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Genetic Linkage of IgA Deficiency to the Major Histocompatibility Complex: Evidence for Allele Segregation Distortion, Parent-of-Origin Penetrance Differences, and the Role of Anti-IgA Antibodies in Disease Predisposition

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Summary

Immunoglobulin A (IgA) deficiency (IgAD) is characterized by a defect of terminal lymphocyte differentiation, leading to a lack of IgA in serum and mucosal secretions. Familial clustering, variable population prevalence in different ethnic groups, and a predominant inheritance pattern suggest a strong genetic predisposition to IgAD. The genetic susceptibility to IgAD is shared with a less prevalent, but more profound, defect called "common variable immunodeficiency" (CVID). Here we show an increased allele sharing at 6p21 in affected members of 83 multiplex IgAD/CVID pedigrees and demonstrate, using transmission/diseqilibrium tests, family-based associations indicating the presence of a predisposing locus, designated "*IGAD1*," in the proximal part of the major histocompatibility complex (MHC). The recurrence risk of IgAD was found to depend on the sex of parents transmitting the defect: affected mothers were more likely to produce offspring with IgAD than were affected fathers. Carrier mothers but not carrier fathers transmitted *IGAD1* alleles more frequently to the affected offspring than would be expected under random segregation. The differential parent-of-origin penetrance is proposed to reflect a maternal effect mediated by the production of anti-IgA antibodies tentatively linked to *IGAD1*. This is supported by higher frequency of anti-IgA–positive females transmitting the disorder to children, in comparison with female IgAD nontransmitters, and by linkage data in the former group. Such pathogenic mechanisms may be shared by other MHC-linked complex traits associated with the production of specific autoantibodies, parental effects, and a particular MHC haplotype.

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Introduction

Selective immunoglobulin A (IgA) deficiency (IgAD [MIM 137100]) is the most common primary immunodeficiency (PID), with a prevalence of ~1/600 in whites. The affected individuals lack IgA in serum and mucosal secretions and may suffer from frequent respiratory and gastrointestinal infections (for review, see Burrows and Cooper 1997). A markedly differing population prevalence among ethnic groups (Burrows and Cooper 1997), strong familial clustering of the disorder (Koistinen 1976; Oen et al. 1982; Schaffer et al. 1989; Vořechovský et al. 1995), a predominant inheritance pattern in multiple-case families that is compatible with autosomal dominant transmission, and a high relative risk for siblings (λ_s) (Vořechovský et al. 1995) suggest the involvement of thus-far-unidentified genetic factors that are responsible for the deficient production of isotypes.

Previous studies (Schaffer et al. 1989; Vořechovský et al. 1995) of close relatives of patients with IgAD suggest a frequent occurrence of common variable immunodeficiency (CVID [MIM 240500]). This PID is more severe than IgAD but is much less prevalent in the general population. Apart from IgA deficiency, CVID patients have decreased levels of IgG and, often, IgM, resulting in frequent infections. Our previous systematic study (Vořechovský et al. 1995) indicated a much higher than expected prevalence of CVID among close relatives of patients with IgAD, suggesting a shared pathogenesis of the two distinguishable PIDs. A substantial proportion of families containing cases of both IgAD and CVID was found in multiplex families obtained by screening for serum Ig levels in family members of patients with IgAD (Vořechovský et al. 1995). In multiple-case families with a dominant transmission of CVID/IgAD, CVID was usually present in the parental generation, followed by IgAD in the descendants (Vořechovský et al. 1995). This observation is consistent with the hypothesis that CVID may develop later in life, as a more severe manifestation

of a common, complex genetic defect, most likely involving immunoglobulin class switching. This concept is supported by a description of a gradual decline of serum IgG levels that progresses at similar ages in affected siblings (Johnson et al. 1997). Furthermore, IgG-subclass deficiencies have been found in a subset of IgAD patients (Oxelius et al. 1981). CVID may develop from IgAD over time (Ishizaka et al. 1989; Espanol et al. 1996; Johnson et al. 1997), and, occasionally, vice versa (Seligmann et al. 1991; Johnson et al. 1997). In both diseases, anti-IgA antibodies can be detected. Because (1) the disease phenotype is persistent (Koskinen et al. 1994; Vořechovský et al. 1995), (2) the phenocopy rate is low, and (3) λ_s, estimated at 50 (Vořechovský et al. 1995), indicates a strong degree of familiar clustering, chromosome susceptibility loci underlying this complex trait should be detectable by genetic linkage analysis.

In recent years, advances in statistical methods for linkage analysis, as well as the development of high-density genetic maps, have facilitated the mapping of predisposing loci for a number of polygenic and multifactorial diseases (Lander and Schork 1994; Dib et al. 1996). This methodology requires a well-defined and sufficient collection of family material. In the present study, we describe a large set of families with IgAD/CVID, which was analyzed for genetic linkage and family-based allelic associations at 6p21.3, containing the major histocompatibility complex (MHC), a region previously implicated in case-control association studies (Ambrus et al. 1977; Schaffer et al. 1989; Olerup et al. 1990; Volanakis et al. 1992). We also investigate both a parental allele–transmission distortion discovered at this locus and associated parent-of-origin differences in the disease penetrance. It is proposed that the parental transmission/penetrance bias is caused by a maternal effect involving the production of anti-IgA antibodies found significantly more frequently in affected mothers transmitting the defect to the offspring than in female IgAD nontransmitters.

Subjects and Methods

Ascertainment of Probands and Their Offspring

Diagnosis of patients with IgAD and CVID was established in accordance with accepted recommendations (Burrows and Cooper 1997), on the basis of measurements of Ig levels in multiple independent blood samples. The identification of index cases has been described elsewhere (Vořechovský et al. 1995). Multiple-case families were ascertained through the proband. In the offspring analysis of male and female patients, the available relatives of Swedish probands with either IgAD (64 females and 52 males) or CVID (16 females and 7 males) were contacted; all who responded and gave informed consent were analyzed for serum Ig levels. Of 259 ascertained children of affected parents, 14 were not available for sampling. Approval to contact and sample family members was obtained from local ethics committees.

