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An african-specific functional polymorphism in *KCNMB1* shows sex-specific association with asthma severity

Max A. Seibold[1,2,*,†], Bin Wang[7,†], Celeste Eng[1,3], Gunjan Kumar[1], Kenneth B. Beckman[8], Saunak Sen[4], Shweta Choudhry[1,3,5], Kelley Meade[8], Michael Lenoir[9], H. Geoffrey Watson[10], Shannon Thyne[6], L. Keoki Williams[11], Rajesh Kumar[12], Kevin B. Weiss[13], Leslie C. Grammer[14], Pedro C. Avila[14], Robert P. Schleimer[14], Esteban González Burchard[1,2,3,5,†] and Robert Brenner[7,†]

[1]Department of Medicine, [2]Biopharmaceutical Sciences, [3]Lung Biology Center, [4]Department of Epidemiology and Biostatistics, [5]Institute for Human Genetics and [6]Department of Pediatrics, University of California San Francisco (UCSF), San Francisco, CA 94143-2911, USA, [7]Department of Physiology, University of Texas Health Science Center, San Antonio, TX, USA, [8]Children's Hospital Oakland Research Institute (CHORI), Oakland, CA, USA, [9]Bay Area Pediatrics, Oakland, CA, USA, [10]The James A. Watson Wellness Center, Oakland, CA, USA, [11]Department of Internal Medicine, Henry Ford Hospital, Detroit, MI, USA, [12]Children's Memorial Hospital, [13]Institute for Health Care Studies and [14]Department of Medicine of Northwestern University, Chicago, IL, USA

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A highly heritable and reproducible measure of asthma severity is baseline pulmonary function. Pulmonary function is largely determined by airway smooth muscle (ASM) tone and contractility. The large conductance, voltage and calcium-activated potassium (BK) channel negatively regulates smooth muscle tone and contraction in ASM. The modulatory subunit of BK channels, the β1-subunit, is critical for proper activation of BK channels in smooth muscle and has shown sex hormone specific regulation. We hypothesized that *KCNMB1* genetic variants in African Americans may underlie differences in bronchial smooth muscle tone and thus pulmonary function, possibly in a sex-specific manner. Through resequencing of the KCNMB1 gene we identified several common variants including a novel African-specific coding polymorphism (C818T, R140W). The C818T SNP and four other *KCNMB1* variants were genotyped in two independent groups of African American asthmatics ($n = 509$) and tested for association with the pulmonary function measure – forced expiratory volume (FEV_1) % of predicted value. The 818T allele is associated with a clinically significant decline (−13%) in FEV_1 in both cohorts of asthmatics among males but not females ($P_{combined} = 0.0003$). Patch clamp electrophysiology studies of the BK channel expressed with the 140Trp variant of the β1-subunit demonstrated significantly reduced channel openings, predicted by the loss of pulmonary function observed. African American male asthmatics carrying the 818T allele (10% of population) are potentially at risk for greater airway obstruction and increased asthma morbidity. Female asthmatics may be insulated from the deleterious effects of the 818T allele by estrogen-mediated upregulation in BK channel activity.

*To whom correspondence should be addressed. Tel: +1 4155149929; Fax: +1 4155144365; Email: mseibold@medsfgh.ucsf.edu
†The authors wish it to be known that, in their opinion, these authors should be regarded as joint Authors.

INTRODUCTION

Asthma is a complex disease characterized by persistent airway inflammation giving rise to bronchial hyperresponsiveness and reversible airway obstruction. The degree of airway obstruction is an important criterion for classifying disease severity (1,2). In fact, severe airway obstruction is a significant risk factor for near fatal asthma (3). Therefore, understanding factors contributing to differences in pulmonary function is important to the management of asthma morbidity and mortality. Airway obstruction varies greatly among subjects. Both family-based and twin studies have established a strong heritability of pulmonary function (4–7). Genetic studies have associated multiple loci and individual variants with airway obstruction or pulmonary function (8–11).

Airway smooth muscle (ASM) tone and thus pulmonary function is largely determined by cholinergic stimulation, which is mediated through muscarinic receptors (12). Muscarinic receptors stimulate contraction by increasing intracellular calcium concentrations. An important negative regulator of this process is the large conductance, Ca^{2+} and voltage-dependent K^+ (BK) channel (13). BK channel potassium conductance is stimulated by an increase in local calcium concentration, thereby moderating depolarization, deactivating voltage-dependent calcium channels and thus reducing overall calcium influx and the resultant airway constriction (14). BK channels are formed by pore-forming α-subunits alone, or with modulatory β1-subunits. Sensitivity of the BK channel to Ca^{2+} influx is modulated by the β1-subunit (15). The β1-subunit is abundantly expressed in bronchial smooth muscle (14). The β1-subunit is encoded for by the *KCNMB1* gene, which is located in the 5q34 chromosomal region, a locus that has been frequently linked with asthma and baseline lung function as denoted by forced expiratory volume (FEV_1) (8,10,16,17). We hypothesized that genetic variation in the *KCNMB1* gene would alter the function and/or expression of the β1-subunit, which could then modulate pulmonary function among subjects with asthma.

This hypothesis is supported by the phenotype observed from β1-subunit knockout mice which display reduced BK channel activity resulting in increased smooth muscle tone in the vasculature (15,18), bladder (19) and trachea (14). In fact, disruption of vascular tone in β1-knockout mice results in hypertension and left-ventricular hypertrophy (15,18). Based on the functional significance of the β1-subunit on vascular smooth muscle tone, genetic studies have been conducted on the *KCNMB1* gene for association with hypertension and related cardiovascular phenotypes. A German twin study associated both intronic and coding variants with baroreflex and regulation of blood pressure (20). Most notably, a large study identified the E65K (a gain-of-function) coding variant in *KCNMB1* as protective against high diastolic blood pressure in a Spanish cohort (21). Furthermore, in a separate study the protective effect of the E65K variant on blood pressure and cardiovascular events was determined to be largely dependent on sex and age, with post-menopausal women showing the strongest effect of the variant on hypertension (22). This result raises another important aspect of BK channels, in that their activity and expression is upregulated by female sex hormones including estrogen and estradiol (23,24). In fact, the protective effect of estrogen pretreatment on murine ASM against hyperresponsiveness, was determined to be primarily mediated by a 50-fold increase in BK channel activity (23,24).

Based on the existing *KCNMB1* genetic results for hypertension coupled with the importance of the β1-subunit in regulation of ASM tone, we undertook a study to determine whether genetic variation in the *KCNMB1* gene modified asthma severity among African Americans and whether there were differences by sex. We chose to study African American subjects with asthma because they have among the highest asthma morbidity and mortality rates in the US.

RESULTS

Identification of a novel KCNMB1 polymorphism in African American subjects

We identified all *KCNMB1* exonic genetic variants and determined their corresponding allele frequencies by resequencing the four *KCNMB1* exons in 24 African American subjects with asthma. We identified eight SNPs including two – 5′-untranslated region (UTR) SNPs, three – coding SNPs, and three – 3′-UTR SNPs (Fig. 1). Interestingly, the polymorphism associated with hypertension (G593A Glu65Lys), had a much lower allele frequency in African Americans (5.2%) than previously reported among Caucasians (17.5%) (25). Additionally, we identified two previously unreported SNPs (A274C and C818T). SNP C818T is a novel African-specific coding polymorphism resulting in an Arginine to Tryptophan change in the *KCNMB1* protein at amino acid position 140. We did not find a single subject heterozygous for the C818T SNP in screening 96 Puerto Rican, 96 Mexican, 86 Caucasian, and seven Asians asthmatics, implying this SNP is specific to populations of African origin.

