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Genome-wide scan for parent-of-origin effects in a sub-Saharan African Cohort with nonsyndromic orofacial clefts

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ABSTRACT

Objective: Nonsyndromic orofacial clefts (NSOFCs) constitute about 70% of all orofacial clefts (OFCs) and present with no additional congenital disorder. NSOFCs have multifactorial etiology where genetic factors, environmental exposure, gene-environment interactions, stochastic factors and gene-gene interactions or epistasis may play cardinal roles. Moreover, NSOFCs largely do not follow Mendelian pattern of inheritance, warranting the need to investigate other genetic phenomena such as parent-of-origin (PoO) effects. PoO effects investigate how

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parental origin of alleles differentially impacts the phenotype of the offspring. The aim of this study was to identify genome-wide PoO effects that can increase risk for NSOFCs in humans.

Patients and Method: The samples (174 case-parent trios from Ghana, Ethiopia and Nigeria) included in this study were from the African only GWAS study that was published in 2019. Genotyping of individual DNA using over 2 million multiethnic and African ancestry-specific SNPs from the Illumina Multi-Ethnic Genotyping Array (MEGA) v2 15070954 A2 (genome build GRCh37/hg19) was done at the Center for Inherited Diseases Research (CIDR). After quality control checks, PLINK was employed to carry out PoO analysis employing the pooled subphenotypes of NSOFCs.

Results: We did not observe any genome-wide significant association ($p < 5\times10^{-8}$) in our study cohort. However, we observed possible hints of PoO effects at several interesting loci in the human genome. These include a 1 mega base pair window at the major histocompatibility complex class 1 (MHC1) window on chromosome 6, *ASB18, ANKEF1, AGAP1, GABRD*, *HHAT*, *CCT7*, *DNMT3A*, *EPHA7*, *FOXO3*, long intergenic noncoding RNAs, microRNA, antisense RNAs and a few genes that encode proteins zinc finger DNA-binding motifs, such as *ZNRD1, ZFAT* and *ZBTB16.*

Conclusion: Findings from our study suggest that some loci may increase the risk for NSOFCs through PoO effects. Additional studies are required to confirm these suggestive loci in NSOFC etiology.

Keywords

Parent-of-origin effects, Nonsyndromic cleft lip and/or cleft palate, sub-Saharan Africans, epigenetics, gene-environment interactions

INTRODUCTION

Orofacial clefts (OFCs) are the most common congenital craniofacial anomalies and the second most common structural birth defects after congenital heart defects (Modell and Mossey, 2012; Huang et al., 2019; van Rooij et al., 2019). These craniofacial birth defects have life-long financial and psychosocial repercussions on affected individuals, families, society and healthcare system. These conditions also affect dentition, speech, language, esthetics, feeding and social integration; and may require multiple surgeries and multidisciplinary team to manage (Nidey et al., 2016; Nidey and Wehby 2019). OFCs are usually grouped into cleft lip only (CL), cleft palate only (CPO) and cleft lip and palate (CLP), with about 50% of CPO being syndromic whereas about 70% of CL and CLP cases are nonsyndromic and present with no additional congenital malformation aside the cleft (Carlson et al., 2019). As a complex trait with multifactorial etiology, environmental exposure, genetic factors such as epistasis and parent-of-origin effects, as well as gene-environment interactions may all contribute to the pathogenesis of OFCs (Thomas, 2010; Wei et al., 2014; Haaland et al., 2017; Gjerdevik et al., 2017). This suggests that maternal peri-conception exposure and intrauterine environment may interact with maternal genetic factors to increase susceptibility to NSOFCs in the fetus.

Classical association studies are based on the premise that phenotypic effects of genetic variants do not depend on parental origin. Association studies are thus severely underpowered to detect non-Mendelian effects that may also influence phenotypes that emanate from genetic variants. This notwithstanding, an interesting non-Mendelian genetic phenomenon that has been postulated to probably play a role in the genetic etiology of OFCs is parent-of-origin (PoO) effects. PoO effects are epigenetic phenomena where the phenotypic consequences of genetics variants or alleles depend on whether they are inherited from the father or mother of an individual (Haaland et al., 2017; Gjerdevik et al., 2017; Zeng et al., 2019). The cardinal epigenetic phenomenon that usually manifest as PoO effects is genomic imprinting. Genomic imprinting is when two alleles at a given locus exhibit varied phenotypic effects that is not due to

