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Fine mapping of the myosin light chain kinase gene replicates the association with asthma in Spanish descent populations

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To the Editor

Asthma is a chronic inflammatory disorder mainly characterized by reversible airflow obstruction, bronchial hyperresponsiveness and dyspnea. The functional alterations in the airway smooth muscle usually observed in asthmatics have been associated with increased expression of the smooth muscle myosin light chain kinase isoform (smMLCK).[1] This constitutes a key cytoskeleton effector of the smooth muscle contractile machinery and is encoded by the myosin light chain kinase (*MYLK*) gene. Two *MYLK* single nucleotide polymorphisms (SNPs), which are common in Africans (>30%) but rare in Europeans (≤1%), have been associated with asthma in African Americans and Afro-Caribbeans.[2,3] However, none of the *MYLK* variants have attained the strict Bonferroni-corrected level of genome-wide significance in any genome-wide association study (GWAS) of asthma. This discrepancy may arise from the low coverage of this region on commercial arrays and/or the genetic specificities of the populations studied, since risk factors in one population might not generalize to another.[4] Here we aimed to fine map the association of SNPs from *MYLK* gene with asthma in case-control studies from Spanish (n=3,219) and Latino (n=4,650) populations.

In the discovery stage, DNA samples from 606 physician-diagnosed asthmatics from the Genetics of Asthma (GOA) study in the Spanish population (GOA I) were compared with 1,258 non-asthmatic subjects. Top associated SNPs were then replicated in two independent Spanish studies: GOA II and The Genetics of Asthma study in the Spanish population from Malaga (GOAM). GOA II included 248 asthma cases and 537 controls genotyped with the Axiom® Genome-Wide CEU 1 Array (Affymetrix, Santa Clara, CA), whereas GOAM comprised 320 asthma cases and 250 controls genotyped using TaqMan allelic discrimination assays (Life Technologies, Carlsbad, CA). Further replication was assessed in two Latino studies using existing genome-wide genotyping data: The Genetics of Asthma in Latino Americans (GALA I),[4] consisting of 529 cases and 347 controls, and Genes-Environments & Admixture in Latino Americans (GALA II) study,[5] including 1,893 cases and 1,881 controls. Detailed descriptions of the study design and sample characteristics can be found in Figure E1, Tables E1 and E2, and Text E1 in the Online Repository at www.jacionline.org.

In the discovery stage, a total of 29 tagging SNPs (tSNPs) were selected from HapMap II to analyze variants with minor allele frequency (MAF)≥5% from the *MYLK* gene in European individuals. Genotyping was performed using the iPLEX® Gold assay on MassARRAY® system (Sequenom Inc., San Diego, CA). A total of 26 SNPs passed quality control (after removing monomorphic SNPs or with $p \leq 1.7 \times 10^{-3}$ for Hardy-Weinberg equilibrium in controls) (Table E3). After imputation, 272 SNPs with MAF≥5%, and a squared correlation between imputed and true genotypes (Rsq)≥0.3, were kept for association testing using logistic regression models (88% of the total variants with MAF≥5% in Europeans). Principal components were used as covariates to adjust for population stratification, as previously described.[6]

Three significantly associated SNPs were observed after Bonferroni correction (p-value $\leq 1.8 \times 10^{-4}$). Two intronic SNPs in perfect linkage disequilibrium (LD, $r^2=1$) and with

MAF=9% showed the most significant associations: rs77820417 and rs78442149 (for both SNPs: OR=2.71 [95%CI=1.79–4.11] for the minor allele, $p=2.78\times10^{-6}$) (Figure 1, Table E4). We followed up the SNP rs77820417 for replication in four independent studies. Association was significant in GOAM (OR=2.32, 95%CI=1.23–4.36, $p=4.17\times10^{-3}$), in GALA I (OR=1.73, 95%CI=1.03–2.93, $p=.040$), and in GALA II (OR=1.31, 95%CI=1.05–1.64, $p=.019$), but not in GOA II (OR=0.88, 95%CI=0.48–1.61, $p=.669$). Due to the heterogeneity of effects among studies (Cochran's Q test, $p=.007$), a random effects meta-analysis of the 7,869 individuals was performed, confirming the strong association of rs77820417 with asthma susceptibility (OR=1.66, 95% CI=1.14–2.42, $p=1.57\times10^{-7}$; Table 1). We further explored the association of rs77820417 with asthma exacerbations in GALA II, defined by the presence of ≥1 asthma-related events (hospitalizations, emergency department visits, and oral steroid use) over the 12 months prior to recruitment and adjusting for the use of medication during the same period. The A allele, associated with asthma risk, was also associated with increased risk of asthma exacerbations (OR=1.80, 95% CI=1.08–2.99, $p=.023$). The associated SNP is located within the *MYLK* gene region encoding the smMLCK isoform, which participates in smooth muscle cell contractility. smMLCK activation is a critical step in the cytoskeletal rearrangements, providing dynamic regulation of cell shape, cell motility and adhesion, which are involved in the remodeling processes underlying asthma.[7]

