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Sex-specific linkage to total serum immunoglobulin E in families of children with asthma in Costa Rica

Benjamin A. Raby[1,2,3], Manuel E. Soto-Quiros[4], Lydiana Avila[4], Stephen L. Lake[1,3], Amy Murphy[1,3], Catherine Liang[1], Eduardo Fournier[4], Mitzi Spesny[4], Jody S. Sylvia[1], Andrei Verner[5], Thomas J. Hudson[5], Barbara J. Klanderman[1], Nelson B. Freimer[6], Edwin K. Silverman[1,3] and Juan C. Celedón[1,2,3,*]

[1]Channing Laboratory and Respiratory Disorders Program, Department of Medicine, Brigham and Women's Hospital, 181 Longwood Avenue, Boston, MA 02115, USA, [2]Division of Pulmonary and Critical Care Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA, [3]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA, [4]Division of Pediatric Pulmonology, Hospital Nacional de Niños, San José, Costa Rica, and, [5]Genome Québec Innovation Centre, McGill University, Montreal, Canada and [6]Department of Psychiatry, University of California at Los Angeles, Los Angeles, California

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Serum total immunoglobulin E (IgE) is a critical intermediate phenotype of allergic diseases. Although total IgE exhibits sexual dimorphism in humans (with males demonstrating higher IgE than females), the molecular basis of this difference is unknown. A genome-wide scan of 380 short-tandem repeat (STR) markers was performed in eight extended pedigrees of asthmatic children ($n = 655$) from the Central Valley of Costa Rica. Genome-wide linkage analysis of total IgE was performed by variance component models. Among all subjects, only one genomic region (chromosome 7p15) showed modest evidence of linkage to total IgE (LOD = 1.60). In contrast, a sex-stratified analysis revealed distinct genetic architectures of total IgE in males and females and identified significant linkage to total IgE on a novel male-specific locus on chromosome 20p12 (LOD = 3.63 at 36 cM). Genotyping of additional STRs on chromosome 20 resulted in improved evidence for linkage (LOD = 3.75 at 33 cM) and a 1.5 LOD-unit support interval for the linkage peak between 26 and 38 cM. Three polymorphisms in two genes on chromosome 20p12 (*JAG1* and *ANKRD5*) were then found to be associated with total IgE in 420 nuclear families of Costa Rican children with asthma. Two of these polymorphisms (in *JAG1*) were significantly associated with total IgE in families of boys ($n = 264$) but not in families of girls ($n = 156$) with asthma. *JAG1* is a hematopoetic cell growth factor that may regulate normal B-cell development. This is the first demonstration of a possible genetic basis for differences in total IgE between sexes.

INTRODUCTION

Allergic diseases are a major public health problem in industrialized countries. In individuals with allergic diseases such as atopic asthma, production of interleukin (IL) 4 and IL-13 by T-helper 2 (Th2) cells results in increased production of serum total IgE. Total IgE is recognized as an important intermediate phenotype in the pathogenesis of asthma (1,2), with heritability estimates as high as 0.61 in twin studies (3). However, significant evidence of linkage (LOD $\geq$ 3.3) (4) has been observed in only two of eleven genome-wide linkage analyses of total IgE (on chromosomes 7p14–15 and 7q21) (5,6). Association mapping of the 7p14–15 region and others has identified several genes that may influence both total IgE and asthma, including *GPR154* and *PFH11* (7,8). Nevertheless, a substantial proportion of the genetic variance of total IgE remains unexplained.

Although population surveys repeatedly demonstrate higher total IgE in males than in females (9–12), the etiology of this difference is not clear. Early reports implicated higher rates of

*To whom correspondence should be addressed. Tel: +1 6175250964; Fax: +1 6175250958; Email: juan.celedon@channing.harvard.edu

smoking among men as a cause, but subsequent surveys in larger populations confirmed sex differences in total IgE after adjustment for smoking status and found that these differences are also observed among lifelong nonsmokers (11,13). Although it has been suggested that hormonal immuno-regulatory effects explain the sexual dimorphism of total IgE (14), empirical evidence for these effects is lacking. Because sex differences in total IgE are established early in life (12,15,16), they may be due to prenatal factors, including genetics.

Sex-specific linkage has been demonstrated for complex traits in both animal models (17–19) and humans (20,21). In a recent study of an isolated population of European descent (the Hutterites), there were sex-specific differences in heritability or linkage for 12 of 17 sexually dimorphic quantitative phenotypes (22). Of note, sex-specific linkage to total IgE was not detected in that study, perhaps because the population was not specifically ascertained through atopic probands.

Most of the ~2.85 million current residents of the Central Valley of Costa Rica descend from ~4000 individuals counted in the census of 1697. Studies of genome-wide background linkage disequilibrium (LD) (23) and genetic demography (24) suggest that the population of the Central Valley is a genetic isolate of predominantly mixed Spanish and Amerindian ancestry that experienced rapid growth. Moreover, the prevalence of asthma in Costa Rica is among the highest in the world (25), making this population highly suited for studies of the genetics of asthma in Hispanics. Herein we report the identification of a novel male-specific locus for total IgE (chromosome 20p12) in eight extended pedigrees (655 subjects, 133 asthmatics) ascertained through young asthmatic probands from the Central Valley. In addition, we provide evidence of association between variants in two candidate genes on chromosome 20p12 (*JAG1* and *ANKRD5*) and total IgE in 420 Costa Rican children with asthma and their parents.

RESULTS

Characteristics of members of extended families of children with asthma

Total serum IgE levels were missing for 12 of the 667 members of extended pedigrees with genotypic data. Table 1 shows the characteristics of the 655 members of the eight families included in the linkage analysis. There was significant variability among participating families with regard to number of members, total IgE and percentages of ever-smokers and subjects with asthma.

