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Genome-wide search for asthma susceptibility loci in a founder population

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Founder populations offer many advantages for mapping genetic traits, particularly complex traits that are likely to be genetically heterogeneous. To identify genes that influence asthma and asthma-associated phenotypes, we conducted a genome-wide screen in the Hutterites, a religious isolate of European ancestry. A primary sample of 361 individuals and a replication sample of 292 individuals were evaluated for asthma phenotypes according to a standardized protocol. A genome-wide screen has been completed using 292 autosomal and three X–Y pseudoautosomal markers. Using the semi-parametric likelihood ratio χ^2 test and the transmission–disequilibrium test, we identified 12 markers in 10 regions that showed possible linkage to asthma or an associated phenotype (likelihood ratio *P* < 0.01). Markers in four regions (5q23–31, 12q15–24.1, 19q13 and 21q21) showed possible linkage in both the primary and replication samples and have also shown linkage to asthma phenotypes in other samples; two adjacent markers in one additional region (3p24.2–22) showing possible linkage is reported for the first time in the Hutterites. The results suggest that even in founder populations with a relatively small number of independent genomes, susceptibility alleles at many loci may influence asthma phenotypes and that these susceptibility alleles are likely to be common polymorphisms in the population.

INTRODUCTION

The advantages of inbred or founder populations for genetic studies were first noted >30 years ago (1–3). Interest in these populations recently has been renewed as a result of the explosion of molecular biological techniques and the initiation of the Human Genome Project (4–6). The relatively small number of founders and recent ancestries that are characteristic of these populations facilitate the search for human disease genes and make them particularly amenable to novel analytical strategies. Indeed, founder populations have proven useful for mapping genes that underlie Mendelian disorders (e.g. 7–11) and multigenic disorders that segregate as Mendelian conditions in specific populations (12,13). The same features of population history that advanced the search for Mendelian conditions in founder populations should also facilitate the search for genes that influence susceptibility to complex traits.

The Hutterites are a religious sect that originated in the Tyrolean Alps in the 1500s. Between the mid 1700s and mid 1800s, during their tenure in Russia, the population grew in size from ~120 to >1000 members (14). In the 1870s, ~900 of these members migrated to what is now South Dakota, and roughly half settled on three communal farms. Due to a high natural fertility rate and the proscription of contraception among communal Hutterites (15), the population expanded dramatically since migrating to the USA. Today there are >35 000 Hutterites living on >350 communal farms (called colonies) in the northern USA and western Canada. Genealogical records, collected by Steinberg and his students in the 1950s and 1960s (2,16,17), trace all extant Hutterites to <90 ancestors who lived in the early 1700s to the early 1800s (18). The relationships between these ancestors are unknown, but some of them may have been related. The three original South Dakota colonies have given rise to the three major subdivisions of Hutterite population structure, called the Schmiedeleut (S-leut), Dariusleut (D-leut) and Leherleut (L-leut), and members of each leut have remained reproductively isolated from each other since 1910 (17).

The subjects of our studies, the S-leut Hutterites of South Dakota, are descendants of 64 Hutterite ancestors. The average

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coefficient of inbreeding among the subjects in our study is 0.0327 (SD 0.016), slightly greater than that of first cousins once removed. Thus, the small number of ancestral genomes present in the current population should result in a smaller number of asthma susceptibility alleles at any given locus, and possibly a smaller number of loci, than in outbred populations, and facilitate the search for genes underlying this complex phenotype. The relatively recent origins of the population enhance the ability to detect linkage disequilibrium between asthma susceptibility alleles and marker alleles over ~5–10 cM distances (19). Furthermore, the Hutterite communal lifestyle ensures that all members are exposed to a relatively uniform environment. In particular, the Hutterites prepare and eat meals in a communal kitchen and dining room, respectively, and smoking is prohibited (and rare) in the population. Thus, primary or secondary exposure to cigarette smoke does not complicate diagnoses of asthma or lung disease in the Hutterites as it does in other populations. Lastly, the Hutterites have been enthusiastic participants in our genetic studies of fertility (20–22) and epidemiologic studies of pulmonary disease (23–25) for more than a decade.

We present here results of a genome-wide screen for genes that confer susceptibility to asthma and the asthma-associated phenotype, bronchial hyperresponsiveness (BHR), in the Hutterites using two measures of linkage: the semi-parametric likelihood ratio χ^2 test (LR) (26) and the transmission–disequilibrium test (TDT) (27,28).

