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Report

Hereditary Vascular Retinopathy, Cerebroretinal Vasculopathy, and Hereditary Endotheliopathy with Retinopathy, Nephropathy, and Stroke Map to a Single Locus on Chromosome 3p21.1-p21.3

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We performed a genomewide search for linkage in an extended Dutch family with hereditary vascular retinopathy associated with migraine and Raynaud phenomenon. Patients with vascular retinopathy are characterized by microangiopathy of the retina, accompanied by microaneurysms and telangiectatic capillaries. The genome search, using a high throughput capillary sequencer, revealed significant evidence of linkage to chromosome 3p21.1-p21.3 (maximum pairwise LOD score 5.25, with D3S1578). Testing of two additional families that had a similar phenotype, cerebroretinal vasculopathy, and hereditary endotheliopathy with retinopathy, nephropathy, and stroke, revealed linkage to the same chromosomal region (combined maximum LOD score 6.30, with D3S1588). Haplotype analysis of all three families defined a 3-cM candidate region between D3S1578 and D3S3564. Our study shows that three autosomal dominant vasculopathy syndromes with prominent cerebroretinal manifestations map to the same 3-cM interval on 3p21, suggesting a common locus.

Hereditary vascular retinopathy (HVR), Raynaud phenomenon, and migraine have been reported in a large Dutch family (Terwindt et al. 1998). HVR shows autosomal dominant inheritance and is characterized by microangiopathy of the retina, accompanied by microaneurysms and telangiectatic capillaries, that appears preferentially around the macula (Storimans et al. 1991). Abnormalities can be detected by use of fluorescein angiography in otherwise asymptomatic family members who are 25–30 years old, suggesting an age of onset in young adulthood. Later stages of the disease involve occlusion of branches of large retinal arteries, avascular areas in the retinal periphery, and, sometimes, proliferative retinopathy with extensive avascular areas, even close to the optic disk. Of the affected members of this family, 80% also have Raynaud phenomenon, a pathological vasomotor reaction of the digital blood vessels to cold exposure, which causes numb and extremely pale fingers. Migraine is present in 70% of individuals with HVR, and a combination of migraine and Raynaud phenomenon is observed in 55% of patients with HVR.

Vascular retinopathy is also a prominent feature in two other conditions, cerebroretinal vasculopathy (CRV [MIM 192315]) and hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS) (Grand et al. 1988; Gutmann et al. 1989; Jen et al. 1997). Grand et al. (1988) described CRV in a large white kindred, and Jen et al. (1997) described HERNS in a family of Chinese descent. These families showed autosomal dom-

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inant inheritance of a syndrome characterized by retinal capillary obliteration and CNS vasculopathy. A distinctive feature of both families was the presence of progressive subcortical contrast-enhancing lesions with surrounding edema, a condition that mimics tumors and prompts biopsy in numerous affected family members. These individuals have progressive visual loss that begins in the third or fourth decades of life and is followed by focal neurological deficits and death within 10 years. Other symptoms commonly observed in our subjects were stroke, dementia, and migrainelike headaches. In the family with HERNS, electron microscopy showed distinctive multilamination of subendothelial basement membranes of capillaries in the brain and other tissues (Jen et al. 1997).

There are, however, distinctions in the clinical manifestation and severity of HVR, CRV, and HERNS. For example, Raynaud phenomenon has been reported only in patients with HVR (Terwindt et al. 1998), whereas prominent pseudotumors, seen in both CRV and HERNS, have not been reported in HVR. These pseudotumors develop when individuals are <50 years old and are the major cause of morbidity in these families. Renal involvement has been described only in HERNS (Jen et al. 1997), but two affected members of the CRV kindred also have prominent renal disease (J.P.A. and M.L.B.L., unpublished data). Furthermore, although HVR has no apparent impact on life expectancy, patients with CRV or HERNS die of neurological complications at $\leqslant$55 years of age. Of note, patients with CRV or HERNS often develop migrainelike headaches.

