Fine Mapping of IGADI in IgA Deficiency and Common Variable Immunodeficiency: Identification and Characterization of Haplotypes Shared by Affected Members of 101 Multiple-Case Families

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Fine Mapping of *IGAD1* in IgA Deficiency and Common Variable Immunodeficiency: Identification and Characterization of Haplotypes Shared by Affected Members of 101 Multiple-Case Families

Igor Vořechovský, † Michael Cullen, ‡ Mary Carrington, ‡ Lennart Hammarström, * and A. David B. Webster †

To limit the region containing a mutation predisposing to selective IgA deficiency (IgAD) and common variable immunodeficiency (CVID), 554 informative members of 101 multiple-case families were haplotted at the *IGAD1* candidate locus in the MHC. Microsatellite markers were placed onto the physical map of *IGAD1* to establish their order and permit rapid haplotype analyses. Linkage analysis of this extended family set provided additional support for a strong susceptibility locus at *IGAD1* with a maximum multipoint nonparametric linkage score in excess of 3. Although the transmission of maternal *IGAD1* haplotypes from unaffected heterozygous parents to the affected offspring was in excess, this was not apparent in multiple-case families with a predominance of affected mothers, suggesting that this parental bias is influenced by the affection status of transmitting parents and supporting a maternal effect in disease susceptibility. Of 110 haplotypes shared by 258 affected family members, a single haplotype (H1) was found in 44 pairs of affected relatives, accounting for the majority of the *IGAD1* contribution to the development of IgAD/CVID in our families. The H1 allelic variability was higher in the telomeric part of the class II region than in the distal part of the class II region in both single- and multiple-case families. Incomplete H1 haplotypes had most variant alleles in the telomeric part of the analyzed region in homozgyous IgAD/CVID patients, whereas this was not observed in unaffected homozygotes. These data suggest that a telomeric part of the class II region or centromeric part of the class III region is the most likely location of *IGAD1*. The Journal of Immunology, 2000, 164: 4408–4416.

Immunglobulin A deficiency (IgAD) is the most frequent primary immunodeficiency in humans (1). Affected individuals lack IgA in serum and mucosal secretions and may suffer from susceptibility to infections (1). IgAD shares a putative MHC-linked genetic defect with common variable immunodeficiency (CVID), a more severe disorder of Ig production characterized by a lack of IgG, IgA, and often IgM (2–4).

Numerous case-control studies of this multifactorial disorder have previously shown HLA associations (2, 5–12), suggesting the existence of a predisposing locus or loci in this region. Further support for the presence of a susceptibility mutation(s) in the MHC (designated *IGAD1*) awaited the evidence for genetic linkage as a significantly increased allele sharing in affected individuals and positive family-based allelic associations, as demonstrated by transmission disequilibrium tests (TDT) (4). TDTs reduce the confounding effect of the population structure on putative allelic associations in case-control studies and should be positive only if the genetic linkage is present (13).

Although a number of allelic associations were reported in IgAD/CVID using serology, RFLP, PCR using sequence-specific oligonucleotide primers (PCR-SSP), and microsatellite markers, no reliable haplotype data have been available that would permit fine mapping of the *IGAD1* locus. Fine mapping of *IGAD1* would facilitate a search for a genuine predisposing mutation(s), but this has been hindered by a high gene density in the region, excessive polymorphism, lower than average recombination fractions and several hot spots for meiotic crossover events in the MHC. The location of *IGAD1* in the class III region has been supported by the observed absence of IgAD among 43 Sardinian DR3 homozygotes who share class II but not class III alleles with the IgAD-associated Northern European haplotype (14). The same region has been favored by an earlier family study (15). A recent analysis of ancestral haplotypes in a large kindred with three CVID patients, three cases with IgAD, and two cases with lower IgA levels (16) suggested that the region between the class III microsatellites designated 821/823 (17) and the HLA-B locus contained the susceptibility gene. However, fine mapping of complex genetic traits based on putative ancestral haplotype fragments shared by few affected family members, some of them with an intermediate phenotype of partial IgA

