Replication of HLA class II association with podoconiosis in diverse Ethiopian ethnic groups

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Introduction

- Podoconiosis: non-filarial elephantiasis
- Etiology remains unknown - Silicon and Aluminum from volcanic rich soils have been implicated (*Price and Henderson, 1978*).
- Host genetic factors play an import role in disease pathogenesis.
  - Cases cluster in families (*Price, 1972*).
  - Autosomal co-dominant pattern of inheritance with sibling recurrence ratio and heritability as 5.07 and 63%, respectively (*Davey, 2007*).
  - First GWAS in Wolaita identified one significant Single-Nucleotide Polymorphism (SNPs) ($p < 5e\text{-}8$) and five suggestive SNPs in the HLA class II region (*Tekola et al, 2012*).
    - Finding have to be replicated in an independent, larger and diverse populations.

Material and Methods

- **Ethical consideration**: Approval was obtained from appropriate ethical committees; Written informed consent was obtained from each participants before enrollment.
- **Study population and case-control definition:** 1920 eligible participants were enrolled from 3 ethnic groups (Wolaita, Oromo and Amhara) in Ethiopia.
  - Cases were adults (aged 18 and above) with lymphoedema typical of podoconiosis stage II and above; resident of podoconiosis endemic area for at least 5 years.
  - Controls were healthy adult farmers aged 50 and above; resident of podoconiosis endemic area for minimum of 25 years; no family history of podoconiosis; no consistent shoe wearing habit.
- **Genotyping and Quality Control (QC)**: DNA was extracted from saliva and genotyped on high-density chip at Wellcome Trust Sanger Institute (WTSI), UK.
  - After QC, 1892 individuals (943 cases and 949 controls) and 2210858 SNPs remained for analysis.

Results: GWA Analysis found association of SNPs on Chromosome 6 with podoconiosis

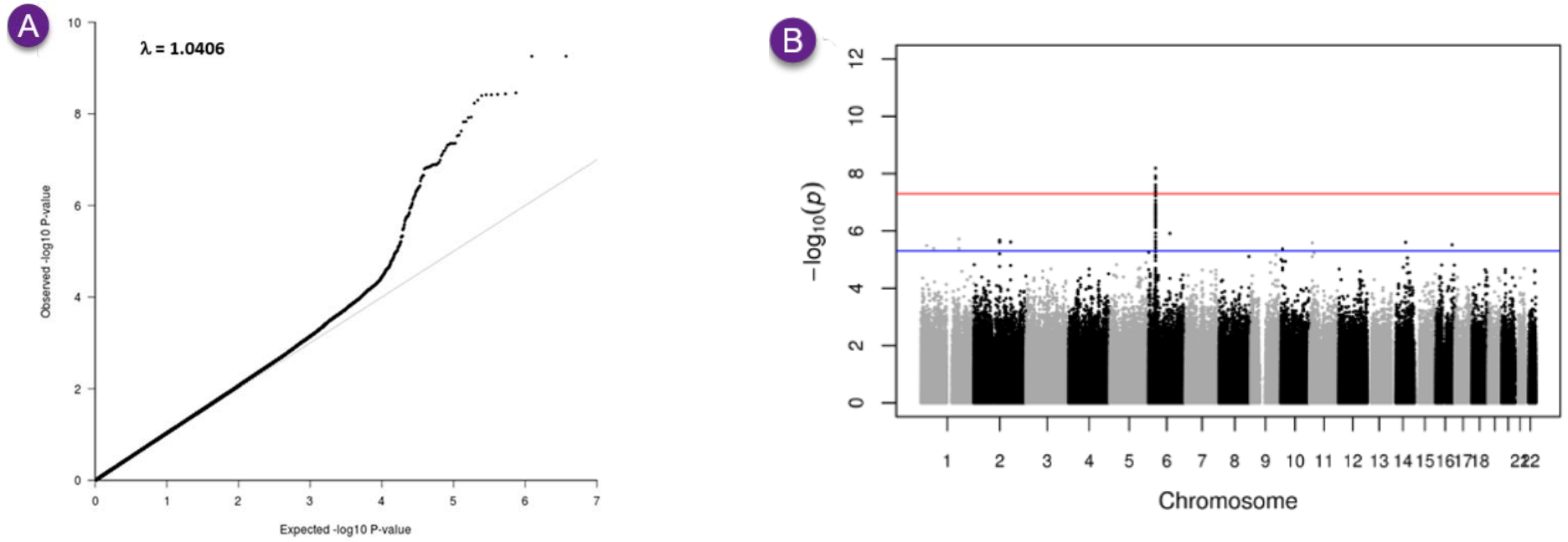


Figure 1: (A) The QQ plot compares expected versus observed –log10 (p-values) for all SNPs included in the GWA analysis (B) Manhattan plot for GWAS podoconiosis.