variation in DNA nucleotide sequence but emanate from epigenetic events (Lawson et al., 2013; Peters, 2014). Recently, a study (Zeng et al., 2019) demonstrated that PoO effects in humans may impact on DNA methylation patterns as well as their proximal and distant regulators, which may inadvertently impact on complex trait phenomics. Some complex genetic syndromes that may present with oral and craniofacial malformations, such as Angelman syndrome, Beckwith-Wiedemann syndrome and Prader-Willi syndrome, have been shown to result from genomic imprinting events that manifest in PoO dependent manner (Lawson et al., 2013).

Several genetic studies on OFCs have attempted to tease out PoO effects, howbeit, with varied outcomes. The studies reviewed here were carried out in populations of Asian and European ancestries, with data on population of African ancestry conspicuously lacking. A study that employed genome-wide association analysis of over 2,000 OFC case parent trios observed no genome-wide level significant PoO effects on risk of orofacial clefting (Shi et al., 2012). Other studies have demonstrated the probability of PoO effects, largely with increased maternal over-transmission, increasing the risk to NSOFCs. *DMD, FGF13, EGFL6* and Xp22.2 have been shown to increase the risk to NSOFCs through PoO effects with either maternal or paternal over-transmission in a study that analyzed SNPs on the X-chromosome for PoO effects (Skare et al., 2018). Other studies have also demonstrated parent-of-origin effects with excess maternal transmission in a few genes or loci that influence the risk of NSOFCs. These maternal PoO effects include genes such as *RUNX2* gene (Sull et al., 2008), *BCL3* (Park et al., 2009), *TGFα* (Sull et al., 2009a), *PDGF-C* (Wu et al., 2012), *VAX1* (Butali et al., 2013), as well as *PAX7* and *PAX3* (Sull et al., 2009b). Some studies could not replicate the maternal PoO effects in *RUNX2* (Jung et al., 2014), *MTHFR* (Boyles et al., 2008), *ROR2* (Wang et al., 2012), *TGFβ1* (Raju et al., 2017) and *LOXL3* (Khan et al., 2018). Interestingly, only few studies have demonstrated PoO effects with paternal over-transmission, including *TGFβ3* (Reutter et al., 2008) and *MSX1* (Suazo et al., 2010). A common observation for all these PoO effects analysis is the specificity of the association signals regarding ethnicity and cleft subphenotypes. Since

data from populations of African ancestry is conspicuously missing and an opportunity for discovery in this ancestral population is possible, we conducted PoO effects studies in our African GWAS samples from Ghana, Ethiopia and Nigeria (Butali et al., 2019). We performed our PoO analysis using PLINK and observed several novel and potential PoO effects in our study cohort.

MATERIALS AND METHODS

Study population and ethics approval

We had earlier published the characteristics of the study population, the genotyping platform used as well as the quality control criteria employed for data cleaning (Butali et al., 2019, Oseni et al., 2018). In summary, we recruited case parent trios from Ghana, Ethiopia and Nigeria; these included individuals born to Ghanaian, Ethiopian and Nigerian parents. No Caucasian or Asian case families were included in this study. The study was reviewed and approved by various institutional review boards: Kwame Nkrumah University of Science and Technology (CHRPE/RC/018/13), Addis Ababa University (003/10/surg), Lagos University Teaching Hospital (ADM/DCST/HREC/VOL.XV/321), Obafemi Awolowo University Teaching Hospital (ERC/2011/12/01) and the NIH Office of Human Subjects Research (OHSRP 11631). Cheek swab and saliva samples were collected from various centers in Africa and shipped to the Butali Lab at the University of Iowa for DNA extraction, quantification and XY-genotyping as a quality control step to verify the sexes of participants.