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*Abbreviations used in this paper: IgAD, selective IgA deficiency; CVID, common variable immunodeficiency; TDT, transmission disequilibrium test; NPL, nonparametric linkage; PCR-SSP, PCR using sequence-specific oligonucleotide primers.*
deficiency, may not be reliable. A single affected family member with a different haplotype fragment does not necessarily limit the \textit{IGAD1} locus and the excess sharing of an IgAD/CVID-associated MHC haplotype in affected heterozygotes may suggest a location of \textit{IGAD1} centromeric to the \textit{821/823} locus (16). Although the relative risk of developing IgAD conferred by the homozygous B8-DR3 haplotype was estimated to be as high as 75 (16), screening of 30 Scandinavian B8-DR3 heterozygotes did not reveal any IgAD (L. Hammarström et al., manuscript in preparation), indicating a lower risk. A central MHC location for \textit{IGAD1}, which contains genes coding for products influencing Ab production, including complement proteins (18), has been disputed by pointing to a primary association of IgAD with \textit{DQB1*0201} (19). The proposal of neutral amino acids of the codon 57 in the \textit{DQ} chain as the only major MHC determinant of IgAD susceptibility has not been accepted (11, 20), and there is currently no consensus where \textit{IGAD1} is a strong susceptibility locus for the most prevalent human Ig deficiencies.

### Materials and Methods

#### Family material

The complete set of 101 multiple-case pedigrees is at http://www.cbt.ki.se/fam/set3–7/scan_set.html. The ascertainment of probands and family members was described previously (4, 22). The families were recruited through the European Society for Immunodeficiency Registry in Sweden (\(n = 49\)) and the U.K. (\(n = 34\)). Samples from small multiple-case families were obtained from Italy (\(n = 4\)), the Czech Republic (\(n = 5\)), Belgium (\(n = 2\)), Poland (\(n = 5\)), Belgium (\(n = 1\)), Turkey (\(n = 1\)), Finland (\(n = 2\)), Poland (\(n = 1\)), Russia (\(n = 2\)), and Spain (\(n = 2\)). Single-case families were from Sweden and the U.K.

#### Genotyping

Genotyping at microsatellite loci was conducted using fluorescent-labeled primers on an ABI 377-Sequence using the Genescan 672 and Genotyper 2.0 packages (Applied Biosystems Division, Perkin-Elmer, Norwalk, CT). The primer sequences used for amplifying novel or previously reported microsatellite loci are shown in Table I. HLA typing at \(HLA-B, DRB1, DQA1,\) and \(DQB1\) loci was performed using PCR-SSP as described previously (8). Family MHC recombinants were further analyzed using microsatellites at \(DNRG4031\) (23), G2722258 (5’–CCT GCC AAT GGA ATG TGA GC–3’), 5’–GAG ACA AAC ACT GAA GCC TC–3’ and \(DQCAR\) (24). These loci were typed with radioactively labeled oligonucleotide primers as previously described (25). The genetic or physical map for a subset of the marker loci used in the present study is earlier described (26, 27) or is shown in Fig. 1.

#### Linkage analysis

A total of 554 pedigree members from 101 apparently unrelated multiplex families were analyzed for linkage, consisting of 258 affected, 231 unaffected and 65 family members with unknown phenotype (http://www.cbt.ki.se/fam/set3–7/scan_set.html). The family material contained 94 affected pairs of siblings, 41 unaffected sib pairs, and 8 half-sibs. Only 30 affected children had affected fathers as compared with 118 affected children with unaffected fathers. In contrast, and in line with our previously described parent-of-origin penetrance effect (4), 75 IgAD/CVID children were born to affected mothers as compared with 95 affected children born to the unaffected mothers, a proportion more than doubled in our family material (http://www.cbt.ki.se/fam/set3–7/scan_set.html). Allele numbers at each locus, their population frequencies, observed heterozygosity, and polymorphism information content are shown at http://www.cbt.ki.se/fam/mhcghdat.htm. Full genotypes of all family members are shown at http://www.cbt.ki.se/fam/mhcppre.htm. Nonparametric linkage (NPL) analysis was

