Genomic Insights into Variants Underlying Syndromic Orofacial Clefts with Limb Defects in the Ghanaian Population

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BACKGROUND

- Orofacial clefts (OFCs) are the most frequent congenital craniofacial anomalies that occur during embryonic development and are characterised by incomplete fusion of the palate, lip, or both.
- Incidence is ~1/700 live births.
- Congenital limb malformations are the second most prevalent birth defect, affecting ~4.48/10,000 live births globally; it can occur in isolation or as part of a syndrome.

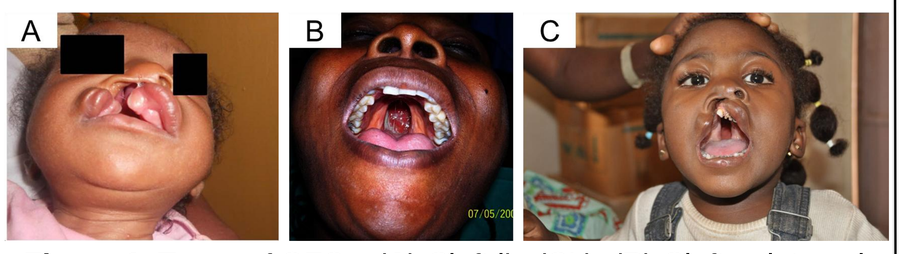

Figure 1: Types of OFCs. (**A**) Cleft lip (CL). (**B**) Cleft palate only (CP). **(C)** Cleft lip and Palate (CLP) [Gowans *et al*., 2018]

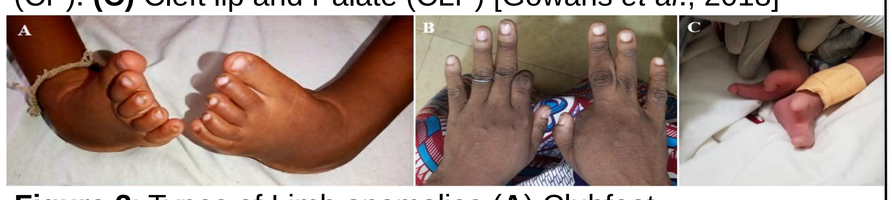

Figure 2: Types of Limb anomalies (**A**) Clubfoot (**B**) Symbrachydactyly/syndactyly **(C)** Ectrodactyly [Gowans *et al.*, 2025]

STUDY AIM

To determine the genetic etiology of syndromes associated with OFCs co-occurring with limb abnormalities in a Ghanaian cohort employing whole exome sequencing

METHODS

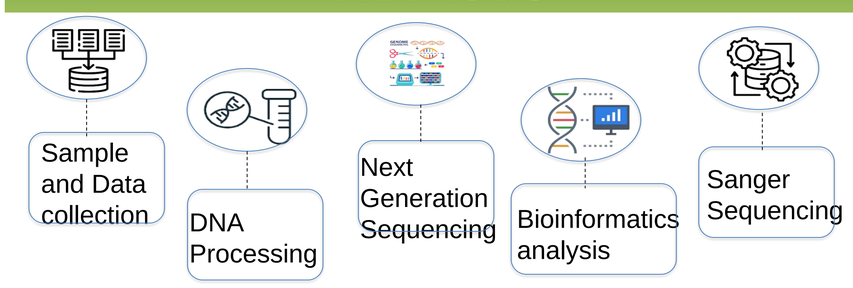

Figure 3: Overview of the entire workflow.

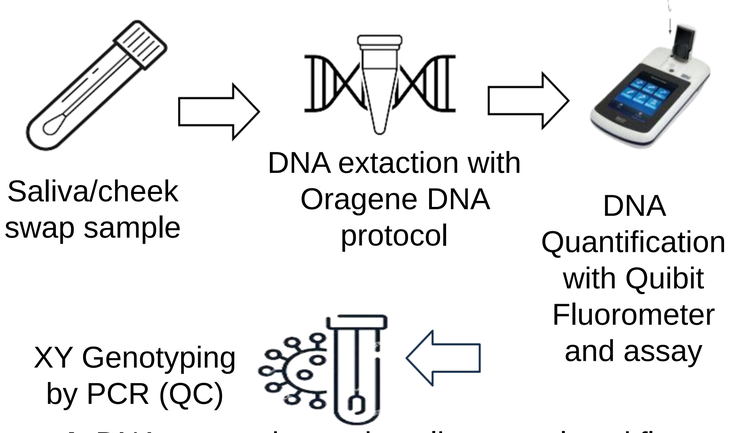

Figure 4: DNA processing and quality control workflow.

