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Association of ABCA4 and MAFB with nonsyndromic cleft lip with or without cleft palate

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To the Editor

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common isolated orofacial birth defect occurring in 1 in 700 to 1000 births in the United States each year and affecting 135,000 newborns worldwide [Murray, 2002]. The etiology of NSCLP is complex with both genetic and environmental factors implicated, but to date approximately only 20% of the genetic susceptibility has been identified [Vieira, 2008]. Recently, two genome-wide association studies (GWAS) found strong evidence for an association to rs987525 in the 8q24 chromosomal region ($p = 3.34\times10^{-24}$ and $p = 9.8\times10^{-8}$) [Birnbaum et al., 2009; Grant et al., 2009]. This association was independently validated using the same SNPs ($p = 3.0\times10^{-6}$ for rs987525) in our nonHispanic and Hispanic family datasets [Blanton et al., 2010]. The genotypes AA and AC for rs987525 conferred an almost 2-fold increased risk (odds ratio = 1.962 95%CI 1.382–2.785, $p = 1.4\times10^{-4}$) in a recent association study [Mostowska et al., 2010]. The chromosomal region containing rs987525 is a gene desert suggesting that this SNP may be in linkage disequilibrium (LD) with the causative gene or it is a regulatory variant for a gene yet to be identified. A third GWAS found significant associations with SNPs in/near *MAFB* (v-maf musculoaponeurotic fibrosarcoma oncogene homolog B; 20q11.2-q13.1), *ABCA4* (ATP-binding cassette, sub-family A, member 4, 1p22.1), *IRF6* and 8q24 [Beaty et al., 2010]. The association with rs987525 at 8q24 ($p = 1.11\times10^{-16}$) was found in both European and Asian datasets. Significant associations were found for rs13041247 which is downstream of *MAFB* ($p = 1.44 \times 10^{-11}$) and rs560426 which is in intron 6 of *ABCA4* ($p = 5.01 \times 10^{-12}$). *MAFB* encodes a basic leucine zipper (bZIP) transcription factor that plays an important role in the regulation of lineage-specific hematopoiesis [Gemelli et al., 2006]. It also functions as an oncogene responsible for the transformation of MAF expressing myeloma cells [van Stralen et al, 2009]. Recent studies found expression of Mafb in the secondary palate of developing mouse embryos suggesting a role in lip and palate morphogenesis [Beaty et al., 2010]. The *ABCA4* gene, encoding a membrane-associated protein, is a member of the superfamily of ATP-binding cassette (ABC) transporters, which transport various molecules across extra- and intracellular membranes [Molday et al., 2009]. While *ABCA4* was originally found to be exclusively expressed in rod and cone photoreceptors of the vertebrate retina, recent studies show expression in the murine brain [Azarian et al., 1997; Bhongsatiern et al., 2005; Tachikawa et al., 2005]. Mutations of *ABCA4* are associated with a spectrum of autosomal recessive retinal degenerative diseases including Stargardt macular degeneration, cone-rod dystrophy and retinitis pigmentosa (OMIM #601691). Expression of Abca4 has not been detected in the palate of E13.5–E14.5 mouse embryos [Beaty et al., 2010].

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To validate the findings of Beaty et al., 2010, we evaluated four SNPs, rs560426 and rs481931 in *ABCA4* and rs13041247 and rs11696257 downstream of *MAFB* to determine whether they were associated with NSCLP in our family-based dataset. The dataset was composed of 182 multiplex (positive family history) NSCLP families (121 non-Hispanic white (NHW) and 60 Hispanic) and 464 simplex (no family history) case-parent duos/trios (294 NHW and 170 Hispanic). We genotyped 734 affect and 1164 unaffect individuals. SNPs were genotyped using the Taqman SNP genotyping assay (Applied Biosystems, Inc, Foster City, CA) and detected with the AB 7900HT Sequence Detection System. The data were imported into Progeny Lab (South Bend, IN, USA) and examined for Mendelian inconsistencies using PedCheck [O'Connell et al., 1998]. Allele frequencies and Hardy-Weinberg Equilibrium (HWE) were calculated using SAS (v9.1). Chi-square analysis was performed to evaluate ethnic differences in allele frequencies. Pedigree Disequilibrium Test (PDT), Geno-PDT and Association in the Presence of Linkage (APL) were used to test for association [Chung et al., 2006; Martin et al., 2000; Martin et al., 2006]. APL was used to test for overtransmission of pairwise haplotypes. The data were first analyzed by ethnicity (NHW and Hispanics) because of allele frequency differences and then stratified by the presence or absence of family history (FH).