The ascertainment of the family material used for TDT was described previously (4). A total of 109 single-case-father-mother trios were analyzed together with 13 families containing 2 affected offspring and 1 family with 3 affected offspring. All index cases included in TDT had only unaffected parents as determined by repeated nephelometric measurements of serum Ig levels (4).

**Haplotype analysis**

Haplotypes were constructed using a maximum-likelihood method implemented in the Genehunter program available at http://waldo.wi.mit.edu/ftp/distribution/software/genehunter/gh2/ (28). The Genetic Analysis System (ftp://ftp-p.ox.ac.uk/pub/users/ayoung) was used for converting Genotyper peak labels into allele numbers and analyzing parental allelic segregation differences.

The ascertainment of the family material used for TDT was described previously (4). A total of 109 single-case-father-mother trios were analyzed together with 13 families containing 2 affected offspring and 1 family with 3 affected offspring. All index cases included in TDT had only unaffected parents as determined by repeated nephelometric measurements of serum Ig levels (4).

**Results**

**Physical map of microsatellite loci in the IGAD1 candidate region**

To facilitate haplotype analysis of IGAD1, oligonucleotide primers flanking simple sequence repeats were designed and tested for polymorphisms and mendelian inheritance using a panel of 12 DNA samples from different ethnic populations (Caucasian, African, Mongoloid) and 3 families. Novel polymorphic loci (Table I) and those previously reported were placed on to the physical map (Fig. 1) available as a contiguous overlapping sequence in public databases. The map covers the IGAD1 candidate region in the telomeric part of the MHC class II region and the class III region (Fig. 1). The map will facilitate a rapid and inexpensive haplotype analysis of large numbers of individuals and will be useful in genetic studies of MHC-linked complex traits, including a number of autoimmune diseases.

**Genetic linkage of IgAD/CVID to MHC in 101 multiple-case families with IgAD/CVID**

The results of NPL analysis on the extended family sample are shown in Tables II and III. Single-point analyses were significant for closely linked markers located in or flanking the MHC. Multipoint analyses showed maximum NPL/\(Z\) scores in excess of 3 at about 5.6 cM telomeric of D6S1610 for both the pairs of affected family members and all informative individuals (Table III). Higher NPL/\(Z\) scores were observed for affected pairs of relatives as compared with all individuals informative for linkage, possibly reflecting an effect of unknown age-dependent penetrance. These results further strengthen the evidence for genetic linkage with this extended sample of families.

**Identification of haplotypes shared by 258 affected members of multiple-case families**

Altogether, 110 haplotypes were shared by at least 2 affected members of multiple-case families. Maximum likelihood haplotypes for all analyzed pedigree members are shown at http://www.cbt.ki.se/fam/mhchaplo.htm.
over 0.25 megabase proximal of G51152, was represented by four alleles, indicating the centromeric border of IGAD1 (Fig. 1). The most common RING3 allele 3 (231 bp) was found on 27 of 44 H1 haplotypes, with alleles 2, 4, 5, and 6 found on 5, 7, 4, and 1 occasions, respectively (Table IV). Although there was no such obvious telomeric border in the class III region, the allelic variability at the central MHC loci between 9N2 and TNF was higher than that at the G51152-DQCARII region, with a variant allele(s) in 12 families (Table IV). Seven of 12 families with variant H1 alleles/haplotypes in IGAD1 were Swedish, whereas families cv82 and cv114 with a variant allele at D6S273 came from Northern Italy and families cv131, cv135, and cv145 were from the British Isles (Table IV). A number of families with different haplotype fragments at the telomeric part contrasted with a single variant allele in the distal part of the class II region in one family (Table IV), despite the highest observed heterozygosity, polymorphism information content, and allele number at DQCARII (http://www.cbt.ki.se/fam/mhcghdat.htm). Although the families with variant H1 alleles did not come from a single population, a number of Swedish families sharing identity-by-descent H1 haplotype with positive NPL scores (cv6, cv18, cv15, and cv107 in Table IV) support a distal part of the class II region rather than the previously proposed telomeric part of the class III region as containing an IGAD1 mutation.

