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Clinically actionable secondary findings in 130 triads from sub-Saharan African families with non-syndromic orofacial clefts

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Abstract

Introduction: The frequency and implications of secondary findings (SFs) from genomic testing data have been extensively researched. However, little is known

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about the frequency or reporting of SFs in Africans, who are underrepresented in large-scale population genomic studies. The availability of data from the first whole-genome sequencing for orofacial clefts in an African population motivated this investigation.

Methods: In total, 130 case-parent trios were analyzed for SFs within the ACMG SFv.3.0 list genes. Additionally, we filtered for four more genes (*HBB*, *HSD32B*, *G6PD* and *ACADM*).

Results: We identified 246 unique variants in 55 genes; five variants in four genes were classified as pathogenic or likely pathogenic (P/LP). The P/LP variants were seen in 2.3% (9/390) of the subjects, a frequency higher than ~1% reported for diverse ethnicities. On the ACMG list, pathogenic variants were observed in *PRKAG* (p. Glu183Lys). Variants in the *PALB2* (p. Glu159Ter), *RYR1* (p. Arg2163Leu) and *LDLR* (p. Asn564Ser) genes were predicted to be LP.

Conclusion: This study provides information on the frequency and pathogenicity of SFs in an African cohort. Early risk detection will help reduce disease burden and contribute to efforts to increase knowledge of the distribution and impact of actionable genomic variants in diverse populations.

KEYWORDS
ACMG guideline, orofacial clefts, secondary findings, sub-Saharan Africa, whole genome sequencing

1 | INTRODUCTION

Whole-exome and genome sequencing (WES/WGS) have been used to successfully investigate clinically actionable mutations, rare Mendelian diseases, and complex conditions, including novel orofacial cleft (OFC) risk genes (Williams et al., 2015). A distinctive characteristic of NGS is the vast amount of sequence data generated. The broad range of genomic findings includes those directly related to the primary indication for the test and additional results, both anticipated and unexpected, uncovered during sequencing (Kalia et al., 2017). Secondary findings (SFs) could have profound health implications for the tested individual of their family, even though they are out of the scope of the test. SFs may cause additional challenges for the patient and the healthcare provider. The early identification of these risks could be vital for disease prevention and intervention, but it also raises issues of what and how to report these SFs (Chen et al., 2018).

While there are established international guidelines on the handling of SFs, none exists in Africa (Van Der Merwe et al., 2022). Also, the inadequate validation of genomic findings from African populations despite the existing genetic diversity limits the return of SFs (Van Der Merwe et al., 2022). In 2013, the American College of Medical Genetics and Genomics (ACMGs) recommended a minimum of 56 genes be reported as incidental or SFs (Kalia et al., 2017). This was done to provide a standard for the return of clinically actionable results. However, the ACMG list has been described as limited, representing only a fraction of genes known to cause Mendelian disease in humans (Green et al., 2013; Kalia et al., 2017; Miller et al., 2022; Rego et al., 2018). Another concern for the curated list is that it may not be as comprehensive for persons of non-European ancestry; most cohorts in genomic research consist of individuals of European ancestry (Popejoy et al., 2018, 2020; Wonkam & de Vries, 2020). Additionally, the discourse around SFs and their management has focused primarily on Caucasians and high-income populations (Sullivan & Berkman, 2018). Thus, identifying the range of SFs in populations historically underrepresented in genetic research will help improve the understanding of variant interpretation and standardise the presentation and utilisation of ethnicity and ancestry in clinical genomics. Additionally, it could impact local decision-making as it concerns the cost-effectiveness of returning SFs, public health program design and policy formulation (Jain et al., 2018; Popejoy et al., 2018; Tang et al., 2018).

