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Article

Variation at *DENND1B* and Asthma on the Island of Tristan da Cunha

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Abstract

A high prevalence of asthma has been documented among the inhabitants of Tristan da Cunha, an isolated island in the South Atlantic. The population derives from just 28 founders. We performed lung function testing, including methacholine inhalation challenge, allergen skin prick testing, and collected DNA from essentially all of the current island population (269 individuals), and genotyped a panel of 43 single-nucleotide polymorphisms (SNPs) reported as associated with asthma and atopy. We carried out a mixed-model association analysis using the known pedigree. There were 96 individuals diagnosed as asthmatic (36%), and heritability estimates were similar to those from nonisolated population samples (multifactorial threshold model, $h^2 = 48\%$). The first component from a genetic principal components analysis using the entire SNP panel was nonlinearly associated with asthma, with the maximum risk to those intermediate to reference (Human Genome Diversity Project) European and African samples means. The single most strongly associated SNP was rs2786098 ($p = 5.5 \times 10^{-5}$), known to regulate the gene *DENND1B*. This explained approximately one-third of the trait heritability, with an allelic odds ratio for the A allele of 2.6. Among A/A carriers, 10 out of 12 individuals were asthmatic. The rs2786098*A variant was initially reported to decrease the risk of childhood (atopic) asthma in European but slightly increase the risk in African-descended populations, and does significantly alter Th2 cell function. Despite an absence of overall association with this variant in recent asthma genome wide association studies meta-analyses, an effect may exist on the particular genetic background of the Tristan da Cunha population.

Keywords: Asthma; genetics association

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Asthma is a common disease that continues to increase in incidence. It is characterized by reversible narrowing of the airways, often associated with allergic inflammatory processes in the airway wall.

Asthma is also a prototypical complex genetic disease. A strong familial component to the occurrence of asthma has been recognized since the time of Cooke (Cooke & Vander Veer, 1916), and genome wide association studies (GWAS) have now identified over 60 individual risk loci (Demenais et al., 2018; Pividori et al., 2019).

One classic approach to genetic disease has been to study isolated populations where the total number of causative loci is likely to be smaller, with risk alleles at higher frequency due to inbreeding and genetic drift, and exposure to environmental risk factors more homogenous (Kenny et al., 2011; Sheffield et al., 1998). The island of Tristan da Cunha offers such a resource for the study of asthma: asthma prevalence is high and the present population is descended from only a small number of founding members. We have previously described (Zamel et al., 1996) a complete population survey of asthma on the island, and here we report the strength of association of asthma with a panel of asthma and atopic disease risk variants previously identified in large outbred population studies (Moffatt et al., 2010; Paternoster et al., 2011; Sleiman et al., 2010).

Methods

Asthma phenotype measurements and blood samples were obtained from the inhabitants of Tristan da Cunha, an isolated island in the South Atlantic (Zamel et al., 1996). The entire island population at the time of examination (1991 and 1996) formed a single large extended family descended from 28 original founders. Settlement of Tristan da Cunha occurred beginning in 1817 with soldiers who remained behind when a British garrison was withdrawn from the island, followed by the survivors of several shipwrecks. In 1827, five women from St Helena, one with children, emigrated to Tristan da Cunha and married island men. William Glass and his wife of African descent are said to have been asthmatic and could be the origin of a genetic founder effect for asthma in this population. Inbreeding has resulted in kinship resemblances of at least first cousin levels for all individuals ($F = .04$). A high prevalence of asthma has been noted on the island over at least 80 years, as well as on St Helena, where many residents are related to the Tristan da Cunha population.

The Tristan da Cunha family pedigrees were ascertained through review of baptismal, marriage and medical records, as well as reliably accurate historical records of the early inhabitants (Roberts, 1968; Zamel et al., 1996). The prevalence of asthma on

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Tristan da Cunha has been reported to be high in several reports (Black et al., 1963; Wild, 1923; Zamel et al., 1996) spanning 80 years — Macklin comments on 'a condition they call "ashmere", meaning asthma. This seems to be the most prevalent complaint on the island' (Wild, 1923, p. 233). In the present population, one-third met strict criteria for diagnosis of asthma.