Sexual dimorphism and estimates of heritability for total serum IgE

We compared the distribution of total IgE in males and females (Fig. 1). As expected, total IgE exhibited sexual dimorphism ($P = 0.001$), with consistently higher levels in males than females across pedigrees (with the exception of pedigree 5, which had only one male member—the father). The estimated narrow-sense heritability (h^2_N) of total IgE was influenced by sex. Among all subjects, the estimated h^2_N

Table 1. Characteristics of members of extended pedigrees of asthmatic children in Costa Rica

Family	Number of subjects (% of males)	Mean age, yrs (range)	Former and current smokers, *n* (%)[a]	Asthma, *n* (%)[b]	Total serum IgE (IU/ml) geometric mean (interquartile range)
1	34 (52.9)	43.9 (8.5–91.6)	8 (23.5)	12 (35.3)	91.7 (24.3–412.0)
2	20 (45.0)	33.7 (11.1–76.0)	4 (21.1)	8 (40.0)	129.2 (41.5–513.4)
3	230 (41.3)	29.2 (6.1–77.9)	39 (17.0)	57 (24.9)	209.9 (70.2–729.0)
4	103 (40.8)	32.1 (6.2–77.0)	19 (18.5)	17 (16.5)	66.7 (23.6–151.0)
5	8 (12.5)	36.0 (9.7–71.6)	5 (62.5)	2 (28.6)	75.5 (18.3–382.4)
6	109 (45.0)	29.1 (7.0–71.5)	10 (9.3)	9 (8.3)	28.0 (9.4–83.1)
7	23 (56.5)	32.2 (9.0–71.7)	9 (39.1)	5 (21.7)	101.1 (24.0–347.0)
8	128 (55.5)	28.4 (6.1–88.8)	12 (9.4)	23 (18.0)	91.1 (34.3–243.5)
All	655 (45.5)	30.1 (6.1–91.6)	106 (16.2)	133 (20.3)	96.7 (27.4–342.0)

[a]Of the 655 participating subjects, 3 lacked data on smoking status and 3 lacked information on asthma.
[b]Physician-diagnosed asthma and wheezing in the previous year.

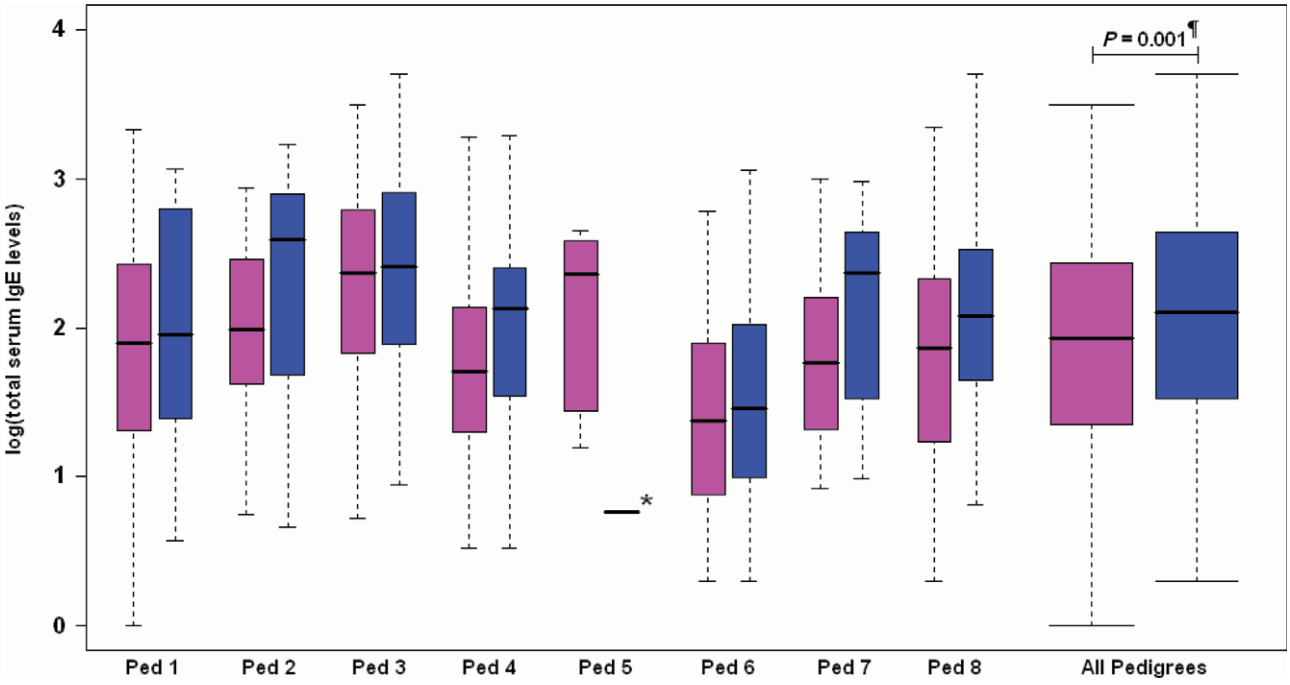

Figure 1. Sex-specific distributions of total serum IgE levels in eight Costa Rican asthma pedigrees. Box-plots of female (pink) and male (blue) log-transformed total serum IgE levels are presented for each family pedigree and for all pedigrees. Only one male (a father) was available for analysis in Pedigree 5 (*). ¶*P*-value for comparison of male and female total serum IgE levels including all subjects, adjusted for age.

of total IgE (adjusted for sex and age) was 0.57 (standard error [SE] = 0.065; $P = 1.1 \times 10^{-35}$), consistent with reports in other populations (26). After stratification by sex, however, the heritability of total IgE was substantially higher in males ($h^2_N = 0.83$, SE = 0.10; $P = 1.1 \times 10^{-16}$) than in females ($h^2_N = 0.63$, SE = 0.10; $P = 1.4 \times 10^{-15}$). To our knowledge, this male-specific estimate of narrow-sense heritability for total IgE is the highest ever observed and suggests that, in these families, total IgE is under strong genetic regulation in males.