The prevalence of SFs in WES and WGS data sets has been explored in several studies. Many used the ACMG guideline as a reporting guide to return clinically actionable findings (Kalia et al., 2017). In 2018,

(Yamaguchi-Kabata et al., 2018) conducted a study on a cohort of 2049 subjects from the Tohoku Medical Megabank Project in Japan and reported that 21% of the individuals had at least one pathogenic allele. Another study examined the frequency of clinically actionable SFs in 954 East Asians (subjects and matched controls) in a Hirschsprung disease study with WGS data and reported that 2.5% of the participants had variants classified as P/LP on the ACMG SF v2.0 (Tang et al., 2018). Similarly, a study by Chen et al. (2018) reported a frequency of 2.85% in a cohort of 421 Chinese. This frequency is higher than reported in the Caucasian population (Chen et al., 2018). Kwak and colleagues evaluated the WES data from 303 individuals from the Korean whole Exome sequencing (KOEX) study and reported a frequency of 2.46% (Kwak et al., 2017). Also, Choudhury et al., in their study exploring genomic diversity in Africa, conducted WGS analyses on 426 subjects from 50 distinct ethnolinguistic groups across Africa. The authors reported that 8 out of the 426 (1.88%) participants had reportable variants on the ACMG SFv2.0 list. However, almost all the subjects belonging to the high-depth coverage WGS cohort had at least one variant annotated as 'pathogenic' in the ClinVar Database (Choudhury et al., 2020).

Additionally (Dorschner et al., 2013), in their study which analyzed 1000 Exomes from individuals of European and African ancestry enrolled in the National Heart, Lung and Blood Institute Exome Sequencing Project for actionable mutations on a list contained in 114 genes (including the 53 genes in the ACMG SFv1.0) reported a frequency of 1.2% for the individuals with African ancestry and 3.4% individuals with European ancestry (Dorschner et al., 2013). More recently, a Thai study reported a frequency of 11.9% for P/LP variants in this cohort (Chetruengchai & Shotelersuk, 2022).

Due to the recent update in the number of actionable genes by the ACMG, the variation in the genetic makeup across different populations and the ethical and social issues surrounding the report of results from genomic testing procedures, it became imperative to contribute to the knowledge base of genetics and genomics research to aid the reporting of secondary genetic findings from genomic data while increasing the utility of genetic testing in resource-limited settings such as that present in Africa. In this study, we analyzed WGS data from 390 Sub-Saharan African individuals from 130 families with non-syndromic OFCs (only the probands had OFCs) and calculated the frequency of clinically actionable SFs. This analysis was done following the updated list of genes recommended by the ACMG for reporting SFs (ACMG SF v3.0). The results presented in this study follow the extensive analysis of the sub-Saharan WGS dataset for the SFs. This will give a broad view of the rate of SFs in the African population and provide a better understanding of the relationship between SFs and preexisting disease conditions.

2 | MATERIALS AND METHODS

2.1 | Study participants

Parent and child case trios ($n = 450$ individuals; 150 families) were selected from two participating countries (Ghana and Nigeria) from an ongoing large African OFC cohort study. All the participants included were of African ancestry (Ghanaians and Nigerians, respectively). Only the probands were diagnosed with non-syndromic cleft lip(nsCL) or cleft lip/palate – (nsCLP) (51 had non-syndromic cleft lip – nsCL and 79 had non-syndromic cleft palate – P). Following the quality control process, which included gender consistency check, missingness, variant HWE, heterozygosity, relatedness and Mendelian errors, 130 triads passed quality control and were analyzed.

This WGS study of nsCL/P case–parent trios was part of the Gabriella–Miller KidsFirst (GMKF) Pediatric Research Consortium. The Gabriella Miller Kids First Pediatric Research Program (Kids First) initiated a genomic sequencing effort to promote research into the biology of childhood cancer and structural birth defects (including nsCL/P), aiming to discover shared genetic pathways between these disorders.

2.2 | DNA extraction and XY genotyping

DNA extraction was done using the Oragene DNA extraction protocol on saliva samples shipped to the Butali Laboratory at the Iowa Institute for Oral Health Research, University of Iowa. Pre-sequencing quality control for sex confirmation was done in-house using the TaqMan XY genotyping. After confirming that all samples were labelled appropriately, samples were sent to the Broad Institute for WGS using the working aliquots (25 µL) with a DNA concentration of ≥250 ng.