All SNPs were in HWE. The results for association analysis are summarized in Table I. For *ABCA4*, only rs481931 had evidence for association in the NHW dataset ($p = 0.00003$ APL, 0.004 PDT and 0.021 Geno-PDT), whereas both rs481931 and rs560426 were associated in the Hispanic dataset ($p < 0.05$). When stratified by family history, the NHW negative family history subset was responsible for most of the association, while in the Hispanics there was evidence for association to rs560426/*ABCA4* in the negative family history subset and to rs481931/*ABCA4* in the positive family history subset. Marginal associations were detected for rs11696257/*MAFB* and rs13041247/*MAFB* in the Hispanic dataset, while no association was found for the NHW dataset. This is in contrast to the results of Beaty et al. [2010] who found no association in South/Central Americans to *MAFB* or *ABCA4*. This is most likely related to population differences. APL analysis of the 2-SNP haplotypes in each gene also found evidence for association to *ABCA4* in both the NHW and Hispanic datasets, while there was no evidence for association to the *MAFB* haplotypes in either dataset (Table II).

These results provide support for the association of *ABCA4* and NSCLP and minimal support for *MAFB*. This is particularly interesting as there is little biological evidence to support a role of *ABCA4* in craniofacial morphogenesis, whereas *MAFB* is expressed in the mouse palate during development. Additional expression studies of *ABCA4* covering a broad range of developmental stages from early orofacial structure initiation to late stage lip/palatal formation are needed to establish whether Abca4 is transiently expressed during development. It is possible that the SNPs in *ABCA4* are tagging for another NSCLP gene near or within the 1p22.1 chromosomal region that is yet to be discovered.

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Table I

Single SNP association by ethnicity and family history

SNP	Gene	All Families			Family History			No Family History		
		PDT	Geno- PDT	APL	PDT	Geno- PDT	APL	PDT	Geno- PDT	APL
nonHispanic White										
rs560426	ABCA4	0.379	0.129	0.075	0.718	0.409	0.324	0.347	0.234	0.113
rs481931	ABCA4	**0.004**	**0.021**	**0.00003**	0.169	0.395	**0.019**	**0.005**	**0.022**	**0.0009**
rs13041247	MAFB	0.535	0.559	0.325	0.219	0.415	0.411	0.756	0.128	0.487
rs11696257	MAFB	0.537	0.64	0.422	0.321	0.53	0.408	0.939	0.225	0.563
Hispanic										
rs560426	ABCA4	**0.026**	0.108	**0.006**	0.303	0.344	0.191	**0.046**	0.15	**0.024**
rs481931	ABCA4	**0.007**	0.063	**0.049**	**0.035**	0.194	**0.033**	0.085	0.297	0.36
rs13041247	MAFB	0.158	0.248	0.091	0.114	0.351	**0.02**	0.558	0.458	0.696
rs11696257	MAFB	0.092	0.132	**0.027**	0.123	0.351	**0.025**	0.346	0.239	0.348

P< 0.05 in bold

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Table II

2-SNP haplotype association by ethnicity

Gene	SNP (Alleles)	Haplotype	nonHispanic White		Hispanic	
			Transmitted	Global *p* value	Transmitted	Global *p* value
ABCA4	rs560426 (C/T)	T,G	Under	**0.001**	Over	**0.025**
		T,T	Over		Over	
	rs481931 (G/T)	C,G	Under		Under	
		C,T	Over		Over	
MAFB	rs13041247 (C/T)	T,T	-	0.130	-	0.055
		C,C	-		-	
	rs11696257 (C/T)	C,T	-		-	
		T,C	-		-	

P< 0.05 in bold, - transmission is not significantly altered