Genome-wide linkage analysis of total IgE

Total IgE was $\log_{10}$-transformed to approximate a normal distribution (residual kurtosis = −0.53). To assess for sex-specific linkage, we performed variance component linkage analyses in all subjects (adjusted for age and sex) and then separately in males and females (adjusted for age; Fig. 2 and Table 2). Among all subjects, there was only modest evidence of linkage to total IgE (highest LOD = 1.60 on chromosome 7p15, at 23 cM). In contrast, the sex-stratified analysis revealed distinct genetic architectures of total IgE in males and females and showed significant linkage of a novel male-specific locus on chromosome 20p12 to total IgE (LOD = 3.63 at 36 cM). In total, there was suggestive or significant evidence of linkage to total IgE for four distinct loci (chromosomes 3q21 and 7p21 in females, and chromosomes 17q12 and 20p12 in males; Fig. 3), of which only two (on chromosomes 7p21 and 20p12) would have been identified in the analysis of all subjects using a liberal LOD score threshold of 1.0. Although smoking-related covariates were not significantly associated with total IgE, we repeated the analyses including covariates with $P < 0.25$ (ever-smoking) because of the known relationship between smoking and increased total IgE and the higher prevalence of smoking in men than in women (11,13). These adjustments consistently resulted in similar, yet stronger evidence for linkage. For example, the LOD score for the male-specific locus on chromosome 20p12 increased from 3.63 to 3.81 after additional adjustment for ever-smoking.

Fine-mapping linkage analysis of total IgE on chromosome 20p

After genotyping 26 additional STR markers on chromosome 20p, a repeat analysis showed slightly improved evidence of linkage to total IgE in males (LOD = 3.75 at 34 cM), with a relatively narrow 1.5 LOD-unit support interval for the linkage peak (26 to 38 cM, corresponding to a physical distance of ∼5.28 Mb) (Fig. 4). Interestingly, this analysis also resulted in the emergence of a second linkage peak at 65 cM (LOD = 3.09, 1.5 LOD-unit support interval from 59 to 79 cM). Similar to the results with the original marker set, there was no evidence of female-specific linkage to total IgE across chromosome 20.

Examination of alternative explanations for linkage findings

Because the Central Valley population is a genetic isolate, we evaluated whether founder effects could explain the linkage to total IgE on chromosome 20p12 in males. Whereas five