2.3 | Sequencing and variant calling

The WGS was sequenced at 30X coverage. WGS data allows for identifying novel rare coding and non-coding variants across the genome and an opportunity for identifying SFs. Paired-end sequence reads were processed according to the GenomeAnalysisToolKit (GATK) best practice recommendations at the Broad Institute

(https://software.broadinstitute.org/gatk/best-practices/workflow) (DePristo et al., 2011) and aligned to the human reference genome (GRCh38). Variant calling was done using the HaplotypeCaller in GVCF mode and GenotypeGVCFs for single-sample variant calling and the multiple-sample joint variant calling, respectively. Additionally, variants were filtered based on Genotype Quality (GQ) ≥20 and read depth (RD) ≥10, to remove sites with low depth. Finally, the variants were stored in a variant call format (VCF) file. Additional information about this data set is provided in a previous publication (Awotoye et al., 2022).

2.4 | Guideline for the report of SFs

Variant extraction was done, focusing on the 73 genes on the ACMG SFv3.0 (Miller, Lee, Chung, et al., 2021; Miller, Lee, Gordon, et al., 2021). This was followed by extracting variants with MAF <1%. The clinical significance of each variant was evaluated according to the 2021 ACMG guidelines. Additionally, we filtered for variants in four genes (*HBB*, *HSD32B*, *G6PD* and *ACADM*) not present on the ACMG list. These variants were included due to the associated disease burden and clinical significance in the African population.

3 | RESULTS

A total of 25,937,518 genetic variants were reported for the 130 trios from the WGS data set from the Ghanaian and Nigerian populations. Three hundred fifty-four variants were annotated as loss of function (LOF) and missense mutations. When checked against the genes on the updated ACMG list, 246 variants were identified for further analysis. Out of the 246 unique variants in 55 genes, one (*PRKAG2*) was annotated as pathogenic (P), while four other variants (*PALB2*, *LDLR* and *RYR1*) were annotated as likely pathogenic (LP, Table 1). The remaining variants were classified as variants of unknown significance (VUS). Interestingly, heterozygous missense variants in *HBB* were found to be pathogenic; however, two individuals (mother and proband from separate families) were homozygotes for the pathogenic *HBB* variants.

Pathogenic variants of *HBB* were identified in 45 individuals (11.5%) from 27 families, while a VUS in *ACADM* was identified in 3 individuals (0.77%) from 2 families (Table 2). No variants were identified in *G6PD* and *HSD32B*. The P/LP variants on the ACMG SF v3.0 were seen in 9 out of the 390 individuals from 5 separate families (2.3%); a frequency higher than about 1% was reported

TABLE 1 List of pathogenic and likely pathogenic variants according to the ACMG SFv3.0 criteria identified in the 390 whole-genome data set from a sub-Saharan African Cohort.

Gene	SeQ ontology	Count	Disease	Ref seq mRNA	Nucleotide change	ClinVar variant ID	Amino acid change	Zygosity	ACMG classification	Proband cleft phenotype
PALB2	Stop gain	2	Familial cancer of breast	NM_024675.3	c.475G > T	548,756	p. Glu159Ter	Heterozygous	LP	CLP
LDLR	Missense	1	Familial hypercholesterolemia	NM_000527.4	c.1691A > G	224,616	p. Asn564Ser	Heterozygous	LP	–
		2		NM_000527.4	c.1291G > A	3695	p. Ala431Thr	Heterozygous	LP	CLP
PRKAG2	Missense	2	Hypertrophic cardiomyopathy	NM_016203.4	c.547G > A	430,968	p. Glu183Lys	Heterozygous	P	CL
RYR1	Missense	2	Malignant hyperthermia	NM_000540.2	c.6488G > T	590,571	p. Arg2163Leu	Heterozygous	LP	CLP

Note: Gene = The genes on the updated ACMG SF v3.0 list in which unique variants were identified. Count: The number of individuals in which the unique variants were identified. ACMG Classification: LP – Likely Pathogenic, P-Pathogenic.

TABLE 2 Variants from the additional genes included in the analysis of 390 whole-genome data set from a sub-Saharan African Cohort.