Genotyping protocol and quality control

A total of 25 µl of each DNA sample at a concentration of ≥50 ng/µl was shipped to the Center for Inherited Disease Research (CIDR) for Multi-Ethnic Genotyping Array (MEGA) genotyping employing the expanded Illumina MEGA v2 15070954 A2 (genome build GRCh37/hg19). The genotyping platform contained over 2 million multi-ethnic SNPs as well as about 60,000 rare

variants that were specifically identified in populations of African ancestry. A total of 174 case-parents trios were successfully genotyped (Table 1), including HapMap controls (70 unique samples and 9 duplicates) for quality control purposes. The detailed quality control (QC) processes had been published earlier (Oseni et al., 2018; Butali et al., 2019). The QC included establishment of continental ancestry with respect to HapMap samples, Hardy-Weinberg equilibrium test, confirmation of relatedness, identification of large chromosomal anomalies, sex chromosome anomalies, missing call rates and batch effects. The cleaned data was deposited in the database of genotypes and phenotypes (dbGaP) with accession ID phs001090.v1.p1.

Parent-of-origin analysis

The parent-of-origin (PoO) effects analysis was performed using PLINK (Purcell et al., 2007) which separately considered the transmission of allele from heterozygous fathers versus heterozygous mothers to affected offsprings. Thus, the PLINK PoO analysis investigated the effect of a risk variant based on whether it was inherited from the mother or the father. This PLINK PoO analysis is a modification of the traditional family-based transmission disequilibrium test (TDT) which does not separately consider transmissions from heterozygous mothers versus heterozygous fathers. The outcomes of PLINK PoO analysis include both paternal and maternal p-values resulting from paternal Chi-squared test and maternal Chi-squared test respectively. The PLINK PoO analysis also returns asymptotic overall p-values of parent-of-origin tests for difference in paternal versus maternal odds ratios.

We applied PoO analysis on data consisting of 2,036,060 variants and obtained 1,062,324 SNPs with valid parent-of-origin tests. Sequel to this, all SNPs with both paternal and maternal p-values less than 0.05 were removed, as these SNPs did not depict PoO effects. This cut-off point resulted in 64,109 SNPs, of which 20,258 had overall p-values less than 0.05. We did not carry out separate PoO effects analysis for each of the NSOFC subphenotypes due to the limited number of case-parent trios employed in this study (Table 1). We did not correct for

multiple testing in our analysis though separate p-values were generated for mothers and fathers, as well as the overall p-value, because these three tests are not true independent tests. For example, Bonferroni correction and multiple different or less conservative methods could have been employed in our analyses to correct for the effect of multiple testing. However, utilizing the false discovery rate (FDR) resulted in all adjusted p-values being 1 due to there being so many SNPs. In order to ascertain whether a given SNP was located within or near a gene, SNPs were viewed in UCSC genome browser (www.genome.ucsc.edu) in a 100 kb genomic region around the SNP in question. The nearest gene to the SNP was presumed to be the tagging gene. All SNPs that were not located within or near a gene within the 100 kb window were classified as intergenic. To confirm the probable gene implicated by the UCSC genome browser, we searched for topologically associated domains (TADs) for each genomic locus by visualizing in the human reference genome (hg19) the interaction domain for the index SNP ID in 880kb genomic window (http://promoter.bx.psu.edu/hi-c/view.php).

RESULTS

Deciphering parent-of-origin effects

Though this study was a genome-wide scan for PoO effects, none of the p-values observed reached genome-wide significant value of $5.0\times10\text{-}^{8}$. This notwithstanding, we observed very interesting trends towards genome-wide significant level which suggest subtle hints of possible PoO effects in our NSOFCs cohort from Ghana, Ethiopia and Nigeria. SNPs which were significant for either maternal (P_MAT) or paternal (P_PAT) p-values, as well as overall PoO p-values (P_POO), were considered as exhibiting possible PoO effects. This was to determine which parent was transmitting the allele differently or contributing mostly to the overall parent-of-origin p-value. A total of 12,156 SNPs exhibited both P_MAT or P_PAT < 0.05, and P_POO < 0.05; however, we report here SNPs with overall p-values of $\leq 2.32 \times 10^{-3}$ (Table 2). The table also contains the Z-score which shows the number of standard deviations a given data point lies

from the mean. It is known that in most large data sets, about 99% of the values have Z-scores ranging from -3 to 3. This suggests that the p-values lie within three standard deviations above and below the mean. The z-scores scores observed in Table 2 demonstrate the quality of the data used for our analyses, as all the z-scores were within -3 to 3.

