clinically diagnosed with non-syndromic cleft lip and/or palate (CL ± P). Eligible individuals were those clinically diagnosed with non-syndromic cleft lip and/or palate from 2012 to 2019. The selection of participants was based on records of standardized examinations performed by trained surgeons who participated in the Pan-African Association of Cleft Lip and Palate Network for the repair of orofacial clefts in Africa. Clinical information, including a detailed description of the phenotype, parental age, and clinical photographs recorded in the database was utilized. All records of patients who met the inclusion criteria during the study period were selected for this study.

Study location: The records of eligible individuals in this study were obtained at the Cleft Clinic of Oral and Maxillofacial Surgery at the Lagos University Teaching Hospital, Lagos. Eligible individuals were patients who presented at the Cleft clinic over the study period. The Research and Ethics Committee of Lagos University Teaching Hospital was informed, and ethical approval was obtained before commencing the study.

Methods: Data was obtained from the AfriCRAN project database on Nigerian non-syndromic orofacial cleft cases. All infants born with orofacial clefts were clinically examined with the overall goal of measuring and characterizing craniofacial morphology and development, and data on parental age were also included. The parental age was calculated by using the parents' date of birth and compared with that of their children who had non-syndromic orofacial clefts. The infants were classified according to whether they were unilateral (left [L] or right [R]-sided) or bilateral.

For this study, the groups with CL ± P and isolated cleft palates were considered two separate populations because of their different embryological origins.

The CL ± P population comprised:

1. unilateral incomplete cleft lip (UICL),
2. bilateral incomplete cleft lip (BICL),
3. unilateral complete cleft lip (UCCL),
4. bilateral complete cleft lip (BCCL),
5. unilateral incomplete cleft lip and palate (UICLP),
6. bilateral incomplete cleft lip and palate (BICLP),
7. unilateral complete cleft lip and palate (UCCLP) and
8. BCCLP (bilateral complete cleft lip and palate).

In this study, all cleft types were classified in severity as: incomplete (IC), i.e., less severe clefts, vs. complete (CC), i.e., more severe clefts.

The parental age was classified into younger fathers, older fathers or younger mothers, and older mothers based on the median ages of the parents. Younger fathers are categorized as those below 35 years, while older fathers are greater than or equal to 35 years older; and younger mothers are categorized as those below 30 years, while older mothers are greater than or equal to 30 years older. The risk of orofacial clefts was analyzed based on these groups.

For de novo mutations, stored saliva samples from the ongoing genetic studies were used. Consent was obtained prior to the collection of saliva samples. For this project, there was no interaction with patients. The stored saliva samples were anonymized and cannot be traced to any individual participant.

DNA extraction was carried out at the Butali laboratory using the Murray Laboratory protocol (genetics@uiowa.edu). Extracted DNA samples were quantified using Qubit (http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/Qubit.html; Thermo Fisher Scientific, Grand Island, NY). Stocks and working aliquots were made for downstream analyses. We confirm the reported sex using Taqman XY genotyping. This is one of the quality controls (QC) analyses.

We created the 0.2X PreAmp Cocktail by combining the 40X assay with the low-TE buffer. The 40X assay contained 1.5 l of each of the 24 assay markers. The 0.2X PreAmp Cocktail was combined with the PreAmp Master Mix (Qiagen product) to make the sample pre-mix. This is then added to the DNA before running the amplification program. Positive controls are included in the wells before the pre-amplification. The negative controls are, however, not amplified. These controls help to test the quality of the results of calls. Each well contains 4 l of the sample pre-mix and 1.3 l of DNA. The DNA samples and positive controls are then amplified. Details of the PCR reaction (used to amplify the DNA) conditions (denaturing, annealing, and extending temperatures) are available from Butali laboratories upon request. Each well is then diluted by adding 20 l of low-TE buffer.