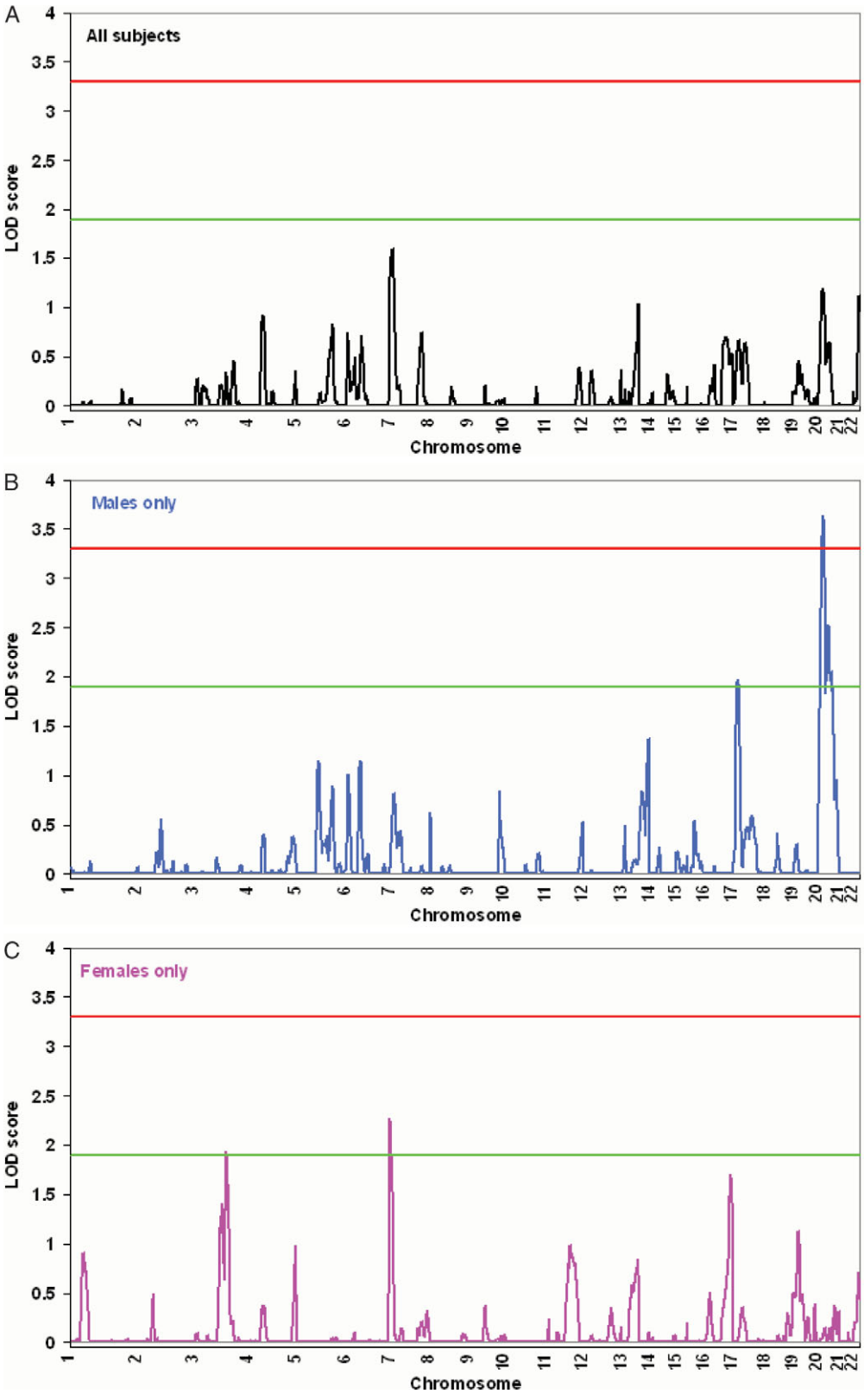

Figure 2. Results of genome-wide multipoint linkage analysis for total serum IgE levels in eight Costa Rican asthma pedigrees. Results presented for all subjects (panel a) and after stratification by sex (panels b and c). Green and red lines denote suggestive (LOD $\geq$ 1.90) and significant (LOD $\geq$ 3.3) LOD scores, as suggested by Lander and Kruglyak (4). Total serum IgE was $\log_{10}$- transformed for analysis, and final models were adjusted for covariates significant at $P < 0.05$ (age and sex for the analysis of all subjects, and age for the analysis of males and females).

Table 2. Genome-wide linkage analysis[a] of total serum IgE[b]

Chromosome	Location (cM)	All subjects[c]		Males		Females	
		LOD score[d]	Empirical *P*-value	LOD score[d]	Empirical *P*-value	LOD score[d]	Empirical *P*-value
3	127	—		—		1.40	0.02
	146	—		—		1.93	0.002
5	105	—		1.15	0.01	—	
6	23	—		1.00	0.02	—	
	73	—		1.15	0.01	—	
7	12	1.16	0.01	—		2.27	0.0007
	23	1.60	0.004	—		1.59	0.004
13	79	1.03	0.02	—		—	
	127	—		1.37	0.006	—	
16	129	—		—		1.70	0.003
17	34	—		1.98	0.001	—	
20	36	1.16	0.01	3.63	<0.0001	—	
22	57	1.11	0.01	—		—	

[a]Multipoint linkage analysis by variance component models, as implemented in the SOLAR program.
[b]Transformed to logarithmic scale for data analysis.
[c]All models were adjusted for covariates significant at $P < 0.05$ (age and sex for all subjects, and age for analysis of males and females).
[d]Multipoint LOD scores greater than or equal to 1 are reported.

families demonstrated evidence of male-specific linkage to total IgE—Pedigrees 1 (LOD = 0.31), 3 (LOD = 1.22), 4 (LOD = 0.37), 6 (LOD = 1.00) and 7 (LOD = 1.14)—, three families did not (LOD = 0)—Pedigrees 2, 5 (only one male) and 8. Among the five families demonstrating linkage to total IgE, there was no evidence of excess allele sharing for those markers within the 1.5 LOD-unit support interval for the linkage peak. Specifically, there was no allele or haplotype shared by most probands in families with linkage to chromosome 20 (data not shown), suggesting that the observed linkage is not due to a common ancestral haplotype.

We also examined whether reduced statistical power could explain the lack of significant evidence of linkage to total IgE among all members of our extended pedigrees. Given an estimated h^2_N of 0.57 and a single quantitative trait locus (QTL) for total IgE, our power to detect a LOD score ≥ 3 was $>99\%$. Even if we assumed that total IgE was controlled by two QTLs, each with trait-locus h^2_N of 0.28, our power to detect a LOD score ≥ 3 would be $>80\%$. It is plausible, however, that our power could have been reduced by genetic heterogeneity (different QTLs influencing total IgE in males and females), which was minimized after stratification by sex.

Positional candidate-gene association studies

Of the 16 known genes mapping to the male-specific locus for total IgE on chromosome 20p12, two were of particular interest: jagged 1 precursor (JAG1) and ankyrin repeat domain protein 5 (ANKRD5). JAG1, an important growth factor implicated in hematopoietic stem cell self-renewal (27,28), is within 0.5 Mb of the STR demonstrating maximal linkage to total IgE (D20S189). JAG1 is expressed in mature B-cells (29), and is a principal ligand for the NOTCH1 receptor, which (in conjunction with Delta-1) has been implicated in lymphocyte precursor stem cell differentiation into T- or B-cell lineages (30,31). ANKRD5 contains eight ankyrin repeat domains and a calcium binding EF-hand domain, and is expressed in both the bone marrow and spleen (32). STR marker D20S162—adjacent to D20S189 and strongly linked to total IgE (LOD = 3.1)—resides within the 3′ untranslated region of *ANKRD5*.

We tested for genetic association between variants in *JAG1* and *ANKRD5* and total IgE in 420 unrelated asthmatic children from the Central Valley of Costa Rica and their parents. Of the 420 index children (ranging in age from 6 to 14 years), 264 (62.9%) were male. Among index children, boys (geometric mean [IQR] = 387.6 IU/ml [163.0–1038.0 IU/ml]) had higher total IgE than girls (geometric mean [IQR] = 263.3 [82.3–809.4]) (age-adjusted P for comparison = 0.008).