The SNP that exhibited the highest PoO effect (P_POO = 5.12×10^{-4}) was rs57792200. This SNP showed paternal over-transmission PoO effect (P_PAT = 6.58×10^{-4}) and is located within an intron of ankyrin repeat and SOCS [suppressor of cytokine signaling] box containing 18 (*ASB18*) gene. Apart from *ASB18* gene, other genes with ankyrin repeat motif, including *ANKEF1* (rs6057132) and *AGAP1* (rs4233622), showed maternal PoO effects. Both *ASB18* and *AGAP1* are located within the same topologically associated domain or TAD (Figure 1). Three loci that exhibited the highest level of maternal PoO effects, including chr2:213801332, chr4:115282539 and rs10058073, are all located in intergenic regions of the human genome. Other interesting candidate PoO effects loci that we observed include gamma-aminobutyric acid (GABA) A receptor delta subunit (*GABRD*), hedgehog acyltransferase (*HHAT*), a chaperon protein coding gene (*CCT7*), DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*), EPH receptor A7 (*EPHA7*), forkhead box O3 (*FOXO3*), long intergenic noncoding RNAs, amicroRNA, antisense RNAs and a number of genes that encode proteins zinc finger DNA-binding motifs, such as *ZNRD1, ZFAT* and *ZBTB16*. Within the TAD where *GABRD* is located is the *PRMD16* gene which has been associated with gene-environment interaction in NSCPO etiology (Yin et al., 2018). The SNP tagging the *HHAT* gene is also situated in the same TAD as both *IRF6* and *SYT14*, important genes that have been associated with OFCs in both DNA sequencing studies and GWAS (Duan et al., 2019). We also observed an interesting hint of PoO effects within a 1 mega base (mb) genomic region localized to the major histocompatibility complex (MHC) class I region on chromosome 6 (Figure 2). This region contains interesting candidate genes such as zinc ribbon domain containing 1 antisense, noncoding RNA (*ZNRD1-AS1*), major

histocompatibility complex, class I, G (*HLA-G)*, HLA-F antisense RNA 1 (*HLA-F-AS1*) and tripartite motif containing group of proteins (*TRIM10, TRIM26, TRIM15* and *TRIM40*). Most interestingly, all the associated loci in this genomic region exhibited maternal PoO effects. The 1 mb MHC Class 1 locus implicated by our study is found in a TAD that harbors *PBX2*, a gene that has been associated with NSOFCs (Maili et al., 2019).

There was no large scale evidence of genomic imprinting in our study cohort since only about 11% of SNPs were within CpG islands (Table 2). Moreover, we observed interesting distribution of the suggestive paternal and maternal PoO effects on the various human chromosomes (Figure 3). On some of the chromosomes, the PoO effects were either solely maternal or paternal, whereas other chromosomes displayed missed paternal and maternal PoO effects. Overall, more maternal PoO effects, comprising over 58% of the associated loci, were observed than paternal PoO effects.

Probable etiologic loci for NSOFCs that do not exhibit unidirectional PoO effects

In situations where both P_PAT and P_MAT, as well as P_POO showed $p<0.05$, we considered this to mean over-transmission of these alleles in both parents (TDT) and may suggest probable loci for NSOFCs that may not exhibit unidirectional PoO effects. We observed 311 of such SNPs with all three p-values, P_MAT, P_PAT, AND P_POO, being less than 0.05 (supplementary Table S1). However, Table S1 contains SNPs with overall p values of $\leq 1.10 \times 10^{-3}$. Interesting, as many as 9 SNPs at the 1mb genomic region localized to the MHC class I region on chromosome 6 once again, suggesting a potential hot spot for NSOFCs risk through non-unidirectional PoO effects. These SNPs included chr6:29781010, chr6:29735532, chr6:29737161, rs1611185, rs2735048, chr6:29834198, rs2517930, chr6:29924625 and chr6:29733837. These SNPs are either near or lie within introns of genes such as *HLA-G, HLA-F, HLA-F-AS1, HCG4* (Supplementary Table SI). Though these 9 SNPs exhibited both P_PAT and P_MAT less than 0.05, the P_MAT values were predominantly and far lower than P_PAT

values, indicating predominant maternal over-transmission. Other interesting loci that showed possible association with NSOFCs but lacking unidirectional PoO effects included cadherin 10, type 2 [*CDH10*] (rs11950412 and rs75699124), solute carrier organic anion transporter family, member 2A1 [*SLCO2A1*] (chr3:133671881), lysine (K)-specific demethylase 4C [*KDM4C*] (rs818882), SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2 [*SMARCA2*] (rs111870807), PHD and ring finger domains 1 [*PHRF1*] (chr11:599653) and solute carrier family 7 (cationic amino acid transporter, y+ system), member 1 [*SLC7A1*] (rs678844).