Pairwise linkage disequilibrium (LD) analysis of the identified SNPs by r^2 statistic revealed limited correlation between the eight SNPs as shown in Table 1. The exceptions were the adjacent SNPs C1054A and A1055C, which were in complete LD among African Americans. Additionally, we found SNP G728C and A1273G to be moderately correlated with an r^2 of 0.43.

KCNMB1 SNPs in African Americans are associated with pulmonary function in males

Based upon allele frequencies and LD patterns determined in the resequencing analysis, we selected SNPs G593A, G728C, C818T, C1054A and A1273G for genotyping in 261 African Americans from the Study of African Americans, Asthma, Genes, and Environments (SAGE) (Supplementary Material, Tables S1 and S2) (Table 2). We first tested for association between the quantitative measure of pulmonary function, FEV_1 % of predicted value, and the five *KCNMB1* SNPs by multiple linear regression correcting for potential confounding factors (Table 3). We did not observe any significant associations in the combined sex analysis. Because of the known effects of female sex hormones on BK channels, we performed a sex-stratified analysis to determine if there was effect modification by sex on the risk associated with *KCNMB1* variants. In the sex-stratified analysis, we observed significant associations between FEV_1 % of predicted value and SNPs C818T ($P = 0.018$) and A1273G ($P = 0.054$), only among males sub-

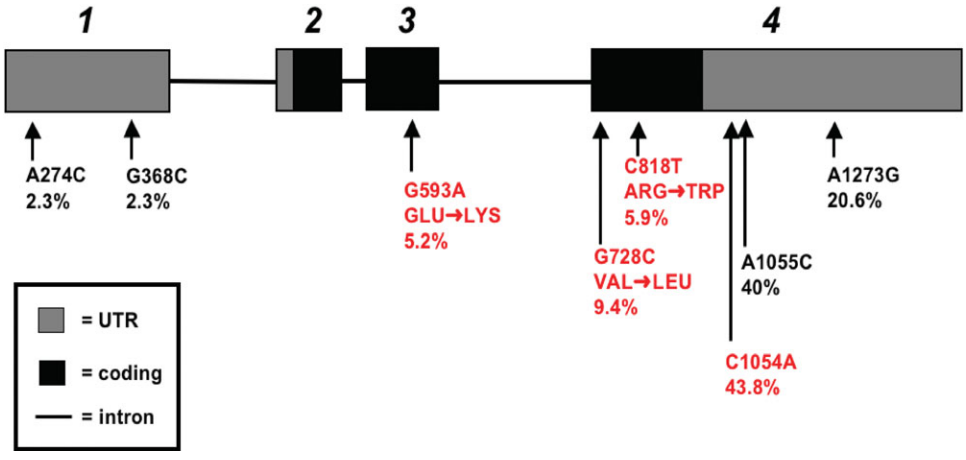

Figure 1. Structure of the human KCNMB1 gene with African American SNPs and their characteristics overlaid. SNPs are identified by mRNA NCBI no. NM_004137 position. Higher frequency allele for each SNP is listed first and minor allele last. Allele frequency among African Americans is listed. Amino acid changes are listed for coding SNPs. SNPs displayed in red were selected for genotyping in asthmatics cohorts.

Table 1. Pairwise linkage disequilibrium (LD) between KCNMB1 SNPs. LD values were determined by the r^2 statistic for African Americans. LD statistics were determined from SAGE genotyping data, and from sequencing data for SNPs not genotyped. The r^2 statistic ranges from a value of 0–1.0, with a value of 1.0 indicating complete LD or correlation between SNPs and a value of 0 indicating no correlation between SNPs

SNP	274	368	*593*	*728*	*818*	*1054*	1055	*1273*
274	*							
368	0	*						
593	0	0.49	*					
728	0	0	0	*				
818	0	0	0	0	*			
1054	0	0.04	0.03	0.15	0.06	*		
1055	0	0.04	0.08	0.15	0.18	1	*	
1273	0.09	0.09	0.01	0.43	0.01	0.29	0.12	*

Table 2. Sex-stratified genotyping results from SAGE and CHIRAH subjects. Numbers listed are frequencies for minor allele indicated

rs no.	SNP	Minor allele	Allele frequency SAGE Males (%)	SAGE Females (%)	CHIRAH Males (%)	CHIRAH Females (%)
rs11739136	593	A	5.4	4.3	5.4	5.9
rs2301149	728	C	11.1	9.7	9.1	8.1
Novel	818	T	3.9	5.3	7.6	6.9
rs2656842	1054	A	43.2	43.5	45.1	43.8
rs314156	1273	G	24.5	19	21.2	19.2

jects (Table 3). The regression coefficient for C818T was −13.73 (T allele confers a decrease in lung function), while the coefficient was positive 5.38 (an increase in lung function) for SNP A1273G (Table 3). No association with any of the five SNPs was observed among female subjects. To confirm our results with an independent group of African American subjects with asthma, we genotyped 248 subjects from the Chicago Initiative to Raise Asthma Health Equity (CHIRAH) study (Supplementary Material, Table S1). The same five *KCNMB1* SNPs examined in the SAGE asthmatics were also genotyped in the CHIRAH asthmatics (Table 2). As in the SAGE analysis, the sex-stratified analysis in CHIRAH yielded significant results with SNP C818T ($P = 0.003$) and borderline significant results with SNP A1273G ($P = 0.093$) only among male subjects (Table 3). The direction of the association with both SNPs was replicated with similar coefficients, namely the 818T allele conferred lower pulmonary function (Coeff. = −13.22), and the 1273 G allele conferred greater lung function (Coeff. = 4.46) (Table 3). We did not observe any significant associations between *KCNMB1* SNPs and FEV_1 % of predicted value among female asthmatics (Table 3).

Combined SAGE and CHIRAH analysis of pulmonary function

In an attempt to increase the power of our stratified analysis of FEV_1 and refine our genetic coefficient estimates for replicated SNPs C818T and A1273G, we analyzed these SNPs in the combined group of 509 SAGE and CHIRAH asthmatics. Multiple linear regression of FEV_1 % of predicted value yielded significant results among males for both SNP C818T (Coeff. = −13.1, $P = 0.0003$) and SNP A1273G (Coeff. = 4.96, $P = 0.01$) (Table 3). Based on these results we tested the interaction term between C818T and sex, which we found to be significant as well ($P = 0.002$). We performed many analyses, which are important on their own; however it is important to assess the burden of multiple comparisons. We performed $3 \times 5 \times 3 = 45$ tests in all (three for sex: males, females, both sexes; five SNPs; three for cohorts: SAGE, CHIRAH, both cohorts). Disregarding the correlations between the tests, applying the Bonferroni correction, the adjusted *P*-value of C818T association with FEV_1 is still significant ($45 \times 0.0003 = 0.0135$). Moreover, male asthmatics with the CC genotype for SNP C818T have a mean FEV_1 % predicted of 92.0%, whereas asthmatics in the CT and TT genotype group (combined due to low numbers of TT homozygotes) have a mean FEV_1 % predicted of 80.2% reflecting the regression results and implying a dominant effect of the T allele ($P = 0.002$) (Fig. 2A). Among male subjects, the

Table 3. Linear regression results for association between KCNMB1 genetic variants and FEV_1 % predicted in all subjects and stratified by sex. Results are displayed as labeled for the SAGE asthmatics, CHIRAH asthmatics, and the combined analysis of both CHIRAH and SAGE asthmatics. Coefficient with standard error in parenthesis (SE) and *P*-values listed are for the respective genotype term in the linear regression model