We selected 35 single nucleotide polymorphisms (SNPs) in *JAG1* and *AKRD5* for our association studies. Of these 35 SNPs, 33 SNPs were successfully genotyped, each with $>98\%$ completion rates and few pedigree inconsistencies. There was no evidence of LD between the two genes (Online Supplemental Material, Fig. 2). Table 3 shows the results of the family-based analysis of association between SNPs in candidate genes and total IgE. Of the 16 SNPs in *JAG1*, only one (rs6040069) was significantly associated with total IgE in all families ($P = 0.01$). After stratification by gender of the index children, two SNPs in *JAG1* (rs6040069 and rs6040060) were significantly associated with total IgE in families of boys ($P = 0.02$ and $P = 0.01$, respectively). There was no significant association between SNPs in *JAG1* and total IgE in families of girls. Of the 17 SNPs in *ANKRD5*, only one (rs559129) was significantly associated with total IgE in all families ($P = 0.03$). After stratification by gender of the index children, three SNPs in *ANKRD5* were significantly associated with total IgE (rs4813909, rs631317 and rs663380) in families of girls ($P \leq 0.05$ in all cases), and one SNP in *ANKRD5* (rs559129) was significantly associated with total IgE in families of boys ($P = 0.04$). Family-based haplotypic association analysis supported the results of the analysis of single SNPs (Table 4). For *JAG1*, several contiguous 3-SNP haplotype windows demonstrated evidence of association with total IgE in

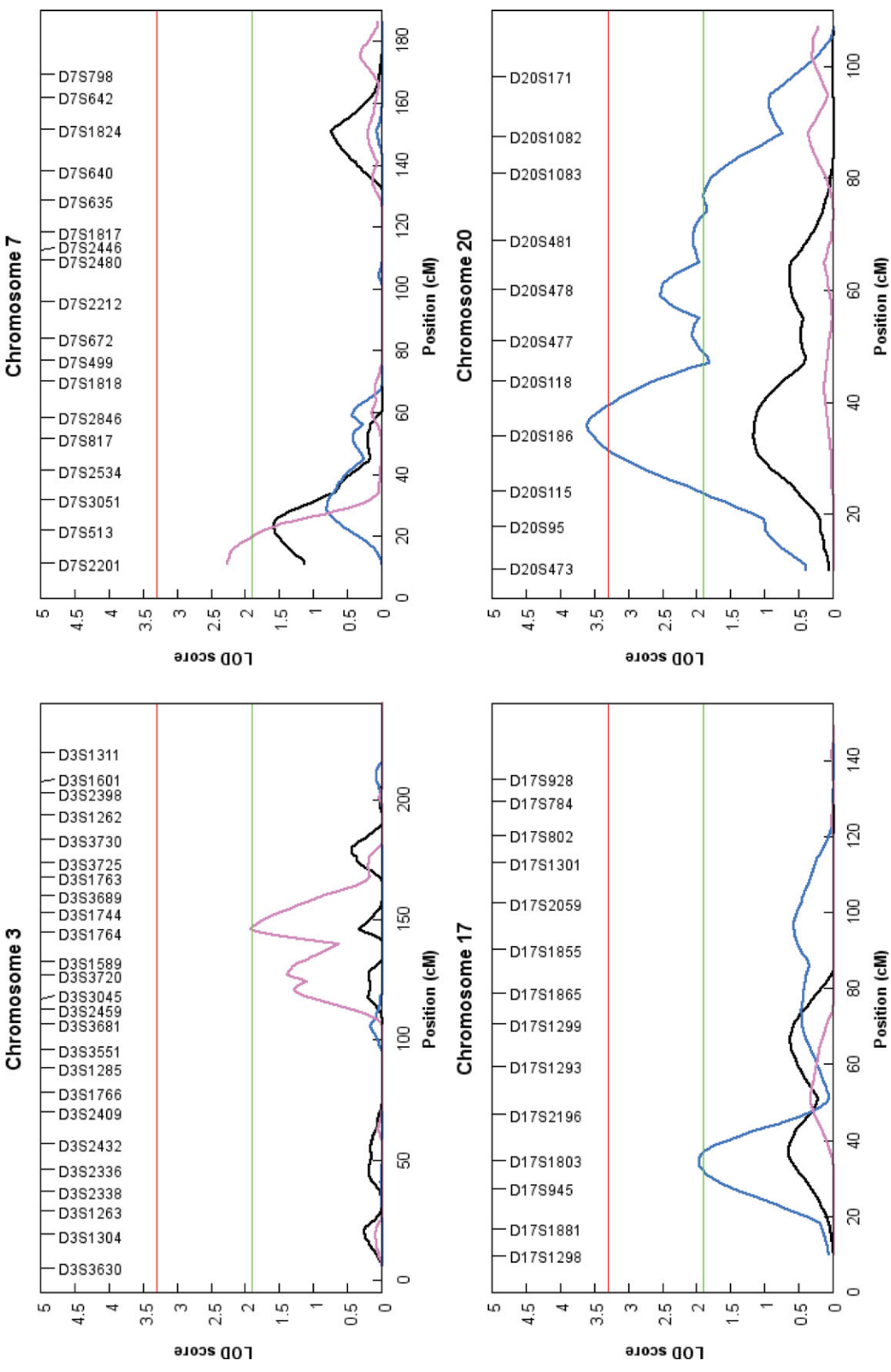

Figure 3. Results of sex-specific analysis for chromosomal regions demonstrating suggestive (LOD $\geq$ 1.90, green line) and significant (LOD $\geq$ 3.3, red line) evidence of linkage to total serum IgE in all subjects (black line), females (pink line) or males (blue line). The relative position of short-tandem repeat markers that were genotyped for each chromosome are displayed. All analyses were adjusted for covariates significant at $P < 0.05$ (age and sex for the analysis of all subjects, and age for the analysis of males and females). Total serum IgE was $\log_{10}$-transformed for analysis.