Detection of Serum IgA and Anti-IgA Antibodies

Serum immunoglobulins were measured by routine nephelometry (Hammarström et al. 1983). The measurements were performed blind to family relationship and affection status. When a multiple-case family was found, the serum Ig measurements were repeated on an independent sample from each family member, to confirm the affection status. The immunoglobulin measurements of samples coming from outside Sweden were repeated in the Swedish laboratory, to guarantee that the same method defined the phenotype. Such design also ensured that a single sample could serve for both the ascertainment of affection status and DNA extraction, thus decreasing the probability of a laboratory mix-up. Anti-IgA antibodies were detected with an ELISA assay, as described elsewhere (Hammarström et al. 1983).

Analysis of Families for Genetic Linkage and Allelic Association

Multiple-case families were collected in Sweden ($n = 47$), the United Kingdom ($n = 21$), and Italy ($n = 4$). In addition, samples from small multiple-case families were obtained from the Netherlands ($n = 2$), the Czech Republic ($n = 2$), Turkey ($n = 1$), Finland ($n = 2$), Poland ($n = 1$), and Spain ($n = 2$). The complete pedigree structure of multiple-case families used for nonparametric genetic linkage analysis is shown at a Website of the Karolinska Institutet Department of Biosciences at NOVUM. The families with multiple sibs contained 59 affected sib pairs and 4 affected sib trios; two families had 4 affected siblings each. Both parental samples for affected siblings were available from 37 families, a single parental sample in 15 families, and no parental sample in 13 families. Of 83 multiple-case families, 48 had only IgAD, 10 families had only CVID, and 25 families (30%) included both IgAD-affected members and CVID-affected members. Among the 25 families with both diseases, 19 were multigenerational; in 16 there was a patient with CVID in the parental generation, followed by IgAD in a subsequent generation. Bilineal multiple-case families, rare cases with previously found homozygous deletions in the immunoglobulin structural genes (Plebani et al. 1993), and those with drug-induced IgAD (Truedsson et al. 1995) were not included in the study. Transmitting parents were defined as subjects who had affected offspring who themselves were affected. The family material used for the transmission/disequilibrium test (TDT), consisting of Swedish and U.K. sporadic cases with unaffected parents (and of

affected sib pairs with unaffected parents, in the TDT as a test of linkage), is shown at a Website of the Karolinska Institutet Department of Biosciences at NOVUM.

There was no overlap between the range of serum IgA level found in unaffected and affected family members, except for three families with IgAD that were excluded from the study. Therefore, the phenotype was considered as a categorical trait defined by serum IgA levels found repeatedly to be lower than a detection limit of 0.05 g/liter (Burrows and Cooper 1997). In addition, IgA levels obtained from two measurements on two independent samples were also analyzed as a quantitative-trait locus (QTL). The means of IgA levels (g/liter) for each family member are shown at a Website of the Karolinska Institutet Department of Biosciences at NOVUM.

DNA was extracted from blood samples, by routine techniques. Polymorphic markers, estimates of their genetic distances, and the frequencies of associated alleles are shown in table 1. Typing was performed with fluorescent-labeled primers (Research Genetics), on an ABI 373A-Stretch DNA Sequencer with GENESCAN 672 and GENOTYPER software packages (Applied Biosystems–Perkin-Elmer). Allelic sizes were converted to allele numbers by the GAS program (A. Young, University of Oxford), and the accuracy of conversion was checked manually. A strict global binning of allele sizes was applied throughout the study. Genotypes of family members are available at the indicated Websites.

The TDT (Spielman et al. 1993; Schaid and Sommer 1994; Sham and Curtis 1995; Spielman and Ewens 1996) was used as a test for genetic linkage (TDT_{link}), both in single-case families with unaffected parents and in multiple-case families each containing two or more affected sibs with unaffected parents. As a test of allelic association (TDT_{assoc}), the TDT was applied only to the firstborn affected individual in multiple-case families and in "single case–father–mother" trios (Spielman and Ewens 1996). To reduce the problem of multiple testing, the three most common alleles at each locus were analyzed by the TDT. The nonparametric linkage (NPL) (Kruglyak et al. 1996) and *Z* statistics (Lange 1986) were computed with the software packages GENEHUNTER (version 1.1) and GAS (version 2.0), respectively. The GENEHUNTER-PLUS program, which incorporates a one-parameter allele-sharing model (Kong and Cox 1997), was used to generate Z_{lr} and LOD* scores. The QTL statistics were analyzed by the GAS modules sibmwu, assmwu (Mann-Whitney U tests for sib-pair analysis and association, respectively), and sibhe (Haseman-Elston multipoint-regression algorithm).

Results

Allelic Associations and Genetic Linkage of IgAD/CVID at 6p21.3

To exclude the possibility that allelic associations in the MHC region in previously reported case-control

Table 1

Results of TDT_{assoc} and TDT_{link} for IgAD/CVID Families

Marker Locus[a]	θ[b]	No. (Size [bp])/ Frequency of Allele	T/NT for TDT_{assoc}	*P*/MGRR[c]	T/NT, for[d] $TDTp_{link}$	$TDTm_{link}$	$TDTall_{link}$	*P*, for TDTp/TDTm/TDTall[c]	TDTall[e]
D6S461	.04			NS				NS	
D6S1621	.005	4 (290)/.227	53/34	.042/4.1	21/18	28/17	60/42	.37/.068/.075	.224
D6S1558	.005	7 (248)/.596	62/40	.029/4.7	29/26	30/18	72/45	.39/.056/.0126	.038
D6S273	.03	1 (140)/.132	35/14	.0027/9.0	15/12	18/4	40/16	.35/.002/.0013	.0039
D6S1583	.03			NS				NS	
D6S291	.04	1 (212)/.103	28/13	.019/5.5	12/10	16/5	32/16	.42/.013/.021	.063
		3 (206)/.042	13/5	.059/3.6	6/4	9/2	15/8	.38/.033/.144	.43
D6S1610		...		NS				NS	

Note.—Allelic frequencies were estimated from the sample of 253 pedigree members without parents (founders and married-ins). The genetic map and θ values are as published elsewhere (Dib et al. 1996) and correspond to those estimated from the pedigree data by the MLINK and ILINK options of the LINKAGE pedigree package (version 5.1). The TDT algorithm as incorporated in the extended TDT (Sham and Curtis 1995) was applied to TDT_{assoc} and $TDTall_{link}$, whereas the GAS asstdt module, which does not deduce incomplete parental genotypes and avoids a potential parental bias, was used for $TDTp_{link}$ and $TDTm_{link}$. The weighted option of the TDT (GAS) yielded comparable *P* values. The Bonferroni correction, which is likely to be conservative for ETDT (Sham and Curtis 1995), was used for presenting corrected *P* values.