Gene	Chr:Pos	SeQ Ont	Count	Disease	Ref seq mRNA	Nucleotide change	ClinVar variant ID	AA change	Zygosity	ACMG classification
HBB	11:5227003	Missense	43	Hb C	NM_000518.5	c.19G > A	15,126	p.Glu7Lys	Heterozygous	P
	11:5225728	Splice_acceptor	2	Beta Thalassemia	NM_000518.5	c.316-2A > G	21,191	NA	Heterozygous	P
ACADM	1:75762896	3_prime_UTR	3	Medium-chain acyl-coenzyme A dehydrogenase deficiency	NM_000016.5	c.*133 T > C	126,567	NA	Heterozygous	VUS

Note: Gene = The genes on the updated ACMG SF v3.0 list in which unique variants were identified. Count = The number of individuals in which the unique variants were identified. ACMG Classification: LP – Likely Pathogenic, P-Pathogenic.

for diverse ethnicities (Green et al., 2013). The LP variants were observed in four families, segregating in either the parents or the children (probands). Only the mother carried the mutation in the fourth family with an LDLR variant. On the contrary, the pathogenic variant of *PRKAG2* observed was seen in one family: affecting the mother and the proband.

All variants identified (P/LP) have an autosomal dominant inheritance pattern.

Additionally, a de novo mutation in *ACTC1* (NM_005159.4:c.*1893C > T) and *PALB2* (NM_024675.3: c.*232G > T) were observed in one proband (Table 3). However, they were of unknown significance. The genes with the highest frequency of variations in this cohort include *STK11*, *HFE, TTN, PALB2, APOB*, *PRKAG2*, *HBB, DSG2*, *LDLR*, *GAA, RPE65, RYR1, DCSC2*, *TMEM4, MYH11* and *TMEM43* (Table S1).

4 | DISCUSSION

This study utilised data from the first WGS for OFCs in an African population. The robustness of the WGS data provides an opportunity to identify 'SFs' that indicate an increased risk for disorders that may have immediate medical implications for study participants or their family members (Kalia et al., 2017). We found that 2.3% of the participants had variants classified as P/LP on the ACMG SFv3.0 list. This frequency is higher than expected from the ACMG guidelines, which anticipated that about 1% of individuals analyzed will have a reportable variant (Green et al., 2013) but similar to findings from other studies in East Asian, Taiwanese, South Korean, Chinese and Lebanese populations (2.5%, 4.97%, 2.5%, 2.85% and 2.1%) (Chen et al., 2018; Jalkh et al., 2020; Jang et al., 2015; Kuo et al., 2020; Tang et al., 2018). (Table 4).

Variations in the estimated carrier frequency of P/LP variants in actionable genes could occur because of the different reasons, which include the characteristics of the cohort being examined, the list of genes used, filtering criteria of clinically actionable variants and methods used to confirm pathogenicity (Elfatih et al., 2021; Kwak et al., 2017). In this study, we restricted our analyses to the 73 genes recommended on the SFv3.0. Concerning study cohort characteristics, a systemic review reported a lower frequency of incidental genetic findings in African cohorts than European cohorts (Amendola et al., 2015; Dorschner et al., 2013; Elfatih et al., 2021; Olfson et al., 2015). The wide gap in carrier frequency might be associated with the limited representation of the African genome in global databases and the lack of consistent guidelines for returning actionable variants specific to the African population, despite the genetic diversity on

TABLE 3 De Novo variants identified in analyzing 390 whole-genome data set from a sub-Saharan African Cohort.

Gene	Chr:Pos	Ref/alt	SeQ Ont	Count	Disease	Ref seq mRNA	ClinVar variant ID	AA change	Zygosity	ACMG classification
ACTC1	15:34788519	G/A	3_prime_UTR	1	Familial hypertrophic cardiomyopathy	NM_005159.4:c.*1893C > T	315,649	NA	Heterozygous	VUS
PALB2	16:23603227	C/A	3_prime_UTR		Familial cancer of the breast	NM_024675.3:c.*232G > T	126,567	NA		

Note: Gene = The genes on the updated ACMG SF v3.0 list in which unique variants were identified. Count = The number of individuals in which the unique variants were identified. ACMG Classification: LP – Likely Pathogenic, P-Pathogenic.

the continent (Bope et al., 2019; Lek et al., 2016; Wonkam & de Vries, 2020).

