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Analysis of Families with Common Variable Immunodeficiency (CVID) and IgA Deficiency Suggests Linkage of CVID to Chromosome 16q

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Abstract

Common variable immunodeficiency (CVID) is an antibody deficiency syndrome that often co-occurs in families with selective IgA deficiency (IgAD). Vořechovský et al. [American Journal of Human Genetics 641999, 1096-1109; Journal of Immunology 1642000, 4408–4416] ascertained and genotyped 101 multiplex IgAD families and used them to identify and fine map the *IGAD1* locus on chromosome 6p. We analyzed the original genotype data in a subset of families with at least one case of CVID and present evidence of a CVID locus on chromosome 16q with autosomal dominant inheritance. The peak (model-based) LOD score for the best marker D16S518 is 2.83 at $\theta = 0.07$, and a four-marker LOD score under heterogeneity peaks at 3.00 with $\alpha = 0.68$. The (model-free) NPL score using the same markers peaks at the same location with a value of 3.38 ($p = 0.0001$).

Keywords

KEYWORDS Common variable immunodeficiency; recurrent infections; IgA deficiency; linkage analysis; primary immunodeficiency disorder; chromosome 16

Introduction

Common variable immunodeficiency (CVID, OMIM 240500) is characterized by recurrent infections, due to the lack of immunoglobulin (Ig) G and IgA. In half of the patients IgM levels are also reduced. The incidence of CVID is estimated at between 1/25,000 and 1/66,000 in different populations (IUIS 1999, Hammarström et al. 2000). Selective IgA deficiency (IgAD) has a higher incidence (1/600 to 1/800), but is often asymptomatic, making CVID the most common primary immunodeficiency in man requiring medical attention. IgAD and CVID often occur in different members of the same family and IgAD occasionally progresses to CVID (Ishizaka et al. 1989; Español et al. 1996; Johnson et al. 1997; Carvalho Neves Forte et al.

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2000) suggesting that they may represent two phenotypic variants in a spectrum associated with the same molecular defect(s).

This hypothesis is supported by the recent discovery of mutations in the *TNFRSF13B* gene, which is located on 17p and encodes the protein TACI (Castigli et al. 2005; Salzer et al. 2005) in some CVID/IgAD families. In both studies, individuals with IgAD and CVID are diagnosed in first degree relatives with the same heterozygous *TNFRSF13B* mutation as the proband.

Besides *TNFRSF13B*, three other genes have recently been found to be mutated in apparently monogenic cases of CVID: *ICOS* on 2q (Grimbacher et al. 2003), *CD19* on 16p (van Zelm et al, and Franco Jl et al, XIth Meeting of the European Society for Immunodeficiency, Versailles 2004, Abstracts #B27 and #B71), and *TNFRSF13C* on 22q, which encodes the BAFF receptor (Warnatz K et al., XIth meeting of the European Society for Immunodeficiency, Versailles, 2004, Abstract #B27). All affected individuals found so far with mutations in these three genes have homozygous mutations, while heterozygote carriers are asymptomatic. Adding to the complexity, some male patients originally diagnosed with CVID have subsequently been found to have the X-linked lymphoproliferative syndrome (OMIM 308240), caused by mutations in *SH2D1A* (Morra et al. 2001, Nistala et al. 2001), or X-linked agammaglobulinemia (OMIM 307200) caused by mutations in *BTK* (Bruton 1952, Saffran et al. 1994, Smith et al. 1994).

Several linkage and association studies have shown an IgAD locus (*IGAD1)* in the MHC region on chromosome 6p (Schaffer et al. 1989; Olerup et al. 1990, 1992; Volanakis et al. 1992; Cucca et al. 1998; Schroeder et al. 1998; Vořechovský et al. 1999, 2000, Braig et al, 2003). Many of these studies combined families with IgAD only and those with IgAD and CVID. In the only study to include large numbers of IgAD-only families (Vořechovský et al. 1999), a transmission disequilibrium test (TDT) with chromosome 6 markers gave significant results when applied to the IgAD-affected individuals and their parents, but did not give significant results when applied to CVID-affected individuals and their parents. In a more recent study, Braig et al. (2003) furthermore suggested linkage of CVID to chromosome 5p in one large family.