DISCUSSIONS

We carried out the first ever genome-wide scan for parent-of-origin (PoO) effects in a NSOFCs cohort recruited from Ghana, Ethiopia and Nigeria. The PLINK tool we used enabled us to perform independent tests for the maternally and paternally over-transmitted alleles (P-PAT and P_MAT). This approach enabled us to directly compare how the relative influences of the two parental genomes increase susceptibility to NSOFCs in case offsprings. We did not observe any genome-wide significant association in our study cohort, probable because PoO effects are difficult to tease out, as other relatively large scale studies could not detect statistically significant PoO effects in European and Asian case-parent trios (Garg et al., 2014; Duan et al., 2017; Moreno Uribe et al., 2017). In the Garg et al (2014) study, which was a genome-wide analysis of about 3,700 NSOFC case parents trios, only suggestive maternal-specific and paternal-specific transmission biases, demonstrating PoO effects on the risk of NSOFCs, were observed at 8q21.3 and 2q25 (*SLC4A3*) loci, respectively. These three studies carried out subphenotype analyses for NSCPO and NSCL/P. Future studies with a larger African cohort may want to carry out separate analyses for these two subphenotypes of cleft. This notwithstanding we observed interesting hints of PoO effects in our study cohort that may predispose offsprings to NSOFCs.

A SNP within the intron of *ASB18* gene showed the highest significance level of P_POO = 5.12×10^{-4} with overall paternal PoO effect. *ASB18* encodes a protein that is a member of the ankyrin repeat and suppressor of cytokine signaling [SOCS] box-containing (ASB) family of proteins. ASB18 protein may be a substrate-recognition component of a SCF-like ECS (Elongin-Cullin-SOCS-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins (Kohroki et al., 2005). *ASB18* has not been previously associated with OFCs or any other craniofacial anomaly, though our observation suggests that post-translation modification of proteins may also be a molecular mechanism that may influence the etiology of NSOFCs. A search through the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/) showed that mutant *ASB18* male mice have aberrant reproductive phenotypes due to abnormal meiosis resulting from Anaphase Bridge. Anaphase bridge occurs when telomeres of sister chromatids fuse together and is unable to segregate into the resultant daughter cells. Interestingly, other genes that encode proteins with ankyrin repeat motif, including *ANKEF1* and *AGAP1*, also gave a hint of maternal PoO effects. *ANKEF1* may play cardinal function in ciliated tissues and during embryonic development (Daniel and Panizzi, 2019) whereas *AGAP1* is known to functionally interact with Kinesin-13 Family Member Kinesin-like Protein 2A (*Kif2A*), with the Kif2A-AGAP1 protein complex participating in the control of cytoskeleton remodeling associated with cell movement (Luo et al., 2016). Recently, a genome-wide scan of NSCL/P trios demonstrated PoO effects for similar genes with ankyrin repeat motif, such as *ANK3* and *ARHGEF10.* The study reported PoO interactions between SNPs in *ANK3, LYZL1, PDGFD, FOCAD* and *FRAS1,* and maternal smoking, as well as between *ARHGEF10* and alcohol consumption (Haaland et al., 2019).

We also observed hints of PoO effects in genes such as *GABRD, HHAT, DNMT3A,* long intergenic noncoding RNAs, amicroRNA and antisense RNAs. Apart from *GABRD*, all the other genes products are involved in epigenetic events. *GABRD* encodes a protein that is a member of gamma-aminobutyric acid (GABA)-A receptors that are ligand-gated chloride channels whose