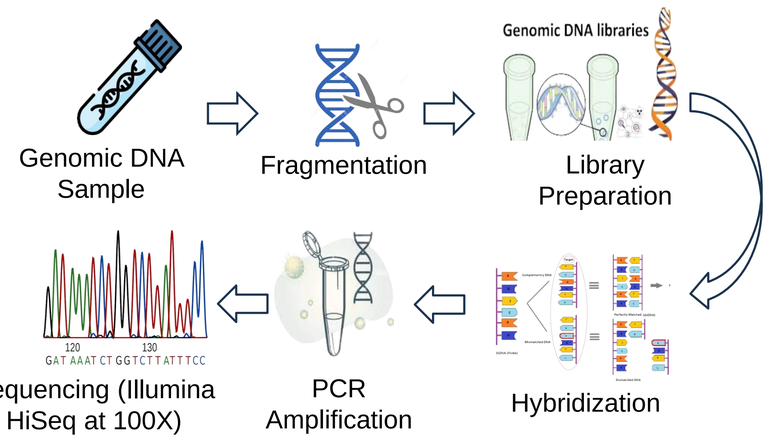

Figure 5: Whole exome sequencing workflow.

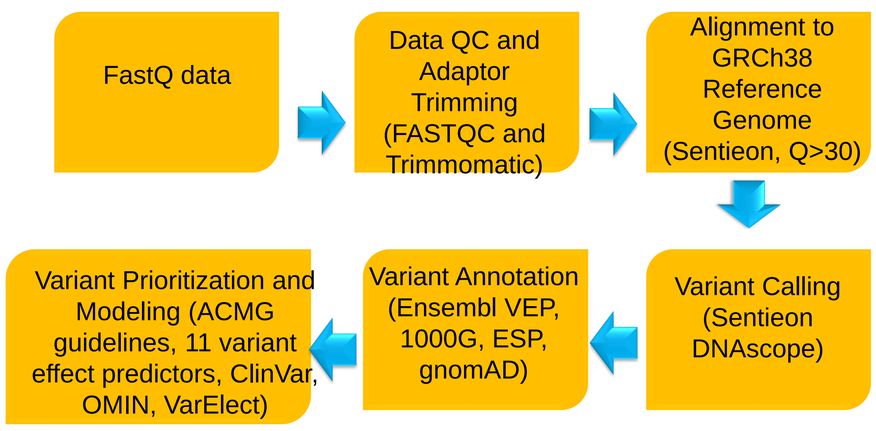

Figure 6: Bioinformatics workflow for WES data.

RESULTS AND DISCUSSION

Table 1: Probable pathogenic genetic variants observed in the affected families.

Family ID	Genetically Determined sex	*Gene*	Ref	Alt	Genotype	HGVSc (Variant)	HGVSp	No. of Tool predicting Pathogenicity	Syndrome/ Expression pattern
GH20130796-1	**M(XY)**	*RGPD5 (De Novo)*	G	A	G/A	c.4708G>A (Missense)	p.Gly1570Arg	7	Nuclear transport
		FAM90A26 (De Novo)	AT	A	A/A	c.10del (Frameshift)	p.Cys4ValfsTer12	N/A	Nucleic acid binding
GH20140599-1	**M(XY)**	*FOXD4L1 (De Novo)*	A	C	A/C	c.329A>C (Missense)	p.Tyr110Ser	9	Regulate neural ectoderm
		FAM170A (De Novo)	T	C	T/C	c.791T>C (Missense)	p.Met264Thr	7	Vater/vacterl Association (**VACTERL**)
		DLG1 (De Novo)	C	T	C/T	c.1730G>A (Missense)	p.Arg577Gln	6	Cleft Lip/palate (**FLP**)
		ANKRD1 (De Novo)	G	A	G/A	c.472C>T (Missense)	p.His158Tyr	11	Dilated Cardiomyopathy (**DCM**)
GH20160199-1	**F(XX)**	*TP63 (De novo)*	C	T	C/T	c.1027C>T (Missense)	p.Arg343Trp	11	Ectrodactyly, Ectodermal Dysplasia, and Cleft Lip/palate Syndrome 3 (**EEC3**)
GH20172514-1	**F(XX)**	*NIPBL(Novel)*	ATG	A	ATG/A	c.7617_7618del (Frameshift)	p.Ser2540ProfsTer21	N/A	Cornelia De Lange Syndrome 1 (**CDLS1**)
GH20207031-1	**F(XX)**	*MYH3 (De novo)*	C	T	C/T	c.2015G>A (Missense)	p.Arg672His	9	Arthrogryposis, Distal, Type 2a (**DA2A**)
GH20207087-1	**F(XX)**	*FGFR2*	G	C	G/C	c.755C>G (Missense)	p.Ser252Trp	10	Apert Syndrome (**APRS**)
GH20218082-1	**M(XY)**	*TRIM74 (De Novo)*	G	A	G/A	c.487C>T (Stop gained)	p.Arg163Ter		Williams-Beuren Syndrome (**WBS**)
		TRIM73 (De Novo)	C	T	C/T	c.487C>T (Stop gained)	p.Arg163Ter		Williams-Beuren Syndrome (**WBS**)
GH20228117-1	**M(XY)**	*PRDM9 (De Novo / Novel)*	C	CTG	C/C	c.2272_2273insTG (Frameshift)	p.Arg758LeufsTer182	N/A	Smith-Magenis Syndrome (**SMS**)
GH20228145-1	**F(XX)**	*TP63*	C	T	C/T	c.952C>T (Missense)	p.Arg318Cys	11	Ectrodactyly, Ectodermal Dysplasia, and Cleft Lip/palate Syndrome 3 (**EEC3**)