[a] Listed in order, from telomere to centromere.

[b] Value shown is that between the marker and that in the row immediately below.

[c] NS = not transmitted significantly more frequently than would be expected by chance.

[d] $TDTp_{link}$ = paternally derived alleles, $TDTm_{link}$ = maternally derived alleles, and TDTall = all transmitted/non-transmitted alleles from heterozygous parents.

[e] Data are corrected *P* values.

studies (Ambrus et al. 1977; Olerup et al. 1990; Volanakis et al. 1992) were caused by a population-structure effect and did not reflect genuine linkage disequilibrium, nontransmitted parental genotypes were used as internal controls in the TDT_{assoc}. A total of 126 unrelated patients (83 with IgAD and 43 with CVID) with either both parents unaffected ($n = 224$) or only one parent unaffected ($n = 14$) and the second parental sample missing were genotyped with microsatellite markers covering 14 cM at and around the MHC region (table 1). The TDT_{assoc} showed that the 140-bp allele at *D6S273* was transmitted from heterozygous parents to the affected offspring more often than would be expected by chance (35 transmissions [T] vs. 14 nontransmissions [NT]). The corresponding matched–genotype relative risk statistic (MGRR) (Schaid and Sommer 1994), was 9.0 ($P = .0027$). The χ^2 for allelewise-extended TDT (ETDT, version 1.8; Sham and Curtis 1995) at *D6S273* was 15.0 (7 df, $P = .036$). Weaker transmission disequilibria and MGRRs were observed at linked loci (table 1). Apart from Swedish and U.K. families, the transmission bias was found in 21 single-case families of northern Italian ancestry (7 T vs. 1 NT of the 140-bp allele at *D6S273* [$P < .05$]).

The TDT was also used as a test of linkage in the presence of allelic association (Spielman and Ewens 1996) in 146 affected individuals (100 with IgAD and 46 with CVID, including multiple affected sibs) with unaffected parents. The TDT_{link} was positive for *D6S273* (the χ^2 for allelewise ETDT was 15.1, 7 df, $P = .035$), with detectable transmission bias at flanking marker loci, indicating that allelic associations are indeed caused by the presence of a disease-susceptibility gene(s)/mutation(s) (termed "*IGAD1*") in the corresponding genomic region. In addition to alleles with an excess of T over NT (table 1), allele 9 (244 bp) at *D6S1558* and allele 4 (134 bp) at *D6S273* were preferentially retained in heterozygous parents (15 T vs. 33 NT [$P = .009$] and 40 T vs. 59 NT [$P = .05$], respectively). The analysis of control DNA samples from 96 child-father-mother trios, ascertained through a child with either cystic fibrosis or phenylketonuria, both of which are recessive diseases mapping to different chromosomes and are not expected to show an allele transmission disequilibrium at 6p21, revealed no segregation bias for any marker alleles (table 2).

The TDT results were consistent with the nonparametric genetic linkage analysis of 83 multiplex IgAD/CVID pedigrees containing a total of 449 individuals (215 affected, 178 nonaffected, and 56 with unknown phenotypes). The results of single-point NPL analysis of all sib-pairs by the GENEHUNTER program are shown in table 3. The maximum single-point NPL score was 2.63 ($P = .001$) at *D6S1583* (table 3). NPL scores at flanking centromeric loci and telomeric *D6S273* were also significant (table 3). In the multipoint GENEHUNTER analysis, the maximum NPL score was 2.4 ($P = .003$; proportion of *IGAD1*-linked families [α] .40, information content .83) between *D6S1583* and *D6S291*. The GENEHUNTER-PLUS program, which computes Z_{lr} and LOD* scores under a one-parameter allele-sharing model, to eliminate the conservativeness of NPL if the identity-by-descent information is less complete, gave a maximum Z_{lr} of 2.84 and LOD* of 1.75, in the same region. Similarly, the identity-by-state (IBS) sib-pair analysis (Lange 1986) revealed significant allelic sharing at the same marker loci, comparable with that shown by the NPL analysis (table 3).

Table 2

Transmission of Associated Alleles in 96 Genotyped Control Child-Father-Mother Trios

Marker Locus	θ	No. (Size [bp]) of Allele	T/NT, for			
			TDTp	TDTm	TDTall	*P*
D6S1621	.005	4 (290)	17/14	14/13	31/26	NS
D6S1558	.005	7 (248)	19/16	24/18	43/34	NS
D6S273	.06	1 (140)	9/8	8/8	17/16	NS
D6S291		1 (212)	8/8	10/9	18/17	NS
		3 (206)	6/5	4/5	10/10	NS

Note.—Data are as explained in the footnotes to table 1.

Although there was no overlap, in serum IgA levels, between IgAD and unaffected individuals in any family included in the linkage study, the mean serum IgA levels in family members were also analyzed as a QTL. In accordance with single-point analysis of IgAD as a categorical trait, the sib-pair analysis with the Mann-Whitney U-test showed the strongest QTL sharing at *D6S1583* and also showed significant allele sharing at flanking loci (data not shown). The test also confirmed both a significant positive association ($P < .0001$) between the 140-bp allele at *D6S273* and low levels of IgA and significant negative association ($P < .0005$) between the 134-bp allele and low levels of IgA, indicating a protective effect. The Haseman-Elston multipoint-regression analysis on weighted sib pairs showed maximum sharing closer to *D6S291*, an area ~2 cM centromeric to that obtained by multipoint NPL analysis.

