Online Supplement

Subject Recruitment

Study of African Americans, Genes, and environments

Eligible asthmatic subjects met the following criteria: (1) between the ages of 8 and 40 years (2) physician-diagnosed asthma (3) symptoms including coughing, wheezing, or shortness of breath for at least 2 years. Asthmatic cases were enrolled only if both biological parents and all biological grandparents self-identified as African American. Exclusion criteria included >10 pack-year smoking history, smoking in the previous 12 months, and/or history of other lung diseases. A modified version of the 1978 American Thoracic Society-Division of Lung Diseases Epidemiology Questionnaire was administered at the time of enrollment to assess individual asthma characteristics and confounders (1).

Chicago Initiative to Raise Asthma Equity (CHIRAH)

The CHIRAH cohort is a longitudinal population based cohort of asthmatics collected from the urban Chicago school system. CHIRAH was designed to examine the impact of socioeconomic and psychosocial stressors on asthma status. Subjects were enrolled from February 2004 to July 2005. The cohort was established by a broad community-based screening for households with persons with asthma using a school based sampling technique. Children were eligible if they were age 8–14 years old and had persistent, symptomatic asthma, defined as requiring at least 8 weeks of asthma medication over the previous 12 months. Exclusion criteria included: caregiver parents not fluent in spoken English (many of the measurement instruments were only validated in English); no telephone (needed for follow-up); or not planning on living in Chicago for the duration of the study follow-up (18 months). Adult asthmatics were recruited if there was no

child in the household with asthma. Inclusion and exclusion criteria are the same for adults with the age range between 18 and 40 years.

Resequencing

Resequencing of 48 chromosomes provided power of > 90% to find all genetic variants of 5% or greater frequency in the general population. Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and the reactions were processed on the ABI Prism 3700 sequencher. PCR primers used to produce *KCNMB1* PCR products are listed in Table S2.

Genotyping

KCNMB1 SNPs G593A, G728C, C818T, C1054A, and A1273G were genotyped in the SAGE and SAGE asthmatics using the AcycloPrime-FP-TDI (PerkinElmer) method (2). The PCR cocktail included: 3.0-5.0 ng genomic DNA, 0.1-0.2 µM primers, 2.5 mM MgCl2, 50 µM dNTPs, 6 µl volume with Platinum Taq PCR buffer and 0.1-0.2 units Platinum Taq (Invitrogen) plus 1 µl extra water to counteract evaporation. PCR cycling conditions were as follows: 95°C for 2 minutes, 40 cycles of 92°C for 10 seconds, 58°C for 20 seconds, 68°C for 30 seconds, and final extension at 68°C for 10 minutes. We used AcycloPrime-FP kits for enzymatic cleanup and single base extension genotyping reactions. Plates were read on an EnVision fluorescence polarization plate reader (PerkinElmer). Genotyping of SNPs G728C and A1273G in the CHIRAH asthmatics was accomplished by the same FP-TDI assay used to genotype this SNP in the SAGE asthmatics. PCR primers and FP allele-specific primers and Fluorescent tagged terminator nucleotides are listed in Table S3.

KCNMB1 SNPs G593A, C818T, and C1054A and were genotyped in the CHIRAH asthmatics

using iPLEX reagents and protocols for multiplex PCR, single base primer extension (SBE) and generation of mass spectra, as per the manufacturer's instructions (for complete details see iPLEX Application Note, Sequenom, San Diego). Multiplexed assays were designed using MassARRAY Designer v 3.1 (Sequenom). Multiplexed PCR was performed in 5-µl reactions on 384-well plates containing 5 ng of genomic DNA. Reactions contained 0.5 U HotStar Taq polymerase (QIAGEN), 100 nM PCR primers (see Table S4), 1.25X HotStar Taq buffer, 1.625 mM MgCl2, and 500 µM dNTPs. Following enzyme activation at 94 °C for 15 min, DNA was amplified with 45 cycles of 94 °C x 20 sec, 56 °C x 30 sec, 72 °C x 1 min, followed by a 3-min extension at 72 °C. Unincorporated dNTPs were removed using shrimp alkaline phosphatase (0.3 U, Sequenom). Single-base extension was carried out by addition of SBE primers (see Table S4) at concentrations from 0.625 µM (low MW primers) to 1.25 µM (high MW primers) using iPLEX enzyme and buffers (Sequenom) in 9-µl reactions. Reactions were desalted and SBE products measured using the MassARRAY Compact system, and mass spectra analyzed using TYPER software (Sequenom), in order to generate genotype calls and allele frequencies. All plates genotyped contained no template controls to ensure genotyping accuracy and lack of contamination. PCR primers and mass-extension SBE primers are listed in Table S4.

References

1. Ferris, B.G. (1978) Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis,* **118,** 1-120.
2. Chen, X., Levine, L. and Kwok, P.Y. (1999) Fluorescence polarization in homogeneous nucleic acid analysis. *Genome Res,* **9,** 492-8.

Table S1.

	SAGE		CHIRAH	
Characteristics	Males	Females	Males	Females
N	105	156	94	154
Age (yr)	16.8 (7.9)	21.1 (9.9)	15.2 (9.2)	23.0 (10.9)
African Ancestry (%)	79.3 (7.8)	77.2 (10.8)	79.9 (12.8)	79.8 (11.0)
FEV_1 % of Predicted	88.9 (16.2)	94.3 (16.8)	92.7 (17.2)	91.9 (20.8)

Table S1. Characteristics of the two case groups genotyped for KCNMB1 genetic variants. Values listed represent mean measurements with the standard deviation in parenthesis.