Although parental samples of the affected siblings in the Swedish family cv15 (Table IV) were not available, additional family members including their unaffected siblings and the offspring could be typed using markers TAP1CA, 7-8601, 8-105224, LH1, 11-36252 and 17-86995 (Fig. 1). These results indicated that the affected siblings shared only a centromeric part of the H1 haplotype up to a recombination event in a 40-kb region between 8-105224 and LH1, whereas a distal part contained different alleles (Table IV). Furthermore, these class III alleles these were strongly negatively associated with the disease in both the single- and multipoint TDT of our family material (data not shown). This family suggests that IGAD1 is centromeric to the LH1 locus.

Representative carriers of the most commonly shared haplotypes were typed using PCR-SSP at HLA-B, DRB1, DQB1, and DQBI loci. The H1 haplotype was invariably found to contain disease-associated alleles HLA-DQB1*02, HLA-DRB1*03, and HLA-DQA1*05 (Table IV). Haplotype H2 (alleles 244–200 bp at the G51152-DQCARII loci, respectively; HLA-DQB1*05, DQA1*01, DRB1*01) was identified in 18 affected H1/H2 individuals. Multipoint TDT analysis revealed 28 transmissions vs 13 nontransmissions from parents heterozygous for this haplotype, confirming previously identified allelic associations in case-control studies (1) in a family-based design. Haplotype H3 (alleles 226–192 bp at G51152-DQCARII; HLA-DQB1*02, DQA1*03) was identified in 4 affected homozygotes and 18 H1/H3 heterozygotes with IgAD/CVID; however, multipoint and single-point TDT analysis showed no significant excess of transmissions of this haplotype/allele. Haplotype H4 (alleles 214–216 bp at G51152-DQCARII; HLA-DQB1*03, DQA1*03, DRB1*07) was found in 10 heterogeneous H1/H4 carriers. Although multipoint TDT suggested an excess of transmissions vs nontransmissions from heterogeneous parents (25 vs. 16), single-point TDT was negative for each allele (26 vs 23 for the 216-bp allele at DQCARII, 47 vs 64 for the 214-bp allele at G51152).

Haplotypes H1-H4 were found in the majority of affected family members. Ninety-nine of 258 affected family members possessed only haplotypes H1-H4 at the G51152-DQCARII loci, while an additional 113 carried one of the four haplotypes, giving a total of 82% (212/258) affected pedigree members carrying at least one of these haplotypes. This figure suggests a restricted repertoire of the MHC molecules in affected individuals and further strengthens the role of the MHC locus in IgAD/CVID susceptibility.