IgAD versus CVID

In separate association analyses of IgAD and CVID, the TDT_{assoc} was positive for IgAD (25 T vs. 8 NT of the 140-bp allele at *D6S273* [$P = .003$] and 19 T vs. 8 NT of 212-bp allele at *D6S291* [$P = .03$], based on 83 affected offspring with 158 unaffected parents and 8 parents with unknown phenotype) but not for CVID (10 T vs. 6 NT, based on 43 affected individuals with 79 unaffected parents and 7 parents with unknown phenotype). Although TDT that was performed on all subjects with IgAD/CVID did not show a statistically sig-

Table 3

Single-Point IBS and NPL Analysis for 6p21.3 Markers and IgAD/CVID

		IBS Sharing					NPL Analysis		
Marker Locus	θ	No. of Affected Sib Pairs	2-1-0 Sharing (Observed/Expected)	One-Sided *P* for Affected Sib Pairs	Total No. of Sib Pairs	One-Sided *P* for All Sib Pairs	NPL_{all} (*P*/ Information Content)	Z_{lr}	LOD*
D6S461	.04	66	26.5-33.5-6.0/25.1-34.2-6.7	.35	85	.23	.65 (.23/.59)	1.01	.222
D6S1621	.005	64	29.3-31.0-3.7/27.0-31.9-5.0	.23	85	.22	.31 (.36/.54)	.48	.051
D6S1558	.005	68	37.5-25.5-5.0/34.4-30.1-3.5	.38	89	.16	.07 (.47/.46)	.12	.029
D6S273	.03	68	30.8-32.8-4.3/24.9-35.7-7.4	.044	89	.026	1.62 (.032/.64)	2.36	1.21
D6S1583	.03	67	33.7-28.7-4.7/22.6-35.6-8.8	.0024	87	.0043	2.63 (.001/.71)	3.45	2.58
D6S291	.04	69	41.5-23.2-4.3/29.8-34.1-5.1	.0074	89	.022	1.72 (.025/.52)	2.73	1.623
D6S1610		65	32.8-24.8-7.3/24.3-34.0-6.7	.061	84	.17	1.61 (.033/.59)	2.18	1.033

nificant transmission disequilibrium at *D6S1583*, the separate analysis of only the families with IgAD did show a weak transmission distortion of a 191-bp allele (30 T vs. 16 NT [$P = .04$]). In addition, the 133-bp allele at *D6S1610* showed 32 T from heterozygous parents, although on 18 occasions it was not passed on to the offspring who had IgAD ($P = .047$); the same allele also showed a positive QTL U-test association ($P = .027$).

Similarly, TDT_{link} was positive for IgAD only. The analysis of a total of 267 individuals in 84 families with 100 IgAD patients showed 30 T versus 10 NT of the 140-bp allele at *D6S273* ($P = .0016$) and 23 T versus 10 NT of the 212-bp allele at *D6S291* ($P = .024$). In CVID, however, there was a tendency of the frequent, 248-bp allele at *D6S1558* to be transmitted to the offspring more often than expected (24 T vs. 14 NT).

Parental Differences in Segregation of Associated Alleles at IGAD1

The TDT_{link} showed that, at *D6S273* and flanking markers, the transmission of IgAD-associated alleles from *unaffected* heterozygous mothers to the *affected* offspring was significantly in excess of what would be expected by chance; alleles transmitted from unaffected heterozygous fathers exhibited random segregation at these loci (table 1). Both allelewise ETDT and genotypewise ETDT (Sham and Curtis 1995) were positive for maternally transmitted alleles at *D6S273* ($\chi^2 = 20.8$, 7 df, $P = .004$, P_m[±standard error {SE}] = .0050 ± .0022 and $\chi^2 = 36.3$, 19 df, $P = .010$, $P_m = .0340 \pm .0057$, respectively, where P_m is the empirical *P* value obtained by Monte Carlo simulations with the MCETDT program, version 1.2 (J. Zhao, D. Curtis, and P. Sham; see the University College London on-line site listed in the Electronic-Database Information section) and at *D6S291* ($\chi^2 = 18.4$, 7 df, $P = .01$, $P_m = .0100 \pm .0031$ and $\chi^2 = 25.6$, 14 df, $P = .03$, $P_m = .0600 \pm .0075$, respectively). At *D6S1558*, a similar tendency was found ($\chi^2 = 14.6$, 7 df, $P = .067$ and $\chi^2 = 24.4$, 16 df, $P = .08$, respectively), although neither allelewise ETDT nor genotypewise ETDT was significant for paternally transmitted alleles. The observed difference between parental transmissions was significant for the 140-bp allele at the *D6S273* locus (GAS TDT algorithm, Fisher's exact test, $P < .05$). The sex of affected recipients of this allele was equally represented.

In contrast, neither maternal nor paternal transmission distortion was observed by typing of 96 unaffected control probands and their biological parents, ascertained through the index case with an unlinked recessive disease (table 2). Because the 140-bp allele at *D6S273* was found to be on the B8-DR3 haplotype, previously associated with IgAD (Ambrus et al. 1977; Schaffer et al. 1989; Olerup et al. 1990) and a number of autoimmune diseases, we also analyzed child-father-mother trios, for a possible segregation bias of this haplotype. These trios were selected from a large sample of children and both parents, which previously had been typed, for MHC specificities, at the Huddinge Hospital. The survey of several hundred consecutively typed index cases revealed 38 heterozygous B8-DR3 carriers (17 females and 21 males) with heterozygous parental haplotypes. All 38 index cases were free of IgAD/CVID, as documented by normal serum Ig measurements. Eighteen individuals inherited the B8-DR3 haplotype from heterozygous mothers, 20 from heterozygous fathers ($P > .05$).