RESULTS

The clinical characteristics of the primary and replication samples are shown in Tables 1 and 2. The samples and methods of analysis are described in detail in Materials and Methods. Briefly, the evidence for linkage between genome-wide markers and four diagnostic groups was assessed using the LR test (26). The four diagnostic groups were: (1) 'strict' asthma (group D); (2) BHR (groups C + D); (3) asthma symptoms (groups B + D); and (4) 'loose' asthma (groups B + C + D). All markers showing some evidence for linkage by the LR test ($P < 0.01$) were then studied in the primary and replication samples using the TDT (27).

The results of the LR test in the primary sample and the TDT in the primary and replication samples are shown in Table 3. In the primary sample, 12 markers in 10 regions showed some evidence for linkage ($P \leq 0.01$) to specific clinical groups by the LR test; five markers met the criteria for suggestive linkage defined by Lander and Kruglyak (29). Of the 12 markers, four also showed evidence for linkage ($P < 0.05$) by the TDT in the primary sample (*D3S1768*, *D12S375*, *D13S787* and *D19S178*). The specific allele that was over-transmitted at two of these four loci also showed evidence for linkage by the TDT in the replication sample (*D12S375*) or in the pooled primary and replication samples (*D19S178*); alleles at the other two loci (*D3S1768* and *D13S787*) did not show evidence for linkage by the TDT in the replication or pooled samples. Two loci, *D5S1480* and *D21S1440*, showing evidence for linkage by the LR test in the primary sample did not show evidence for linkage by the TDT in the primary sample but the most commonly transmitted allele showed evidence for linkage by the TDT in the pooled samples. In only one region, two adjacent markers (*D3S2432* and *D3S1768*) showed some evidence for linkage. None of the 'candidate' loci examined showed evidence for linkage in this sample (see Materials and Methods).

Table 1. Characteristics of the primary sample

	Group A	Group B	Group C	Group D
No. of subjects	246	35	30	50
Mean age (years) (SD)	30.2 (15.9)	32.8 (14.05)	26.0 (21.1)	25.5 (17.9)
Sex ratio (M:F)	106:140	16:19	14:16	30:20
Mean % fall in FEV1 at maximum dose (SD)	6.0 (3.7)	9.4 (5.4)	26.9 (5.0)	30.9 (10.9)
Geometric mean IgE (IU)	20.88	37.41	14.94	76.51
% Positive multi-RAST IgE	18.2	32.4	19.2	48.7
% Positive ≥1 skin test allergen	39.0	45.7	40.0	54.6
% Positive for house dust mite allergen	17.0	11.4	33.3	29.6

The average inbreeding coefficient in the primary sample was 0.0327 (SD 0.016). Group A, unaffected; group B, asthma symptoms, equivocal BHR; group C, BHR, equivocal history; group D, affected. See text for details.

Table 2. Characteristics of the replication sample

	Group A	Group B	Group C	Group D
No. of subjects	177	38	51	26
Mean age (years) (SD)	27.7 (15.5)	29.8 (15.0)	23.8 (18.2)	19.5 (12.3)
Sex ratio (M:F)	79:98	19:19	25:26	14:12
Mean % fall in FEV1 at maximum dose (SD)	7.7 (4.6)	10.0 (5.5)	29.8 (7.8)	31.5 (11.5)
% Positive ≥1 skin test allergen	45.8	57.9	56.1	50.0
% Positive for house dust mite allergen	27.1	31.6	32.0	30.8

The average inbreeding coefficient in the replication sample was 0.0325 (SD 0.014). Group A, unaffected; group B, asthma symptoms, equivocal BHR; group C, BHR, equivocal for symptoms; group D, affected. See text for details.