### Table II. Results of nonparametric linkage analysis of 101 IgAD/CVID families: single-point NPL analysis

<table>
<thead>
<tr>
<th>Marker Locus</th>
<th>NPL_all ($p$)</th>
<th>Zu_all (LOD$_u$)</th>
<th>NPL_pairs ($p$)</th>
<th>Zu_pairs (LOD$_u$)</th>
<th>$\alpha$, HLOD</th>
<th>Information Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>D6S1610</td>
<td>1.37 (0.05)</td>
<td>2.05 (0.92)</td>
<td>1.40 (0.05)</td>
<td>2.07 (0.93)</td>
<td>0.34, 0.41</td>
<td>0.52</td>
</tr>
<tr>
<td>D6S291</td>
<td>1.56 (0.03)</td>
<td>2.58 (1.44)</td>
<td>1.72 (0.02)</td>
<td>2.72 (1.61)</td>
<td>0.19, 0.06</td>
<td>0.51</td>
</tr>
<tr>
<td>D6S1383</td>
<td>2.97 (0.003)</td>
<td>4.08 (3.49)</td>
<td>3.21 (0.0001)</td>
<td>4.33 (4.13)</td>
<td>0.49, 1.19</td>
<td>0.68</td>
</tr>
<tr>
<td>RING3CA</td>
<td>1.81 (0.02)</td>
<td>2.58 (1.45)</td>
<td>2.10 (0.006)</td>
<td>2.94 (1.88)</td>
<td>0.37, 0.68</td>
<td>0.63</td>
</tr>
<tr>
<td>G51152</td>
<td>2.19 (0.005)</td>
<td>3.10 (2.13)</td>
<td>2.43 (0.002)</td>
<td>3.41 (2.52)</td>
<td>0.50, 1.14</td>
<td>0.63</td>
</tr>
<tr>
<td>DQCARII</td>
<td>2.65 (0.001)</td>
<td>3.40 (2.51)</td>
<td>2.78 (0.0007)</td>
<td>3.47 (2.61)</td>
<td>0.40, 0.78</td>
<td>0.75</td>
</tr>
<tr>
<td>9N2</td>
<td>2.29 (0.004)</td>
<td>3.11 (2.10)</td>
<td>2.50 (0.001)</td>
<td>3.28 (2.34)</td>
<td>0.41, 0.78</td>
<td>0.71</td>
</tr>
<tr>
<td>9N1</td>
<td>1.24 (0.07)</td>
<td>2.14 (0.99)</td>
<td>1.30 (0.06)</td>
<td>2.13 (0.98)</td>
<td>0.45, 0.60</td>
<td>0.50</td>
</tr>
<tr>
<td>D6S273</td>
<td>1.40 (0.05)</td>
<td>1.96 (0.84)</td>
<td>1.60 (0.04)</td>
<td>2.36 (1.21)</td>
<td>0.12, 0.03</td>
<td>0.67</td>
</tr>
<tr>
<td>821</td>
<td>1.24 (0.07)</td>
<td>1.84 (0.74)</td>
<td>1.46 (0.04)</td>
<td>2.10 (0.95)</td>
<td>0.33, 0.44</td>
<td>0.62</td>
</tr>
<tr>
<td>62</td>
<td>1.73 (0.02)</td>
<td>2.28 (1.13)</td>
<td>1.96 (0.01)</td>
<td>2.52 (1.37)</td>
<td>0.26, 0.31</td>
<td>0.68</td>
</tr>
<tr>
<td>D6S1558</td>
<td>0.25 (0.38)</td>
<td>0.40 (0.07)</td>
<td>0.44 (0.30)</td>
<td>0.68 (0.10)</td>
<td>0.01, 0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>D6S1621</td>
<td>0.94 (0.18)</td>
<td>1.92 (0.19)</td>
<td>1.26 (0.07)</td>
<td>1.84 (0.73)</td>
<td>0.11, 0.01</td>
<td>0.56</td>
</tr>
<tr>
<td>D6S461</td>
<td>0.54 (0.26)</td>
<td>0.92 (0.19)</td>
<td>0.58 (0.25)</td>
<td>0.97 (0.20)</td>
<td>0.15, 0.12</td>
<td>0.48</td>
</tr>
</tbody>
</table>

* NPL score, $\alpha$ (proportion of IGAD1-linked families) and HLOD (heterogeneity LOD score) was as previously defined (28); Zu score was described by Kong and Cox (32). The scores were computed for pairs of affected pedigree members only (pairs) and for all family members (all) using the Genehunter 2 (28) and Genehunter Plus 1.2 algorithms (32).