Because there was no parental transmission bias of microsatellite alleles/MHC haplotypes, either in unaffected controls or in previous studies of the MHC in unaffected individuals (Klitz et al. 1987), we conclude that the parental allele segregation distortion was confined to the IgAD phenotype. This indicates that the contributions that parental haplotypes at *IGAD1* make to the development of the disorder are not equal. One explanation for this finding is putative paternal gametic imprinting at the *IGAD1* locus, which is supported by the structure of some pedigrees with IgAD. In four ascertained large, multigeneration pedigrees with IgAD skipping one generation, it was always a nonmanifesting woman who transmitted the phenotype to the offspring

(data not shown). In addition, a survey of published multiple-case pedigrees with IgAD/CVID (table 4) also showed two such pedigrees (family 50 in a study by Oen et al. [1982] and family 15 in a study by Volanakis et al. [1992]). These pedigrees had been proposed as being indicative of paternal gametic imprinting in a disease-predisposing locus (Hall 1990). Direct testing of this hypothesis, however, awaits the identification of the susceptibility-gene(s)/mutation(s) at *IGAD1*.

Penetrance of IgAD in the Offspring: Dependence on Gender of Transmitting Parent

If there is a parental allele transmission bias at *IGAD1,* and if this locus contributes substantially to the IgAD susceptibility, then one should see parent-of-origin penetrance differences in families. We therefore studied the offspring of Swedish index cases and individuals transmitting the disease to the next generation. A total of 87 women with either IgAD or CVID as index cases gave birth to 159 offspring, and a total of 52 affected men as index cases produced 86 offspring; 32 (20.1%) of the children born to affected women were found to be affected, whereas only 5 (5.8%) of the children born to affected men had IgAD ($P < .005$). To address the possibility that the ascertainment of paternal descendants was less complete, the offspring of an additional 26 male patients with IgAD were screened for serum Ig levels. In 42 analyzed children, only a single case of IgAD was identified.

This differential disease recurrence risk for children of women index cases versus children of men index cases was consistent with previous reports of multiple-case pedigrees, in which the total number of affected offspring of women with IgAD was much higher than the total number of affected offspring of men with IgAD (table 4). Although the numbers of men and women as index cases of multiplex families published to date are similar (28 vs. 23, respectively), which is consistent with the approximately equal sex ratio of our probands, the number of female patients transmitting the disease was higher than the number of affected male transmitters (45 vs. 26). The survey of multiplex pedigrees in 29 pub-

Table 4

Number of Female/Males, as Index Cases in Reported Multiple-Case Families with IgAD/CVID, as Parents Transmitting the Disease to Their Offspring, and as Children of Transmitting Parents

Source	No. of Females/Males[a]		No. of Affected Children of Females/Males with IgAD[a]
	Index Cases	Transmitting IgAD	
Ammann and Hong (1971)	?/?	2/1	3/1
de Asis et al. (1996)	1/0	1/0	2/0
Bach et al. (1971)	1/0	0/1	0/2
Beermann and Holm (1974)	1/0	1/0	2/0
Buckley (1975)	1/1	1/1	1/1
Cleland and Bell (1978)	1/0	2/0	10/0
Cuccia-Belvedere et al. (1989)	?/?	4/1	?/?
Cunningham-Rundles et al. (1991)	2/1	3/1	4/1
Douglas et al. (1971)	1/0	1/0	1/0
Gudmundsson and Jensson (1977)	0/1	1/0	1/0
Hilman et al. (1969)	1/0	1/0	2/0
Hobbs (1968)	1/3	2/2	4/2
Huntley and Stephenson (1968)	3/1	2/1	3/1
Kirkpatrick and Ruth (1966)	1/0	1/0	1/0
Koistinen (1976), S. Koskinen (personal communication)	6/6	3/5	5/7
de Laat et al. (1991)	2/0	2/0	2/0
Lakhanpal et al. (1988)	1/0	2/1	4/1
Lawton et al. (1972)	?/?	0/1	0/1
Levitt and Cooper (1981)	1/0	1/0	2/0
van Loghem (1974)	1/0	1/0	6/0
Natvig et al. (1971)	1/0	1/0	1/0
Nell et al. (1972)	1/2	2/1	3/1
Oen et al. (1982)	3/4	5/3	14/3
Rosner et al. (1978)	1/0	0/1	0/1
Schwartz et al. (1969)	0/1	0/1	0/2
Stocker et al. (1968)	1/0	2/0	3/0
Tomkin et al. (1971)	1/0	1/0	2/0
Vassalo et al. (1970)	0/1	2/1	5/1
Volanakis et al. (1992)	0/1	1/4	1/5

[a] A question mark (?) denotes that the relevant numbers could not be obtained from the reference.

lished reports (table 4) also showed that, although the affected women gave birth to a total of 82 children with IgAD, only 30 affected children of male transmitters were ascertained. The total numbers of affected children of women and men with IgAD, including those in the Swedish data set, were 123 and 46, respectively, indicating that mothers with IgAD are more likely to transmit the defect to their offspring than are the affected fathers. This difference could be explained by several factors, including ascertainment bias, feto-maternal interactions, and paternal genomic imprinting at one or more IgAD/CVID-susceptibility loci.

Serum Anti-IgA Antibodies: More Prevalent in Females Transmitting IgAD than in Nontransmitters

To assess a possible maternal effect exerted by IgG autoantibodies in disease susceptibility (Petty et al. 1985), we analyzed the frequency of carriers with anti-IgA antibodies in Swedish single- and multiple-case families. The overall frequency of Swedish IgAD patients without a family history of IgAD/CVID who were positive for anti-IgA antibodies (anti-IgA$^+$) was 22.2% (36/162 tested). Anti-IgA$^+$ females with IgAD (20/88 tested) and anti-IgA$^+$ males with IgAD (16/74 tested) were equally represented (22.7 vs. 21.6%; $P > .05$). In contrast, 22 (20 with IgAD and 2 with CVID) of 36 affected females (31 with IgAD and 5 with CVID) transmitting the disease were anti-IgA$^+$ ($\chi^2 = 17.9$ for females, $P < .00005$). This difference remained significant when only women with anti-IgA$^+$ IgAD who do not have a family history of IgAD/CVID and who gave birth to unaffected offspring (8/37 tested) were considered versus those with affected offspring (20/31 tested; $\chi^2 = 12.8$, $P < .0005$). Although the presence of anti-IgA antibodies in mothers with IgAD could theoretically be attributed to previous therapy with IgA-containing gammaglobulin preparations, only 1/20 mothers with IgAD anti-IgA$^+$ had been treated.