To date, GWAS have firmly identified susceptibility genes underlying asthma risk, although most of the studies were performed using HapMap-based inferences, where the coverage for genetic variation is limited compared to the information provided by the 1000 Genomes Project (1KGP). In fact, neither the top hit observed in the current study nor its proxy (rs78442149) were tested for association in the GABRIEL (http://www.cng.fr/gabriel/results.html) or the EVE consortia, the largest GWAS meta-analyses in asthma performed in Europeans[8] and multi-ethnic groups.[9] No other variants from the 1KGP are in moderate LD with those two SNPs (highest $r^2=0.25$). However, one *MYLK* variant was associated with asthma in the GABRIEL consortium (rs7633133, $p=1.01\times10^{-7}$) (Figure 1). This SNP shows a MAF=1% in 1KGP Europeans and, therefore, was not tested in our study. In the EVE consortium, although no *MYLK* SNP showed an outstanding significance (minimum $p=.01$) (Figure 1), a 4-fold enrichment of significant associations was observed in Latinos (Fisher exact test $p=7.03\times10^{-5}$), but not in European or African Americans ($p=.720$ and $p=1.0$, respectively) (Table E5 and Text E1).

One striking aspect of our study is the large effect sizes found for the association of rs77820417 with asthma susceptibility and exacerbations, which is only comparable to the effect reported for a SNP in *GSDMB* with early childhood asthma with severe exacerbations.[10] However, this effect size may be confounded by the large gender and age differences among cases and controls in the discovery sample. Despite this, the validation across multiple studies of both children and adults with different gender balance suggests that the result from the discovery study is not a false positive. The catalog of asthma susceptibility genes could be more comprehensive if imputation based on 1KGP data would be exploited to meta-analyze existing GWAS data. In addition, analysis of diverse populations also contributes with new susceptibility loci, as many GWAS hits are not

transferable to all populations.[4] In fact, higher North African ancestry is detected in southwestern Europe and is decreased in northern latitudes.[11] Therefore, novel susceptibility loci for asthma could be revealed in Spanish-descent populations.

In summary, we identified a *MYLK* SNP association with asthma in Spanish descent individuals, showing suggestive genome-wide significance. Future studies will be needed to confirm its importance in other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

1KGP	1000 Genomes Project
CI	confidence interval
LD	linkage disequilibrium
MLCK	myosin light chain kinase
MYLK	myosin light chain kinase gene
GWAS	genome-wide association study
GALA I	The Genetics of Asthma in Latino Americans
GALA II	Genes-Environments & Admixture in Latino Americans
GOAM	Genetics of Asthma study in the Spanish population from Malaga
GOA I	Genetics of Asthma study in the Spanish population
GOA II	Genetics of Asthma study in the Spanish population II
OR	odds ratio