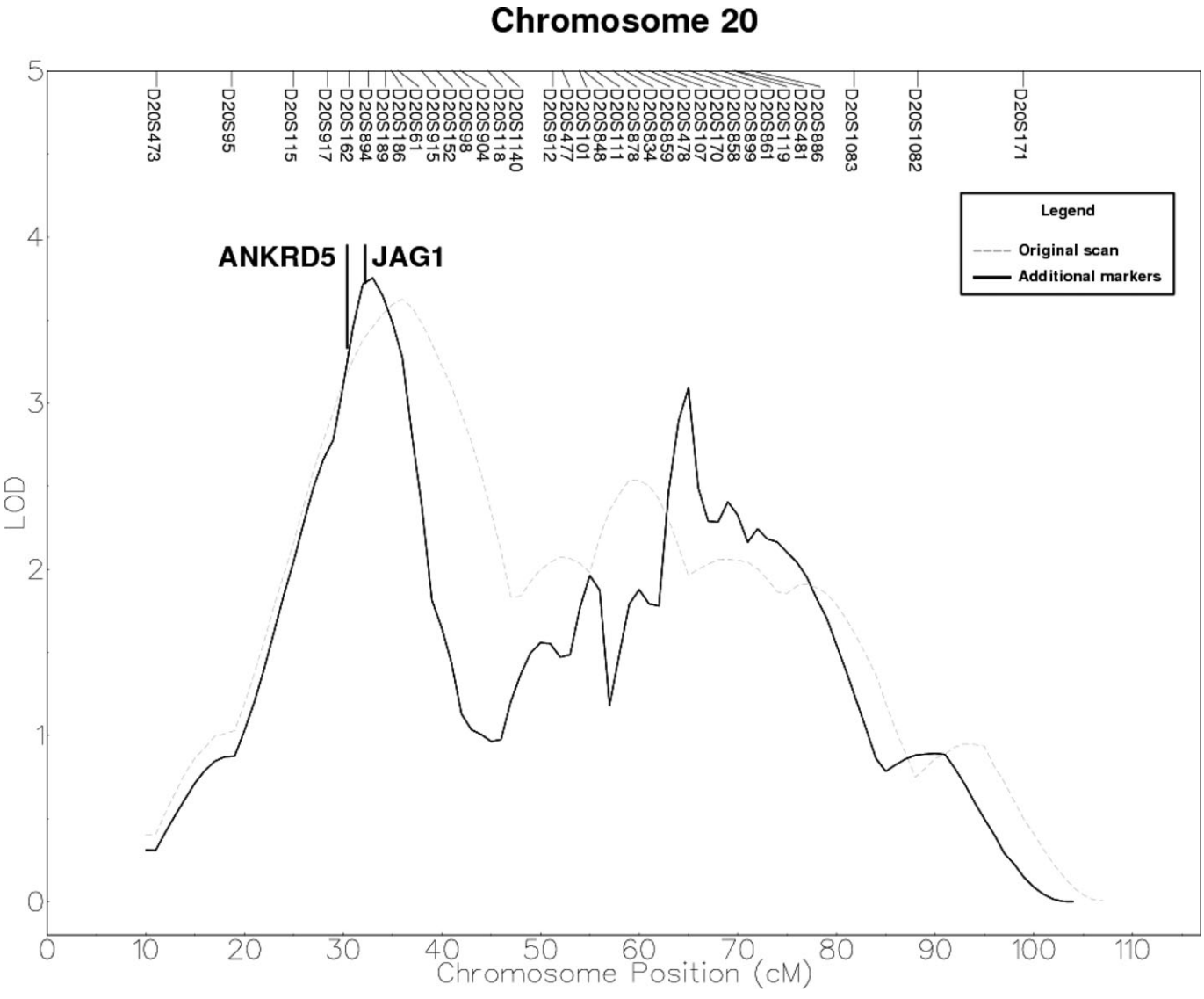

Figure 4. High-resolution linkage mapping with additional STR markers resulted in narrowing of male-specific total IgE locus on chromosome 20p12 at 34 cM and emergence of a second peak at 65 cM. The relative location of two positional candidate genes is demonstrated: *ANKRD5* (ankyrin repeat domain protein 5) and *JAG1* (jagged-1 precursor).

families of boys, whereas no haplotypic associations with total IgE were observed in families of girls. For *ANKRD5*, there was little evidence of haplotypic association with total IgE in families of boys, yet some evidence of haplotypic association was observed in families of girls. None of the single SNP or haplotypic associations was significant after correction for multiple comparisons.

DISCUSSION

In a genome-wide linkage analysis of extended pedigrees of asthmatic children in Costa Rica, we found no suggestive or significant evidence of linkage to total IgE. Because of sexual dimorphism of total IgE, we repeated the analysis after stratification by sex. We found significant (chromosome 20p12 in males) or suggestive (chromosomes 3q21 and 7p21 in females, and chromosome 17q12 in males) evidence of linkage to total IgE on four genomic regions. To our knowledge, this is the first report of significant evidence of linkage to total IgE or any intermediate phenotype of asthma on chromosome 20p12. An adjacent genomic region on chromosome 20p (20p13) that does not overlap with the 1.5 LOD-unit support interval for our peak LOD score for total IgE in males showed significant evidence of linkage to asthma in Caucasian families in the USA and the UK (33). In that study, there was suggestive evidence of linkage to asthma and elevated total IgE (LOD = 2.3) but no separate analysis of total IgE.

Chromosome 20p12 has recently been linked to systemic lupus erythematosus (SLE), an autoantibody-mediated disease (34), suggesting a possible shared genetic determinant of B-cell antibody production for asthma and SLE. Consistent

Table 3. Results of family-based association analysis of candidate genes on chromosome 20p12 and total IgE in 420 Costa Rican children with asthma and their parents

Gene	Marker	Position (bp)	Allele	MAF (parents)	Genetic Model	PBAT *P*-value		
						All families ($n = 420$)	Families of boys ($n = 264$)	Families of girls ($n = 156$)
JAG1	rs6040060	10588201	A	0.186	dom	—	0.01	—
					add	—	0.04	—
	rs6040069	10593197	A	0.182	dom	0.01	—	—
					dom	—	0.02	—
ANKRD5	rs4813909	9971689	A	0.371	dom	—	—	0.03
	rs6141102	9979138	G	0.223	add	—	—	0.05
	rs559129	9989304	C	0.402	dom	0.03	—	—
					dom	—	0.04	—
	rs631317	9990319	C	0.180	dom	—	—	0.03
	rs663380	9992849	G	0.267	dom	—	—	0.04

All results are adjusted for age. Analysis in all families also adjusted for sex. dom = dominant model, add = additive model. For clarity, only markers with $P \leq 0.05$ are presented.