Furthermore, the overrepresentation of variants found in individuals of European ancestry in international genomic databases limits the rate/accuracy of identification variants that are only found in the African Population, thus under- or overestimating the frequency of population-specific variants, including incidental findings (Elfatih et al., 2021; Wonkam & de Vries, 2020). Indeed, one of the crucial steps of improving the practice of genomic medicine in the African population is variant review, as this helps to strengthen the information database. Another barrier to incorporating WES in practice described among African professionals is the absence of guidelines for handling IFs and the difficulties associated with variant interpretation so much that providers avoid receiving them (Van Der Merwe et al., 2022). Thus, it becomes imperative to have African-specific variant characterization criteria providing extensive evidence for variant pathogenicity and clinical actionability in African genomic research (Van Der Merwe et al., 2022; Wonkam & de Vries, 2020).

Of the predicted P/LP variants on the ACMG SFv3.0, a pathogenic variant of *PRKAG2* was identified in two individuals. Variants in *PRKAG2* are associated with the autosomal dominantly inherited Wolff Parkinson White Pattern (WPWP) (Chhabra et al., 2023). WPWP is a congenital anomaly affecting the normal conduction of the electrical impulses between the atria and ventricles. Commonly associated symptoms include chest pain, palpitations, progressive shortness of breath, syncope and, in rare cases, sudden cardiac death (Talle et al., 2019). The information on the epidemiology of WPW has been limited in sub-Saharan Africa by inadequate resources, diagnostic equipment and screening programs (Ogunlade & Asafa, 2017; Talle et al., 2019). A prevalence of 0.11% in Nigeria was reported in a cohort of young adults aged 15–40 (Ogunlade & Asafa, 2017). Another case report by Talle et al. provided information from a patient who was diagnosed with WPW IN Nigeria, whose evaluation and management were hindered by a lack of facilities and the required expertise (Talle et al., 2019); furthermore, the authors stress that the availability of diagnostic options such as ambulatory ECG, cardiac magnetic resonance imaging (MRI), electrophysiological study (EP study) and genetic testing would have allowed for better management (Talle et al., 2019). Overall, early risk assessment may result in no intervention in asymptomatic individuals, preventive antiarrhythmic medications, to prophylactic accessory pathway ablation based on the level of risk (Chhabra et al., 2023).

Likely pathogenic heterozygous variants were identified in *PALB2, RYR1* and *LDLR*. *PALB2* variants have been reported to be associated with an elevated risk of breast cancer and other tumor types (Adedokun et al., 2020;

TABLE 4 Summary of SF reporting rates from previous studies.

Author and publication year	Title	Sample size	Frequency of SFs	Gene list
Chetruengchai & Shotelersuk (2022)	Actionable secondary findings (SFs) in the 73 ACMG-recommended genes in 1559 Thai exomes	1559	11.9%	ACMG SF v3.0
Choudhury et al. (2020)	High-depth African genomes inform human migration and health	426	1.9%	ACMG SF v2.0
Kuo et al. (2020)	Frequency and spectrum of actionable pathogenic SFs in Taiwanese exomes	161	4.97%	ACMG SF v2.0
Jalkh et al. (2020)[a]	Actionable Exomic SFs in 280 Lebanese Participants	280	2.1%	ACMG SF v2.0
Yamaguchi-Kabata et al. (2018)	Evaluation of reported pathogenic variants and their frequencies in a Japanese population based on a whole-genome reference panel of 2049 individuals	2049	21%	ACMG SF v2.0
Tang et al. (2018)	Actionable SFs from whole-genome sequencing of 954 East Asians	954	2.5%	ACMG SF v2.0
Chen et al. (2018)	SFs in 421 whole-exome-sequenced Chinese children	421	2.85%	ACMG SF v2.0.
Thompson et al. (2018)	Genomic sequencing identifies SFs in a cohort of parent study participants	789	3.2%	ACMG SF v2.0.
Kwak et al. (2017)	Findings of a 1303 Korean whole-exome sequencing study	1303	2.46%	ACMG SF v1.0.
Amendola et al. (2015)	Actionable exomic incidental findings in 6503 participants: challenges of variant classification	6503	1.7%	112 genes (including ACMG SF v2.0)
Dewey et al. (2016)	Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study	50,726	3.5%	Geisinger-76 (G76) – ACMG SFv 1.0 & 20 additional genes
Jang et al. (2015)	Frequency and spectrum of actionable pathogenic SFs in 196 Korean exomes	196	7% for the control subjects & 6% for test group	ACMG SF v1.0.
Dorschner et al. (2013)	Actionable, Pathogenic Incidental Findings in 1000 Participants' Exomes	1000	~3.4% for Europeans & ~1.2% for Africans	114 genes selected by an expert panel

[a]Population comparison of the frequency of individuals with SFs significantly differed when compared to frequency reported in this study, $p = 0.0002196$.