The only published large cohort of multiplex IgAD families was described at three stages of ascertainment (Vořechovský et al. 1995, 1999, 2000). Most of the families were previously genotyped at markers spanning the human genome and model-free linkage analysis methods were used to find the *IGAD1* locus on 6p (Vořechovský et al. 1999). The 18 most recently ascertained families were previously genotyped both on 6p (Vořechovský et al. 2000) and with a subset of markers used in the 1999 study. Forty families had at least one case of CVID. In this study, we reanalyzed the existing genotype data for these 40 families, and we extended the genotyping in 32 families where samples were available. The aim was to search for loci that show linkage to CVID.

Patients, Materials, and Methods

Patients

Informed written consent was obtained from each individual prior to participation under the internal ethics review board-approved clinical study protocol (#239/99 for B.G. and 435/99 for L.H.). The initial 101 families included 43 families with at least one case of CVID. For these families, a very strict upper limit of 0.05 g/L of IgA was used to diagnose IgAD, which is one reason that model-free linkage analysis was used in the previous study. One of the several ascertainment strategies was to identify CVID patients with IgA levels below the limit and then measure IgA levels in their first degree relatives. Therefore, the IgAD family cohort is enriched for families with at least one CVID case.

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Between the time of the initial analysis and the analysis shown here, we found that one family was a duplicate (cv73 and cv94 in Vořechovský et al. 1999) and two families have mutations in *TNFRSF13B*. These two families are family A (cv79 in Vořechovský et al. 1999) with a homozygous S144X mutation and family C (cv22 in Vořechovský et al. 1999) with a heterozygous A181E mutation in Salzer et al. (2005). Both families show variable penetrance/severity among individuals with the same mutation. Excluding these two families slightly weakens the results (e.g., single-marker LOD scores) shown below. Of the 40 families used for the analysis here, we had sufficient sample material from 32 of the families to do follow-up genotyping using a set of new markers.

Genotyping

Genotypes from a genome-wide scan published by (Vořechovský et al. 1999) were reanalyzed by different methods in this study. Based on promising preliminary analysis on chromosomes 7 and 16, genotypes for the new markers D16S3018 and D16S3049 were determined on all the available family members from families with at least one case of CVID. We also determined some genotypes for two new markers on chromosome 7, but the additional markers gave negative scores and are not discussed below.

For the new genotyping, primers and other reagents were purchased from Invitrogen Research Genetics (Karlsruhe, Germany) and biomers.net GmbH (Ulm, Germany) and Qiagen GmbH (Hilden, Germany). The polymerase chain reactions (PCR) for genotyping were performed according to the protocols accompanying the reagents. The PCR products were sequenced on an ABI377 sequencer (PE Applied Biosystems, Foster City, USA) using the COLLECTION and ANALYIS software. Integer allele lengths were assigned using the GENOTYPER (PE Applied Biosystems) software package.

Linkage Analysis

For this study, we initially used the model-based LOD score method, as implemented in FASTLINK (Lathrop et al. 1984; Cottingham et al. 1993; Schäffer et al. 1994). This was followed by genotyping a set of new markers on the most promising regions on chromosome 16 . We assessed the significance of the LOD scores at the most promising marker with the simulation package (FAST)SLINK (Ott 1999; Weeks et al. 1990; Cottingham et al. 1993). We also evaluated the data for this marker with the model-free method implemented in SimIBD (Davis et al. 1996). For the final chromosome 16 data, we also computed LOD scores under heterogeneity and model-free NPL scores with the GENEHUNTER software package (Kruglyak et al. 1996; Kruglyak and Lander 1998)

For model-based LOD score analysis, we used the penetrance function shown in Table 1. In addition, some individuals who were not examined have unknown (0) disease status. We used dominant inheritance because that appears to be far more common in CVID/IgAD than recessive inheritance (Vořechovský et al. 1995). The unaffected individuals in class 2, who have a non-negligible probability of carrying the disease allele, are assigned an equivocal penetrance function, making the analysis close to “affecteds only”. The disease allele frequency was set to 0.001, as in Vořechovský et al. (1999); marker allele frequencies were estimated from the data using the downfreq program (Terwilliger 1995).