Results: A number of SNPs were found to be associated with podoconiosis.

Chr	SNP	Position	A1	A2	Gene	Consequence	MAF	Allelic Model: Additive	
								P-value	OR (95 % CI)
6	rs9270911	32572202	T	C	-	regulatory_region_variant	0.4413	5.512E-10	1.53 (1.34 -1.74)
6	rs6906021	32626311	C	T	HLA-DQB1	downstream_gene_variant	0.4406	3.478E-09	1.50 (1.31 -1.72)
6	rs1129740	32609105	G	A	HLA-DQA1	missense_variant	0.4889	3.657E-09	0.67 (0.59 - 0.77)
6	rs482205	32576009	G	T	-	intergenic_variant	0.3621	3.754E-09	1.51 (1.32 - 1.73)
6	rs1063355	32627714	T	G	HLA-DQB1	3_prime_UTR_variant	0.4894	3.831E-09	0.67 (0.59 - 0.77)
6	rs9273349	32625869	T	C	HLA-DQB1	downstream_gene_variant	0.4894	3.831E-09	0.67 (0.59 - 0.77)
6	rs643889	32575918	T	A	-	intergenic_variant	0.3614	3.996E-09	1.51 (1.32 - 1.74)
6	rs477515	32569691	A	G	-	intergenic_variant	0.3338	5.033E-09	1.51 (1.32 - 1.74)
6	rs2516049	32570400	C	T	-	intergenic_variant	0.3343	5.847E-09	1.51 (1.32 - 1.74)
6	rs17205647	32637418	A	G	HLA-DQB1	upstream_gene_variant	0.3721	1.177E-08	1.48 (1.30 - 1.69)
6	rs1071630	32609126	T	C	HLA-DQA1	missense_variant	0.4862	1.207E-08	0.68 (0.60 - 0.78)
6	rs6928482	32626249	C	T	HLA-DQB1	downstream_gene_variant	0.4447	1.482E-08	1.48 (1.29 - 1.69)
6	rs17843604	32620283	T	C	-	intergenic_variant	0.4888	1.497E-08	1.47 (1.29 - 1.68)
6	rs4538748	32657505	C	T	-	intergenic_variant	0.3743	2.377E-08	1.46 (1.28 -1.67)

