

## **Supplementary Methods**

### Study Population:

The description of the study population has been described in our previously published study(Butali et al. 2019). In summary, ethical approvals were obtained at the Local Institution Review Boards (IRBs): Lagos University Teaching Hospital (ADM/DCST/HREC/VOL.XV/321), Obafemi Awolowo University Teaching Hospital (ERC/2011/12/01), Kwame Nkrumah University of Science and Technology (CHRPE/RC/018/130) and Addis Ababa University (003/10/surg). Cases were among those who participated in the Pan-African Association of Cleft Lip and Palate network for free surgical repair of clefts in Africa.

They were recruited across the 3 countries (Nigeria, Ghana and Ethiopia) by surgeons at cleft clinics and during surgical missions. Standardized phenotyping protocol was used by the surgeons for physical examination, taking clinical photographs and detailing the cleft phenotypes in a clinical database. Echocardiography was used to rule out congenital cardiac defects. Controls were recruited at immunization clinics and dental clinics at the same centers as cases and were age-, gender- and geographical location- matched. These controls were individuals born without any congenital defects. All individuals (cases and controls) recruited in this study were of African ancestry and indigenous to the 3 countries (Nigeria, Ghana and Ethiopia). All the families recruited were assigned a unique identifier (UNID) number and their data were remotely entered into a secured REDCap database(Harris et al. 2009). Deidentified samples were shipped to the Butali laboratory, United States.

### Genotyping and Data Cleaning:

The description of the methods for genotyping has been described in our previously published study (Butali et al. 2019). Briefly, over 2 million common SNPs and over 87, 000 rare coding variants in the African population were genotyped using the expanded Illumina MEGA v2 15070954 A2 (genome build 37). There were 3178 unique and 70 duplicates successfully genotyped samples. For the quality control (QC) process, we also genotyped 70 unique samples and 9 duplicates which were the HapMap controls.

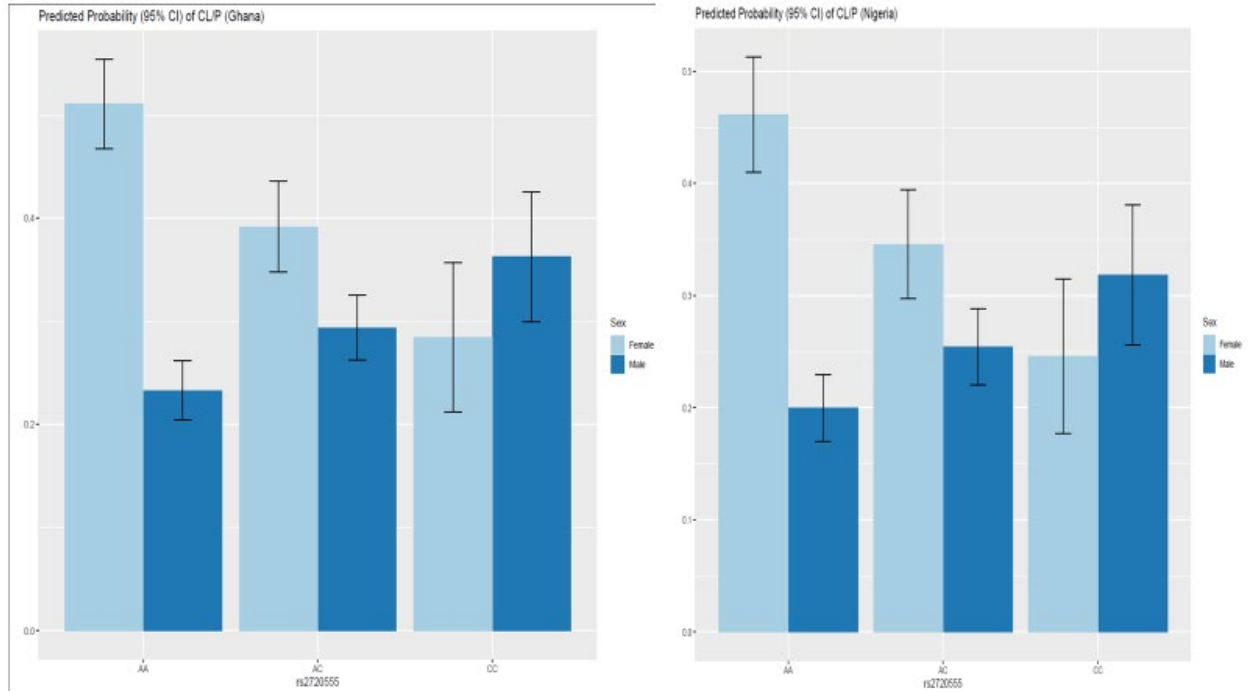
A detailed description of the data cleaning process have been published (Oseni et al. 2018). Briefly, we checked for chromosomal anomalies, missingness, batch effects, relatedness (IBD/IBS) and confirmed the continental ancestry of our cohorts with respect to the HapMap samples using the methods published in Laurie et al. 2010 (Laurie et al. 2010). This was executed using the R package, SNPRelate, and GENESIS.