Statistical analysis: Data collected were entered into Microsoft Excel® Sheet 2016 (Microsoft, Raymond, WA) for sorting and subsequently transferred to Statistical Package for Social Sciences Software for Windows (IBM SPSS® Statistics for Windows, Version 21.0; Armonk, NY: IBM

Corp.) for analysis. A binary outcome variable was defined with two values (0 = IC, 1 = CC) for the primary analysis. A logistic regression model was used to analyze the parental age and risk of a complete or incomplete cleft. For this model, the dependent variable is the cleft severity (complete or incomplete cleft), while the independent variable is the parental age. Based on logistic regression, the odds ratio with confidence interval was calculated between the severity of orofacial clefts and parental age. The statistical significance for this study was stated as $p \leq 0.05$.

RESULTS

The total number of non-syndromic orofacial cleft cases analyzed was 267, with 202 CL ± P and 65 CP cases. There is an almost equal sex distribution, with 137 females and 130 males (1:1.05 male to female ratio). Table 1 shows the parental age distribution of the cleft cases. Most fathers were above the age of 35 for both unilateral CL±P and cleft palate only. For both groups, most participants have older fathers and older mothers, although in the younger fathers' group, most participants have younger mothers.

Generally, there are more complete cleft CL ± P than incomplete cases (Table 1). There is no statistically significant association between the type of CL ± P and parental age in younger fathers ($p = 0.93$). When older fathers were considered, the percentage of complete CL ± P cases increased, especially in older mothers, and this was statistically significant at $p = 0.036$. These findings indicate that the older father-older mother combination may be more associated with more severe CL ± P.

Table 1 shows the relationship of parental age to cleft lip and palate cases

Fathers' age based on median			Type of Cleft lip and palate		Total (%)	p-value
			IC (%)	CC (%)		
< 35	Mothers age based on median	< 30	13 (72%)	42(71.2%)	55(71.4%)	0.930
		> 30	5 (28%)	17(28.8%)	22(28.6%)	
	Total		18(100%)	59 (100%)	77(100%)	
≥35	Mothers age based on median	< 30	12(54.5%)	32 (31%)	44(35.2%)	0.036
		> 30	10(45.5%)	71 (69%)	81(64.8%)	
	Total		22(100%)	103 (100%)	125(100%)	

The severity of CL ± P: There was no increased risk of severity of CL ± P in younger fathers (OR: 1.05, CI: 0.3-3.4), and there was no increased risk for any subtype of CL ± P (Table 2). In older fathers, there was over a two-fold statistically significant increase in the odds of having children with incomplete CL ± P (OR: 2.66, CI: 1.04-6.80). This indicates that older fathers are more at risk of having less severe (incomplete) cases.

Table 2: Relative risk of severity of CL ± P about parental age.

Risk Estimate				
Fathers' age based on median		Odds ratio	95% Confidence Interval	
			Lower	Upper
< 35	Odds Ratio for Mothers age based on median (< 30 / > 30)	1.052	.325	3.409
	For cohort Type of Cleft lip = IC	1.040	.421	2.571
	For cohort Type of Cleft lip = CC	0.988	.754	1.295
≥ 35	Odds Ratio for Mothers age based on median (< 30 / > 30)	2.663	1.043	6.797
	For cohort Type of Cleft lip = IC	2.209	1.039	4.699
	For cohort Type of Cleft lip = CC	0.830	.680	1.012
Total	Odds Ratio for Mothers age based on median (< 30 / > 30)	1.982	.974	4.035
	For cohort Type of Cleft lip = IC	1.734	.973	3.090
	For cohort Type of Cleft lip = CC	0.875	.761	1.006

Risk of a left or right-sided cleft in unilateral CL ± P: There was no associated increase in the risk of unilateral CL ± P for either the left or right side in younger fathers (Table 3). In older fathers, there was a significantly increased risk of developing right-sided CL ± P (OR: 1.61, CI: 1.0–2.59). Generally, there was a slightly increased risk of developing a right-sided CL ± P when both paternal and maternal ages were considered, and this was statistically significant (OR: 1.43, CI: 1.02-2.03).