Results

Various methods of linkage analysis suggest linkage of CVID to chromosome 16q. Peak single-marker LOD scores for chromosome 16q markers are shown in Table 2. The marker D16S518 achieves the best score (2.83), and the positive scores at consecutive markers suggest that this is a true positive locus. We assessed the empirical significance of the 2.83 LOD score by using

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the simulation package (FAST)SLINK to generate 3000 unlinked replicates of the 40 families using the same disease parameters and D16S518 allele frequencies. The highest-scoring replicate achieved a score of 3.34, while the second best replicate achieved a score of 2.59. According to the method described in Ott (1991, p. 191), one unlinked replicate in 3000 with a score above the true score implies that the latter is significant at $p < 0.0017$.

The two-marker LOD score using D16S515 and D16S518 peaks at 3.53, but places the putative disease gene at $\theta = 0.06$ below D16S518, which would suggest that the gene is located below D16S3049. This result and the far right column of Table 2 with all recombination fractions > 0.05, suggest locus heterogeneity. This is hardly surprising since four genes that cause CVID have already been discovered. We computed 4-marker (D16S3018, D16S515, D16S518, D16S3049) LOD scores under heterogeneity (HLOD) using the GENEHUNTER software package The HLOD peaks at 3.00 between D16S518 and D16S3049, where the estimate of the fraction of linked families (α) is 0.68.

GENEHUNTER also computes the model-free NPL score, and for the same 4-marker run the NPL score again peaks between D16S518 and D16S3049 at 3.38 ($p=0.0001$). The p-values associated with the moving NPL scores are all < 0.001 from D16S515 and below. We also tested for linkage of D16S518 using another model-free method, SimIBD. Using the default recommended parameters, SimIBD yields $p = 0.043$ for the data at D16S518, which is considered significant.

Discussion

In summary, we have presented several genetic linkage analyses suggesting a locus for CVID/IgAD on chromosome 16q, near the marker D16S518. We identified one possible candidate gene, *WWOX* (WW-domain containing oxidoreductase), which is located near D16S518. *WWOX* has been considered as a candidate for somatic mutation in cancer, partly because it is present within the fragile site FRA16D. One study in mice shows that the WWOX protein participates in the TNFα signaling pathway (Chang et al. 2001), which suggests that *WWOX* may have a role in immunity. We therefore sequenced all coding regions of *WWOX* in six families with positive multipoint scores on 16q (cv125, cv46, cv4, cv72, cv134, cv147 of Vořechovský et al. 1999), but found no mutations.

Interestingly, there is precedent for two genetic loci on chromosome 16 predisposing to a complex immune-related disease. The *NOD2/CARD15* gene on 16q is mutated in some patients with Crohn's disease, and in some patients with other types of inflammatory bowel disease (IBD; Hugot et al. 2001; Ogura et al. 2001), and there appears to be a second locus for inflammatory bowel disease on 16p (Hampe et al. 2002). Both IBD and CVID are complex diseases for which some susceptibility genes have been found, but the majority of patients have no mutations in the known genes. We hope that the identification of this CVID-predisposing locus on 16q leads to the identification of another CVID susceptibility gene.

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Table 1

Penetrance function in LINKAGE notation for model-based linkage analysis of 40 multiplex CVID/IgAD families.

Class	Description	Affection Status	Penetrance		
1	unaffected, married in	1	0.00	1.00	1.00
2	unaffected, not married in	1	0.50	0.50	0.50
3	affected with IgAD only	2	0.01	1.00	1.00
4	affected with CVID	2	0.00	1.00	1.00

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Table 2

Markers in the linkage region on chromosome 16 and peak single marker LOD scores under the model in Table 1 and locus homogeneity. Markers marked with * were genotyped for this study in 32/40 families.

Marker	Sequence Position(Mb)	Peak LOD score	Recombination fraction(θ)
D16S503	62.2	1.14	0.17
D16S2624	70.3	0.96	0.17
D16S3018*	72.7	1.29	0.17
D16S515	75.1	0.76	0.16
D16S518	76.7	2.83	0.07
D16S3049*	77.5	0.35	0.21
D16S516	77.7	0.27	0.22