SNPs with minor allele frequency (MAF) < 3%, missingness  $\geq$  2%, discordant call in the 70 study duplicates >1, Mendelian errors >1 in 890 duos and trios or those deviating from Hardy-Weinberg equilibrium (HWE of  $p < 1.00 \times 10^{-3}$ ) were excluded from the analysis. A total of 3178 individuals (814 cases of CL/P, 205 cases of CPO and 2159 related and unrelated controls) made up of 1133 males and 2045 females (Table 1), passed these QC filters and were used in our analyses.

We did preimputation and postimputation quality control (QC) following genotyping of the cases and controls. Preimputation QC included filtering out genotypes with missing call rates  $\geq$ 2%, >1 discordant call in 70 study duplicates, >1 Mendelian errors in 890 duos and trios, an Hardy

Weinberg equilibrium (HWE) of  $P < 10^{-3}$  and a minor allele frequency (MAF) of  $< 0.01$ , among others. Our imputed SNPs have a MAF of  $\geq 0.01$  and a quality metric (INFO, score is an estimated quality measurement of imputation) of  $\geq 0.3$ . Choosing this threshold of 0.3 retained 69.5% of all imputed variants for downstream analyses. Imputation was carried out using IMPUTE2 (a genotype imputation and phasing program) into the 1KG Phase 3 reference imputation panel. The final data set comprised 3178 (1133 male and 2045 female) participants enrolled from Ethiopia (30%), Ghana (43%) and Nigeria (27%). The imputation yield was a total of  $\sim 45$  million SNPs out of which  $\sim 17$  million passed our QC and were used in our association analyses.

## Supplementary Figures



**Appendix Figure 1** : The 95% Confidence interval of the predicted probabilities (which estimated the odds) of NSCL/P for an additional alternate allele in the Ghanaian and Nigerian population. It is observed that for every additional minor allele, the odds of clefting in male increases while the effect is opposite for females.

## Supplementary Tables

	nsCL/P	nsCPO	Control
<b>Female</b>	377	133	1535
<b>Male</b>	437	72	624
<b>Total</b>	<b>814</b>	<b>205</b>	<b>2159</b>

**Appendix Table 1A:** The sex distribution of the cases (nsCL/P and nsCPO) and controls in the discovery samples. The controls are unaffected consisting of related and unrelated individuals of the same ancestry as the cases.

	nsCL/P	nsCPO	Control
<b>Female</b>	173	52	734
<b>Male</b>	172	31	696
<b>Total</b>	<b>345</b>	<b>83</b>	<b>1430</b>

**Appendix Table 1B:** The sex distribution of the cases (nsCL/P and nsCPO) and controls in the replication samples. The controls are unaffected consisting of related and unrelated individuals of the same ancestry as the cases.

