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Molecular analysis of the *ICAM4* gene in an autochthonous East-African population

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The Intracellular Adhesion Molecule 4 *(ICAM4)* gene, located on chromosome 19p13.3, encodes a transmembrane glycoprotein expressing the Landsteiner-Wiener (LW) blood group antigens.[1,2] Only one study has systematically analyzed the *ICAM4* gene at the allele level in Caucasians and African Americans.[2] This is the first genetic study in an African population to describe the variability of the *ICAM4* gene and identify prevalent alleles.

Study subjects and methods

DNA was extracted from EDTA-anticoagulated whole blood samples from 57 individuals from Gambela, a southwestern region of Ethiopia,[3] and the 1,920 nucleotides of *ICAM4* gene were sequenced as described previously.[2] Nucleotide sequences were aligned (CodonCode Aligner; CodonCode, Dedham, MA) to NCBI RefSeq NG_007728.1 and nucleotide positions defined using the first nucleotide of the coding sequence (CDS) of NM_001544.5 (ICAM4 isoform 1). For comparison, 3 Ethiopian samples drawn in Addis Ababa[3] were also tested.

Results and discussion

We identified 2 known and 1 novel allele of the *ICAM4* gene, without ambiguity. No SNP encoding a missense, non-sense or frameshift mutation was found.

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Author contribution. A.T.M conducted and A.G. contributed to the sample collection. W.A.F and K.S designed the experiments. J.B.S performed the molecular testing. Q.Y., K.S., and W.A.F. analyzed the data and wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Alleles.

We observed 3 alleles (Table 1) which occurred in 4 genotype patterns (Table S1). All alleles carried the variant (c.299G; p.Arg100) specific for the common LW(a+b-) phenotype. The reference *ICAM4* allele NG_007728.1, which is shorter than our sequenced region by 348 nucleotides, was confirmed to be the most common allele in Ethiopia (KF712272; Table 1). KF725837 has previously been observed[2] and the third allele was novel (MK138571; Table 1).

Nucleotide variations and genotype patterns.

Among 57 indigenous southwestern Ethiopians analyzed and 104,440 nucleotides of the *ICAM4* gene sequenced, we observed only 2 nucleotide positions with single-nucleotide polymorphisms (SNPs). One SNP occurred in the promoter region (rs3093030) and the other in intron 1 (rs5030384; Table 2), which were in Hardy-Weinberg equilibrium (HWE).

Impact of population substructure.

When including data from 3 Addis Ababa individuals, the SNP rs3093030 showed statistically significant deviation from the HWE ($p<0.05$; Table S2). The variant allele frequency (VAF) of this SNP in the Ethiopian population (0.042; Table 2) was similar to that of the African population in the 1000Genomes Project (0.047).[4] The deviation from HWE was due to the presence of an individual homozygous for rs3093030, showing the effect of population substructure (stratification) as reported before.[5] Further studies in other regions of Ethiopia[2] may be instrumental to molecularly define differences among subpopulations in Ethiopia, which may have a role in pathogenesis of endemic diseases such as malaria.

Conclusion.

Only 3 *ICAM4* alleles were observed in 120 Ethiopian chromosomes, although they represent a population with deep ancestry. This is in accordance with our previous study[2] where 5 *ICAM4* alleles were observed among 182 African American chromosomes. This low degree of genetic variation in *ICAM4* gene may be due to the small size of the *ICAM4* gene or its importance in human development and cellular function. The data generated in our study will be useful in determining the evolutionary history of the *ICAM4* gene and can be applied to develop, evaluate, and validate next-generation sequencing techniques and precision medicine.[3,6]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

ICAM4 allele distribution in 57 Ethiopian individuals from Gambela

		Allele frequencies								
		Present study (Gambela)			Caucasian (USA)[2]			African American (USA)[2]		
Allele	**Sequence***	**Observed (n)**	**Mean (%)†**	**95% CI‡**	**Observed (n)**	**Mean (%)†**	**95% CI‡**	**Observed (n)**	**Mean (%)†**	**95%CI‡**
KF712272	CG	109	95.6	78.3–114.6	120	62	51.3–74	147	81	68–95
KF725837	TG	3	2.6	0.7–7.1	70	36	28.1–45.6	26	14	9.3–21
MK138571	CA	2	1.8	0.3–5.9	0	NA	NA	0	NA	NA

*The nucleotides at the 2 SNP positions are shown in 5'- to 3'- orientation (see Table 2). The remaining 1,918 nucleotide positions that we determined had no variation relative to the reference sequence NG_007728.1.

†Number of observed alleles x 100/Total number of alleles

‡95% confidence interval (CI), Poisson distribution, two sided

NA – Not applicable

Table 2.

Genetic variations detected in *ICAM4* gene in 57 Ethiopian individuals from Gambela

Location	Nucleotide change*	dbSNP reference no.	Observations in present study (n=57)					Caucasian VAF[2]	African American VAF[2]	Global VAF†
			Homozygote reference	Heterozygote variant	Homozygote variant	VAF	HWE (p)			
Promoter	c.−286C>T	rs3093030	54	3	0	0.042	0.838	0.361	0.143	0.320
Intron 1	c.394+7G>A	rs5030384	55	2	0	0.017	0.896	0.000	0.000	0.004

*Nucleotide substitutions are shown relative to the reference sequence (NG_007728.1). Nucleotide positions are defined using the first nucleotide of the coding sequence (CDs) of NM_001544.5 isoform 1 as nucleotide position 1

†Global VAF from 1000Genome, TOPMed (nhlbiwgs.org) and gnomAD (http://gnomad.broadinstitute.org/) databases

VAF - variant allele frequency, HWE – Hardy-Weinberg equilibrium