Table S2

Amplicon	Exons	Forward Primer	Reverse Primer
1	1	AGAGAGGTGCCATAACTTGCTC	CCGGTCTTCAGAGAAATCACTT
2	2	CTCTTTCTGTGGATCACCTG	GCTCAGTTCATCCTACCTTC
3	3	TGTGTATGTGTGCATGTGTG	CAGGGATAAAGGGATGTGT
4	4(5' end)	ATCAAGCCCTTGGCTAAGAA	TCAAAGGTTAGTCCTGCAAC
5	4(3' end)	AAGTAGAGCCATCCATCCAT	CCACTTCCATCTTTTTCTGT

Table S2**.** PCR primers for KCNMB1 sequencing analysis.

Table S3

SNP	dbSNP rs #	Forward PCR Primer	Reverse PCR Primer	FP Primer	Dye
G593A	rs11739136	TGTGTATGTGTGCATGTGTG	CAGGGATAAAGGGATGTGT	CACCTTCTTGCCCTTCAGCT	C/T
G728C	rs2301149	GCCCGACTACAAAGCTGGTAT	ACCATGGCGATAATGAGGAG	CTCCTACATCCCAGGCAGC	G/C
C818T	Novel	ATCAAGCCCTTGGCTAAGAA	TCAAAGGTTAGTCCTGCAAC	TCTACTGCTTCTCCGCACCT	C/T
C1054A	rs2656842	AAGTAGAGCCATCCATCCAT	CCACTTCCATCTTTTTCTGT	GGCTGCTCCCCACTTGCAG	C/A
A1273G	rs314156	CATCCATGCCATACCACTTG	AGGGAGAACTCAGGCACAGA	TGTTTCCGGCTGCATCACT	A/G

Table S3. PCR and Single Base pair extension primers used in FP-TDI genotyping assays.

Table S4

SNP	dbSNP rs#	Forward PCR Primer	Reverse PCR Primer	Unextended SBE Primer
G593A	rs11739136	ACGTTGGATGACACGT TGACCCACAGGCAT	ACGTTGGATGGATTGAGA CCAACATCAGGG	tggaaAACATCAGGGAC CAGGAG
C818T	Novel	ACGTTGGATGGGTCAG AGCCAAATTCCAAG	ACGTTGGATGTGGAATAG GACGCTGGTTTC	tgacaCTGGTTTCGTTCCC CC
C1054A	rs2656842	ACGTTGGATGTGGACT GGAAGAGTGGGAGG	ACGTTGGATGTACCACTTG TCAGGGCACAG	gGCTCCCCACTTGCAG

Table S4. PCR and mass-extension SBE primers used in Sequenom iPLEX genotyping assays.

Figure S1

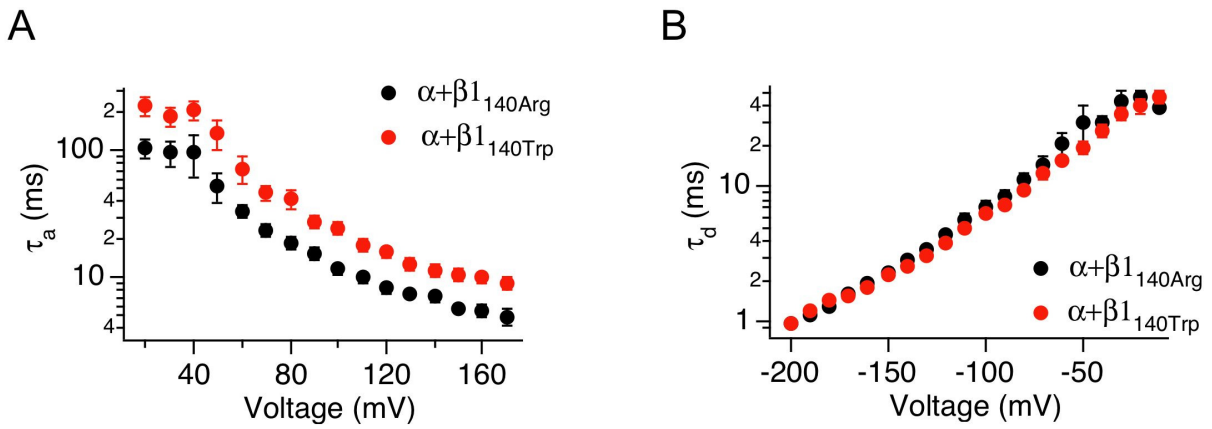

Figure S1. Effects of Arg140Trp polymorphism on BK channel gating kinetics. **(A)** Comparison of channel activation time constant at 1.7 μM Ca^{2+}. The Arg140Trp polymorphism slows channel opening. **(B)** Comparison of channel deactivation time constant at 1.7 μM Ca^{2+}. The Arg140Trp polymorphism does not alter deactivation kinetics. Symbols represent mean data for $\alpha+\beta1_{140Arg}$ (black) and $\alpha+\beta1_{140Trp}$ (red) and error bars represent SEM.