SNP	Gender	SAGE Coefficient (SE)	*P*-value	CHIRAH Coefficient (SE)	*P*-value	Combined Coefficient (SE)	*P*-value
G593A	All	1.75 (3.5)	0.613	−0.33 (3.8)	0.93	0.42 (2.6)	0.871
	Females	1.54 (4.8)	0.75	−3.23 (5.2)	0.535	−0.85 (3.6)	0.812
	Males	0.72 (5.1)	0.888	7.61 (5.3)	0.152	3.02 (3.7)	0.413
G728C	All	−0.86 (2.6)	0.744	1.98 (3.3)	0.548	0.5 (2.1)	0.81
	Females	−5.03 (3.6)	0.166	0.52 (4.6)	0.909	−2.18 (2.9)	0.452
	Males	4.36 (3.8)	0.252	3.48 (4.3)	0.419	4.24 (2.8)	0.137
C818T	All	−4.91 (3.7)	0.182	−0.48 (3.6)	0.893	−2.24 (2.5)	0.377
	Females	−2.1 (4.7)	0.654	7.33 (4.9)	0.14	3.64 (3.4)	0.287
	Males	*−13.73 (5.7)*	*0.018*	*−13.22 (4.4)*	*0.003*	*−13.1 (3.5)*	*0.0003*
C1054A	All	1.91 (1.6)	0.221	0.74 (1.7)	0.672	1.51 (1.2)	0.196
	Females	0.9 (2.0)	0.655	1.42 (2.4)	0.556	1.36 (1.6)	0.386
	Males	3.92 (2.5)	0.117	−0.06 (2.3)	0.98	2.08 (1.7)	0.222
A1273G	All	0.54 (1.8)	0.766	3.84 (2.0)	0.062	2.31 (1.4)	0.096
	Females	−2.66 (2.4)	0.274	3.06 (2.9)	0.285	0.41 (1.9)	0.829
	Males	*5.38 (2.8)*	*0.054*	*4.46 (2.6)*	*0.093*	*4.96 (1.9)*	*0.01*

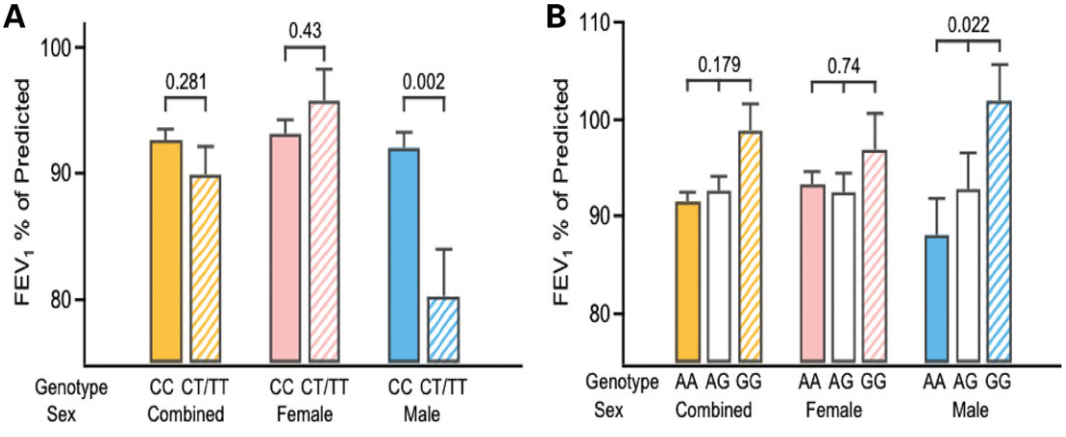

Figure 2. Mean FEV_1 % predicted by genotype and gender among combined SAGE and CHIRAH subjects (**A**) C818T (**B**) A1273G. Due to low numbers of 818TT homozygotes these subjects were combined with the CT heterozygotes. Error bars represent SEM. *P*-values listed are for the students *t*-test (C818T) and one-way ANOVA test (A1273G).

mean FEV_1 % for genotypes AA, AG and GG of the SNP + A1273G were 88.1, 92.9 and 101.9%, respectively. These results are consistent with an additive effect of SNP A1273G ($P = 0.022$) (Fig. 2B).

PCA analysis of multiple pulmonary function measures

Based upon these results, we chose to explore the effect of these SNPs on other relevant measures of airway obstruction including, forced expiratory flow in 25–75 s (FEF_{25-75}), Forced Vital Capacity (FVC%) and FEV_1/FVC ratio. To avoid multiple testing problems and to take advantage of the correlated nature of these lung function measures we used principal components analysis (PCA) to create a new variable representing pulmonary function in general. PCA yielded two components with Eigen values >1. The first component accounted for 67% of the total variance in these pulmonary function measures (Table 4). Linear regression was used to test for association of the first principal component and SNPs C818T and A1273G in the combined group of asthmatics (SAGE and CHIRAH). We found no association with either SNP among all asthmatics or among females. However, similar to the genotype analysis for FEV_1, both C818T and A1273G SNPs were associated with the first principal component of pulmonary function ($P = 0.001$ negative coefficient and $P = 0.011$ positive coefficient, respectively) only among male subjects. These results strongly support the

Table 4. Principal component loading with % variance explained for the pulmonary function measures

Pulmonary function measures	Principal components Component 1	Component 2
FEV_1	0.58	0.28
FVC	0.36	0.73
FEF_{25-75}	0.57	−0.28
FEV_1/FVC	0.46	−0.56
Eigen value	2.67	1.25
% of Total variance	67	31

involvement of *KCNMB1* genetic variants in male subjects on multiple measures of airway obstruction.

Functional studies demonstrate C818T reduces BK channel activation

The C818T allele results in a change from Arginine to Tryptophan at amino acid 140 of the *KCNMB1* protein. Alignment of mammalian *KCNMB1* sequences indicates that this polymorphism is not observed in other mammalian *KCNMB1* sequences (data not shown). The non-conserved change from Arginine, a basic amino acid, to tryptophan, an amino acid with a bulky non-polar side chain, suggests that such a change may have effects on *KCNMB1* function. To investigate the consequence of the C818T polymorphism on BK channel function, the human *KCNMB1* 140Arg (818C) or 140Trp (818T) cDNAs were coexpressed with the BK channel pore-forming α-subunit in HEK293 cells and studied using inside-out, excised patch clamp recording. Figure 3 shows representative current traces for α-subunit alone, $\alpha + \beta1_{140Arg}$ and $\alpha + \beta1_{140Trp}$ evoked by a series of voltage steps. α-Subunit currents alone have relatively fast activation and deactivation (Fig. 3A). Plots of normalized conductance demonstrate half-maximal openings ($V_{1/2}$) at 79.9 ± 5.9 mV (Fig. 3B). As shown previously (26), the $\beta1_{140Arg}$-subunit slows activation and deactivation of BK channels (Fig. 3C) and shifts the $V_{1/2}$ by 50 mV to more negative voltages (Fig. 3D, $V_{1/2}$ is 30.6 ± 2.7 mV). In contrast, $\beta1_{140Trp}$-subunit confers a smaller G–V shift of 34 mV (Fig. 3F) resulting in BK channels that require a greater depolarization to open (average $V_{1/2}$ is 46.0 ± 2.9 mV). Table 5 shows a plot of average $V_{1/2}$ values measured across a range of calcium concentrations. The effect of the Trp140 residue is significant at intermediate calcium concentrations of 1.7 (shown in Fig. 3) and 7 μM where there is a 12–15 mV positive shift of the $V_{1/2}$ relative to 140Arg containing channels. The apparent voltage dependence (Q) is not altered by the polymorphism (Table 5). A comparison of activation and deactivation kinetics of the *KCNMB1* polymorphism demonstrates that the 140Trp polymorphism causes >2-fold slowing of activation time over 140Arg containing subunits (e.g. at +50 mV and 1.7 μM Ca^{2+}: $\beta1_{140Arg}$ is 53 ± 14 ms versus $\beta1_{140Trp}$ 135 ± 34 ms, $P = 0.02$) (Supplementary Material, Fig. S1A) without affecting deactivation (Supplementary Material, Fig. S1B).