Results: The majorities were non-coding regulatory variants

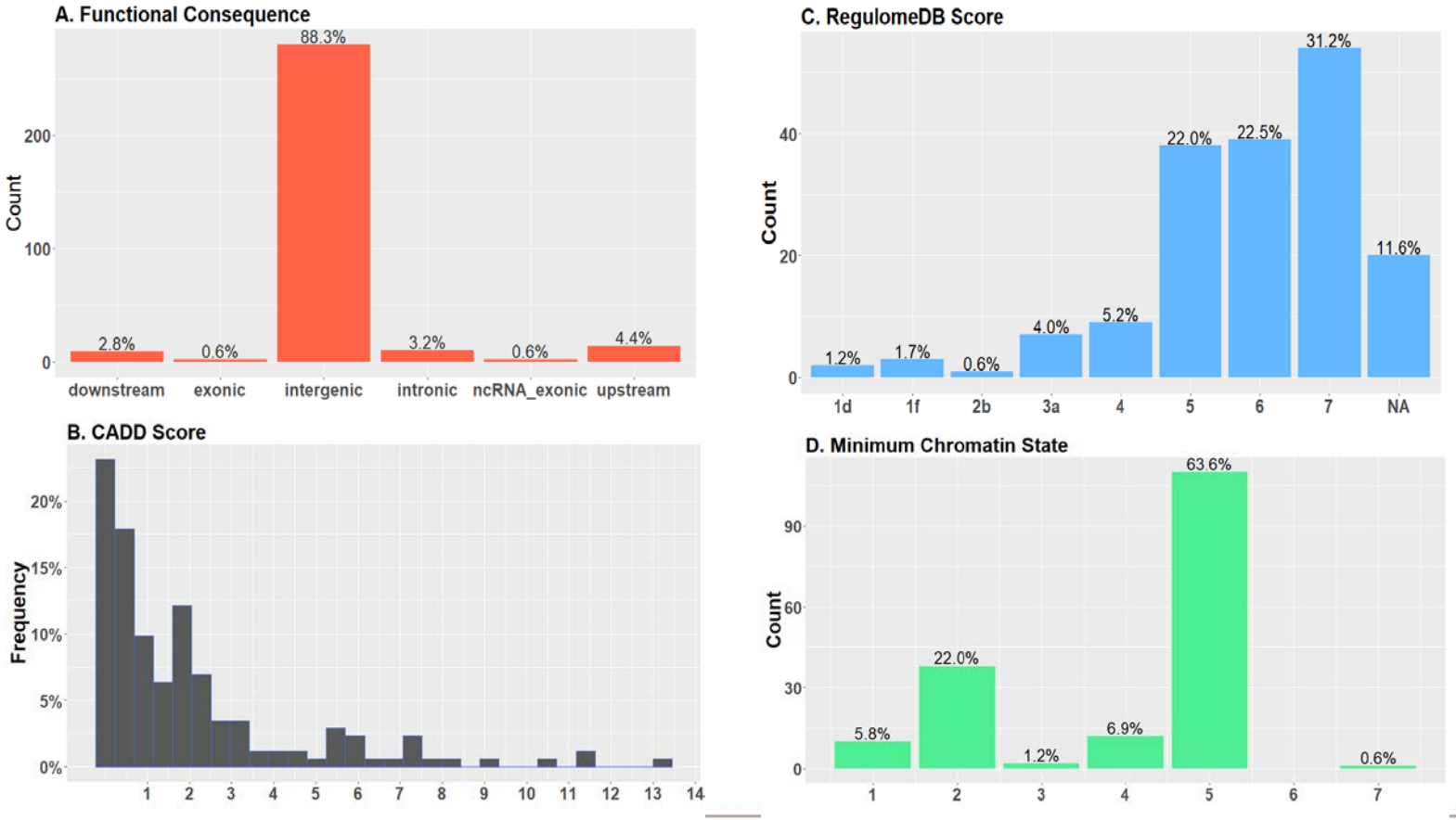
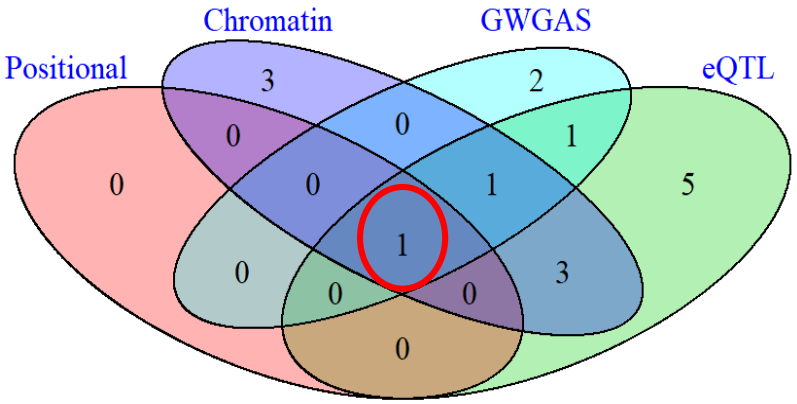


Figure 2: *Functional annotation of the annotated SNPs that are in LD (r2>=0.6) with one of the GWA significant SNPs*

Results: Different gene mapping strategies map associated SNPs to genes

Table 1: List of genes mapped using the four gene-mapping strategies

Positional	eQTL	Chromatin	GWGAS
HLA-DRB1	HLA-DRA	BTNL2	HLA-DQB1
	HLA-DRB9	HLA-DRA	HLA-DRB1
	HLA-DRB5	HLA-DRB6	HLA-DQA1
	RNU1-61P	HLA-DRB1	HIST1H2AI
	HLA-DRB6	HLA-DQB1	HIST1H3H
	HLA-DRB1	HLA-DQB1-AS1	
	HLA-DQA1	MTCO3P1	
	HLA-DQB1	HLA-DOB	
	HLA-DQB1-AS1		
	HLA-DQA2		
	HLA-DQB2		



eQTL: Expression quantitative trait loci (eQTLs); **GWGAS**: genome-wide gene-based association

Results: Gene-set association study identified immune-related pathways.

- At least one SNP mapped to each of a total of 18251 genes (out of 19427).
- Two gene-sets were found to be significantly associated (p < with podoconiosis.
  - **GO: MHC Class II Protein Complex** (MAGMA competitive P = 1.08e-06; corrected P=0.007) - A transmembrane protein complex composed of an MHC class II alpha and MHC class II beta chain, and with or without a bound peptide or polysaccharide antigen.
  - **KEGG: Cytosolic DNA Sensing Pathway** (MAGMA competitive P = 1.3e-05; corrected P= 0.016) - Specific families of pattern recognition receptors are responsible for detecting foreign DNA from invading microbes or host cells and generating innate immune responses.

Conclusion/Summary

- Our findings suggested the role of variants in the major histocompatibility complex (MHC) locus as determinants of podoconiosis.
  - Antigen presentation and T cell mediated response
- Thus, future studies must be conducted to elucidate immunological mechanisms of disease development and understand functional consequences of the variants in podoconiosis susceptibility.

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footwork
The International Podoconiosis Initiative

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