Here, we report the mapping of the gene for HVR to the 3p21.1-p21.3 region in an extended Dutch kindred. In addition, we show that CRV (Grand et al. 1988) and HERNS (Jen et al. 1997) map to the same chromosomal location. Detailed clinical data on the extended Dutch family with >300 individuals have been published elsewhere (Storimans et al. 1991; Terwindt et al. 1998). In the present study we performed genomewide genotyping only of the 21 family members with the most distinctive phenotype (i.e., HVR), together with their first-degree relatives, for reconstruction of linkage phase. Genotyping was performed using 400 evenly distributed markers from the ABI Prism Linkage Mapping Set, version 2, analyzed via a new high throughput capillary electrophoresis system (ABI Prism 3700 DNA analyzer, Applied Biosystems). PCR reactions were performed under standard conditions, using an ABI Prism 877 Integral Thermal Cycler (Applied Biosystems). All genotypes were independently scored by two of us (J.D.Y. and R.A.O.), using GENOTYPER 2.1 software, and consensus tables were created for each marker. Discrepancies were corrected by assigning the genotype status "unknown" to ambiguous genotypes. Genotyping for follow-up and high-resolution mapping was performed using PCR, under standard conditions, with PE 9700 instruments. PCR products were detected by use of an ABI 377 sequencer and were analyzed using GENESCAN and GENOTYPER software (Applied Biosystems).

Linkage analysis was performed under the assumption of a dominant model for vascular retinopathy with 90% penetrance for this phenotype and .0001 frequency of the disease allele. The phenocopy rate was set at 0.1%. The affected status of family members without HVR symptoms was considered to be unknown. The data were checked for Mendelian inheritance, using the UNKNOWN program (Lathrop et al. 1984), and two-point linkage analyses were performed using MLINK program of the LINKAGE package, version 5.1 (Lathrop et al. 1984). Multipoint LOD scores were computed by the VITESSE algorithm (O'Connell and Weeks 1995). Marker-allele frequencies were estimated on the basis of data on the founders and spouses of the families used in this study. The Marshfield and Généthon genetic maps were used for selection of additional markers and to determine the genetic distances (Broman et al. 1998; Dib et al. 1996).

Initial results from the two-point genomewide linkage analysis produced no conclusive proof of linkage (data not shown). However, three chromosomal regions were identified for further analysis (one of which was on chromosome 3p), with marker D3S1289 yielding a LOD score of 1.83. A single chromosome 3p haplotype consistently cosegregated with the retinopathy phenotype. Genotyping of additional markers and additional family members (fig. 1) further supported linkage to this region. Two-point LOD scores for the closely spaced markers on chromosome 3p in the more-extended pedigree are shown in table 1. A maximum two-point LOD score (Z_{max}) of 5.25 was obtained at a recombination fraction (θ) of 0 for marker D3S1578. Six flanking markers also yielded LOD scores with statistical significance for linkage (LOD > 3) (table 1). Multipoint analysis with the most informative markers confirmed the assignment of the HVR locus to this region on chromosome 3p (data not shown).

Given the overlap of the cardinal clinical feature, microangiopathic retinopathy, we tested the microsatellite markers from chromosome 3p, which corresponds to the HVR candidate region, in the families with CRV or HERNS (Grand et al. 1988; Jen et al. 1997) (fig. 2). Because the trait appeared to be a highly penetrant autosomal dominant disease in both families, LOD scores were calculated under a more stringent genetic model, assuming full penetrance and not allowing for phenocopies; the frequency of the disease allele was set at .0001. Under this more stringent model, Z_{max} in the family with CRV (family 2) was 4.35 with locus D3S1588, at $\theta = 0$; in the family with HERNS (family 3), $Z_{max} = 1.98$ at $\theta = 0$ of locus D3S1578 (table 2). Multi-