### Table III. Results of nonparametric linkage analysis of 101 IgAD/CVID families: Multipoint NPL analysis

<table>
<thead>
<tr>
<th>Score</th>
<th>All Individuals ($\theta$)</th>
<th>Pairs of Affected Members ($\theta$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPL$_{max}$, significance ($p$)</td>
<td>3.049 (5.6), 0.0002</td>
<td>3.196 (5.6), 0.00009</td>
</tr>
<tr>
<td>Z$<em>{u</em>{max}}$</td>
<td>3.545 (5.6)</td>
<td>3.656 (5.6)</td>
</tr>
<tr>
<td>LOD$<em>{u</em>{max}}$</td>
<td>2.728 (5.6)</td>
<td>2.902 (5.5)</td>
</tr>
<tr>
<td>Information content at $\theta_{max}$</td>
<td>0.926</td>
<td>0.926</td>
</tr>
</tbody>
</table>

* $\theta$, genetic distance in centimorgans from the D6S1610 locus; NPL score, $\alpha$ (proportion of IGAD1-linked families) and HLOD (heterogeneity LOD score) was as previously defined (28); Zu score was described by Kong and Cox (32). The scores were computed for pairs of affected pedigree members only and for all family members using the Genehunter 2 (28) and Genehunter Plus 1.2 algorithms (32).
family members typed for HLA specificities using PCR-SSP were found to be homozygous for DQB1*02, DRB1*03, DQA1*05 alleles. Of 16 homozygotes, 6 family members from altogether 4 families were unaffected, whereas 9 cases from 6 families had IgAD and 1 patient had CVID. However, seven more affected (six IgAD and one CVID) cases, but only one unaffected family member (homozygous in the class III, but not in the class II region, data not shown), were homozygous for H1 alleles in at least two loci (Table V). Similarly, haplotyping of single case-parent trios revealed two homozygous IgADs and one homozygous CVID carrier of the full H1 haplotype. In addition, two affected index cases were found to carry this haplotype with a variant allele at the DQCARII loci (Table V).

These data indicate that the affected homozygotes carrying a portion of the H1 haplotype shared common alleles at the telomeric region of the class II region, while possessing variant haplotype fragments at the telomeric part of the class III region (Table V and Fig. 1). This pattern corresponded to the H1 haplotypes shared by heterozygous individuals (Table IV). These results suggest that the IGAD1 mutation is at the centromeric part of the class III region or in the telomeric part of the class II region.

Identification of class II and class III recombinants

The recombinant families were detected in the process of genomewide linkage mapping of 83 multiple-case families containing a total of 449 pedigree members, 215 of them affected (I.V. et al., manuscript in preparation). Initially, seven families were identified with recombinant meioses between the proximal D6S1583 and distal D6S461 marker loci, located about 6 cM apart and flanking the MHC (26). These families were typed using a set of closely spaced markers within the MHC to identify those with a recombination event in the IGAD1 candidate region. Two such families were found, giving a recombination fraction estimate of 0.4% (2 of 501 meioses) in the proximal half of the MHC (23). Fine mapping of the crossover in family cv29 placed the breakpoint telomeric of TNFd, whereas in family cv5 the markers DNRNG4031 and DNRNGCA (25) placed the crossover to a 4-kb area between the HLA-DNA and RING3 genes (data not shown), a region that contains...
a hot spot for recombination events (25). Although there were both affected and unaffected descendants of recombinant and nonrecombinant meioses, making each family potentially informative for narrowing the location of a predisposing mutation, the cosegregation of phenotypes and haplotypes provided no conclusive evidence as to the location of IGAD1.

**Transmission-disequilibrium analysis**

The single-point TDT showed the most significant transmission distortion at DQCARII in multiple-case families (Table VI). In single-case families, the most significant disequilibrium was observed for a negatively associated allele at LH1 and the 216-bp allele at G51152 (Table VI); the latter allele was found on all H1 haplotypes shared by affected family members (Table IV). The G51152 and DQCARII loci showed association with more than one allele/haplotype: the H1 haplotype (alleles 216–202 bp, respectively) was transmitted on 36 occasions whereas it was retained 17 times in single-case families; these numbers were 48 and 24, respectively, in multiple-case families. The number of transmissions vs. nontransmissions for the H2 haplotype 244–200 (226–200) was 25/13 (7/2) in single-case families and 28/13 (12/4) in multiple-case families. The telomeric part of class III or centromeric part of class II region showed the most significant family-based allelic associations of all markers in both the single- and the multiple-case pedigrees.