NB: Top candidate genes in each family have been highlighted in green. There were no paternal samples for families 4 and 9. All other samples and data were from case parent trios.

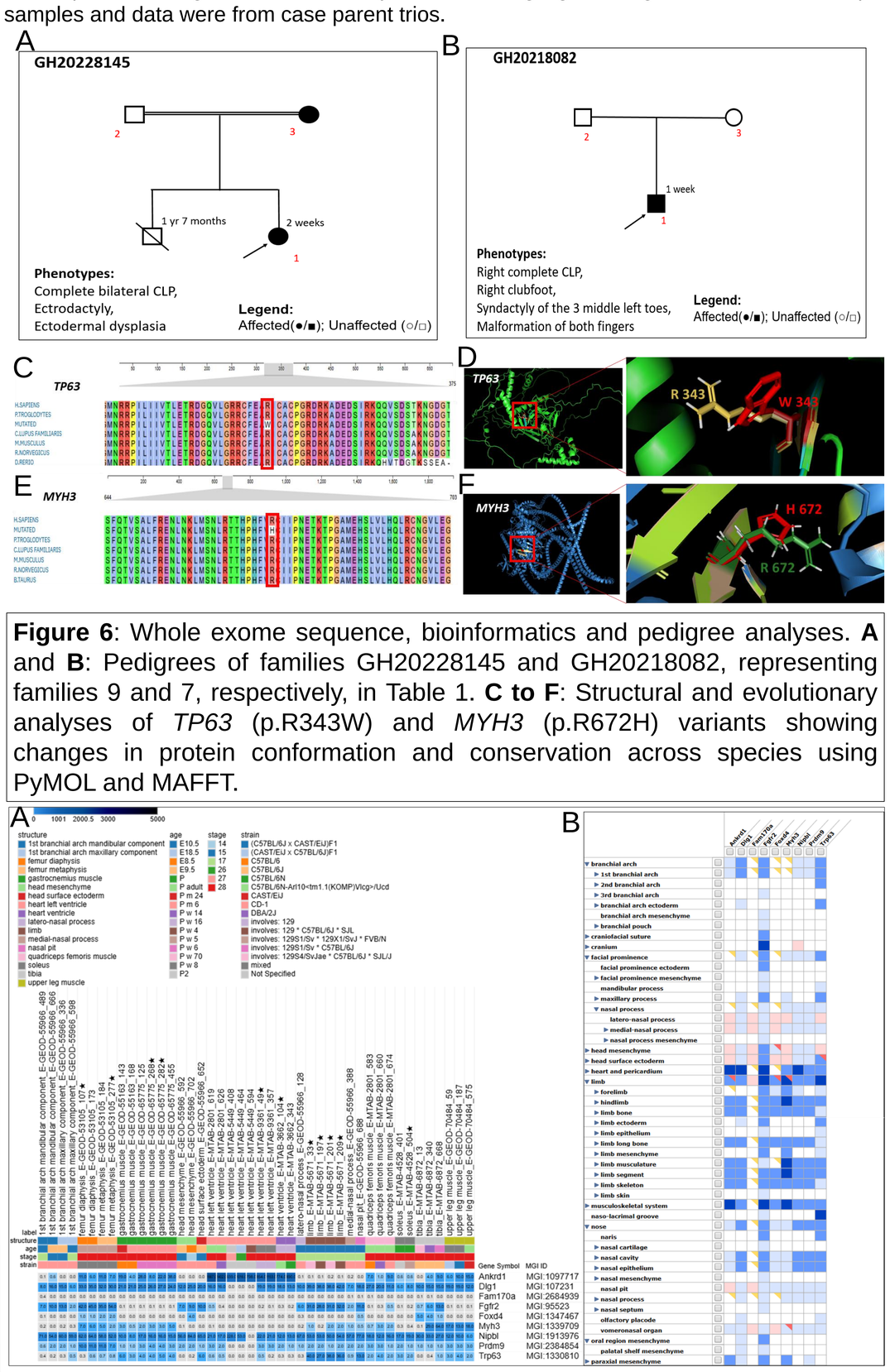

Figure 6: Whole exome sequence, bioinformatics and pedigree analyses. **A** and **B**: Pedigrees of families GH20228145 and GH20218082, representing families 9 and 7, respectively, in Table 1. **C to F**: Structural and evolutionary analyses of *TP63* (p.R343W) and *MYH3* (p.R672H) variants showing changes in protein conformation and conservation across species using PyMOL and MAFFT.