Clinical Characterization

Standardized questionnaires based on that of the American Thoracic Society were used to record the presence of respiratory symptoms such as cough, sputum and wheezing; the presence of other chest disorders, including recent upper respiratory tract infection, allergic history; asthmatic attacks, including age at onset, offset, confirmation by a physician, prevalence, severity and precipitating factors; other illnesses and smoking history; and all medications used within the previous 3 months. A physician-confirmed asthmatic attack was the principal criterion for diagnosis of asthma.

Skin atopy was determined by skin prick tests to common allergens: *Aspergillus fumigatus*, *Cladosporium*, *Alternaria*, egg, milk, wheat, tree, dog, grass, horse, house dust, cat, feathers and house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Saline and histamine controls were also performed (Bencard Laboratories, Mississauga, Ontario). Antihistamines were withdrawn for at least 48 h prior to testing. Wheal diameters were corrected by subtraction of the saline control wheal diameter, and a corrected wheal size of >3 mm recorded 10 min after application was considered a positive response.

Airway responsiveness was assessed by a methacholine challenge test using the tidal breathing method (Cockcroft et al., 1977). Doubling doses of methacholine from .03 to 16 mg/ml were administered using a Wright nebulizer at 4-min intervals to measure the provocative concentration of methacholine, producing a 20% fall in FEV1 (PC20). In those subjects with a baseline FEV1 (forced exhalation volume in 1 s) >70% of predicted (Crapo et al., 1981), a bronchodilator response to 400-mg salbutamol aerosol was used to determine airway responsiveness. Both methacholine challenges and bronchodilator responses were measured using a computerized bronchial challenge system (S&M Instrument Co. Inc., Doyleston, PA) consisting of a software package and interface board installed in a Toshiba T185C laptop computer and connected to a flow sensor (RS232FS). The power source for instruments used on Tristan da Cunha has been described (Zamel et al., 1996).

Increased airway responsiveness was defined as a PC20 <4.0 mg/ml or a >15% improvement in FEV1 15-min postbronchodilator. Participants were asked to withhold bronchodilators at least 8 h before testing; inhaled or systemic steroids were maintained at the usual dosage.

Genotyping

Single-nucleotide polymorphism (SNP) genotyping was carried out using the Sequenom MASSarray system. A set of 43 candidate SNPs in 37 genes was selected from the published literature, especially the meta-analysis of Moffatt et al. (2010; see Table 1). The genotyping assay failed for two SNPs. The Human Genome Diversity Project (HGDP; Li et al., 2008) sample genotypes at the same panel of SNPs provided external controls to assess the relationship of the Tristan da Cunha population with those from other regions.

Statistical Genetic Analysis

Descriptive statistics and plots were generated using the R Statistical Computing Environment (R Core Team, 2019). Various genetic analyses were performed using the sib-pair package (Duffy, 1997, 2019). We estimated the heritability of asthma based on the recorded pedigree under the multifactorial threshold model using the R *MCMCglmm* package (Hadfield, 2010). A mixed-model genetic association analysis of onset of asthma under the Cox proportional hazards was performed using the R *coxme* package (Therneau, 2015; Therneau and Grambsch, 2000), including the kinship matrix based on the recorded pedigree and including the genotypes at each SNP in turn along with the first five principal components (including a quadratic fixed effect for the first principal component, PC1) from a genetic principal components analysis of the panel of candidate SNPs: Given that these are known asthma candidate variants, we also calculated a polygenic risk score (PRS) based on the effect sizes reported in the GABRIEL asthma meta-analysis (Moffatt et al., 2010).

Results

As previously described (Zamel et al., 1996), at the time of the fieldwork for this study, the population of the Island of Tristan da Cunha was 299 individuals, of whom 282 individuals (97%) provided medical histories and underwent skin prick allergen testing. A total of 102 had a medical history consistent with a diagnosis of asthma plus significant airway hyperresponsiveness to inhaled methacholine. Among the 269 individuals successfully genotyped, 96 (36%) met these criteria. The median age of onset was 10 years (interquartile range 5–21).