Table 4. Results of family-based haplotypic association analysis of candidate genes on chromosome 20p12 and total IgE levels in Costa Rican children with asthma and their parents

Markers	Alleles	Haplotype frequency	Model	All families ($n = 420$)	Families of boys ($n = 264$)	Families of girls ($n = 156$)
JAG1						
rs6040055-rs2273060-rs6040060	C:G:G	0.467	dom	—	0.04	—
rs2273060-rs6040060-rs7269017	A:A:A	0.169	dom	0.03	0.005	—
rs2273060-rs6040060-rs7269017	A:A:A	0.169	dom	—	0.005*	—
rs6040060-rs7269017-rs11907050	A:A:A	0.175	dom	—	0.01	—
rs17536052-rs6040068-rs6040069	G:C:A	0.173	dom	0.009	0.006	—
rs6040068-rs6040069-rs7271215	C:A:G	0.173	dom	0.009	0.006	—
rs6040069-rs7271215-rs910119	A:G:T	0.164	dom	0.003*	0.009	—
ANKRD5						
rs582827-rs641648-rs4813909	C:G:A	0.336	dom	—	—	0.03
rs641648-rs4813909-rs6039663	G:G:G	0.314	dom	—	—	0.02
rs4813909-rs6039663-rs656111	A:G:C	0.329	dom	—	—	0.01
rs4813909-rs6039663-rs656111	G:G:C	0.114	dom	—	0.04	—
rs6141102-rs681750-rs6087123	G:G:C	0.208	dom	—	—	0.03
rs559129-rs631317-rs6039665	C:T:A	0.298	dom	0.05*	—	—
rs631317-rs6039665-rs6516548	T:A:A	0.811	add	—	—	0.04
rs6039665-rs6516548-rs663380	A:A:G	0.264	dom	—	—	0.05*
rs6516548-rs663380-rs674630	A:G:T	0.244	dom	—	—	0.04*

* denotes haplotypes groups with global (multiallelic) $P < 0.05$. All results are adjusted for age. Analysis in all families also adjusted for sex. dom = dominant model, add = additive genetic model. Haplotypes with $P \leq 0.05$ and haplotype frequency > 0.05 are presented.

with this possibility, we have demonstrated preliminary evidence suggesting male-specific associations of total IgE with polymorphisms in *JAG1*, a principal ligand of NOTCH1 that has been implicated in hematopoetic stem cell differentiation and B-cell development (27,31). While the observed SNP associations were of borderline statistical significance and could thus be due to chance, and the lack of association in females could be due to reduced statistical power in the relatively small group of families of girls, they are intriguing and merit further exploration in other cohorts. Weaker associations were demonstrated for SNPs in the second positional candidate gene tested—*ANKDR5*. Like the associations with *JAG1*, no findings were significant following correction for multiple comparisons. Moreover, the lack of association in the larger subset of male trios for all but one marker suggests that variants in this gene are unlikely to explain the male-specific linkage observed in the extended pedigrees.

This study is the largest genome-wide linkage analysis of total IgE in Hispanics and the first in a Hispanic population in Latin America. Although asthma is a significant cause of morbidity in Hispanics (35), only one genome scan for asthma or its intermediate phenotypes (conducted by the Collaborative Study on the Genetics of Asthma [CSGA]) has included Hispanic individuals (36–38). The CSGA (36), which included 205 subjects in 32 families of siblings with asthma in New Mexico (USA), did not find any genomic region showing suggestive or significant evidence of linkage

to total IgE in Hispanics (highest LOD scores for total IgE: 1.51 on chromosome 9q and 1.48 on chromosome 12p). In addition to differences in statistical power and lack of sex-stratified analysis in the CSGA, potential reasons for the discrepant findings between that study and ours are differences in ethnic composition and environmental exposures.

Most—but not all—Hispanics have variable proportions of European, Amerindian and African ancestry (39), and there is marked variation in asthma morbidity among Hispanic subpopulations. To reduce this heterogeneity, we are studying the genetics of asthma in a relatively homogeneous Hispanic population in the Central Valley of Costa Rica. Total IgE levels in this population do not reflect parasitic infection, as the prevalence of helminthiasis in Costa Rica is very low (40) because of good hygienic conditions and widespread use of antihelminthic treatment (41). We found no evidence of helminthiasis after examination of single-stool specimens for ova and parasites in 137 of the 420 Costa Rican children with asthma included in our association studies (unpublished data).

In conclusion, we have identified different genetic architectures of total IgE in males and females, as well as a novel male-specific locus for total IgE on chromosome 20p12. Our findings provide a possible genetic basis for the observed sex-related differences in total IgE and should motivate sex-specific linkage analysis of total IgE in other populations. We have also shown preliminary evidence of male-specific association of polymorphisms in *JAG1* with total IgE. We will follow-up these results with high-resolution SNP association mapping across chromosome 20p12 to attempt to identify the variant(s) responsible for the observed male-specific linkage. It is interesting to note that sexual dimorphism in total IgE have also consistently been observed in BALB/c murine models of pulmonary allergic response, with higher levels in female mice (42,43). Due to a lack of similar data in other strains, it is unclear whether this difference is strain- or species-specific. It is conceivable that the QTL underlying this response in the BALB/c strain overlaps with the female-specific loci identified here. Future comparative mapping approaches should consider these effects.