To understand the effect of the 140Trp polymorphism at physiological voltages, we measured channel open probability at voltages where ASM fluctuates (27). Figure 4A shows an example of single-channel recordings of $\alpha + \beta1_{140Arg}$ and $\alpha + \beta1_{140Trp}$ channels. We found that $\beta1_{140Trp}$ has a somewhat smaller single-channel current than $\beta1_{140Arg}$ at negative voltages (15.6 ± 0.8 pA for $\beta1_{140Trp}$ versus 17 ± 0.7 pA for $\beta1_{140Arg}$ at −80 mV, $P < 0.005$) (Fig. 4B). Consistent with the positive shift of the $V_{1/2}$, the 140Trp polymorphism reduces the BK channel open probability (P_o) relative to $\beta1_{140Arg}$ containing channels (Fig. 4C). For example, there is a 3.3-fold decrease in P_o of $\alpha + \beta1_{140Trp}$ versus $\beta1_{140Arg}$ ($P = 0.027$) at −80 mV and 1.7 μM Ca^{2+}. Interestingly, there is no difference in open probability between $\beta1_{140Arg}$ and $\beta1_{140Trp}$ channels at 0.3 μM calcium (Fig. 4D). The calcium-dependent effect on open probability indicates that the 140Trp polymorphism reduces BK channel activity through an effect on the apparent calcium sensitivity. In summary, these results suggest that individuals carrying the 818T allele (140Trp polymorphism) will have BK channels with reduced openings which will likely result in membrane depolarization and therefore more constricted airways.

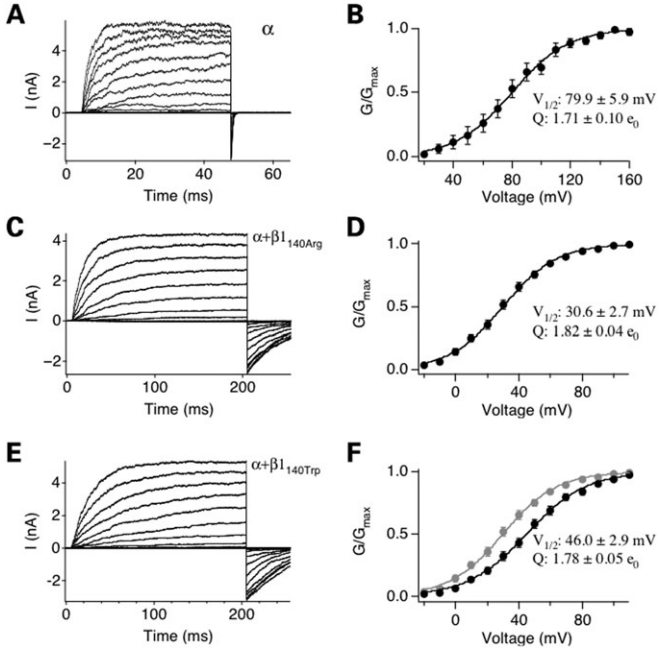

Figure 3. $\alpha + \beta1_{140Trp}$ channels have lower steady-state opening than $\alpha + \beta1_{140Arg}$ channels. Example current traces recorded from patches containing BK channels composed of α alone (**A**), $\alpha + \beta1_{140Arg}$ (**C**) and $\alpha + \beta1_{140Trp}$ (**E**). Mean G–V relations for α alone (**B**), $\alpha + \beta1_{140Arg}$ (**D**) and $\alpha + \beta1_{140Trp}$ (**F**) in 1.7μM Ca^{2+}. Error bars represent SEM. Solid curves represent fits to a Boltzmann function (see Materials and Methods). Co-expression of $\beta1_{140Arg}$ enhances channel opening by shifting the G–V relationship by ∼50 mV to negative potentials [(D) versus (B)]. $\beta1_{140Trp}$ causes a delayed rise in current in the first 40 ms (E) and a smaller negative shift (35 mV) of the G–V curve (F). Gray trace in panel (F) is $\alpha + \beta1_{140Arg}$ (from D) for comparison.

DISCUSSION

We have demonstrated significant associations between multiple exonic variants in the *KCNMB1* gene and asthma severity as defined by baseline pulmonary function in two independent groups of African American asthmatics. Our results indicate a complex and important role of *KCNMB1* genetic variation in pulmonary function, which is sexually dimorphic. Namely, we have observed a novel amino acid changing variant in the *KCNMB1* gene, C818T R140W, which is strongly associated with a clinically significant decrease in FEV_1 % of predicted value only among African American male subjects in two independent groups of asthmatics. In fact, subjects either heterozygous or homozygous for the 818T allele had a mean FEV_1 of 80.2%, indicating moderate airway obstruction, compared with the mild airway obstruction exhibited by the 818 CC genotype group with a mean FEV_1 of 92.0%. Furthermore, the *KCNMB1* 3′-UTR SNP, A1273G, was associated

Table 5. Conductance–voltage relationships of α alone, and α + β1$_{140Arg}$ (818C), and α + β1$_{140Trp}$ (818T) BK channels. The values shown are Boltzmann-fit parameters to determine voltage of half-maximal conductance ($V_{1/2}$) and equivalent gating charge (Q). Values are mean ± SEM. *P*-values shown are from student unpaired *t*-test of $V_{1/2}$ values between α + β1$_{140Arg}$ and β1$_{140Trp}$ measured at the same Ca^{2+} concentration

[Ca^{2+}] (mM)	α			α + β1$_{140Arg}$			α + β1$_{140Trp}$			*P*-value
	$V_{1/2}$ (mV)	Q (e0)	N	$V_{1/2}$ (mV)	Q (e0)	N	$V_{1/2}$ (mV)	Q (e0)	N	
0.3	127 ± 4	1.9 ± 0.2	6	94 ± 3	1.7 ± 0.1	12	98 ± 4	1.6 ± 0.1	15	3
1.7	80 ± 6	1.7 ± 0.1	12	31 ± 3	1.8 ± 0.1	45	46 ± 3[a]	1.8 ± 0.1	48	<0.0001
7	41 ± 3	1.8 ± 0.1	9	−24 ± 3	1.9 ± 0.1	29	−12 ± 3	1.8 ± 0.4	21	0.003
18.5	9 ± 2	1.6 ± 0.1	7	−84 ± 2	1.8 ± 0.1	16	−82 ± 2	1.8 ± 0.1	9	0.6
100	−26 ± 3	1.7 ± 0.1	6	−115 ± 3	1.8 ± 0.1	13	−108 ± 4	2.1 ± 0.2	9	0.1

[a]*P* value less than 0.05.

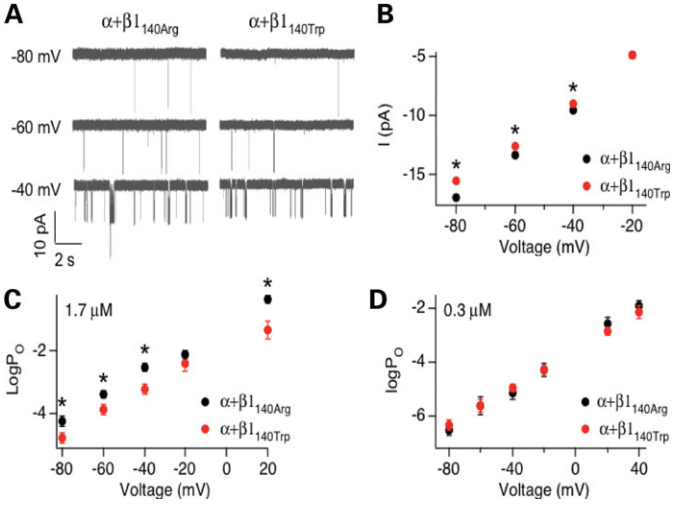

Figure 4. At 1.7 but not 0.3 μM Ca^{2+}, the Arg140Trp polymorphism decreases channel open probability over physiologically relevant voltage ranges. (**A**) Representative single-channel BK currents recorded at 1.7 μM Ca^{2+} from patches containing ∼25 α + β1$_{140Arg}$ (left panel) and ∼64 α + β1$_{140Trp}$ (right panels) channels. Traces were low-pass filtered at 4 kHz. (**B**) Single-channel current amplitude plotted as a function of voltage. (**C**) Averaged Log P_o–V relations at 1.7 mM [Ca^{2+}]. Asterisks represent statistically significant difference $P < 0.05$ in unpaired student *t*-test. (**D**) Averaged Log P_o–V relations at 0.3 mM [Ca^{2+}]. Symbols represent mean data for α + β1$_{140Arg}$ (black symbol) and α + β1$_{140Trp}$ (red symbol) and error bars represent SEM. In (B), the error bars are smaller than the symbols.