Table 3. Significance levels and clinical groups for markers showing some evidence for linkage by the LR test ($P \leq 0.01$) in the primary sample

Locus	LR *P*-value in primary sample	TDT *P*-value in primary sample	Clinical group	TDT *P*-value in replication sample	TDT *P*-value in pooled sample
D2S1328	0.0040	>0.10	B + C + D	>0.10	>0.10
D3S2432[a]	0.0010	>0.10	C + D	0.083	>0.10
D3S1768[a]	0.0012	0.035	B + D	>0.10	>0.10
D3S2427	0.0053	>0.10	C + D	0.083	>0.10
D5S1480	**0.0079**	**>0.10**	**B + C + D**	**>0.10**	**0.00091**
D9S925[a]	0.00028	>0.10	C + D	>0.10	>0.10
D9S922	0.0093	>0.10	B + C + D	>0.10	0.078
D12S375	**0.0081**	**0.033**	**B + C + D**	**0.033**	**0.0025**
D13S787	0.0025	0.00067	C + D	>0.10	0.048
D19S178[a]	**0.0005**	**0.032**	**D**	**>0.10**	**0.010**
D21S1262[a]	0.0012	>0.10	C + D	>0.10	>0.10
D21S1440	**0.0096**	**>0.10**	**D**	**0.08**	**0.033**

[a]Markers meeting criteria for suggestive linkage (29).

Loci showing evidence for linkage by both the LR test in the primary sample and the TDT in the pooled primary and replication samples are shown in bold. In the replication and pooled samples, the TDT was performed using the most commonly transmitted allele in the primary sample in the same clinical group showing evidence for linkage in the primary sample. The four clinical groups were: (1) 'strict' asthma (group D); (2) bronchial hyperresponsiveness (BHR) (groups C + D); (3) asthma symptoms (groups B + D); and (4) 'loose' asthma (groups B+ C+ D). See Materials and Methods for details.

Among the 12 markers showing some evidence for linkage by the LR test in the primary sample, four were with the loosest definition of asthma (groups B + C + D), two were with asthma symptoms (groups B + D), four were with BHR (groups C + D) and two were with strict asthma (group 4).

DISCUSSION

The well-documented history of the Hutterite population that indicates a uniquely small number of founder chromosomes allowed us to consider the genetic bases of asthma at the population level. The small number of founders and communal lifestyle provide a model for studying the genetic basis of complex phenotypes in a relatively homogeneous context, with respect to both background genes and environmental factors. In contrast to the prior expectation that relatively few genes would be segregating for complex traits in the population, no single strong linkages were detected despite the relative genetic simplicity of the Hutterite population. Thus, even in founder populations with a small number of unrelated genomes (maximum of 128 haploid genomes in the Hutterite pedigree), susceptibility alleles at many loci may be present and the effects of alleles at each of these loci on susceptibility may be small. If multiple susceptibility alleles are indeed present in the Hutterites, these alleles may be very common in the outbred European population.

Furthermore, susceptibility alleles that are present in the Hutterites are likely to represent most of the alleles present in outbred populations, which is consistent with the fact that the population grew exponentially after its founding. As a result, most of the effects of random genetic drift would have occurred in the first few generations, with little random allele loss after this time. Therefore, most of the alleles that were present in the ancestral population should still be present in the contemporary Hutterites. In fact, it is likely that not only are the same alleles present in Hutterites and outbred Europeans but that frequencies of asthma susceptibility alleles in the Hutterites are similar to frequencies in European populations. To investigate this further, we took advantage of the extensive short tandem repeat polymorphism (STRP) genotype data available in the Hutterites and genotype data available through the Centre d'Etudes du Polymorphisme Human (CEPH) database (www.cephb.fr). STRPs are ideal for this type of analysis because they have many alleles at each locus, allowing for the investigation of allele frequency dynamics over the full spectrum of frequencies. We compared the alleles at 32 STRP loci on 21 autosomes in both samples. One hundred alleles were present in both the Hutterite and CEPH samples (mean frequency = 0.15; SD 0.12; range 0.005–0.57) and an additional 64 alleles were present only in the CEPH families (mean frequency in CEPH = 0.03; SD 0.05; range 0.005–0.26). Among the alleles that were present in both samples, there was a remarkably high correlation between the allele frequencies ($R^2 = 0.759$; $P < 0.001$). The frequencies of the CEPH alleles that were either present or absent in the Hutterites differed most at the two tails of the frequency distribution, as expected (Fig. 1). For example, 94% of alleles that are absent in the Hutterites (hatched bars in Fig. 1) have frequencies of <0.10 in the CEPH sample, whereas 90% of CEPH alleles with frequencies >0.10 (filled bars in Fig. 1) are present in the Hutterites. Extrapolating from this observation, we would suggest that asthma susceptibility alleles that are present in both the Hutterites and outbred samples (e.g. possible loci on 5q, 12q, 19q and 21q) occur at similar frequencies in both populations and are likely to have frequencies >0.10. High frequency susceptibility alleles with modest effects on the phenotype will make it difficult to confirm their role as asthma susceptibility alleles in family studies. Population-based association studies may be more suitable for these investigations, as suggested previously (30).