For genetic analysis, these 269 individuals were incorporated into a 559-member, 10-generation pedigree. The mean inbreeding coefficient for the asthma cases was .045, and that of unaffected individuals .046. While 15% of offspring were asthmatic when neither parent was affected, this rose to 30% when one parent was affected and 50% when both parents were affected. Based on the entire pedigree, the heritability of asthma under the multifactorial threshold model (using *MCMCglmm*) was .51 (95% highest posterior density interval [.21, .76], see Table 2).

Of the 41 successfully genotyped SNPs, the five (uncommon) *FLG* coding variants were all monomorphic (see Table 1). The multilocus genetic distances between the Tristan and HDGP continental populations were roughly equal, with the Americas slightly closer (Europe multilocus F statistics = .16; Africa .20; Americas .13; Asia .16; see Figure 1). On plotting the relationship between asthma risk and loading on the first genetic principal component (Figure 2), a significant quadratic relationship was found, with risk greatest for those individuals intermediate between African and European mean values. This is why we chose to adjust for a quadratic effect of PC1 in the individual SNP association analyses.

Only one SNP met the Bonferroni-corrected significance threshold for association to asthma — rs2786098 (*coxme* crude allelic $p = 5.5 \times 10^{-5}$; $p = .002$ corrected for 37 tests, see Table 3). The allelic odds ratio for rs2786098*A was 2.6, and of 12 A/A homozygotes, 10 were asthmatics. Age at onset was 8 years in A/A homozygotes versus 19 years in C/C homozygotes, but the Age × Genotype interaction term in the mixed Cox model was not statistically significant (main effect of age, $p = .06$; interaction $p = .60$). The frequency of the rs2786098*A allele in cases was 31%, and 13% in unaffecteds (Table 4). In Western Europe,

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Table 1. Panel of previously reported asthma-associated SNPs tested in the Tristan da Cunha sample, and results for *coxme* and *MCMCglmm* tests of allelic association to asthma

SNP	Chr	Position (b37 bp)	Gene	Minor allele (major)	Minor allele frequency	*MCMCglmm* association *p*-value	*coxme* association *p*-value
rs9050	1	152079314	*TCHH*	T(G)	.0057	.085	6.5e-03
S3247X	1	152277622	*FLG*	W	1.0000	–	–
R2447X	1	152280023	*FLG*	W	1.0000	–	–
3702delG	1	152284217	*FLG*	W	1.0000	–	–
2282del4	1	152285637	*FLG*	W	1.0000	–	–
R501X	1	152285860	*FLG*	W	1.0000	–	–
rs4129267	1	154426264	*IL6R*	T(C)	.2567	.20	.19
rs1101999	1	158932555	*PYHIN1*	C(T)	.0189	.97	.55
rs2786098	1	197325908	*DENND1B*	A(C)	.1977	**.00067**	**5.5×10^{-5}**
rs3771180	2	102953617	*IL1RL1*	C	1.0000	–	–
rs9807989	2	102971200	*IL18R1*	C(T)	.0698	.33	.60
rs7686660	4	144003159	*LOC729675*	G(T)	.2898	.84	.89
rs1588265	5	59369794	*PDE4D*	G(A)	.4981	.20	.41
rs1837253	5	110401872	*TSLP*	T(C)	.3434	.055	.027
rs2073643	5	131723288	*SLC22A5*	C(T)	.3755	.25	.27
rs2244012	5	131901225	*RAD50*	C(T)	.3000	.43	.21
rs1295686	5	131995843	*IL13*	T(C)	.3340	.076	.040
rs2897442	5	132049027	*KIF3A*	T(C)	.3931	.24	.15
rs6867913	5	141445980	*NDFIP1*	–	–		–
rs3853601	6	31499603	*DDX39B*	C(G)	.3258	.061	.15
rs404860	6	32184345	*NOTCH4*	C(T)	.2216	.24	.47
rs3129943	6	32338695	*C6orf10*	–	–	–	–
rs3117098	6	32358513	*BTNL2*	C(T)	.1379	.95	.79
rs9268516	6	32379489	*BTNL2*	T(C)	.3604	.75	.88
rs3129890	6	32414273	*HLA-DRA*	C(T)	.1160	.98	.84
rs9273349	6	32625869	*HLA-DQ*	–	–	–	–
rs7775228	6	32658079	*HLA-DQB1*	C(T)	.1521	.83	.42
rs9275698	6	32687973	*HLA-DQA2*	G(A)	.2774	.56	.48
rs9500927	6	32961361	*HLA-DOA*	A(G)	.2472	.039	.16
rs987870	6	33042880	*HLA DPB1*	C(T)	.2838	.045	.13
rs7000782	8	81308150		T(A)	.4981	.61	.32
rs3019885	8	118025645	*SLC30A8*	G(T)	.2906	.46	.84
rs1342326	9	6190076	*IL33*	G(T)	.2321	.51	.48
rs10508372	10	8972018	*LOC338591*	–	–	–	–
rs7922491	10	53493473	*PRKG1*	A(G)	.1302	.60	.86
rs479844	11	65551957	*OVOL1*	C(T)	.4583	.35	.93
rs7130588	11	76270683	*LRRC32*	G(A)	.2928	.082	.049
rs1701704	12	56412487	*IKZF4*	G(T)	.1189	.78	.81
rs744910	15	67446785	*SMAD3*	G(A)	.4072	.21	.33
rs7216389	17	38069949	*ORMDL3*	C(T)	.2774	.60	.33
rs2164983	19	8789381	*ACTL9*	A(C)	.0758	.55	.53
rs4821544	22	37258503	*NCF4*	C(T)	.2453	.39	.49
rs2284033	22	37534034	*IL2RB*	G(A)	.4809	.09	.32