Chromosome	BP	SNP	Ref	Alt	P_2DF	P_GxSEX	OR_GxSEX
8	17350807	rs2720555	C	A	1.82E-08	2.43E-09	0.4511
8	1.46E+08	exm732028	G	C	7.94E-08	6.12E-08	1.876
23	1.21E+08	rs16996667	A	G	2.05E-07	4.61E-08	0.3957
12	26287230	rs1383112	A	G	2.51E-07	4.04E-08	1.877
2	1.98E+08	rs7575389	G	A	3.12E-07	8.94E-07	0.5863
7	47214444	rs1347801	A	G	3.12E-07	5.90E-08	0.5466
15	91515638	rs28393051	G	A	3.57E-07	7.17E-08	2.567
8	1.26E+08	rs17663005	G	A	4.57E-07	2.16E-07	2.251
5	72451309	rs6881806	A	G	4.76E-07	7.19E-08	2.276
11	60841281	exm2259827	A	C	5.87E-07	1.20E-07	2.034
23	87228407	rs12388812	C	A	7.43E-07	2.31E-05	1.822
11	94620479	rs518361	G	A	8.25E-07	2.56E-07	0.401
19	51829311	rs73551994	A	G	9.31E-07	1.38E-07	0.5278
18	74313259	JHU_18.74313258	A	G	9.94E-07	1.86E-07	0.5536
11	1.35E+08	JHU_11.134769410	C	G	1.06E-06	6.54E-07	1.729
3	1.6E+08	rs6781583	A	G	1.32E-06	3.14E-07	1.764
15	92088994	rs4129637	G	A	1.35E-06	7.51E-07	0.5315
8	1.3E+08	rs17242358	A	G	1.43E-06	0.00065	1.905
5	74692776	5:74692776-A-G	A	G	1.48E-06	2.24E-07	2.044
1	1.5E+08	exm93887	G	A	1.61E-06	2.47E-07	3.159
1	78976570	rs6701594	A	G	1.64E-06	4.65E-07	2.493
16	83058508	rs11860936	G	A	1.69E-06	5.51E-07	3.333
17	65000219	exm2264647	C	A	1.73E-06	7.02E-07	1.708
1	2.49E+08	rs6695844	A	G	1.80E-06	3.41E-07	1.816
1	2.49E+08	rs11205415	A	C	1.99E-06	3.85E-07	1.753
11	1.08E+08	rs17693854	A	T	2.03E-06	3.26E-07	2.726
12	26265632	rs7977928	A	G	2.21E-06	6.62E-07	1.921
10	1.08E+08	rs10884283	G	A	2.22E-06	9.12E-07	1.992
9	27295857	JHU_9.27295856	A	G	2.25E-06	6.03E-07	0.5724
10	95790063	rs12254065	A	G	2.28E-06	5.26E-07	1.842
6	39324581	rs6924090	A	G	2.50E-06	4.46E-07	1.833
7	1.08E+08	rs2193241	A	G	2.55E-06	4.30E-07	1.762
16	83004620	rs4782739	A	G	2.67E-06	5.71E-07	2.925
18	74869242	rs2850325	A	T	2.88E-06	4.53E-07	0.4165
8	62795087	rs16928045	G	A	2.97E-06	7.58E-07	1.717
5	1.02E+08	rs6878284	G	A	3.12E-06	0.000282	1.542
23	71198116	kgp22775447	G	A	3.18E-06	7.00E-07	2.022