Although genome-wide association-based studies are not yet practical in most populations, the extensive linkage disequilibrium in the Hutterites makes them extremely well suited for such studies. For example, a study of 15 STRP loci on chromosome 4 in the Hutterites detected significant linkage disequilibrium (Fisher exact

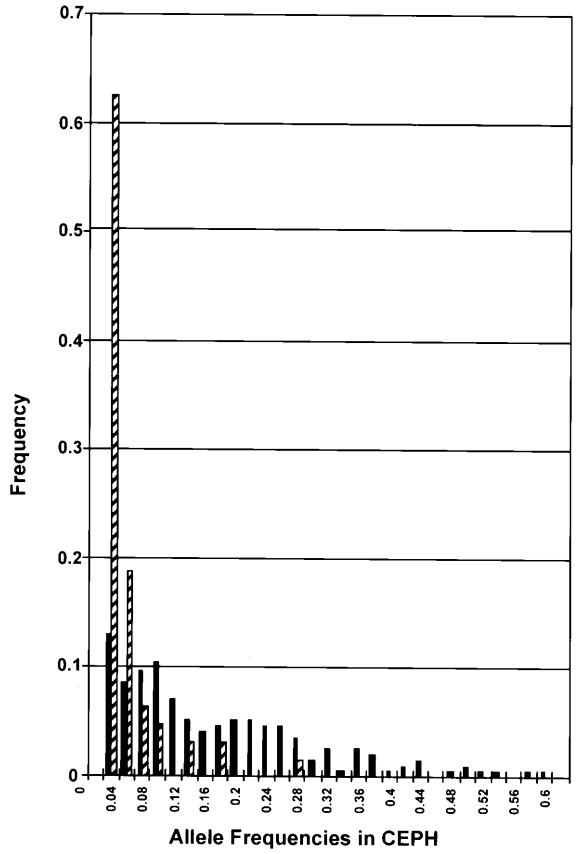

Figure 1. The distribution of allele frequencies in CEPH families. Alleles at 32 STRP loci on 21 autosomal chromosomes were compared between CEPH families and Hutterites. There were 100 alleles that were present in both samples (filled bars) and 64 alleles (at 32 loci) that were present in CEPH but absent in the Hutterites (hatched bars).

test, $P < 0.05$) at 75% of marker pairs spaced <5 cM apart, 60% of marker pairs spaced 6–10 cM apart and 15% of marker pairs spaced 11–16 cM apart (19). Among marker pairs that included only loci with heterozygosity levels ≥0.62, significant linkage disequilibrium was detected uniformly up to 10 cM. In our study, only 14% of markers had heterozygosities <0.62, so it is likely that we would be able to detect linkage disequilibrium over a significant portion of the genome. Furthermore, a ≤10 cM map using markers with high heterozygosity levels could be used for genome-wide association-based studies in this population. In fact, even with a less perfect map, we were able to detect associations with particular alleles at four of 12 loci showing evidence for linkage by the LR test. Thus, association-based mapping methods that would require <1 cM maps in most populations, can be used in the Hutterites to localize complex trait genes.

The results of this and other previously published studies together provide suggestive evidence for asthma genes in four chromosomal locations: 5q23–31, 12q15–24, 19q13 and 21q21. These four regions showed evidence for linkage in the Collaborative Study on the Genetics of Asthma (CSGA) clinic families, in whom identical protocols were used to evaluate subjects (31), and two of these regions (5q and 12q) have shown evidence for linkage in independent samples that were evaluated using different measures of BHR and/or atopy (32–36). Lastly, two adjacent loci in the novel region, 3p24.2–22, showed possible evidence for linkage and may also contain asthma susceptibility loci in the Hutterites.