**Parental bias in transmission of IgAD-associated alleles is influenced by parental affection status**

The TDT analysis using a large set of single-case families with unaffected parents was conducted separately for each parental transmission. In accordance with our previously reported data (4), we found a bias in the transmission of maternal vs paternal alleles/
haplotypes from unaffected heterozygous parents to the affected offspring (Table VI). In multiple-case families, we also found a significant TDT for most IGAD1 loci studied, although no clear parental bias was observed. This suggests that the parental transmission bias of associated IGAD1 alleles to the affected offspring is influenced by the parental phenotype. Because affected mothers transmitting the phenotype to the offspring are overrepresented as compared with affected male transmitters in our data set, it is the maternal phenotype that is likely to contribute more to this effect.

**Discussion**

**Location of IGAD1**

The results of our present study suggest that the most likely location of the IgAD/CVID susceptibility mutation is in the telomeric part of the class II region or centromeric part of the class III region. This is supported by 1) the most significant family-based allelic associations in the class II loci in TDT (Table VI) and in previous case-control studies (8, 29); 2) the allelic uniformity at DQCARII, and particularly at G51152 on the H1 haplotype, which shows the most significant association with IgAD/CVID in the population studied; 3) the existence of variant H1 haplotypes in the telomeric part of the class III region in a number of heterozygous multiple-case families, which are shared in excess by the affected family members; 4) the allelic variability of haplotype fragments at the telomeric part of IGAD1 in affected H1 homozygotes as compared with the centromeric part; and 5) no such bias in nonaffected individuals.

Although our data do not support the previously suggested class III location for the IgAD/CVID susceptibility gene (14–16), we believe that they do not warrant the exclusion of the suggested region between the G1 gene and the 821/823 loci. Fine mapping of susceptibility loci in complex traits cannot be reliably based on a single or even a few ancestral recombination events because the phenotype in such families may have been determined by different etiological factors. Although there were many more families with variant H1 fragments in the previously implicated class III region than in the opposite part of IGAD1 (Tables IV and V), the shared haplotype may not have been a major susceptibility factor for the development of the phenotype in a particular family. This uncertainty is magnified by zero or negative NPL scores in some families with a variant H1 haplotype, their diverse population of origin and a possibility that a single variant allele in a number of H1 haplotypes could have arisen due to a marker locus mutation rather than an ancestral recombination event (Table IV). However, the existence of several Swedish families with clear ancestral H1 variants and positive NPL scores does provide a support for the telomeric part of the class II region rather than the telomeric end of the class III region (Table IV). A dense coverage of the candidate region would raise the proportion of MHC-linked families to the IgAD/CVID susceptibility? Assuming a penetrance ratio of 20, a dominant model, and a frequency of disease allele of 0.1%, ~40% of families showed linkage to the MHC, contributing to the heterogeneity LOD score of ~1.36. With a phenocopy rate of 1% and penetrance ratio of 20, the proportion of MHC-linked families in our sample would drop to about 23%. However, if the number
of phenocopies is higher and the disease penetrance is lower, the contribution of the MHC to the overall disease prevalence could exceed 40% (e.g., 3% phenocopies and 40% IgAD/CVID penetrance in our families would raise this estimate to 49% under the same mode of inheritance). Although it will be interesting to assess the contribution of the MHC more accurately using the data of our genome-wide scan with multiple-case families (I. Vořechovský, L. Hammarström, A. D. B. Webster, et al., manuscript in preparation), it is becoming increasingly apparent that IGAD1 is a major susceptibility locus for common human Ig deficiencies.