Note: Bold type indicates the most statistically significant result.

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Table 2. Asthma heritability estimates based on pedigree and SNP-derived kinship matrices, and contribution of rs2786098 to diagnosis of asthma

Program	Model	Pedigree h^2 (95% CI)	Total SNP h^2	rs2786098*A		*p*-value
				β	h^2	
MCMCglmm	MFT	.51 [.21, .76][a]	–			
		.31 [0, .63][a]		1.05 (.30)		7×10^{-4}

[a]Highest posterior density (HPD) interval.

the A frequency is usually ~22%, but is ~8% in African-descended populations.

For a PRS based on all these asthma candidate genes and the estimates from the GABRIEL asthma meta-analysis, unaffected individuals had a significantly higher mean PRS than asthmatics (*coxme* $p = .02$). This is not due to rs2786098, because in the GABRIEL meta-analysis, over all studies this SNP was not associated with asthma ($OR = 1.04$).

Conclusions

Among the candidate SNPs for asthma that we tested, the most strongly disease-associated allele was rs2786098*A, with a large effect size (allelic $OR = 2.6$) in the Tristan da Cunha population. The SNP rs2786098 on chromosome 1q31 was originally reported to be associated with childhood asthma in 2010 by Sleiman et al. (2010). The major C allele was increased in asthmatics in the European origin discovery (with an allele frequency of 8D1C 5% vs. 78% in controls) and replication (82% vs. 77%) sets, but the direction of association was reversed in African–Americans (90% vs. 93%, $p = 4 \times 10^{-5}$). Those authors reported the association peak included multiple additional SNP spanning an interval of 400 kbp, and flagged the *DENNDB1* in this interval as a plausible candidate, given that it was known to be a TNF-α binding protein, and expressed in particular dendritic cell subtypes.

In several subsequent large GWAS meta-analyses (Demenais et al., 2018; Moffatt et al., 2010; Zhu et al., 2018), there was no evidence of association of rs2786098 to asthma. For example, analyzing 15,500 (doctor-diagnosed) asthmatics and 106,000 controls from the UK Biobank, the allelic odds ratio for rs2786098*A is .9999, with a 95% confidence interval (95% CI [.9962, 1.0036]; Canela-Xandri et al., 2018). Very recently, Ortega et al. (2019) have reported a relationship between a coding change (rs35173535) in *DENND1C* and acute exacerbations of chronic obstructive pulmonary disease ($p = 7.4 \times 10^{-7}$), which they thought to further implicate this family of genes in lung disease.