Table 3: Risk of Left or Right-sided Unilateral CL ± P

Risk Estimate				
Fathers' age based on median		ODDS RATIO	95% Confidence Interval	
			Lower	Upper
< 35	For cohort Cleft Lip Details = Left	1.026	.548	1.919
	For cohort Cleft Lip Details = Right	.981	.618	1.558
≥ 35	For cohort Cleft Lip Details = Left	.714	.493	1.033
	For cohort Cleft Lip Details = Right	1.614	1.006	2.588
Total	For cohort Cleft Lip Details = Left	.733	.549	.978
	For cohort Cleft Lip Details = Right	1.443	1.028	2.025

Cleft palate: There were reduced odds of having children with CP in younger fathers (OR: 0.36, CI: 0.07–1.71), but the risk increases (not statistically significant) when considering complete cleft palates (OR: 1.63, CI: 0.7–1.7) (table 4). A non-significant increase in the odds of having CP was also observed in older fathers.

Table 4: Risk of parental age and severity of cleft palate only

Risk Estimate				
Fathers' age based on median		Odds ratio	95% Confidence Interval	
			Lower	Upper
<35	Odds Ratio for Mothers age based on median (<30 / >30)	0.359	.075	1.714
	For cohort Populations = ICP	0.583	.267	1.276
	For cohort Populations = CCP	1.625	.713	3.706
≥35	Odds Ratio for Mothers age based on median (<30 / >30)	0.593	.149	2.365
	For cohort Populations = ICP	0.781	.395	1.545
	For cohort Populations = CCP	1.316	.646	2.680
Total	Odds Ratio for Mothers age based on median (<30 / >30)	0.445	.165	1.200
	For cohort Populations = ICP	0.663	.398	1.106
	For cohort Populations = CCP	1.492	.904	2.462

Risk of De Novo Mutations: The average age of fathers with no de novo mutations were 34.3 years, while that of mothers was 27.6 years. The average age of fathers with one or more de novo mutations was 37.0 years, while that of mothers was 29.8 years. For both parents, as the age increases, the number of de novo mutations increases (Fig. 1). When comparing the age difference between the parents (father's age and mother's age), there were more de novo mutations as the gap in age increased (Fig. 2). This shows that the father's increasing age increases the risk of de novo mutations in their offspring.

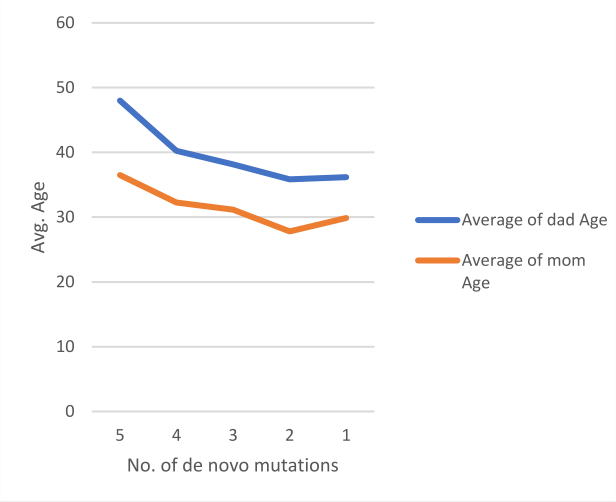

FIGURE 1: Diagram showing the relationship between the average age of mother and father and the number of de novo mutations in offspring.

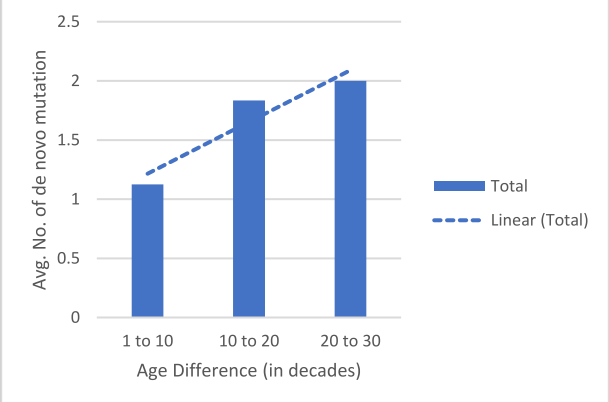

FIGURE 2: Bar chart showing a linear increase with de novo mutation in offspring with an increasing age gap between the ages of the father and the mother.