Two regions (5q23–31 and 12q15–24) that were linked to our 'loosest' definition of asthma (asthma groups 2 + 3 + 4) have been linked to a variety of asthma phenotypes in other populations, such as total IgE and BHR in the Dutch (33,36), total IgE in the Amish (32), asthma and total IgE in a Barbados population (34) and asthma in the CSGA families (31). Our findings are consistent with genes in this region conferring susceptibility to less specific 'atopic' phenotypes, rather than asthma *per se*. On the other hand, linkages of genes in 19q13 and 21q21 to 'strict' asthma have been reported previously only in the outbred CSGA families. Evidence for linkage to asthma was reported with markers in 19q13 in the outbred Caucasian CSGA families ($P = 0.0013$) and in 21q21 in the outbred Hispanic CSGA families ($P = 0.0040$). Similarly, in this study, markers in these regions (*D19S178* and *D21S1440*) showed evidence for linkage to 'strict' asthma (asthma group 4). Further studies in these and other well-defined populations will help clarify the relative contributions of genes in each of these regions to asthma and to each of the asthma-associated phenotypes.

MATERIALS AND METHODS

Composition and clinical evaluation in the primary sample

As part of our participation in the CSGA, we studied asthma and atopic phenotypes in the Hutterites between January and April 1994, using the same protocols that were used to study the outbred CSGA families (31).

Four related colonies were selected for these studies because of the high prevalence of asthma and atopy in the resident families (23). In addition to the members of the colonies and their first degree relatives, 55 Hutterites living in neighboring colonies came to the study colonies for evaluation because of a previous diagnosis of asthma in their family. A total of 361 individuals ≥6 years old were studied. These subjects are members of a large extended genealogy of 1101 individuals spanning 11 generations.

Subjects were evaluated in the colonies according to the CSGA protocol (31), with two exceptions. First, a shortened methacholine challenge protocol was used in which the first dose of methacholine was 25 mg/ml, similar to screening protocols used by others (37). During subsequent visits to each colony, standard methacholine challenge tests were administered to 42 of the 110 individuals who previously demonstrated a ≥12% fall in baseline forced expiratory volume at 1 s (FEV1) at the maximum dose of methacholine. Second, the CSGA pulmonary history questionnaire was modified to reflect Hutterite lifestyle more closely.

In addition to standard spirometry (38) and methacholine challenge testing, as described above, skin prick testing was performed to a standardized set of allergens, which included mites, animals danders, mold, insects, pollen and a negative (saline) and positive (histamine) control. Subjects were considered skin test positive if the wheal was at least 3 mm greater than the negative control. Blood samples were collected from all subjects for DNA studies and for measurements of serum IgE.

Questionnaires were reviewed by a pulmonologist (R. Parry) and subjects were classified into three categories based on a history of asthma symptoms (wheeze, cough, shortness of breath)

and a physician diagnosis of asthma. Category 1 included individuals with neither current symptoms nor a history consistent with asthma ($N = 223$), category 2 included individuals with an equivocal history of asthma or asthma symptoms ($N = 57$) and category 3 included individuals who reported a current or past history of symptoms and/or a previous diagnosis of asthma ($N = 81$). Our final diagnostic categories for asthma that were used for linkage studies utilized both the categories 1–3 and results of methacholine challenge tests or studies of airway reversibility as follows. Group A included individuals in clinical category 1 with a drop of <20% baseline FEV1 at the maximum dose of methacholine ($N = 246$). These were presumed to be 'unaffected' individuals. Group B included individuals in clinical category 3 with a <20% drop in baseline FEV1 at the maximum dose of methacholine or in clinical category 2 with a >10% but <20% drop in baseline FEV1 at the maximum dose of methacholine ($N = 35$). These individuals were considered to have symptoms consistent with asthma but do not meet the criteria for 'definite' asthma. Group C included individuals in clinical category 1 with a ≥20% drop in baseline FEV1 at the maximum dose of methacholine ($N = 27$) or with evidence of reversibility of airway obstruction ($N = 3$). These individuals were considered to have BHR or reversible obstructive airway disease, respectively, but an equivocal diagnosis of asthma. Group D included individuals in clinical category 3 with a ≥20% drop in baseline FEV1 at the maximum dose of methacholine ($N = 49$) or evidence of airway reversibility ($N = 1$). Group D individuals meet our 'strict' criteria for asthma and are equivalent to the probands in the CSGA clinic families (31). Because *a priori* we did not know if the underlying pathogenesis of asthma symptoms and BHR were due to common or distinct genes, all analyses were performed using four diagnostic groups as 'affected': (1) group D (strict asthma); (2) groups B and D combined (asthma symptoms); (3) groups C and D combined (BHR); and (4) groups B, C and D ('loose' asthma). Only individuals in group 1 were considered unaffected in all analyses. For example, in analyses considering only group 4 individuals as affected, group B and group C individuals were considered of unknown status.