MATERIALS AND METHODS

Study population

Adult participants gave written informed consent. Written assent and parental consent were obtained for participating children. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, MA).

Families were recruited as part of an ongoing study of asthma genetics in Costa Rica. For the extended pedigrees, probands were identified from index children in this genetics study ($n = 7$) and from children who participated in Phase II of the International Study of Asthma and Allergies in Childhood ($n = 1$) in Costa Rica (44). Enrollment criteria for these eight probands included age ≥ 6 years but < 15 years, physician-diagnosed asthma, at least two respiratory symptoms (cough, wheezing, or dyspnea) or a history of asthma attacks in the previous year, increased airway responsiveness (a dose of methacholine causing a 20% decline in post-saline FEV_1 [PD_{20}] ≤ 8.58 μmoles), ≥ 1 sibling with physician-diagnosed asthma, and at least six great-grandparents born in the Central Valley of Costa Rica. (45) After selection of a proband, all of his/her first- and second-degree relatives who were ≥ 6 years old were invited to participate. Families were further extended through affected relatives by including all of their first-degree relatives who were ≥ 6 years old. For the family-based association studies, children ages 6 to 14 years were eligible for inclusion as probands if they had physician-diagnosed asthma, ≥ 2 respiratory symptoms or a history of asthma attacks in the previous year, and high probability of having ≥ 6 great-grandparents born in the Central Valley (as determined by the study genealogist). All study participants completed a protocol that included a questionnaire, collection of blood samples for DNA extraction, and measurement of serum total IgE, except for parents of probands in the family-based association studies (who only provided DNA).

Questionnaire

Each participant completed a slightly modified version of the questionnaire used in the CSGA (46), which was translated into Spanish. The questionnaire was used in two forms, one for adolescents and adults (> 12 years of age) and one for children (≤ 12 years of age). All children were considered non-smokers. Pack-years of cigarette smoking were calculated as the product of the duration of smoking (in years) and the average number of cigarettes smoked per day, which was divided by 20 to convert to packs.

Measurement of serum total IgE

Serum total IgE levels were determined by the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, MI), with samples measured in duplicate. All values were transformed to a $\log_{10}$ scale for statistical analysis (47).

Genotyping and data management

The Genome Quebec Innovation Centre performed genome-wide genotyping in 671 individuals with Applied Biosystems (AB, Foster City, California) 3700 and 3730 analyzers using DNA that was extracted from blood samples by Puregene Kits (Gentra Systems). 380 autosomal short-tandem repeat (STR) markers with an average spacing of 8.2 cM (ranging from 6.2 cM on chromosome 22 to 9 cM on chromosome 17) were genotyped. An additional 18 STR markers (Figs 2 and 3) were genotyped at the Channing Laboratory (Online Supplementary Material). All marker locations were determined using the deCODE map (48).

The RELPAIR program was used to determine pedigree relationships on the basis of the genome scan marker data (49,50). Four subjects failed to match reported familial relationships and were excluded from the analysis. Mendelian inconsistencies at individual markers were resolved by the PEDCHECK program (51). Pedigree genotype inconsistencies were observed on average $< 0.5\%$ per STR.

Genotyping of SNPs in candidate genes was performed using the Sequenom I-Plex platform (33 SNPs) and by Taqman 5'-nuclease assays (2 SNPs); protocol details are available in the Online Supplementary Data. SNPs were selected from those genotyped for the International HapMap project if they had a minor allele frequency $\geq$10% among CEPH parents. Repeat genotyping of all SNPs in a random sample of $\sim$5% revealed complete concordance among genotypes. Pedigree genotype inconsistencies identified by PEDCHECK were observed on average $<$0.5% per SNP (49–51).

Statistical analysis

Estimates of h^2_N for total IgE and multipoint genome-wide linkage analysis were performed by variance component models implemented in SOLAR (version 2.12) (52). Because of the size of the pedigrees, multipoint IBD matrices were first estimated by a Markov-Chain Monte Carlo algorithm implemented in Loki (version 2.4.7) (53). Multipoint linkage analysis using the estimated IBD was then performed in SOLAR. To simplify the linkage analysis and focus on genetic effects that are transmitted across generations, we assumed that the genetic loci influencing total IgE (a complex trait) only had additive effects. Covariates considered in the linkage analyses included sex, age, ever-smoking, pack-years of cigarette smoking, age^2 and pack-years^2, with those covariates that were significant ($P < 0.05$) retained in the final models. Confirmatory analyses including covariates significant at $P < 0.25$ were then conducted. Empirical *P*-values for the observed multipoint LOD scores were estimated by simulations in SOLAR (54). Genotypes for a fully informative marker were created, and the evidence for linkage was examined in 100 000 replicates. Empirical *P*-values were calculated by comparing the observed multipoint LOD score with the empirical distribution of simulated LOD scores. In addition, 1000 simulations were ran in SOLAR to assess our statistical power to detect linkage (LOD $\geq$3) to one or two bi-allelic QTLs for total IgE in all members of the Costa Rican pedigrees, assuming fully informative marker data for study subjects.

Pair-wise estimates of LD (r^2) and haplotype-block analysis (Gabriel definition (55)) of SNPs in candidate genes were performed using Haploview (56). Sex-stratified family-based single-SNP and haplotypic association analyses of total IgE were performed using PBAT, with adjustment for age (57). A grouped analysis of males and females, adjusted for age and gender, was performed for comparison with the sex-stratified analysis. The haplotypic analysis was performed using a 3-SNP sliding window. Tests for global haplotype significance were performed using the multiallelic test in PBAT.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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