with increased FEV_1 % of predicted value, also among male subjects. The additive increase in FEV_1 by the A1273G variant was observed in both groups of asthmatic males. Additionally, we used PCA to create a summary variable (principal component) of pulmonary function representing FEV_1, FVC, FEF_{25-75} and FEV_1/FVC. Again we observed strong negative effects of the 818T allele on this general measure of pulmonary function, solidifying the effect of this SNP on African American males with asthma. Similar to C818T, SNP A1273G was also associated with the PCA generated measure of pulmonary function among males.

Our exploration of the potential effects of *KCNMB1* genetic variants on pulmonary function was motivated by the vital role that BK channels play in the regulation of arterial and bronchial smooth muscle contraction and the subsequent studies relating genetic variants in the *KCNMB1* gene with modulation of blood pressure. Our results are consistent with functional studies reporting the importance of BK channels in ASM contraction due to their high potassium conductance and abundant expression in ASM (14,23). The ASM of β1 knock-out mice shows increased resting calcium levels and calcium influx during contraction, revealing the importance of this subunit in moderating airway constriction (14).

Sexual dimorphism in asthma and related traits is well established. There are well-documented differences by sex with respect to lung development, age of asthma onset, asthma severity, and rate of pulmonary function decline (28–31). Sex modification of genotype–phenotype associations in asthma has precedence including, male-specific associations between vitamin D receptor variants and both asthma and IgE levels, male-specific association of β2-adrenergic receptor polymorphisms and persistence of asthma, female-specific associations of estrogen receptor polymorphisms with both airway hyperresponsiveness and rate of lung function decline (32–35). Moreover, a prior study of *KCNMB1* G593A E65K variant indicated its effects were sex-specific and primarily present in post-menopausal women (22). We propose that these effects maybe hormonally mediated since BK channel activity is upregulated by female sex hormones including estrogen and estradiol (23) (Fig. 5A). The mechanism behind estrogen activation of BK channels is an acute non-genomic event triggered by a signaling cascade, which has been established in murine ASM cells (23). Namely, estrogen stimulates the production of NO from Nitric oxide synthases, which then activates guanylate cyclases/protein kinase G (PKG) (36). Subsequently PKG phosphorylates BK channels resulting in a dramatic 50-fold increase in BK channel activity (Fig. 5A) (23). Indeed in women low estrogen levels experienced during the premenstrual luteal phase have been associated with both asthma exacerbation and decline in pulmonary function, as opposed to ovulation, when estrogen levels are highest, and asthma severity is decreased (37–39). In our study the median age of females was 22.1 years with the oldest being 40 years, implying that all female participants are pre-menopausal. The upregulation of BK channel activity mediated by female sex hormones may insulate pre-menopausal women from the moderate functional effects of *KCNMB1* variants, whereas these same moderate genetic effects may push males and post-menopausal women over a critical threshold resulting in disease, as observed in this study and in the study of hypertension (22) (Fig. 5B).

Despite the replication of our genetic association with pulmonary function and sex modification, we sought to character-

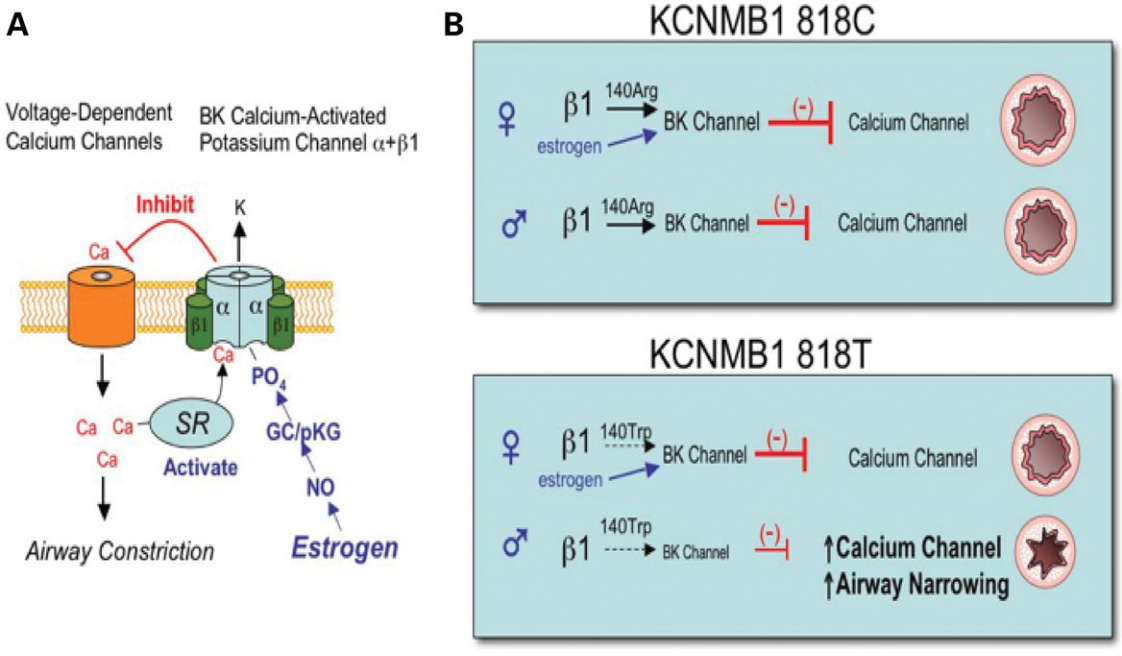

Figure 5. Proposed sex-specific effects of C818T polymorphisms on airway smooth muscle (ASM) contractility. (**A**) BK channel α + β1-subunits are activated by local calcium from the sarcoplasmic reticulum, SR, (calcium-induced calcium release from ryanodine receptors). The β1-subunit is required for BK channel activation in response to local calcium (14,15,18). The $\beta1_{140Arg}$ (818C allele) confers a greater activation of BK channels than the $\beta1_{140Trp}$ (818T allele). Complementing activation of BK channels is an estrogen-signaling cascade that activates nitric oxide (NO), guanylate cyclase (GC) and protein kinase G (PKG)-dependent phosphorylation of BK channels (23,36). (**B**) In 818C individuals, the $\beta1_{140Arg}$ activates BK channels sufficiently to moderate ASM constriction in the absence of estrogen signaling. In African American females carrying the 818T allele, estrogen signaling sufficiently complements defects in BK channel activation caused by $\beta1_{140Trp.}$ In African American 818T males, $\beta1_{140Trp}$ reduced BK channel activation combined with the lack of estrogen signaling significantly enhancing airway constriction.

ize the effect of the C818T coding variant on BK channel function as further validation of the clinical effects observed in this study. We found the $\beta1_{140Trp}$ isoform (encoded by 818T allele) to confer a partial loss of function by significantly reducing open probability of the BK channel compared with the $\beta1_{140Arg}$ isoform (encoded by 818C allele). This is consistent with the loss in pulmonary function observed in carriers of the 818T, since BK channels act as a brake on muscarinic signaling-induced bronchial smooth muscle contraction and tone (14). These results give mechanistic and biologic plausibility to the genetic results observed for the C818T variant (Fig. 5). Based upon these results we hypothesize that the increase in lung function observed for 1273 G allele carriers may be mediated by an upregulation in expression and/or activity of the BK channel. We did not find an association with SNP G593A despite prior reports of channel gain-of-function conferred by this variant and association with hypertension (21). However, these results were obtained in populations of European origin where the G593A allele is present at a much higher frequency. The low allele frequency in African Americans coupled with the loss in power due to stratification by sex likely contributed to this lack of association. Further studies in a European population will be needed to confirm our result.