**Role of IGAD1 homozygosity in susceptibility to IgAD/CVID**

At least six H1 homozygotes were identified among nonaffected family members, indicating that mere H1 homozygosity at the candidate IGAD1 loci is not sufficient for the development of IgAD/CVID and that additional factors must exist in the pathogenesis of the defect. However, the number of H1 homozygotes including H1 variants was almost tripled in affected individuals, possibly suggesting a role in IgAD/CVID predisposition via a putative recessive defect. Although we were not able to type all family members for HLA specificities because of the costs incurred, these results appear to correspond to those of de la Concha et al. (30), who reported an increased number of class II homozygotes among CVID patients. However, this increase may not be a primary contributor to the disease susceptibility due to a restricted diversity of class II molecules as proposed (30) but could reflect the presence of a genuine predisposing mutation in close proximity to the class II genes. This has been observed in a number of families with recessive mendelian disorders and utilized for the autozozygosity mapping of underlying disease genes. The support for mere IGAD1 homozygosity as an important etiological factor in IgAD/CVID susceptibility is further diminished by zero or negative NPL values in families with homozygous H1 carriers (Tables V and VI).

**Family MHC recombinants**

The discovery of disease genes has often been facilitated by the identification of informative multiple-case families with a short cut event such as a cytogenetic abnormality, microdeletion, gene conversion, or informative crossover. IgAD exhibits a high relative risk to siblings, supporting a limited number of predisposing loci (4), and strong familial clustering can therefore make these events informative also in multifactorial disorders. However, both such families in our data set proved to be uninformative in this respect.

In the first family, affected siblings shared both MHC haplotypes identical by descent, but only one full and one recombinant haplotype with a nonaffected sister and nonaffected brother, respectively, consistent with an increased allelic sharing of affected individuals and linkage to the MHC. Assuming the presence of an IgAD-predisposing mutation on the maternally inherited haplotype shared by affected sisters, full penetrance of the mutation in this family, and no trans-acting influence of the paternally inherited haplotype in the son, the location of IGAD1 would be compatible with the class III region distal to the recombination event between MIB and TNFα loci. This area would correspond to that suggested by haplotype analysis of a large family with CVID (16), further limiting the proposed candidate region on its centromeric side by more than 20 kb, excluding 1C7 and a centromeric part of the LST1 gene. However, if IGAD1 were present on the paternally inherited IgAD-associated haplotype, the family would provide no information about the location of IGAD1.

In the second family, containing six affected individuals in two generations, affected daughters shared the IgAD-associated haplotype HLA-B8-D6S273(140)-DR17-DQ2. However, there was no co-segregation of the haplotype with the phenotype in the subsequence generation, arguing for the presence of non-MHC determinants in IgAD susceptibility. Thus, even if IgAD confers a high risk of developing the defect in close relatives, potentially informative family recombinants failed to provide conclusive evidence for the IGAD1 location. These examples illustrate difficulties in fine mapping complex disease genes.

**TDT in the analysis of parental allelic effects**

While single-case-unaffected parent trios showed an excess of the transmission of maternal haplotypes to the affected offspring (Table VI), this was not obvious in multiple-case families with a predominance of IgAD-transmitting mothers (Table VI), although the overall positivity of TDT was clearly observed for both ascertainment schemes. Unlike case-control studies, TDT can distinguish maternal and fetal genotype effects (31) by considering separately maternal genotypes with both maternal grandparents first and then, in a second step, fetal-parental trios. In addition to the impact of the gender of transmitting parents on the phenotypic outcome of the offspring (4), our present study supports the hypothesis that it is also a maternal MHC genotype that influences the risk of IgAD in the offspring. Our results also suggest that the parental phenotype of index cases should be considered when designing pedigree structures for genetic studies on human complex traits with parental allelic bias in susceptibility loci and/or parent-of-origin penetrance differences.

**Acknowledgments**


**References**


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