Even though the production of anti-IgA antibodies observed in a subset of IgAD is considered to be a constant feature over many years (Koskinen et al. 1995), antibodies may occasionally disappear or diminish in titer in mothers after delivery (Petty et al. 1985). Therefore, serum IgA levels were analyzed prospectively in the offspring of those women with IgAD/CVID who had been examined for anti-IgA antibodies during their pregnancies. Our 15-year follow-up of children of 12 such mothers with IgAD (4 with anti-IgA$^-$ and 8 with anti-IgA$^+$) showed (table 5) that 0/8 children born to mothers who were negative for IgAD developed IgAD, although 5/11 children born to mothers with anti-IgA$^+$ have IgAD ($P = .04$, Fisher's exact test). The mean age of the offspring at the time of sampling for serum Ig levels was similar in the two groups (9.4 and 7.5 years, respectively; $P = .26$, *t*-test). These observations indicate that women with IgAD who have affected children are more likely to develop anti-IgA antibodies than are IgAD-nontransmitting mothers, and our prospective study suggests that pregnant women with anti-IgA$^+$ and IgAD tend to produce more affected descendants than are produced by affected mothers with no detectable anti-IgA antibodies during pregnancy. This supports a role for specific autoantibodies in the familial clustering and pathogenesis of IgAD.

Table 5

Prospective Follow-up of Children of Affected Females with Documented Anti-IgA Antibody Titers during Pregnancy

	Status[b]	
Patient[a]	Maternal Anti-IgA/ First Child (age [years])	Maternal Anti-IgA/ Second Child (age [years])
1	++/IgAD (11)	+/NA (8)
2	++/NA (15)	++/NA (13)
3	+/NA (12)	
4	−/NA (8)	−/NA (6)
5	++++/NA (8)	
6	+++/IgAD (12)	
7	−/NA (8)	−/NA (3)
8	−/NA (10)	−/NA (5)
9	++++/IgAD (9)	++/IgAD (7)
10	−/NA (13)	−/NA (7)
11	++++/NA (2)	
12	++/IgAD (6)	

[a] Patients 10 and 11 had CVID; the remaining patients had IgAD.
[b] Titers are represented as follows: + = ⩽1:64; ++ = ⩽1:256; +++ = 1:1,024; ++++ = >1,024. NA = not affected.

Disease Susceptibility Conferred by IGAD1: Involvement of an Autoimmune Component Mediated by Anti-IgA Antibodies

To investigate whether the evidence for linkage to *IGAD1* was greater in multiplex families with women with both anti-IgA$^+$ and IgAD who transmitted the disease to their offspring than it was in the remaining dominant families—in which either the transmitting women were anti-IgA$^-$, the transmitters were men, or the autoantibody status was unknown—separate NPL analyses were performed. At *D6S273,* the NPL score was 1.620 ($P = .02$; LOD score 1.095, $\alpha = .96$, information content .64) in the former group, consisting of 20 families with anti-IgA$^+$, suggesting a linkage to this marker. In contrast, the latter group of 31 pedigrees had an NPL score of .143 ($P = .42$, LOD score -4.843, $\alpha = .002$, information content .64), giving no evidence for linkage. Similar differences between the two groups were observed for other markers in the region (data not shown). These results are not inconsistent with our previous analysis of a subset of 16 dominant families exhibiting no evidence for linkage at 6p21 (Vořechovský et al. 1995):

only 4 of these families were later found to have anti-IgA^+ transmitting women, whereas the remaining pedigrees had anti-IgA^- or male transmitters. These results strongly support genetic heterogeneity of IgAD/CVID, which was, in addition to a small sample size, likely to have contributed to previous negative linkage data. They also show that the predisposition to IgAD in families with females who are anti-IgA^+ transmitters is likely to be determined, to large extent, by *IGAD1*. This locus thus confers susceptibility to the development of anti-IgA antibodies and appears to be a major contributor to parental penetrance effects.

Discussion

Parent-of-Origin Penetrance Differences in IgAD

This report is the first formal demonstration of the differential parent-of-origin penetrance in the offspring of IgAD patients. Previously, Oen et al. (1982) had noticed a higher number of affected children born to mothers with IgAD, compared with the number of affected children born to affected fathers, in one large multiple-case family. Petty et al. (1985) analyzed serum IgA levels in infants and small children at age 2–29 mo who had been born to an affected parent, and they observed that the offspring of mothers with IgAD—but not the offspring of fathers with IgAD—had mean levels of serum IgA that were below normal values. We had considered parental penetrance effects to be due to the ascertainment bias in probands. First, female index cases identified through the clinical chemistry department were overrepresented (Vořechovský et al. 1995) because women either were referred to or sought a routine check-up for serum Ig levels more often than men did. However, affected women ascertained by this referral route were not overrepresented among those transmitting the disease to the offspring, nor did they contribute substantially to the number of probands (Vořechovský et al. 1995). Furthermore, the ascertainment of large numbers of cases by the screening of either infection-prone individuals or the general population yielded approximately equal sex ratios (Vořechovský et al. 1995), consistent with a vast body of data in the literature (Petty et al. 1985; Truedsson et al. 1995; Burrows and Cooper 1997). Second, the ascertainment of the offspring of affected men may have been less complete than the ascertainment of the offspring of affected women. Measurements of serum Ig levels in 42 descendants of additional men with IgAD, however, yielded a similar estimate of the recurrence risk for men, 1/42 versus 5/86 ($P > .05$); both of these estimates are significantly lower than the ~1/5 recurrence risk for descendants of women with IgAD. Third, because the exact age-dependent penetrance is unknown for IgAD, the transmission bias may be explained by an earlier date of birth of children of female transmitters. However, there was no significant difference between the mean ages of female versus male transmitters in our sample. Fourth, parental effects can be seen in a single family with many affected individuals (Oen et al. 1982; Schroeder et al. 1998). Most important, a possible bias in the proband ascertainment cannot account for the clear evidence of parental allele transmission distortion observed at *IGAD1* (table 1), because only unaffected parents of sporadic cases/affected sib pairs were analyzed in the TDT.