chloride conductance may be modulated by agents such as benzodiazepines. The GABA-A receptor is generally pentameric, consisting alpha, beta, gamma, delta (GABRD) and rho subunits, with variants in the genes for these subunits being associated with a number of psychiatric disorders such as epilepsy. In line with our observation that variants in *GABRD* exhibit paternal PoO effects, it has been demonstrated in human and mouse sperms that GABRD may act as progesterone receptor or modulator in spermatozoa, being responsible for the progesterone-induced Ca^{2+} influx needed for acrosome reaction by interacting with the $P2X_2$ receptor (Xu et al., 2017). *GABRD* has not previously been associated with OFCs; however, gamma-aminobutyric acid type A receptor subunit beta3 (GABRB3), a subunit of the GABA-A receptor pentamer, has been associated with cleft palate in mice (http://www.informatics.jax.org/marker/phenotypes/MGI:95621). For example, it has been shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes delayed palatal shelf elevation, ultimately leading to CP, by decreasing the levels of GABRB3 in mice. This implies that *GABRB3* may play an important role in the elevation and fusion phases of the palate development (Lei et al., 2019). A Norwegian study could not establish association between NSOFCs and variants in gamma-aminobutyric acid B receptor 2 (GABBR2), a gene related to *GABRD* but associated with nicotine dependence (Jugessur et al., 2012). *GABRD* and other subunits of the GABA-A receptor pentamer is of particular interest because they demonstrate how the exposome can interact with the genome. Quite recently, it was demonstrated that PoO effects may vary across different environmental exposures, the so–called PoOxE effects. For example, statistically suggestive PoO interaction effects have been shown between SNPs near *ICE1* and *NAALADL2* and maternal smoking which increases risk to NSCPO in Europeans (Haaland et al., 2017). *HHAT* encodes an enzyme that attaches palmitoyl residues crucially needed for multimerization as well as long- and short-range hedgehog signaling. Variants in *HHAT* have been associated with a number of craniofacial anomalies, such as cleft lip (Kurosaka et al., 2014) and holoprosencephaly together with acrania and agnathia in mice

(Dennis et al., 2012), and Nivelon-Nivelon-Mabille syndrome that may present with microcephaly, early infantile onset seizures, and cerebellar vermis hypoplasia (Abdel-Salam et al., 2019). *DNMT3A* encodes a DNA methyltransferase that participates in *de novo* methylation but not maintenance methylation, with the expression of enzyme being developmentally regulated. For instance, TCDD induces global and low CpG methylation level of Dnmt3a by up-regulating the expression of Dnmt3a which may lead to global hypermethylation in fetal palate tissue culminating in palate malformation (Wang et al., 2017).

Other interesting loci that exhibited overwhelming maternal PoO effects was a 1mb genomic window at the MHC Class 1 locus on chromosome 6. As many as 6 SNPs demonstrated hints of maternal PoO effects (P_PAT>0.05, P_MAT<0.05, P_POO<0.05) at this locus which contains genes such as *ZNRD1, ZNRD1-AS1, HLA-F, HLA-G, HLA-F AS1, TRIM26, TRIM15, TRIM10* and *TRIM40*. In the other arm of the analyses that considered SNPs with all P_PAT, P_MAT and P_POO being <0.05, as many as 9 SNPs also showed predominantly maternal PoO effects. This region has not been largely associated with the etiology of OFCs, except that some microsatellite markers in HLA have been associated with CL/P in Indians (Rajendran et al., 2011). Our observation possibly suggests that components of the human adaptive immune system at this MHC Class 1 locus on chromosome 6 may interact with maternal exposome, such as pathogens or allergens, to increase the risk for NSOFCs. Overall, we observed more probable maternal PoO effects (58% of reported SNPs) than paternal PoO effects. This is probably because of the high influence of the intrauterine environment and other maternal factors on fetal development.

CONCLUSION

The multifactorial nature of the etiology of NSOFCs warrants investigations into other genetic phenomena that do not exhibit classical Mendelian pattern of inheritance. Here, we conducted genome-wide scan for PoO effects in a sub-Saharan African cohort with NSOFCs and

demonstrated hints of the possible existence of PoO effects that may influence risk to NSOFCs. Importantly, we have shown that genes that may be involved in epigenetic regulation of the human genome such as *DNMT3A*, those that serves as receptors for environmental agents such as *GABRD* and those involved in maternal immunity such as *HLA-G* may increase risk to NSOFCs through PoO effects. Further studies in larger case-parent trios, as well as subphenotypes analyses, are warranted in order to confirm the roles of the reported loci in the etiology of NSOFCs.

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Declaration of Conflicting Interests

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