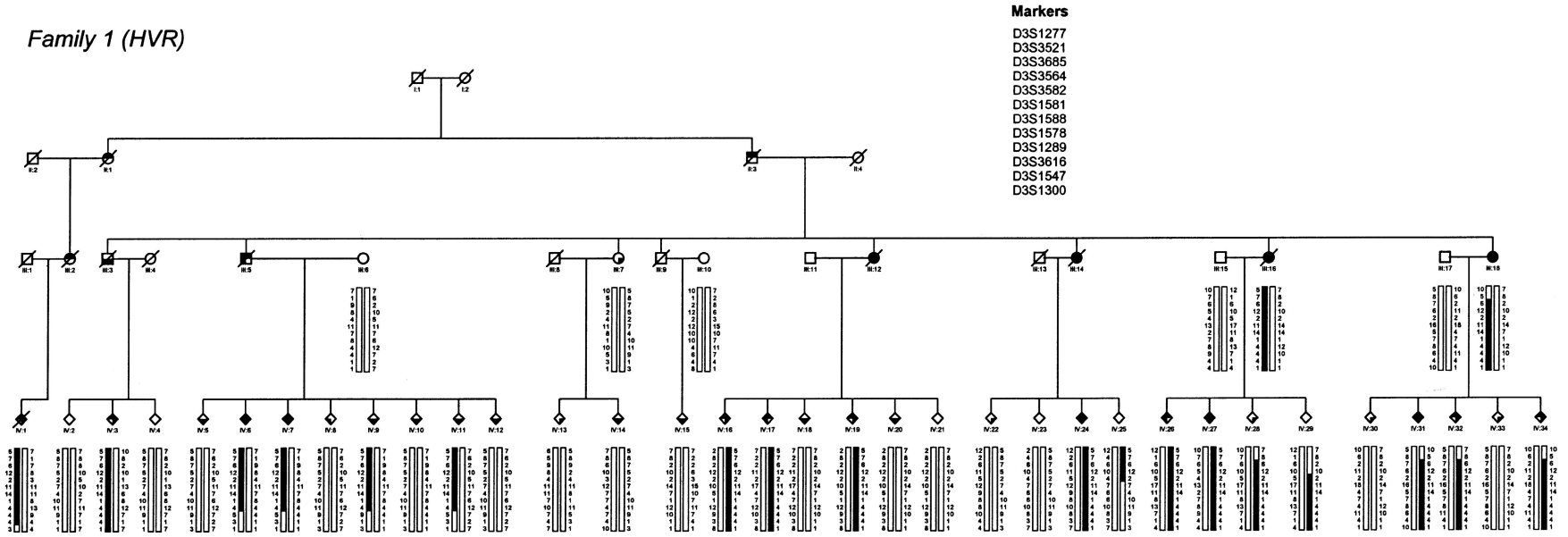

Figure 1 Partial pedigree of the Dutch family with HVR (family 1). In the youngest generation, the order has been randomized, and the gender of all individuals is depicted as unknown. Haplotypes for 12 microsatellite markers spanning ~20 cM on chromosome 3p are shown. Black bars indicate the haplotype segregating with the retinopathy phenotype. Completely blackened symbols indicate individuals affected by all three symptoms (HVR, Raynaud's phenomenon, and migraine); symbols with the upper half blackened indicate HVR; symbols with the bottom left blackened indicate migraine, and symbols with the bottom right blackened indicate Raynaud's phenomenon.

Table 1

Two-Point LOD Score Results between the HVR Locus and Chromosome 3p Markers

	LOD Score at θ =								
Marker	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ (Z_{max})
D3S1277	−4.48	−.25	.89	1.17	1.09	.71	.24	1.19	.13
D3S3521	−.01	2.62	3.00	2.88	2.31	1.54	.66	3.00	.05
D3S3564	4.28	4.20	3.89	3.48	2.61	1.65	.61	4.28	.00
D3S3685	4.48	4.41	4.10	3.70	2.84	1.86	.76	4.48	.00
D3S3582	1.07	1.04	.94	.81	.56	.30	.09	1.07	.00
D3S1581	3.77	3.69	3.40	3.03	2.29	1.54	.73	3.77	.00
D3S1588	1.32	1.28	1.13	.98	.60	.31	.10	1.32	.00
D3S1578	5.25	5.17	4.80	4.33	3.31	2.20	.98	5.25	.00
D3S1289	4.71	4.62	4.29	3.85	2.92	1.90	.79	4.71	.00
D3S3616	.30	2.92	3.28	3.14	2.51	1.67	.71	3.28	.05
D3S1547	−.11	1.93	2.35	2.30	1.85	1.23	.53	2.36	.06
D3S1300	−2.73	−.80	−.23	−.09	−.07	−.08	−.05	.00	.50

point analysis confirmed linkage in both families (data not shown). The two-point LOD score is clearly significant for the family with CRV alone. The a priori evidence of linkage of the HVR disease to chromosome 3p was the reason to evaluate only this chromosomal region in the families with CRV and HERNS. The linkage result in the family with HERNS approximates the standard for significance ($LOD > 2$) for this circumstance. Also, the similarity of the CRV and HERNS phenotypes warrants combining the LOD scores for these two families, resulting in a highly significant value of $Z_{max} = 6.30$ at D3S1588 (table 2).