Despite these unpromising epidemiological replication results, Yang et al. (2016) have shown that Th2 cells from rs2786098*A carriers of European ancestry produce significantly more *DENNDB1* mRNA and protein than those from C/C carriers, as well as lower amounts of IL-4 and IL-13 production following T-cell receptor stimulation. These effects were replicated in rs2786098*A transfection in C/C Th2 cells and siRNA *DENNDB1* knockdown in A/A Th2 cells. In *Dennd1b knockout* mice (close in biochemical effect to the human C/C genotype), there is a parallel increase in airway inflammation following allergen challenge. These authors thought this implied that the variable population level association between rs2786098 and asthma might reflect strong modification by covariates such as age or asthma subtype. One would expect the environment of

Table 3. Results of Cox proportional hazards mixed model (R *coxme* package) for association between rs2786098 and age at onset of asthma

Model term	β	Exp (β)	*SE* (β)	Wald *z*	Likelihood ratio test *p*-value
PC1 (linear)	.09	1.1	2.52	.03	1.6×10^{-4}
PC1 (quadratic)	−7.54	5.3×10^{-4}	2.89	−2.61	
PC2	21.30	1.8×10^{9}	16.67	1.28	.41
PC3	−41.24	1.2×10^{-18}	16.14	−2.55	.037
PC4	10.70	4.4×10^{4}	5.24	2.04	.017
PC5	−6.21	2.0×10^{-3}	5.58	−1.11	.78
rs2786098*A	.95	2.6	.22	4.31	5.5×10^{-5}

Table 4. Allele and genotype frequencies for rs2786098 near *DENNDB1* on Tristan da Cunha and in comparison populations from the 1000 Genomes project

Sample	Total	C/C	A/C	A/A	MAF	HWE *p*
1000G Africa	246	207	38	1	.0813	1.0000
1000G Americas	181	112	59	10	.2182	.5178
1000G Asia	286	171	91	24	.2430	.0244
1000G Europe	379	229	129	21	.2256	.6587
UK Biobank	452,264				.218	
Tristan da Cunha nonasthmatic	172	128	41	3	.1366	1.0000
Tristan da Cunha asthmatic	91	44	37	10	.3132	.6275

Note: MAF = minor allele frequency; HWE = Hardy–Weinberg equilibrium.

Tristan da Cunha differs greatly in terms of risk factor exposures from that of other populations studied, even though, for example, a positive skin prick test to *D. pteronyssinus* house dust mite allergen was seen in 80% of the asthmatics in the sample (Zamel et al., 1996). The latter sensitization is characteristic of atopic asthmatics in most Western environments.

In the Tristan population, we do not see any age interaction effects, and the association is with the A allele that is putatively protective in European populations. Our result does not reach the usual genome wide significance threshold of 5×10^{-8}, and the inclusion of this variant in our analysis was solely based on the original report of association. While the frequency of the rs2786098*A allele in our unaffected controls is close to that in the HGDP African-descended individuals, that in the cases is greater than that in HDGP European samples. We have attempted to exclude ethnic confounding using genetic principal components based on the SNP panel in our association analyses. Given the depth of the pedigree, much confounding from ethnic admixture should have broken down.

We cannot therefore definitely implicate *DENND1B* genotypes in the high rates of asthma in this population, but retain a high index of suspicion of a relationship given the various lines of evidence presented.

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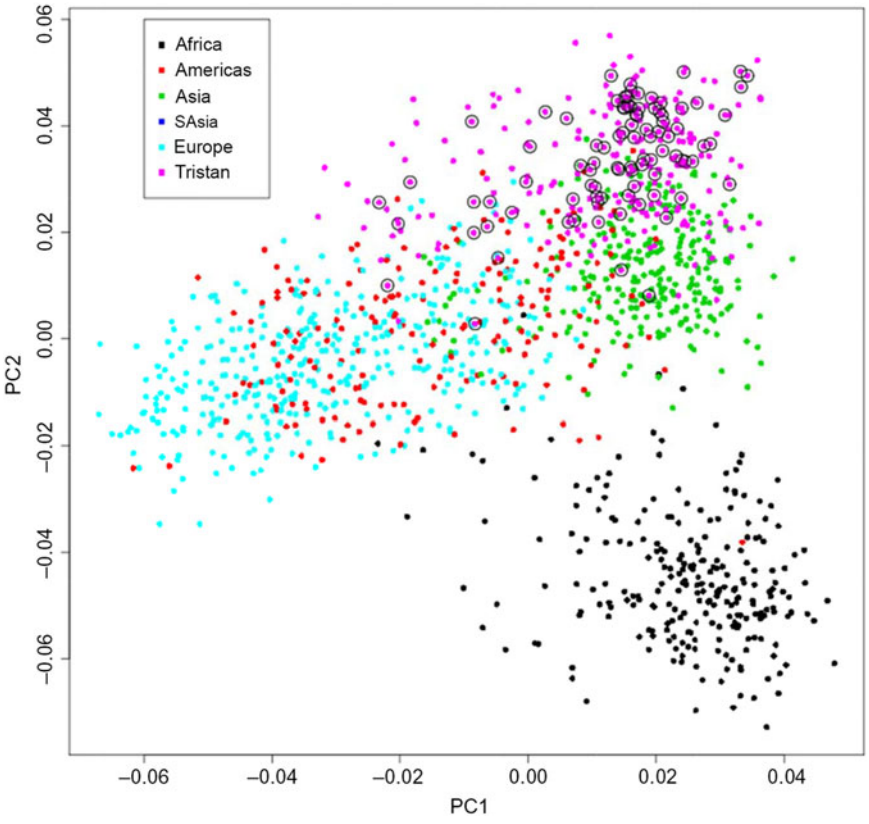