SNP	Single nucleotide polymorphism
tSNPs	tagging SNPs

References

1. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med. 2003; 167:1360–8. [PubMed: 12531777]
2. Flores C, Ma SF, Maresso K, Ober C, Garcia JG. A variant of the myosin light chain kinase gene is associated with severe asthma in African Americans. Genet Epidemiol. 2007; 31:296–305. [PubMed: 17266121]
3. Gao L, Grant AV, Rafaels N, Stockton-Porter M, Watkins T, Gao P, et al. Polymorphisms in the myosin light chain kinase gene that confer risk of severe sepsis are associated with a lower risk of asthma. J Allergy Clin Immunol. 2007; 119:1111–8. [PubMed: 17472811]
4. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS, et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The Genes-environments & Admixture in Latino Americans study. J Allergy Clin Immunol. 2014; 134:295–305. [PubMed: 24406073]
5. Torgerson DG, Gignoux CR, Galanter JM, Drake KA, Roth LA, Eng C, et al. Case-control admixture mapping in Latino populations enriches for known asthma-associated genes. J Allergy Clin Immunol. 2012; 130:76–82. e12. [PubMed: 22502797]
6. Pino-Yanes M, Corrales A, Acosta-Herrera M, Perez-Rodriguez E, Cumplido J, Campo P, et al. HLA-DRB1*15:01 allele protects from asthma susceptibility. J Allergy Clin Immunol. 2014; 134:1201–3. [PubMed: 25034406]
7. Garcia JG, Lazar V, Gilbert-McClain LI, Gallagher PJ, Verin AD. Myosin light chain kinase in endothelium: molecular cloning and regulation. Am J Respir Cell Mol Biol. 1997; 16:489–94. [PubMed: 9160829]
8. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med. 2010; 363:1211–21. [PubMed: 20860503]
9. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet. 2011; 43:887–92. [PubMed: 21804549]
10. Bonnelykke K, Sleiman P, Nielsen K, Kreiner-Moller E, Mercader JM, Belgrave D, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet. 2014; 46:51–5. [PubMed: 24241537]
11. Botigue LR, Henn BM, Gravel S, Maples BK, Gignoux CR, Corona E, et al. Gene flow from North Africa contributes to differential human genetic diversity in southern Europe. Proc Natl Acad Sci U S A. 2013; 110:11791–6. [PubMed: 23733930]

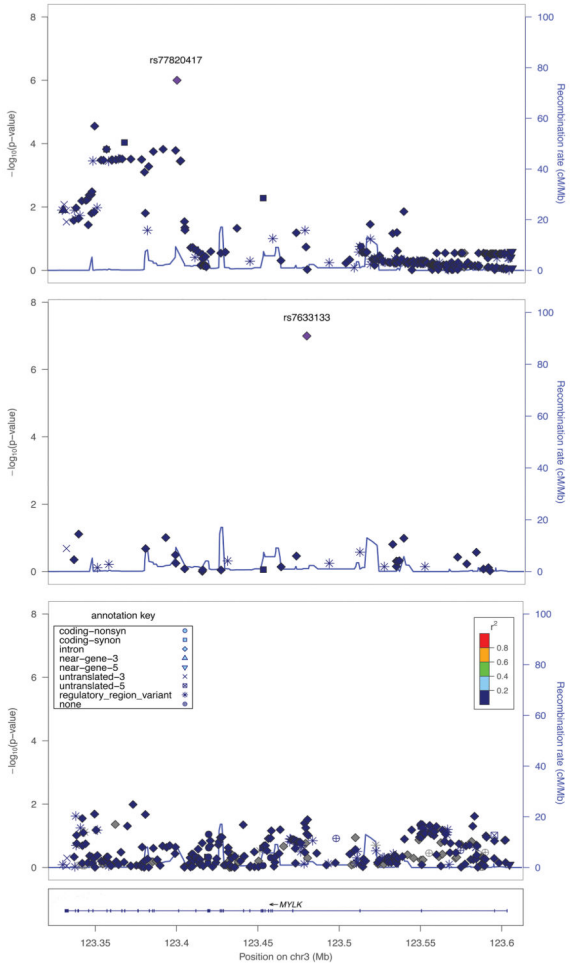

Figure 1. Regional plots of association results in the discovery sample (upper panel), GABRIEL (middle panel), and EVE (lower panel)

The -$\log_{10}$ transformed *p*-values for association tests are plotted as symbols according to their functional annotation. The SNP rs number indicated on the plot denotes the result for the most significantly associated SNP with asthma risk. The results for the remaining SNPs are color coded to reflect their degree of linkage disequilibrium with the most significant SNP based on pairwise r^2 values from the European population of the 1KGP. Estimated recombination rates (light blue line) are plotted on the right y-axis.

Table 1

Summary of association testing of rs77820417 with asthma susceptibility.

Study	Sample size (Cases:Controls)	MAF	OR (95% CI)	*p*-value
GOA I	1,864 (606:1,258)	0.090	2.71 (1.79–4.11)	**2.78×10^{-6}**
GOA II	785 (248:537)	0.084	0.88 (0.48–1.61)	.669
GOAM	570 (320:250)	0.052	2.32 (1.23–4.36)	**4.17×10^{-3}**
GALA I	876 (529:347)	0.073	1.73 (1.03–2.93)	**.040**
GALA II	3,774 (1,893:1,881)	0.044	1.31 (1.05–1.64)	**.019**
Meta-analysis	7,869 (3,596:4,273)	-	1.66 (1.14–2. 42)	**1.57×10^{-7}**

p-values≤.05 are in boldface.