The most likely haplotypes, cosegregating with the disease in the three different families, are displayed in figures 1 and 2, along with the pedigrees. The haplotypes are unique in each family, which suggests independent genetic origin of the disease-causing mutations. Haplotype analysis in the Dutch family with HVR (fig. 1) shows that the entire region delimited by markers D3S3521 and D3S3616 is consistent with linkage to the HVR phenotype, exhibiting recombination with the disease locus in patient III-18 and the offspring of III-5, respectively. Haplotype reconstruction of the kindred with CRV (family 2) showed a recombination event in individual 666, which allowed us to further refine the location of the disease gene distal to marker D3S1578 (fig. 2). In both the CRV and HERNS pedigrees, recombinations were observed in healthy family members who are probably past the age of risk for the respective diseases. Individual 517—who, at age >60 years, is a healthy member of the family with CRV—and individual 316—a healthy 57-year-old member of the family with HERNS—both carry part of the disease-associated haplotype, with recombinants between D3S3564 and D3S3582 (fig. 2). If we assume full penetrance of the disease mutation in these families, the candidate region can be narrowed to an interval of ~3 cM between loci D3S3564 and D3S1578 on chromosome 3p21.2-p21.3 (fig. 3).

Detailed haplotyping in the Dutch family indicates incomplete penetrance of HVR, because several members of the family with the complete disease-containing haplotype (individuals IV-9, IV-11, and IV-28) or parts of the haplotype (individuals IV-25 and IV-29) did not show any abnormalities when tested with fluorescein angiography. As the current ages of these individuals are 35–55 years (and the age at onset of the HVR symptoms is 26–62 years [Terwindt et al. 1998]), the asymptomatic subjects sharing the disease haplotype may still develop the disease.

Haplotype analysis in the family with HVR also reveals that there is no simple relationship between the HVR haplotype and the occurrence of migraine and Raynaud phenomenon. Family members without HVR but with migraine or Raynaud phenomenon did not all share the disease-related haplotype. For example, of the subjects who have the combination of migraine and Raynaud phenomenon (subjects IV-5, IV-10, IV-12, IV-14, IV-15, IV-18, and IV-20) do not have the HVR-related haplotype, whereas subjects IV-9 and IV-11 do carry the disease haplotype. The high prevalence of migraine (Russell et al. 1995) and Raynaud phenomenon (Maricq et al. 1997) in the general population (both >10%) may at least partly contribute to this poor correlation. Fluorescein angiography clearly is crucial in establishing the diagnosis of this syndrome.

The 3-cM candidate region defined by haplotype reconstruction has a high density of known and putative genes ($n > 100$) and is physically large for positional cloning (>10 Mb) (GeneMap'99). Among these genes is collagen type VII α1 (COL7A1) (fig. 3). A host of different types of mutations in the COL7A1 gene have been described in different families, causing dominant and recessive forms of epidermolysis of the skin (MIM