Fig. 1. First and second principal components scores from genetic principal components analysis combining Tristan da Cunha and 1000 Genomes comparison samples for the 43 SNPs. Open circles designate asthma cases.

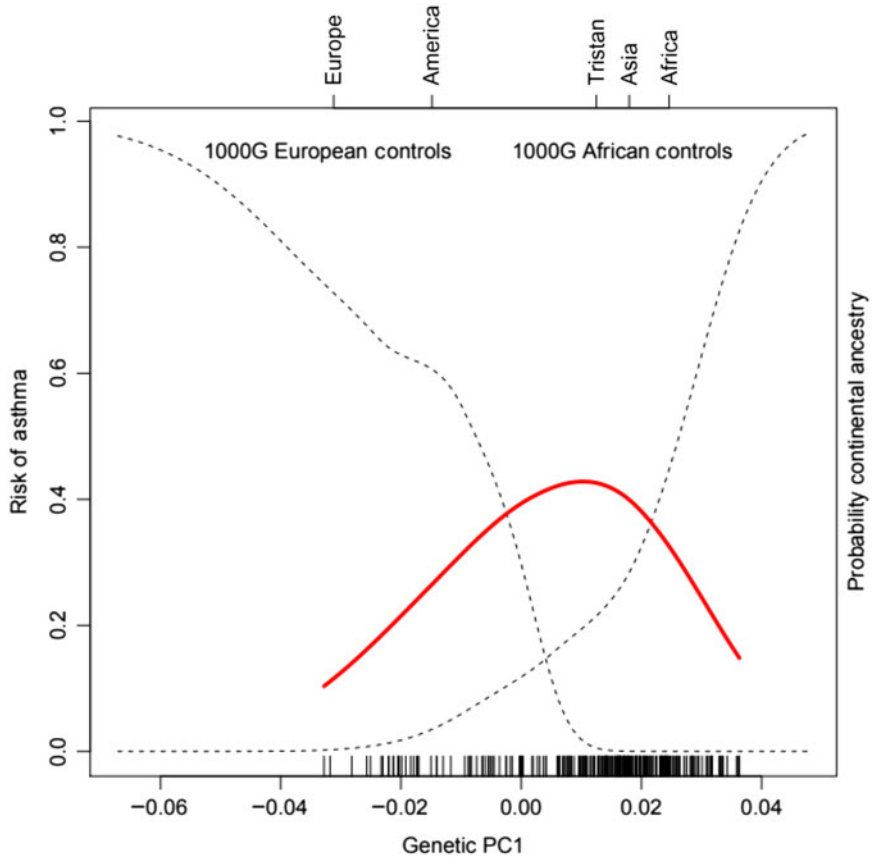

Fig. 2. (Colour online) Risk of asthma (red line) versus average ancestry as measured by first genetic principal component (PC1) for the SNP panel. The mean location of the 1000 Genomes external control populations on PC1 is indicated at the top of the plot, and the two dotted curves represent the probability of membership of the European and African 1000 Genome samples for values of PC1. The smooth non-linear fit for asthma risk is from a localized regression.

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