Interestingly, we found the associated 818T allele to be African-specific. In fact, we were unable to detect the 818T allele after screening large numbers of Caucasian, Asian, Puerto Rican, and Mexican subjects. This explains the undiscovered nature of this variant prior to our study despite multiple genetic studies of hypertension and *KCNMB1*. Our results underscore the need to examine racially and ethnically diverse populations in genetic research. These racial specific variants may explain in part, along with social, economic, and environmental variables the significant disparities in asthma burden observed among African Americans (40,41). Our results have important clinical and public health implications. In the US, African Americans have nearly the highest asthma morbidity and mortality rates with prior studies finding significantly lower FEV_1 values among African American asthmatics compared with other ethnicities (42). Based on our results 10% of African American males with asthma carry at least one copy of the 818T allele, potentially putting them at higher risk for asthma morbidity and mortality. These findings bring us closer to understanding the pharmacogenetic profiles of different populations and create the possibility for more tailored drug design for asthma. Indeed substances that activate BK channels through the β1-subunit, such as dehydrosoyasaponin-1 (43) should be visited as potential asthma therapeutics, particularly for individuals carrying the 818T allele.

Furthermore, the effect of this variant maybe of additional significance in other disease states such as hypertension, which also disproportionately affects African American males (44). The other associated SNP, A1273G, is common among other ethnic groups including Caucasians (11%), Mexicans (15%) and Puerto Ricans (15%); therefore the effects mediated by this SNP may extend to other populations as well (25).

In conclusion, our results indicate that both the C818T and A1273G variants in the *KCNMB1* gene strongly influence pulmonary function among African American males with asthma. We find these genetic effects to be restricted to male subjects, possibly due to hormonal differences. The effects of both the C818T and A1273G variants on baseline pulmonary function are clinically significant and as such are important indicators of asthma severity. Results of the functional studies of the C818T encoded isoform revealed a significant loss-of-function of the BK channel with the (T) allele. Further studies will be needed to establish the role of the A1273G variant in pulmonary function among other populations. Additionally, studies are warranted for bronchial hyperresponsiveness considering the established role of BK channel conductance in controlling cholinergic stimulated contraction of bronchial smooth muscle.

MATERIALS AND METHODS

Study participants

The resequencing analysis was performed on our SNP Discovery Panel, which contains 24 African American asthmatic subjects. Recruitment for the SAGE study is ongoing and includes 261 asthmatic cases from the San Francisco Bay Area (45,46). African American subjects from the CHIRAH were used as an independent replicate population. The CHIRAH cohort is a community-based longitudinal cohort study of urban children and adults with persistent asthma (47). Studies are described in detail in the online supplement with characteristics listed in Supplementary Material, Table S1. Local Institutional Review Boards approved all the studies, and all subjects provided written, age-appropriate informed consent or assent.

Pulmonary function tests

Pulmonary function was evaluated using spirometry performed according to American Thoracic Society standards (48). Subjects were instructed to abstain from all bronchodilator medications for 8 h prior to all pulmonary function tests. Spirometric reference values from Hankinson *et al*. (49) were used to determine percent predicted values.

KCNMB1 sequencing

All *KCNMB1* exons (four) and exon–intron boundaries (20 bp from either end of the exon) were sequenced in the SNP Discovery panel. Sequencing details and primers are listed in the Supplementary Material, Table S2.

Genotyping

SNPs G593A, G728C, C818T, C1054A and A1273G were genotyped in the SAGE and CHIRAH cohorts. All genotyping methods and primers are described in the online supplement (Supplementary Material, Tables S3 and S4).

Statistical analysis of genetic data

The χ^2 goodness-of-fit test was used to determine whether the individual *KCNMB1* SNPs were under Hardy Weinberg equilibrium (HWE). All SNPs genotyped conformed to HWE ($P > 0.01$) in both sets of asthmatic cases. LD patterns were determined by using the r^2 statistic (Table 1). Multiple linear regression was used to test association between *KCNMB1* SNP genotypes and the degree of airway obstruction as determined by FEV in 1 s percent of predicted value (FEV_1 %) in the SAGE and CHIRAH asthma cases. Genotypes were coded in an additive fashion by default for SNPs with large minor allele frequencies (MAF) (C1054A and A1273G), and dominantly coded for SNPs with a small MAF (G593A, G728C, C818T) due to the small number of minor allele homozygotes. Age, sex, and individual ancestry estimates (IAE) were all included as covariates in SAGE and CHIRAH linear regression models. Additionally, we tested use of short-acting β_2-agonists, inhaled corticosteroids, oral corticosteroids (not tested or available in CHIRAH), and asthma duration for their inclusion into the regression models due to their potential for confounding. The *F*-statistic was used to determine if these variables affected the regression models. The IAE variable was generated by genotyping 60 ancestry informative markers in all SAGE and CHIRAH subjects and analyzing this data along with ancestral genotypes in the program STRUCTURE as is described elsewhere (50,51). Sex-stratified analyses were repeated in the same fashion. In the combined analysis of both SAGE and CHIRAH subjects the regression models included age, sex, and IAE and an additional covariate for each specific study (SAGE or CHIRAH).

Measures of pulmonary function are highly correlated. Therefore, we used PCA using the correlation matrix between variables to create a single summary variable representing pulmonary function. The pulmonary function measures included in the PCA are FEV_1, FVC, FEF_{25-75} and FEV_1/FVC. PCA was used to create components of these four variables that together account for the total variance in these measures. Components with Eigen values >1.0 were kept and scores were created for these components with a varimax rotation method. These scores were then tested as a general measure of pulmonary function for association with SNPs C818T and A1273G by linear regression. Regression models were run as described earlier. All statistical analyses were performed using STATA 8.0 S/E statistical software (College Station, TX, USA).

Expression and electrophysiology of BK channels in HEK293 cells

Effects of $\beta1_{140Arg}$ and $\beta1_{140Trp}$ on BK channel gating were characterized in HEK293T cells. The human β1 cDNA (NM_004137) was mutated to introduce the C818T (R140W) polymorphism using the Stratagene Quickchange mutagenesis kit and confirmed by sequencing. The original human α-subunit cDNA (U11058) initiates translation at the third in-frame translation initiation site, methionine-aspartic acid-alanine-leucine (52). The construct was therefore appended at the 5′ end to include the first translation initiation site (methionine-alanine-asparagine) and downstream sequence

that was absent from this expression construct. The human α-subunit, β1-subunit and EGFP-N1 plasmids were cotransfected at a ratio of 1:10:1 to ensure saturation of BK channels with β1-subunits and enable identification of transfected (green fluorescent protein fluorescent) cells.