17	29652456	rs11867465	A	G	3.21E-06	5.22E-07	0.5797
2	2.37E+08	rs6730534	A	G	3.26E-06	6.87E-07	0.565
9	1.17E+08	rs12377342	A	G	3.28E-06	5.82E-07	2.218
1	11711176	JHU_1.11711175	A	C	3.29E-06	5.04E-07	1.702
15	32200523	rs2001347	G	C	3.38E-06	6.97E-07	2.812
11	2298259	rs4929971	A	G	3.48E-06	1.13E-06	2.06
6	33099166	JHU_6.33099165	C	G	3.64E-06	5.59E-07	1.686
10	16088496	rs75544249	G	A	3.68E-06	4.01E-06	2.189
5	1.02E+08	rs1542296	A	G	3.71E-06	0.00031	1.537
11	1.3E+08	rs7125179	A	G	3.72E-06	5.79E-07	1.954
13	47709882	rs2567674	G	A	3.76E-06	5.91E-07	1.69
11	59169295	rs11604216	G	A	3.78E-06	2.31E-06	2.042
11	2297093	JHU_11.2297092	A	G	3.83E-06	1.41E-06	2.17
5	1.02E+08	JHU_5.101796593	G	A	4.07E-06	0.000292	1.54
11	97196848	rs7943263	A	G	4.31E-06	7.45E-07	1.928
17	58872391	rs7210374	A	G	4.40E-06	8.02E-07	1.761
2	1.98E+08	rs787994	G	A	4.45E-06	1.00E-05	1.582
2	12322109	rs12996777	A	G	4.67E-06	7.24E-07	2.707
10	9863986	rs10905625	C	A	4.71E-06	1.37E-06	2.134
3	38847152	rs7627881	A	G	4.89E-06	6.20E-05	1.646
17	29616632	rs8078423	A	G	5.03E-06	7.83E-07	0.5797
2	20304899	rs907864	C	A	5.20E-06	8.28E-07	1.769
19	51886275	JHU_19.51886274	A	G	5.27E-06	8.64E-07	1.835
23	87240701	rs10126101	A	C	5.38E-06	0.00025	1.67
3	1.88E+08	rs9290877	G	A	5.45E-06	3.28E-05	2.139
5	1.02E+08	rs1584717	C	G	5.46E-06	0.000513	1.512
5	1.02E+08	JHU_5.101725341	A	G	5.46E-06	0.000513	1.512
5	1.02E+08	exm-rs1584717	C	G	5.48E-06	0.000499	1.514
5	11742434	JHU_5.11742433	C	G	5.53E-06	8.83E-07	0.5797
15	41421648	JHU_15.41421647	A	G	5.56E-06	1.24E-06	0.5343
5	1.02E+08	JHU_5.101700952	G	A	5.61E-06	0.000523	1.511
16	89763263	16:89763263-TC	G	A	5.79E-06	1.32E-06	1.736
4	1.28E+08	JHU_4.127505484	A	G	5.83E-06	1.74E-06	2.722
19	51886922	rs7250000	G	A	5.88E-06	9.98E-07	1.826
12	65638072	JHU_12.65638071	C	A	5.95E-06	9.42E-07	1.638
14	99480422	rs1873661	A	C	6.39E-06	3.08E-06	2.537
3	42270787	JHU_3.42270786	A	T	6.48E-06	1.02E-06	2.318
6	33132931	rs2855455	G	A	6.48E-06	1.12E-06	0.5822
5	74935825	rs34344	G	A	6.60E-06	1.05E-06	1.9
20	24024681	JHU_20.24024680	A	G	6.71E-06	2.61E-05	1.855
6	32171683	rs2071277	G	A	6.80E-06	1.19E-05	1.632