Hayat et al., 2021; Preobrazhenskaya et al., 2021; Zheng et al., 2018). The prevalence of breast cancer is high in African women and it is the second most common cause of cancer mortality in Africa (Bray et al., 2018; Hayat et al., 2021). It is predicted that the incidence of breast cancer will increase across Africa in the coming decades (Ferlay et al., 2019). Due to limited resources, steps focusing on risk management and early diagnosis should be encouraged, especially for those at high risk; this includes comprehensive-genomic sequencing (Zheng et al., 2018). In fact, it has been proposed that countries with a high burden of breast cancer in SSA include genetic risk assessment in their cancer management protocol (Adedokun et al., 2020; Zheng et al., 2018).

Variants in *LDLR* associated with Familial hypercholesterolemia were identified in three subjects. FH is an autosomal dominantly inherited disorder with cardiovascular manifestation and has a global prevalence of about 1 in 200–250 (Nordestgaard et al., 2013). Affected individuals present with an elevated risk of CVD due to constant exposure to elevated cholesterol. Additionally, it has been described as the 'most

common life-threatening genetic condition,' which is often underdiagnosed and undermanaged (Wilemon et al., 2020). More important is the common lack of awareness in the general public, care providers, professionals and healthcare systems about the prevalence and associated mortality associated with FH in low- to high-income countries (Wilemon et al., 2020). Early identification and treatment, including lifestyle modification, could reduce the incidence of CVD-related events, consequently leading to a reduced burden on African populations (Hayat et al., 2020; Kromberg et al., 2013).

Malignant hyperthermia (MH) is an autosomal dominant muscle disorder characterised by hypermetabolism which arises from mutations in *RYR1* (Foo et al., 2022). The reported incidence is 1 in 5000 to 100,000 individuals and it is triggered by certain anesthetic compounds in affected persons (Denborough, 1998; Krivosic-Horber, 2014; Ndikontar et al., 2020; Nelson & Litman, 2014). Fatality can occur if not properly identified and promptly treated (Foo et al., 2022). Although MH is rare in sub-Saharan Africa, Ndikontar et al. presented three cases post-exposure to general anaesthesia in Cameroon, with two case fatalities. This report highlights the triggering anesthetic agent and the clinical diagnosis process in a limited resource setting.

Furthermore, the authors stressed early diagnosis, management (with dantrolene) and follow-up could help prevent death (Ndikontar et al., 2020). Genetic testing provides a non-invasive and cost-effective method of assessing risk in affected individuals and families; Bennette et al. reported that disclosing IFs from genetic screening in healthy individuals led to reduced cost and increased quality of life years (QALYs) (Bennette et al., 2015; Sei et al., 2004). For the affected family in this cohort (mother and proband), it is essential that the care team be extremely vigilant should they require a procedure that requires general anesthesia with the identified triggering compounds.