Macroscopic current analysis

Ionic current recordings were made using the inside-out patch clamp configuration according to previously described methods (26). Conductance–voltage (G–V) relationships were obtained using test pulses that were followed by a step to a tail voltage (+60 at 0.3 μM Ca^{2+}, −80 at higher Ca^{2+}), and then measuring instantaneous tail current 200 μs after the test pulse. Tail current amplitudes (G) were normalized to tail currents at maximal conductance (G_{max}). In experiments where G_{max} were not reached, G_{max} values at higher Ca^{2+} from the same patch were used. G/G_{max}–V data were fitted with the Boltzman function: $G = G_{max}\{1/[1 + e^{-(V-V_{1/2})QF/RT}]\}$, where V is the test potential, $V_{1/2}$ is the membrane potential at half-maximal conductance, Q is the equivalent gating charge, and F, R and T are constants.

Single-channel analysis

Single-channel opening events were obtained from patches containing tens to hundreds of channels. Recordings were of 20 s to hundreds of seconds in duration. Analysis was performed using TAC and TACFIT programs (Bruxton Corporations). NP_o was determined using either all-point amplitude histogram or by event detection using a 50% amplitude criteria. The probability (P_k) of occupying each open level (k) gives rise to NP_o: $NP_o = \sum_k kP_k$. P_o was then determined by normalizing NP_o values by channel number (N). N was estimated by dividing the maximum instantaneous tail current amplitude by the unitary conductance.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

Conflict of Interest statement. None declared.

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REFERENCES

1. Moore, W.C., Bleecker, E.R., Curran-Everett, D., Erzurum, S.C., Ameredes, B.T., Bacharier, L., Calhoun, W.J., Castro, M., Chung, K.F., Clark, M.P. *et al.* (2007) Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J. Allergy Clin. Immunol.*, **119**, 405–413.
2. Sorkness, R.L., Bleecker, E.R., Busse, W.W., Calhoun, W.J., Castro, M., Chung, K.F., Curran-Everett, D., Erzurum, S.C., Gaston, B.M., Israel, E. *et al.* (2008) Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation. *J. Appl. Physiol.*, **104**, 394–403.
3. Gelb, A.F., Schein, A., Nussbaum, E., Shinar, C.M., Aelony, Y., Aharonian, H. and Zamel, N. (2004) Risk factors for near-fatal asthma. *Chest*, **126**, 1138–1146.
4. Devor, E.J. and Crawford, M.H. (1984) Family resemblance for normal pulmonary function. *Ann. Hum. Biol.*, **11**, 439–448.
5. Hubert, H.B., Fabsitz, R.R., Feinleib, M. and Gwinn, C. (1982) Genetic and environmental influences on pulmonary function in adult twins. *Am. Rev. Respir. Dis.*, **125**, 409–415.
6. McClearn, G.E., Svartengren, M., Pedersen, N.L., Heller, D.A. and Plomin, R. (1994) Genetic and environmental influences on pulmonary function in aging Swedish twins. *J. Gerontol.*, **49**, 264–268.
7. Tager, I.B., Rosner, B., Tishler, P.V., Speizer, F.E. and Kass, E.H. (1976) Household aggregation of pulmonary function and chronic bronchitis. *Am. Rev. Respir. Dis.*, **114**, 485–492.
8. Burchard, E.G., Silverman, E.K., Rosenwasser, L.J., Borish, L., Yandava, C., Pillari, A., Weiss, S.T., Hasday, J., Lilly, C.M., Ford, J.G. *et al.* (1999) Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am. J. Respir. Crit. Care Med.*, **160**, 919–922.
9. Joost, O., Wilk, J.B., Cupples, L.A., Harmon, M., Shearman, A.M., Baldwin, C.T., O'Connor, G.T., Myers, R.H. and Gottlieb, D.J. (2002) Genetic loci influencing lung function: a genome-wide scan in the Framingham Study. *Am. J. Respir. Crit. Care Med.*, **165**, 795–799.
10. Postma, D.S., Meyers, D.A., Jongepier, H., Howard, T.D., Koppelman, G.H. and Bleecker, E.R. (2005) Genomewide screen for pulmonary function in 200 families ascertained for asthma. *Am. J. Respir. Crit. Care Med.*, **172**, 446–452.
11. Zhu, G., Warren, L., Aponte, J., Gulsvik, A., Bakke, P., Anderson, W.H., Lomas, D.A., Silverman, E.K. and Pillai, S.G. (2007) The SERPINE2 gene is associated with chronic obstructive pulmonary disease in two large populations. *Am. J. Respir. Crit. Care Med.*, **176**, 167–173.
12. Billington, C.K. and Penn, R.B. (2003) Signaling and regulation of G protein-coupled receptors in airway smooth muscle. *Respir. Res.*, **4**, 2.
13. Pelaia, G., Gallelli, L., Vatrella, A., Grembiale, R.D., Maselli, R., De Sarro, G.B. and Marsico, S.A. (2002) Potential role of potassium channel openers in the treatment of asthma and chronic obstructive pulmonary disease. *Life Sciences*, **70**, 977–990.
14. Semenov, I., Wang, B., Herlihy, J.T. and Brenner, R. (2006) BK channel beta1-subunit regulation of calcium handling and constriction in tracheal smooth muscle. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **291**, L802–L810.
15. Brenner, R., Perez, G.J., Bonev, A.D., Eckman, D.M., Kosek, J.C., Wiler, S.W., Patterson, A.J., Nelson, M.T. and Aldrich, R.W. (2000) Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature*, **407**, 870–876.
16. Bleecker, E.R., Amelung, P.J., Levitt, R.C., Postma, D.S. and Meyers, D.A. (1995) Evidence for linkage of total serum IgE and bronchial hyperresponsiveness to chromosome 5q: a major regulatory locus important in asthma. *Clin. Exp. Allergy*, **25** (Suppl. 2), 84–88. Discussion 95–96.
17. Meyers, D.A., Postma, D.S., Stine, O.C., Koppelman, G.H., Ampleford, E.J., Jongepier, H., Howard, T.D. and Bleecker, E.R. (2005) Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J. Allergy Clin. Immunol.*, **115**, 1169–1175.