17	58828402	rs11655927	A	G	7.09E-06	1.39E-06	1.737
4	43840953	rs2715650	G	A	7.09E-06	1.13E-06	2.177
11	3128774	rs3741353	G	A	7.13E-06	1.13E-06	2.395
11	758446	JHU_11.758445	G	A	7.19E-06	1.24E-06	2.129
11	1.3E+08	rs620263	A	G	7.27E-06	1.15E-06	1.867
8	281208	JHU_8.281207	A	C	7.29E-06	1.20E-06	1.865
16	73851099	rs8045441	C	A	7.29E-06	1.16E-06	0.5639
5	1.02E+08	rs1562960	G	A	7.29E-06	0.00062	1.503
15	73554544	exm2267843	A	G	7.41E-06	2.12E-06	1.737
9	1.07E+08	JHU_9.107344827	A	G	7.51E-06	2.08E-06	1.685
16	8084800	rs7193708	G	A	7.60E-06	2.67E-06	0.5891
3	88086902	rs9880959	G	A	7.60E-06	1.23E-06	0.56
5	1.02E+08	JHU_5.101763548	G	A	7.67E-06	0.000609	1.504
11	1.3E+08	rs7103088	G	A	7.69E-06	1.43E-06	2.063
14	36964744	rs986877	A	G	7.70E-06	1.37E-06	2.193
14	36964744	JHU_14.36964743	A	G	7.74E-06	1.38E-06	2.192
6	33131734	exm-rs2257126	A	G	7.78E-06	1.29E-06	0.5837
12	26272824	rs10842678	C	A	7.90E-06	4.84E-06	1.804
9	14346208	rs12343468	G	A	7.93E-06	1.65E-06	1.83
11	97128693	rs2555331	A	G	8.05E-06	1.41E-06	1.833
20	6506199	rs75325296	G	A	8.21E-06	1.55E-06	0.4583
23	56079984	JHU_X.56079983	A	C	8.22E-06	4.65E-06	2.001
8	1.46E+08	rs2721193	C	A	8.31E-06	6.28E-06	0.5509
23	1.12E+08	JHU_X.112086858	C	A	8.44E-06	1.86E-05	1.967
11	1.28E+08	rs10790956	A	G	8.63E-06	2.19E-06	1.865
6	75682566	rs4706591	A	G	8.76E-06	2.32E-06	1.792
9	95845152	rs9696357	A	G	8.78E-06	0.4626	1.1
16	84972920	rs17189009	G	A	8.93E-06	1.57E-06	2.296
10	1.3E+08	JHU_10.12959563 5	G	A	9.03E-06	2.05E-06	3.244
1	2.49E+08	rs12029054	A	C	9.06E-06	1.84E-06	1.685
20	36932676	exm2254258	C	G	9.16E-06	2.43E-06	1.844
5	1.02E+08	exm-rs1502844	G	A	9.21E-06	0.000706	1.502
20	24042329	exm2272924	A	G	9.48E-06	7.74E-05	1.856
11	1.3E+08	rs747180	A	G	9.89E-06	1.78E-06	1.704
2	1.45E+08	rs12612659	G	C	9.95E-06	1.68E-06	0.597

**Appendix Table 2:** Table shows the SNPs with suggestive p value at the joint test (P\_2DF) and their corresponding p values at the GxSex (P\_GxSEX) analysis. The odds (risk) of clefting as a result of this SNP is also reported in this table (OR\_GxSEX).



	AA (ADD = 0)	Aa (ADD = 1)	Aa (ADD = 2)
Male Cases	353	138	18
Female Cases	286	181	43
Male Controls	347	234	43
Female Controls	982	477	76

**Appendix Table 3:** Sex specific Allelic distribution of Reference (A) and Alternate (a) alleles in cases and controls for the Genome-wide GxSex Interaction most significant locus (rs2720555).

Cleft type	Chromosome location	SNP	REF/ALT	Females		Males	
				OR [CI]	P-values	OR [CI]	P-values
nsOFC	8: 17493298	rs2720555	T/G	1.30 [1.02, 1.65]	0.037	0.86 [0.65, 1.14]	0.286
nsCLO	8: 17493298	rs2720555	T/G	1.62 [1.09, 2.40]	0.018	0.92 [0.58, 1.47]	0.729
nsCLP	8: 17493298	rs2720555	T/G	1.25 [0.84, 1.85]	0.277	0.79 [0.53, 1.19]	0.262
nsCL/P	8: 17493298	rs2720555	T/G	1.33 [0.999, 1.78]	0.051	0.81 [0.59, 1.13]	0.215

**Appendix Table 4:** Sex-specific replication analysis result showing the risk of cleft due to the loci in females and males.