The penetrance bias may also result from reduced fertility in men with IgAD, compared with that in women with IgAD. This seems to be supported by a maximum allele sharing in the region syntenic to the *t*-complex in mice (Dunn 1957; Ardlie and Silver 1996). Mouse *t*-haplotypes are variants of the proximal third of chromosome 17 that are known by their capacity for segregation distortion and a 50–100-fold reduction in normal levels of recombination (Ardlie and Silver 1996). Although their segregation in females is consistent with Mendelian ratios, *t*/+ heterozygous males transmit the *t*-haplotypes to >95% of their offspring, although homozygous *t*/*t* males are sterile, preventing a fixation of *t*-haplotypes in the population (Dunn 1957; Ardlie and Silver 1996). However, in our study, although the total number of ascertained offspring of men with IgAD was somewhat lower than that of women with IgAD, a significant difference was observed only for the affected offspring. The segregation of the IgAD-associated B8-DR3 haplotype, in which recombination is suppressed relative to that for other haplotypes (Thomsen et al. 1994), as well as the segregation of IgAD-associated microsatellite alleles and HLA specificities (Klitz et al. 1987), did not depart from Mendelian ratios in unaffected individuals, arguing against a "meiotic drive" in controls. At a large number of polymorphic loci throughout the genome, no apparent inheritance errors have been noted in any of the 83 families included in the linkage study, (I. Vořechovský, unpublished data). The possibility that the transmission of some IgAD/CVID-associated MHC haplotypes to the unaffected offspring may still be distorted will need to be addressed in future studies, after the identification of *IGAD1*. With a small number of families, the extended B8-DR3 haplotypes containing the *GLO2* allele—but not *GLO1*—were reported to exhibit an excess transmission from men but not from women (Awdeh et al. 1983).

The inheritance pattern of IgAD/CVID, with a predominance of maternal transmission and different intrafamilial phenotypic manifestations, is reminiscent of that of mitochondrial defect(s). However, although paternal mitochondrial inheritance has been described in experimental populations (Gyllensten et al. 1991; Zouros et al. 1992), it is considered rare in humans, and

there have been few, if any, documented cases of human paternal transmission of mitochondria that has resulted in a disease (Schork and Guo 1993). The existence of a number of affected father-child pairs observed among our IgAD/CVID patients does not support this hypothesis; however, this argument weakens for human complex traits compared with Mendelian diseases, particularly if mitochondrial and nuclear gene products interact.

A number of human phenotypes have been shown to exhibit differential sex-related penetrance risks. In addition to monogenic disorders caused by expansion of repeat sequences (e.g., Huntington disease [Trottier et al. 1994] fragile X syndrome [Loesch et al. 1995], and congenital myotonic dystrophy [Lavedan et al. 1993]) and to defects resulting from differential contributions of parental genomes (e.g., Angelman and Prader-Willi syndromes [Nicholls 1993]), several complex diseases with such a biased pattern have been reported—for example, type 1 diabetes mellitus (Warram et al. 1984), in which parent-of-origin effects have been reported at two susceptibility loci, located in the MHC and in the insulin-gene regulatory region (Vadheim et al. 1986; Julier et al. 1991; Margaritte-Jeannin et al. 1995; Bui et al. 1996; Noble et al. 1996); bipolar affective disorder (McMahon et al. 1995); rheumatoid arthritis (Meyer et al. 1996); and asthma and atopy (Daniels et al. 1996). On the basis of maternal allelic distortion at several predisposing loci, the penetrance of asthma and atopy has been proposed as resulting from feto-maternal interactions (Daniels et al. 1996). For MHC-linked coeliac disease, Petronzelli et al. (1997) suggested that the parental sex influences the risk to affected children that is conferred by the DR3-bearing haplotypes, found their differential transmission in affected but not in unaffected offspring, and mentioned overrepresentation of mothers among transmitting parents. Parent-of-origin penetrance differences have also been reported for inguinal hernia (Gong et al. 1994) and breast cancer (Lindblom et al. 1993).

The proposed hypothesis that the disease susceptibility conferred by *IGAD1* is mediated by anti-IgA antibodies is supported by the observation of their transplacental transport to the offspring who developed both IgAD and anti-IgA antibodies early in life (Weemaes et al. 1982) that were accompanied by T-cell defects (de Laat et al. 1991). These case reports led de Laat et al. (1991) to suggest that IgAD with early-onset anti-IgA antibody production may be a distinct entity. Such an etiological mechanism is not unique, because a transplacental passage of maternal anti-Ro/anti-La antibodies can result in permanent fetal conduction-system damage and congenital heart block in neonatal lupus erythematosus associated with maternal DR3 (Olah and Gee 1993). It would be interesting to test this hypothesis in other MHC-linked autoimmune diseases with reported parental effects in the penetrance and allele segregation.

The IgAD autoimmune component proposed in this study is supported by earlier reports of a high prevalence of antibodies against different autoantigens in IgAD and the multireactivity of these antibodies (Goshen et al. 1989; Barka et al. 1995). Of 21 different autoantibodies, 16 were found in a significantly higher frequency in individuals with IgAD than in unaffected controls (Barka et al. 1995). A higher prevalence of anti-IgA antibodies was found in patients with IgAD who had either systemic lupus erythematosus or rheumatoid arthritis, compared with healthy subjects with IgAD (Petty et al. 1979).

IGAD1 *Locus*

Although the results of linkage analyses indicate a maximum allele sharing just centromeric of the MHC, the TDT clearly points to the proximal part of the MHC region (table 1). In meiotic mapping of Mendelian diseases, the underlying gene is present in the maximum-sharing region delineated by the closest flanking recombinants. In complex traits, however, a lack of maximum allele sharing at a predisposing locus, with a shift of this maximum to an adjacent region, may reflect the fact that one or more affected individuals does not carry the disease-locus mutation. Therefore, *IGAD1* is not likely to be near *D6S1583* but should be located more telomeric, closer to *D6S273,* the marker locus exhibiting the most significant allelic associations and parental effects, which maps to the MHC class III region between *HSP70* and *BAT2* (Martin et al. 1998). Since the allelic associations with distal markers in class I and with those telomeric to the MHC are weaker (table 1), *IGAD1* is likely to reside in the proximal half of the MHC, which contains class II and class III genes.