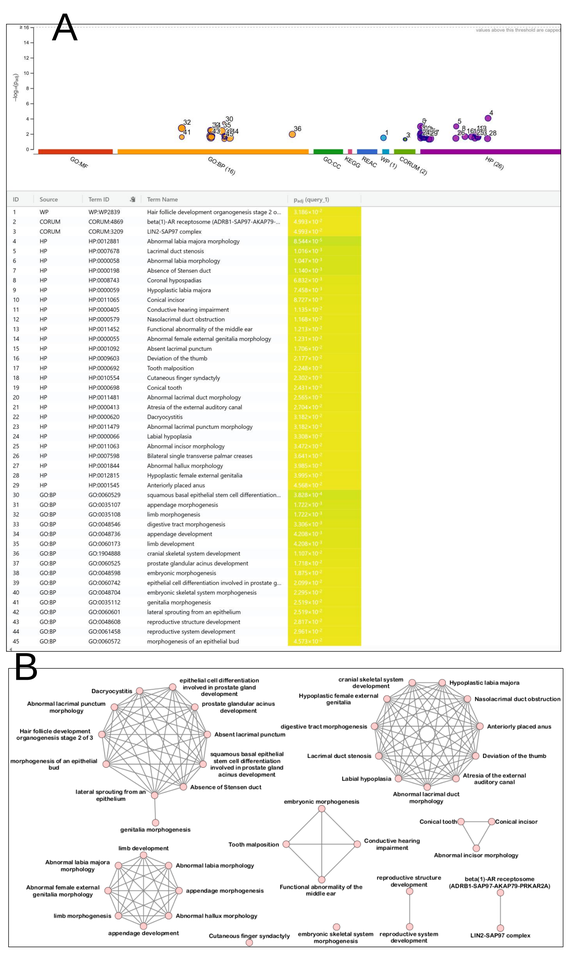

Figure 7: Spatiotemporal gene expression analysis during mouse development **A**: Temporal expression heatmap for candidate genes. **B**: Anatomical expression matrix for candidate genes.
NB: Nine of thirteen candidate genes shown based on MGI database availability.

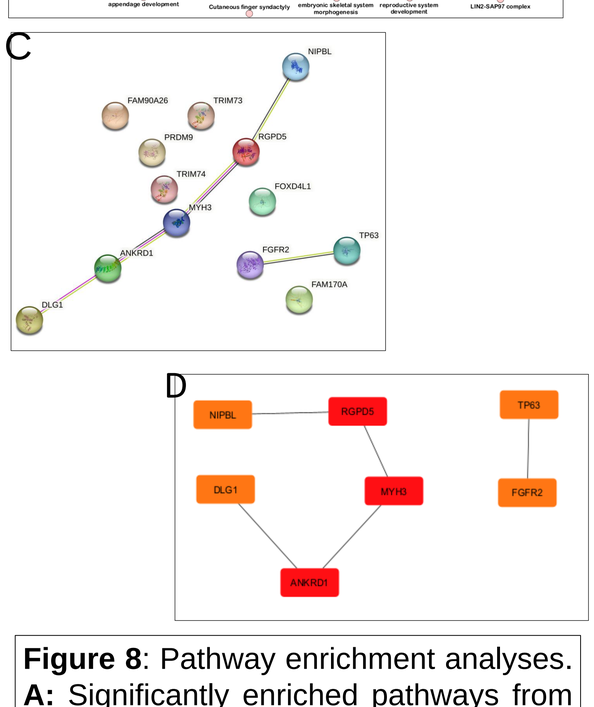

Figure 8: Pathway enrichment analyses. **A:** Significantly enriched pathways from g:Profiler, **B:** Pathway interaction network, **C**: Protein-Protein Interaction (PPI) Network Analysis. **D**: Hub Genes

CONCLUSION

- Multiple genetic syndromes underlie OFCs co-occurring with limb abnormalities in the Ghanaian population.
- Whereas phenotypes can be attributed to single-gene syndromes, such as *NIPBL*-associated Cornelia de Lange Syndrome, in some cases, others may result from the co-occurrence of multiple genetic syndromes.
- These findings will inform recurrence risk estimates, genetic counseling, and other clinical management of OFCs.

RECOMMENDATION

- Implementing genetic testing programmes in Ghana.
- Functional validation of implicated genes and variants.

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