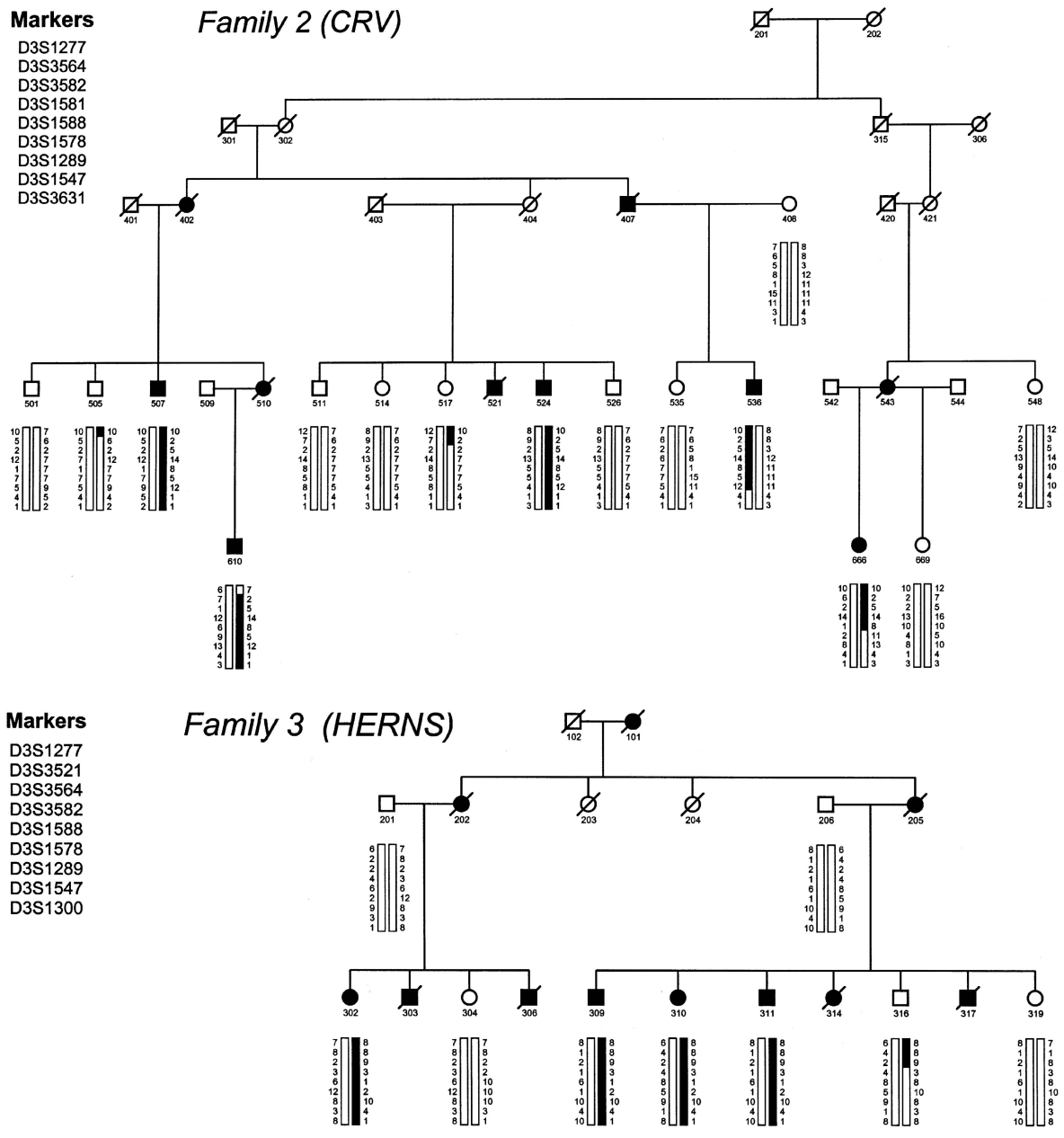

Figure 2 Pedigree structures and haplotypes of a kindred with CRV (*top*, family 2) and a kindred with HERNS (*bottom*, family 3). Blackened symbols indicate individuals affected by CRV or HERNS. Haplotypes for marker loci from the chromosome 3 HVR candidate region are shown. Black bars indicate haplotypes that segregate with the disease.

120120). However, none of the mutation-linked clinical features, as described so far, suggest that they are involved in the etiology of vascular retinopathy, the cardinal feature of HVR, CRV, and HERNS.

The chromosomal 3p region has been implicated in other hereditary disorders that affect the retina. Spinocerebellar ataxia 7 (SCA7 [MIM 164500]) is associated with retinal degeneration in addition to cerebellar degeneration. However, detailed genetic mapping of the SCA7 gene located the gene between D3S1312 and D3S1600 (David et al. 1996), just proximal to marker D3S1300 and outside the HVR/CRV candidate region (fig. 3). Analysis of the SCA7 repeat in the family with HVR confirmed the exclusion of this gene (data not shown). Recently, linkage was found for Usher syndrome type IIB (USH2B [MIM 276905]) to chromosome 3p,

entirely overlapping the HVR locus (Hmani et al. 1999). USH2B is characterized by congenital hearing loss, normal vestibular function, and onset of retinitis pigmentosa in the late second or the third decade of life.

Given the large physical size of the 3-cM region and the high density of genes, further refinement of the candidate region is necessary. Consequently, we are in the process of identifying additional individuals with vascular retinopathy, who are (remotely) related to one of these families. Alternatively, the narrowing of the candidate region may require identification of new families with hereditary vascular retinopathy or related symptoms. There are at least three other families reported with cerebroretinal vasculopathy mimicking a brain tumor, a condition similar to the characteristics described for the families with CRV (Gutmann et al. 1989; Weil et al. 1999; Niedermayer et al. 2000).