The absence of IgAD among 43 Sardinian DR3 homozygotes—who share the class II haplotype, but not the class III haplotype, with the disease-associated northern European extended haplotype B8-DR3 (Cucca et al. 1998*b*)—appears to favor the class III region. This location would correspond to that suggested by a recent haplotype analysis in a single multicase family (Schroeder et al. 1998) and to that proposed earlier by Wilton et al. (1985). However, screening of 24 B8-DR3 Scandinavian homozygotes for serum IgA levels did not reveal any IgAD either (L. Hammarström, unpublished data), perhaps suggesting that the relative risk conferred by this haplotype may have been overestimated. The prevalence of IgAD/CVID in DQB1*0201-DR3-B8-A1 homozygotes has been estimated to be as high as 13% (Schroeder et al. 1998). Although the recombination rate on the associated haplotype B8-DR3 is lower than average (Thomsen et al. 1994), and although the principle of linkage-disequilibrium decline as a monotonic func-

tion of the recombination fraction (θ) appears to be violated in the *IGAD1* candidate region (Klitz et al. 1995), typing of our family material by recently defined microsatellite loci (Martin et al. 1998), as well as by MHC specificities followed by linkage disequilibrium/haplotype analyses, may help us to define the locus further.

CVID versus IgAD

Separate TDT analyses of IgAD versus CVID seem to suggest that *IGAD1* confers susceptibility to IgAD rather than to CVID, whereas loci responsible for the lack of additional immunoglobulin classes in CVID are elsewhere in the genome. This concurs with the observed lack of significant parental effects in CVID. Less convincing data supporting the role of the MHC in susceptibility to CVID are consistent with a previous case-control study (Olerup et al. 1992), which showed that allelic associations in both the class II region and the class III region were much weaker in CVID than in IgAD. However, the number of available patients with CVID was lower than the number of available patients with IgAD. Furthermore, CVID is likely to be more genetically heterogeneous than IgAD, and the probability of misdiagnosis is higher (Spickett et al. 1997).

Candidate Molecules/Mutations at IGAD1

Because the IgAD/CVID phenotype is thought to be limited to lymphocytes/macrophages, candidate molecules defective at *IGAD1* are likely to exhibit a narrow expression, possibly confined to T-cells and/or antigen-presenting cells. The failure of T-cells to respond to antigens in CVID (Kondratenko et al. 1997), as well as the presence of subtle T-cell abnormalities in IgAD (Horowitz and Hong 1975), point to a putative antigen-presentation defect, originally proposed to exist in a patient with CVID (Eibl et al. 1982). This hypothesis is compatible with the observation that very low levels of IgA and other isotypes can temporarily be induced in vivo by lysosomotropic agents such as chloroquine (for review, see Truedsson et al. 1995). This drug selectively inhibits the capacity of splenic accessory cells to process large antigens for T-cell activation and suppresses immune responses to antigens requiring lysosomal processing (Lee et al. 1982). The inhibition of antigen presentation (Ziegler and Unanue 1982) may be elicited by other IgAD-inducing agents, such as gold salts, diphenylhydantoin, or D-penicillamine; the latter drug is known to lead to myasthenia gravis, systemic lupus erythematosus, and, often, multiple autoimmune complications after a short treatment period (for review, see Smith and Hammarström 1985). T-cell responses have been implicated in the adverse effects caused by all these agents (Gleichmann 1981; Schumann et al. 1990; O'Donnell and Coleman 1992). CD4+ cells from patients with CVID have reduced glutathione (Aukrust et al. 1995), a major physiological reducing thiol, and its decreased intracellular level has been linked to impaired antigen presentation (Short et al. 1996). Although a recent study did not reveal a defect in antigen presentation by CVID B-cells and monocytes (Thon et al. 1997), the possibility remains that it is indeed a defective crosstalk between B-cell and T-cells that results in an impaired isotype production in these PIDs.

Parent-of-origin penetrance differences, a maternal allele-transmission bias at *IGAD1,* and the complex genetics of the defect may suggest that the disease-predisposing mutation(s) can lie outside a gene coding sequence. This location could be an altered DNA-binding target or a tandem repeat of a unique sequence, which have been found to be either associated with or contained within imprinted genes. Heritable DNA-methylation changes at CpG dinucleotides of several nucleotide-sequence motifs in the MHC have been shown to alter binding of some DNA-binding proteins to their recognition sequence (Zhang et al. 1990). A recent study has indicated that β-like chains in the MHC class II region have a CpG island over the most polymorphic second exons, and promoter inactivation has been shown to lead to methylation of the mouse class II Aβ CpG island (Macleod et al. 1998).

In conclusion, the present study provides evidence of genetic linkage and population stratification–independent allelic associations in IgAD/CVID, localizing the first PID polygene to a chromosomal region by means of meiotic mapping. Observed parent-of-origin penetrance differences in IgAD associated with allelic segregation distortion at the MHC are proposed to be caused by a maternal effect, which involves the production of anti-IgA antibodies tentatively linked to *IGAD1*. The identification of other susceptibility loci by genome scanning (I. Vořechovský, unpublished data), some of which may be involved in the regulation of serum IgA levels in normal individuals (Wiltshire et al. 1998) or reside on the X chromosome (Cucca et al. 1998*a*), should lead to better understanding of the terminal stages in lymphocyte differentiation and of PIDs.

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Electronic-Database Information

URLs for data in this article are as follows:

University College London, ftp://www.gene.ucl.ac.uk/pub/packages/dcurtis (for ETDT program version 1.8 and MCETDT program version 1.2)

Karolinska Institutet Department of Biosciences at NOVUM, http://www.cbt.ki.se (for linkage pedigree file of multiple-case families [http://www.cbt.ki.se/fam/npl.htm], QTL analysis [http://www.cbt.ki.se/fam/qtl.htm], and family material used for TDT [http://www.cbt.ki.se/fam/tdt.htm])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for CVID [MIM 240500] and IgAD [MIM 137100])

GAS program (A. Young, University of Oxford), ftp://ftp.ox.ac.uk/pub/users/ayoung)

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