18. Pluger, S., Faulhaber, J., Furstenau, M., Lohn, M., Waldschutz, R., Gollasch, M., Haller, H., Luft, F.C., Ehmke, H. and Pongs, O. (2000) Mice with disrupted BK channel beta1 subunit gene feature abnormal Ca(2+) spark/STOC coupling and elevated blood pressure. *Circ. Res.*, **87**, E53–E60.
19. Petkov, G.V., Bonev, A.D., Heppner, T.J., Brenner, R., Aldrich, R.W. and Nelson, M.T. (2001) Beta1-subunit of the Ca2+-activated K+ channel regulates contractile activity of mouse urinary bladder smooth muscle. *J. Physiol.*, **537**, 443–452.
20. Gollasch, M., Tank, J., Luft, F.C., Jordan, J., Maass, P., Krasko, C., Sharma, A.M., Busjahn, A. and Bahring, S. (2002) The BK channel beta1 subunit gene is associated with human baroreflex and blood pressure regulation. *J. Hypertens.*, **20**, 927–933.
21. Fernandez-Fernandez, J.M., Tomas, M., Vazquez, E., Orio, P., Latorre, R., Senti, M., Marrugat, J. and Valverde, M.A. (2004) Gain-of-function mutation in the KCNMB1 potassium channel subunit is associated with low prevalence of diastolic hypertension. *J. Clin. Invest.*, **113**, 1032–1039.
22. Senti, M., Fernandez-Fernandez, J.M., Tomas, M., Vazquez, E., Elosua, R., Marrugat, J. and Valverde, M.A. (2005) Protective effect of the KCNMB1 E65K genetic polymorphism against diastolic hypertension in aging women and its relevance to cardiovascular risk. *Circ. Res.*, **97**, 1360–1365.
23. Dimitropoulou, C., White, R.E., Ownby, D.R. and Catravas, J.D. (2005) Estrogen reduces carbachol-induced constriction of asthmatic airways by stimulating large-conductance voltage and calcium-dependent potassium channels. *Am. J. Respir. Cell Mol. Biol.*, **32**, 239–247.
24. Kundu, P., Alioua, A., Stefani, E. and Toro, L. (2007) Regulation of mouse Slo gene expression: multiple promoters, transcription start sites, and genomic action of estrogen. *J. Biol. Chem.*, **282**, 27478–27492.
25. Gong, Y., Beitelshees, A.L., Wessel, J., Langaee, T.Y., Schork, N.J. and Johnson, J.A. (2007) Single nucleotide polymorphism discovery and haplotype analysis of Ca2+-dependent K+ channel beta-1 subunit. *Pharmacogenet. Genomics*, **17**, 267–275.
26. Wang, B. and Brenner, R. (2006) An S6 mutation in BK channels reveals beta1 subunit effects on intrinsic and voltage-dependent gating. *J. Gen. Physiol.*, **128**, 731–744.
27. Richards, I.S., Miller, L., Solomon, D., Kulkarni, A., Brooks, S. and Sperelakis, N. (1990) Azelastine and desmethylazelastine suppress acetylcholine-induced contraction and depolarization in human airway smooth muscle. *Eur. J. Pharmacol.*, **186**, 331–334.
28. Taussig, L.M., Cota, K. and Kaltenborn, W. (1981) Different mechanical properties of the lung in boys and girls. *Am. Rev. Respir. Dis.*, **123**, 640–643.
29. Hibbert, M., Lannigan, A., Raven, J., Landau, L. and Phelan, P. (1995) Gender differences in lung growth. *Pediatr. Pulmonol.*, **19**, 129–134.
30. Skobeloff, E.M., Spivey, W.H., St Clair, S.S. and Schoffstall, J.M. (1992) The influence of age and sex on asthma admissions. *JAMA*, **268**, 3437–3440.
31. Burrows, B., Bloom, J.W., Traver, G.A. and Cline, M.G. (1987) The course and prognosis of different forms of chronic airways obstruction in a sample from the general population. *N. Engl. J. Med.*, **317**, 1309–1314.
32. Dijkstra, A., Howard, T.D., Vonk, J.M., Ampleford, E.J., Lange, L.A., Bleecker, E.R., Meyers, D.A. and Postma, D.S. (2006) Estrogen receptor 1 polymorphisms are associated with airway hyperresponsiveness and lung function decline, particularly in female subjects with asthma. *J. Allergy Clin. Immunol.*, **117**, 604–611.
33. Guerra, S., Graves, P.E., Morgan, W.J., Sherrill, D.L., Holberg, C.J., Wright, A.L. and Martinez, F.D. (2005) Relation of beta2-adrenoceptor polymorphisms at codons 16 and 27 to persistence of asthma symptoms after the onset of puberty. *Chest*, **128**, 609–617.
34. Raby, B.A., Lazarus, R., Silverman, E.K., Lake, S., Lange, C., Wjst, M. and Weiss, S.T. (2004) Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am. J. Respir. Crit. Care Med.*, **170**, 1057–1065.
35. Hunninghake, G.M., Lasky-Su, J., Soto-Quiros, M.E., Avila, L., Liang, C., Lake, S.L., Hudson, T.J., Spesny, M., Fournier, E., Sylvia, J.S. *et al.* (2008) Sex-stratified linkage analysis identifies a locus for IgE to cockroach in female Costa Ricans. *Am. J. Respir. Crit. Care Med.*, **177**, 830–836.
36. Wellman, G.C., Bonev, A.D., Nelson, M.T. and Brayden, J.E. (1996) Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca(2+)-dependent K+ channels. *Circ. Res.*, **79**, 1024–1030.
37. Pauli, B.D., Reid, R.L., Munt, P.W., Wigle, R.D. and Forkert, L. (1989) Influence of the menstrual cycle on airway function in asthmatic and normal subjects. *Am. Rev. Respir. Dis.*, **140**, 358–362.
38. Hanley, S.P. (1981) Asthma variation with menstruation. *Br. J. Dis. Chest*, **75**, 306–308.
39. Chandler, M.H., Schuldheisz, S., Phillips, B.A. and Muse, K.N. (1997) Premenstrual asthma: the effect of estrogen on symptoms, pulmonary function, and beta 2-receptors. *Pharmacotherapy*, **17**, 224–234.
40. Drake, K.A., Galanter, J.M. and Burchard, E.G. (2008) Race, ethnicity, and social class and the complex etiologies of asthma. *Pharmacogenomics*, **9**, 453–462.
41. Burchard, E.G., Ziv, E., Coyle, N., Gomez, S.L., Tang, H., Karter, A.J., Mountain, J.L., Perez-Stable, E.J., Sheppard, D. and Risch, N. (2003) The importance of race and ethnic background in biomedical research and clinical practice. *N. Engl. J. Med.*, **348**, 1170–1175.
42. Lester, L.A., Rich, S.S., Blumenthal, M.N., Togias, A., Murphy, S., Malveaux, F., Miller, M.E., Dunston, G.M., Solway, J., Wolf, R.L. *et al.* (2001) Ethnic differences in asthma and associated phenotypes: collaborative study on the genetics of asthma. *J. Allergy Clin. Immunol.*, **108**, 357–362.
43. Giangiacomo, K.M., Kamassah, A., Harris, G. and McManus, O.B. (1998) Mechanism of maxi-K channel activation by dehydrosoyasaponin-I. *J. Gen. Physiol.*, **112**, 485–501.
44. Ostchega, Y., Dillon, C.F., Hughes, J.P., Carroll, M. and Yoon, S. (2007) Trends in hypertension prevalence, awareness, treatment, and control in older U.S. adults: data from the National Health and Nutrition Examination Survey 1988 to 2004. *J. Am. Geriatr. Soc.*, **55**, 1056–1065.
45. Battle, N.C., Choudhry, S., Tsai, H.J., Eng, C., Kumar, G., Beckman, K.B., Naqvi, M., Meade, K., Watson, H.G., Lenoir, M. *et al.* (2007) Ethnicity-specific gene-gene interaction between IL-13 and IL-4R alpha among African Americans with asthma. *Am. J. Respir. Crit. Care Med.*, **175**, 881–887.
46. Tsai, H.J., Shaikh, N., Kho, J.Y., Battle, N., Naqvi, M., Navarro, D., Matallana, H., Lilly, C.M., Eng, C.S., Kumar, G. *et al.* (2006) Beta 2-adrenergic receptor polymorphisms: pharmacogenetic response to bronchodilator among African American asthmatics. *Hum. Genet.*, **119**, 547–557.
47. Shalowitz, M.U., Sadowski, L.M., Kumar, R., Weiss, K.B. and Shannon, J.J. (2007) Asthma burden in a citywide, diverse sample of elementary schoolchildren in Chicago. *Ambul. Pediatr.*, **7**, 271–277.
48. (1995) Standardization of spirometry, 1994 update. American Thoracic Society. *Am. J. Respir. Crit. Care Med.*, **152**, 1107–1136.
49. Hankinson, J.L., Odencrantz, J.R. and Fedan, K.B. (1999) Spirometric reference values from a sample of the general U.S. population. *Am. J. Respir. Crit. Care Med.*, **159**, 179–187.
50. Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
51. Tsai, H.J., Kho, J.Y., Shaikh, N., Choudhry, S., Naqvi, M., Navarro, D., Matallana, H., Castro, R., Lilly, C.M., Watson, H.G. *et al.* (2006) Admixture-matched case-control study: a practical approach for genetic association studies in admixed populations. *Hum. Genet.*, **118**, 626–639.
52. Wallner, M., Meera, P. and Toro, L. (1996) Determinant for b-subunit regulation in high-conductance voltage-activated and Ca21-sensitive K1 channels: an additional transmembrane region at the N terminus. *Proc. Natl Acad. Sci.*, **93**, 14922–14927.