Of the additional genes considered in this analysis, mutations in the *HBB* gene are associated with potentially life-threatening haemoglobin disorders, including sickle cell anaemia and beta-thalassemia (Carlice-Dos-Reis et al., 2017). SCD is an autosomal recessive monogenic disease endemic to the African population and about 4/5th of the cases on the continent are seen in sub-Saharan Africa (Williams, 2016). Early diagnosis and intervention have been reported to significantly reduce the very high rate of associated mortality (Grosse et al., 2011; Piel et al., 2013; Sack et al., 2017). The *HBB* variants (rs33930165) identified in this cohort are associated with the haemoglobin C (Hb C) trait commonly seen in malaria-endemic regions in Africa (Kowalski et al., 2019; Modiano et al., 2001). It is a less severe form of hemoglobinopathy (compared to Hb SS) and both the homozygous and heterozygous carriers are protected against *P. falciparum* malaria (Modiano et al., 2001). A unique variant of *ACADM* was identified in two families; however, it was a variant of unknown significance. *ACADM* mutation is associated with Medium-Chain Acyl-CoA dehydrogenase Deficiency (MCAD). No variants were found in *HSD32B* and *G6PD*; these genes are associated with Congenital Adrenal Hyperplasia (CAH) and Glucose-6-phosphate dehydrogenase (G6PD), respectively. These genes were included due to the high prevalence of the associated disorders and public health relevance. CAH is an endocrine disorder; that is potentially life-threatening in the absence of early diagnosis and treatment and typically requires lifelong therapy with steroids. The timely initiation of corticosteroid therapy and regular follow-up can lead to a reduction in associated mortality (Dabas et al., 2020). Even though MCAD is considerably rarer, it can be affordably managed by maintaining a high-glucose diet, avoiding fasting and glucose supplementation during illness (Merritt & Chang, 1993). Although we did not find mutations in all four genes added due to their association with childhood mortality, it is important to note that identifying the risk variants will lead to early interventions. Also, data from studies like this could help detect rare and pathogenic variants and estimate their population frequencies. For example, newborn screening and infection prevention by the prophylactic administration of penicillin and pneumococcal vaccination for SCD has been proven to reduce associated mortality. However, even in the absence of these therapies, health education by raising awareness of fever and splenic sequestration has been proven to effectively reduce SCD mortality in young children (Grosse et al., 2011). Finally, the shortage of support following the return of testing results, including SFs, remains an issue as it concerns the understanding and utility of these finding in resource-limited settings. This is evident of limited genetic knowledge and workforce including genetic counsellors who can help patients understand the impact of testing outcomes on their well-being and that of their families (Abacan et al., 2019; Quinonez et al., 2021; Zhong et al., 2021). The outcome is reduced access to genomic medicine services, further widening the health equity gap in African populations. Our group is currently working on efforts to assess the knowledge and comfort level of cleft-craniofacial providers with returning testing results in addition to the potential role that the community could play as pillars of support to affected families. Lessons learnt will be used to develop intervention strategies to help patients understand the testing process and the outcomes of testing.

5 | LIMITATIONS

The study participants were from a nsOFC cohort in Nigeria and Ghana; hence, the rate of SFs in this study is not representative of the sub-Saharan African

population. Additionally, the higher prevalence of pathogenic variants observed in this cohort could be attributable to this being a cleft cohort. Furthermore, the SFs considered here include only the likely pathogenic and pathogenic variants registered in the ClinVar database. Therefore, some pathogenic variants specific to the African population, which is yet to be registered in these databases, might have been missed. Also, recontacting patients for the return of actionable results has been difficult due to a poor address system and incorrect cell phone contacts. Thus, to ensure that actionable results are successfully returned to the patients and their families in the future, we hope to establish a collaboration with family support organizations to optimise the possibility of returning SFs.

6 | CONCLUSION

We found P/LP SFs in 2.3% of 390 Africans from an nsOFC WGS cohort. To the best of our knowledge, this is the first analysis of SFs in a cleft craniofacial genomic study performed in Africa. Our results further corroborate the ACMG's position on the need to intentionally search clinical genomics sequence data for actionable SFs based on the ACMG's list of actionable genes. Furthermore, our results suggest the need for additional studies to build an information infrastructure of clinically actionable variants for the African population through appropriate variants review and interpretations. The early identification of increased risk can allow patients and healthcare providers to take deliberate actions to reduce risk by implementing lifestyle changes and increasing disease surveillance. This will ultimately improve the practice and delivery of personalised healthcare for the African population.

AUTHOR CONTRIBUTIONS

Material preparation, data collection and analysis were performed by O.A, G.J., A.W and A.B. The first draft of the article was written by O.A and supervised by A.B, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The sequence dataset is publicly available at dbGaP – (dbGaP Accession Number: phs001997).

ETHICS STATEMENT

All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Institutional Review Boards approved the study at the Kwame Nkrumah University of Science and Technology (CHRPE/RC/018/130), Lagos University Teaching Hospital (ADM/DCST/HREC/VOL.XV/321), Obafemi Awolowo University Teaching Hospital (ERC/2011/12/01) and University of Iowa (201101720), respectively. The initial IRB-approved consent allowed for study participation and publication of data generated. An approved IRB amendment permitted the identification of secondary findings and re-contacting of participants for the return of results. Informed consent was obtained from all patients for being included in the study.

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SUPPORTING INFORMATION

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