Hereditary cerebroretinal vasculopathies are uncommon. For this reason, the demonstration that HVR, CRV, and HERNS are all linked to a single 3-cM region on chromosome 3p21.1-p21.3 is consistent with the idea of a common etiology for all three phenotypes. It should be noted that so far all three families have been investigated by different clinicians applying different protocols, and thus further detailed comparative studies are warranted. Like the vascular retinopathy in the Dutch family, CRV and HERNS are characterized by microangiopathy of the posterior pole. In contrast to HVR, however, the retinal periphery is not affected. Other features—such as the presence of a cerebral frontal pseudotumor, progressive cognitive deterioration, psychiatric disturbances, extensive cerebral white matter lesions on MRI, and stroke—distinguish CRV and HERNS from HVR (Grand et al. 1988; Jen et al. 1997). Interestingly, a notable similarity among these three families is the high prevalence of migrainelike headaches. Because a vascular component is part of the etiology, it is conceivable that the retinopathy gene may have a secondary role in the development of migrainelike symptoms and Raynaud phenomenon in these families.

Identification of the causative gene will further help to unravel the pathophysiology of these hereditary cerebroretinal vasculopathies and may shed new light on common neurovascular disorders such as stroke, migraine, and Raynaud phenomenon.

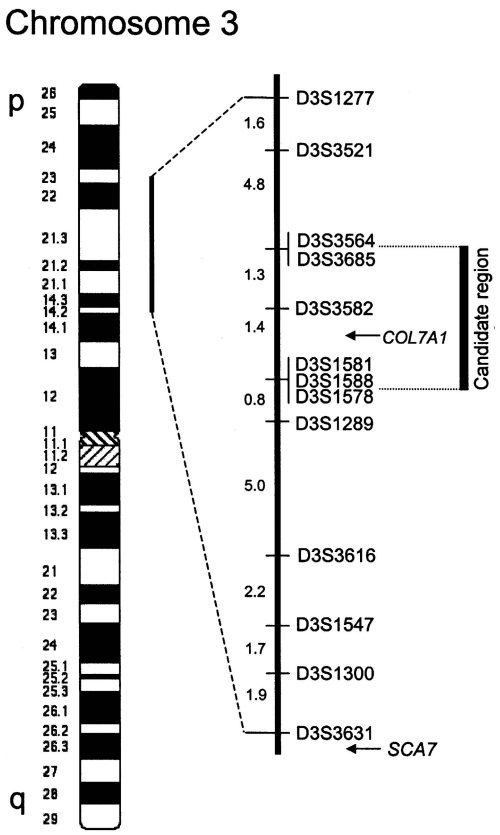

Figure 3 Ideogram of chromosome 3 with the genetic map of the HVR/CRV/HERNS candidate region on 3p21.2-p21.3. Positions of genes are indicated and abbreviations are explained in text; genetic distance is in centimorgans and is based on the Marshfield map (Broman et al. 1998).

Table 2

Two-Point LOD Scores for a Family with CRV (Family 2) and a Family with HERNS (Family 3), Using a Stringent Linkage Model that Assumes Full Penetrance and No Phenocopies

		LOD Score at θ =						
Marker	Family	.00	.01	.05	.10	.20	.30	.40
D3S1277	2	−8	−1.96	−.48	.03	.26	.19	.06
	3	−8	−.04	.53	.66	.59	.39	.13
D3S3521	2	...	...	...	...	...	...	...
	3	−8	−1.05	−.35	−.08	.08	.08	.03
D3S3564	2	−8	1.59	2.04	2.02	1.65	1.12	.50
	3	−8	−.10	.46	.59	.54	.34	.11
D3S3582	2	−1.17	.94	1.44	1.49	1.23	.80	.32
	3	.41	.40	.34	.28	.17	.08	.02
D3S1581	2	3.36	3.29	3.03	2.69	1.99	1.27	.54
	3	...	...	...	...	...	...	...
D3S1588	2	4.35	4.27	3.92	3.47	2.53	1.57	.64
	3	1.95	1.92	1.77	1.58	1.18	.74	.28
D3S1578	2	.28	2.20	2.56	2.43	1.85	1.14	.42
	3	1.98	1.94	1.79	1.59	1.17	.72	.26
D3S1289	2	.65	2.63	2.99	2.87	2.27	1.50	.64
	3	.81	.84	.87	.84	.67	.42	.14
D3S1547	2	−2.06	−.92	.24	.58	.63	.40	.11
	3	1.43	1.40	1.29	1.15	.86	.53	.19

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for selection of additional markers and to determine the genetic distances)

GeneMap'99 and Entrez Map Viewer, http://www.ncbi.nlm.nih.gov/ (for map of the candidate region)

Généthon, http://www.genethon.fr/ (for selection of additional markers and to determine the genetic distances)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CRV [MIM 192315], SCA7 [MIM 164500], and USH2B [MIM 276905])

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