Studies in replication sample

Two hundred and ninety two Hutterites from an additional five South Dakota colonies were studied between October 1996 and February 1997, using protocols identical to those described above for the primary sample except that studies of serum IgE have not been performed in the replication sample. The clinical characteristics of subjects in this sample are described in Table 2. A subsample of 115 subjects in groups B, C or D and their parents (when available) were genotyped for all markers that showed possible evidence of linkage in the primary sample.

Genotyping

STRPs were typed by manual (radiolabeled) methods at the University of Chicago and by automated (fluorescent-labeled) methods at Whitehead Institute/MIT. Genotypes were determined in a total of 292 autosomal and three X–Y pseudoautosomal loci. The marker spacing was on average 11.5 cM (SD = 8.2 cM; range 0–39.1 cM). The average heterozygosity over all loci in the primary sample was 0.71 (SD 0.09; range 0.38–0.96).

Studies of candidate genes

Prior to or during the course of these studies, possible linkages of asthma or atopy to certain regions of the genome were identified by others or ourselves. Candidate genes in some of these regions were examined by genotyping members of the pedigrees either for polymorphisms within the genes themselves or for STRPs near the gene. These include the cytotoxic T lymphocyte antigen-4 gene (*CTLA4*) on chromosome 2q31–32, the interleukin 4 (*IL4*), interleukin 9 (*IL9*) and β_2-adrenergic receptor (*ADRB2R*) genes on 5q31–33, MHC genes (*HLA-DPB1*, *HLA-DRB1*, *TNFα*, *HLA-B*) on 6p21, the interferon α gene (*IFNA*) on 9p23–22, and the β chain of the high affinity Fcε receptor (*FCER1B*) on 11q13.

Linkage studies in Hutterites

A complete genealogy of the 361 individuals was constructed from the 12 903 member genealogy of the Hutterites; this yielded a 1101 member genealogy, which included all known ancestors of the 361 individuals. The ancestry of the 361 individuals was traced back one generation at a time, to link up as many individuals as possible into pedigrees. Three 'lineages' were identified through this process and these were the three subpedigrees used for subsequent linkage analyses by the likelihood ratio χ^2 test, described below.

Evidence for linkage was evaluated in the primary sample using two tests of linkage. First, the semi-parametric likelihood ratio χ^2 test (LR) (26) was used to assess evidence for linkage in the pedigree. Using a modification of the ILINK program from the LINKAGE package, a likelihood ratio χ^2 was calculated. In this approach, the genetic parameters describing the mode of transmission of susceptibility to asthma are not fixed, but rather estimated from the data, constrained by the total population prevalence. First, the likelihood of the data is maximized with the penetrances and disease allele frequency as well as θ as free parameters. Then the likelihood of the data is maximized with the genetic parameters free, but θ fixed at 0.5. Twice the difference in the ln likelihood values is asymptotically distributed as a χ^2 with 1 degree of freedom. A one-tailed test was used to assess significance. Because we could not maintain the integrity of the entire pedigree for analyses, we constructed three subpedigrees from the larger 1101 person pedigree, in which all inbreeding and marriage loops were broken. As a result, the LR test may be overly conservative in this sample. Therefore, as a second measure of linkage, we used the TDT (27).

The TDT examines whether the transmission of specific marker alleles from heterozygous parents to affected offspring is random. A significant excess of transmissions over the 50% (Mendelian) expectations is evidence that the over-transmitted allele is linked to the disease allele. A significant TDT may also reflect association, resulting from the correlated history of the marker locus and the 'disease' locus, and indicates that the marker is in relatively close proximity to the disease locus. However, because these studies were conducted in a single large pedigree, a significant TDT result may merely reflect linkage and not necessarily association. Therefore, we used the TDT as a second (although non-independent) test of linkage and do not make inferences regarding distances between the marker and disease loci or associations with particular alleles on the basis of this test.

All marker loci with an LR *P*-value ≤0.01 in the primary sample were studied in the primary sample, in a subset of the replication

sample and the pooled primary and replication samples using the TDT test. The subset of the replication sample included affected subjects (group B + C + D) and their parents, when available. In the replication sample, we specifically examined the transmission of the allele that was most commonly transmitted to the affected individuals in the primary sample in the same clinical group that showed evidence for linkage in the primary sample.

The overall evidence for linkage was evaluated using the combined results of the LR and TDT tests in the primary and replication samples.

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