DISCUSSION

This study evaluated the relationship between parental age and severity of cleft using data derived from Nigerian patients with cleft lip and palate. This study showed that increased parental age is associated with more severe CL ± P cases, as a combination of older parents produces more severe cases. This aligns with various studies that have reported increased congenital malformations in older parents.[6,16]

A population-based study on the Danish Facial Cleft Database reported that the influence of maternal and paternal ages on the risk of cleft lip with or without cleft palate increases with the advancing age of the other parent and that the influence vanishes if the other parent is young. Though there have been varying reports on whether the maternal or paternal chromosomes are culpable, the exact mechanism of this occurrence has not been elucidated, though single gene mutations are suggested mechanisms.[11]

According to this study, advanced paternal age is associated with an increased risk of less severe unilateral and bilateral CL ± P. This is in agreement with a similar study by Herman et al.[15] which reported that paternal age increases the risk of CL ± P, which is more pronounced with advanced maternal age. The paternal age seems to have a great deal of influence on the prevalence of CL ± P in any population. The influence of paternal age has also been reported for cleft palate[17]. In this current study, paternal age is associated with an increased risk of severe cleft palate.

Though maternal age has been associated with chromosomal abnormalities in some studies, paternal age is usually associated with birth defects.[12,13] It is reported in some literature that the risk of birth defects such as heart malformation, other musculoskeletal anomalies, tracheoesophageal fistula/oesophageal atresia, neurodevelopmental diseases, Down's syndrome, and other chromosomal anomalies increases slightly with advancing paternal age.[5,14] This is in support of the findings of this study and many others,

whereby de novo mutations are driven by the increasing age of the fathers.[17] This is a result of the increased rate of de novo mutations in the father's sperm as age increases. Thus, it predisposes the child to having possible deleterious mutations.

The effect of paternal age on chromosomal abnormalities and other genetic consequences in progeny has been investigated. The amount of DNA damage in the sperm of men aged 36–57 is three times that of men aged 35 years and less.[11] This includes a greater risk of de novo mutations in children of older fathers.[18] This is due to the fact that sperm cells develop repeatedly during a man's life, increasing the likelihood of mutations with each division. Research has indicated a noteworthy rise in the quantity of de novo mutations among the offspring of elderly fathers.[19] Also related to paternal age are autosomal dominant disorders. An association between younger fathers and several selected birth defects, like neural tube defects, has also been published.[17] The association of paternal age with birth defects has been attributed to the accumulation of chromosomal aberrations and mutations during the maturation of male germ cells.[10,18]

The prevalence and pattern of occurrence of OFC in each population are expected to fluctuate as the average parental age changes. Furthermore, an increased occurrence of more severe cleft is expected with advanced parental ages, and this may take a toll on available resources.

Strengths and limitations

The strength of this study is that this is a hospital-based investigation in a location with a diverse population. Thus, it serves as a reflection of the non-syndromic cleft cases seen in the community. Furthermore, only parents of children with non-syndromic clefts were included. The limitation of this study is the small sample size, and other environmental factors like the socio-economic status of the parents, maternal intake of alcohol, and smoking were not considered.

CONCLUSION

Increased parental age may be associated with an increased risk of OFC. In this study, advanced paternal age is associated with an increased risk of less severe unilateral CL ± P but a more severe cleft palate. There is also an increased risk of having a de novo mutation in children with increased paternal age. Future prospective studies on different populations and considering other socioeconomic factors may provide more insights into the influence of parental age on the occurrence and severity of OFC.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

OA was involved in the conceptualization, initial draft, data collection, analysis, and final draft of the manuscript; AVO was involved in the conceptualization, analysis, and review of the manuscript. Authors AA, WA, TB, and ML were involved in the data analysis, review, and final draft of the manuscript. Authors AF, JO, VS, JO, MO, and WA were involved in the manuscript review and final draft of the manuscript. Author AB was involved in the conceptualization, initial draft, review, and final draft of the manuscript.

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