Genetic Linkage of Wagner Disease and Erosive
Vitreoretinopathy to Chromosome 5q13-14

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Background: Wagner disease and erosive vitreoretinopathy are potentially blinding autosomal dominant diseases that share some similarities with Stickler syndrome. However, both disorders have associated retinal pigment epithelial changes, poor night vision, visual field defects, and abnormal electroretinographic findings, which are not found in families with COL2A1-associated Stickler syndrome. In addition, rhegmatogenous retinal detachments are uncommon in Wagner disease but occur in approximately 50% of patients with either Stickler syndrome or erosive vitreoretinopathy.

Objectives: To identify the chromosomal location of the genes involved in Wagner disease and erosive vitreoretinopathy and to distinguish these conditions genetically from Stickler syndrome.

Methods: Fifteen affected members of a family affected with erosive vitreoretinopathy and 24 affected descendants of the pedigree described by Wagner were genotyped with a set of short tandem repeat polymorphisms distributed across the genome.

Results: Significant linkage was observed in each family between the disease phenotype and markers that map to chromosome 5q13-14. The highest lod score for the family affected with erosive vitreoretinopathy was 4.2 and was obtained with marker GATA3H06 (9 q0). The highest lod score for the family affected with Wagner disease was 5.8 and was obtained with marker D5S815 (9 q0). A candidate gene (cartilage link protein) that is known to lie near the linked interval was screened for mutations, but none was found in either family.

Conclusions: These data suggest that erosive vitreoretinopathy and Wagner disease are allelic disorders and demonstrate that they are genetically distinct from COL2A1-associated Stickler syndrome.

METHODS

GENOTYPING

After obtaining informed consent, blood samples were obtained from 39 affected family members and 15 informative spouses. DNA was prepared from whole blood using a nonorganic procedure. A screening panel of over 200 short tandem repeat polymorphisms, distributed across the autosomal genome, was selected from those characterized by the Cooperative Human Linkage Center (CHLC), Iowa City, Iowa; Genethon, Evry, France; or the published literature. The majority of the markers used were GATA tetranucleotide repeats obtained through the CHLC. Oligonucleotide primers flanking each short tandem repeat polymorphism were synthesized using standard phosphoramidite chemistry (Model 391 DNA synthesizer, Applied Biosystems, Foster City, Calif.).

Thirty nanograms of each patient's DNA was used as a template in an 8.35-μL polymerase chain reaction mixture containing the following: 1.25 μL of buffer solution consisting of TRIS hydrochloride (100 mM, pH 8.8), potassium chloride (500 mM), magnesium chloride (15 mM), and gelatin (0.01% wt/vol); 200 μM each of deoxycytidine triphosphate, deoxyadenosine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate; 1 pmol of each primer; and 0.25 U of Taq polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Samples were incubated in a DNA thermocycler (Omigene, Woodbridge, NJ) for 35 cycles at 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds. After amplification, 5 μL of stop solution (95% formamide; sodium hydroxide, 10 mM; 0.05% bromphenol blue; and 0.05% xylene cyanol) was added to each sample. The samples were then denatured and electrophoresed on 6% polyacrylamide gels at 60 W for about 3 hours. Following electrophoresis, gels were silver stained as previously described. Permanent records were created of all gels with EDF film (Eastman Kodak Co, Rochester, NY).

SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS

Single-strand conformation polymorphism analysis was used to screen the majority of the coding sequences of the CRTL1 gene for the presence of mutations in affected individuals from both families. Polymerase chain reaction primer sequences were chosen from the published CRTL1 sequence (Table 1). Each exon was amplified in 8.35 μL of polymerase chain reaction mixture using the same reaction mixture as that used for genotyping (above). Samples were incubated in the DNA thermocycler for 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Following amplification, 5 μL of stop solution was added to each well. Samples were denatured and electrophoresed on 6% polyacrylamide gels (5% glycerol; TRIS hydrochloride, 45 mM, pH 8.0; boric acid, 45 mM; and ethylenediaminetetraacetic acid [EDTA], 0.5 mM) at 20 W for approximately 4 hours at room temperature. Following electrophoresis, gels were silver stained as described above.

LINKAGE ANALYSIS

Linkage analysis was performed using the LINKAGE program package (version 5.1). The MLINK routine was used for the pairwise analysis. For the data given in Table 2, the allele frequencies were assumed to be equal for each marker. The reference genetic maps used for the analysis were obtained from Murray et al and the CHLC. The CHLC map was generated using version 2.5 of combined CEPH data (Center d'Etudes du Polymorphisme Humain, Evry). (These data are available on request from CEPH or electronically at FTP.CEPH.ORG or via Gopher server at Gopher.CHLC.ORG.)

RESULTS

CLINICAL FINDINGS

Twenty-six family members of the erosive vitreoretinopathy pedigree originally reported by Brown and coworkers were studied (Figure 1, left). All were at a 50% risk of having the disease because of a known affected parent or sibling. Fifteen of these patients were found to have marked vitreous syneresis and other typical findings of erosive vitreoretinopathy. A constant finding in affected patients was a transience or erosion of the RPE that allowed clear visualization of the choroidal vessels. In severely affected patients, the RPE was denuded in a geographic, scalloped pattern at the equator. Late in the course of the disease, some patients had profound RPE atrophy and pigment clumping similar to retinitis pigmentosa or choroideremia (Figure 2). Vitreous syneresis was seen in all affected patients as pronounced vitreous sheets, veins, and ropes. Often, vitreous traction was observed at the border between erosive lesions and normal-appearing RPE. Eleven affected patients (73%) had rhegmatogenous retinal detachments, five of which were bilateral. Only two affected patients over the age of 10 years had completely attached retinas. As in Stickler syndrome, the retinal breaks were often posterior and difficult to repair. Visual acuity was reduced to light perception or worse in six (20%) of the 30 affected eyes.

Sixty living descendents of the original pedigree described by Wagner were also available for study. Fifty-two were at a 50% risk of having the disease because of a known affected parent or sibling (Figure 1, right). Twenty-eight were believed to have unmistakable characteristics of Wagner disease, which usually affects the retinas, eyes. An anterior posterior retinal break visual acuity
was a thoughtful plier no-NA 8°C anch on 5% oric acid y 4esis, crossed 2, Gene 2, as pro-vitrous e lesions (73%) in situ hybridization to 5q13-14. A short tandem repeat polymorphism exists within the CRTL1 gene, and genotyping of this marker in the two families revealed no affected recombinants (combined lod score, 7.5; \( \theta = 0 \)). Although no intron sequence data have been published for the CRTL1 gene, the cDNA sequence and positions of the introns are known. These data were used to develop a single-strand conformation polymorphism assay that spans 77% of the coding sequence (Table 1). No mutations were detected in either family using this assay.

No intragenic polymorphisms have been found in the CSPG2 gene, and its precise location on 5q is not known. The structure of the CSPG2 gene is even less well-characterized than that of CRTL1, and, as a result, mutation analysis of this gene was not performed.

**Table 1. Primer Sequences for CRTL1 Assay**

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<tr>
<th>Exon</th>
<th>Primers</th>
<th>Nucleotide Positions</th>
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<tbody>
<tr>
<td>1</td>
<td>5' GCGTGAATTTGACGTTGACGTTGACG 3'</td>
<td>40-44</td>
</tr>
<tr>
<td>1</td>
<td>5' GCGTGAATTTGACGTTGACGTTGACG 3'</td>
<td>224-228</td>
</tr>
<tr>
<td>2</td>
<td>5' TGGTCTGATGCAGCTGACGCTGACG 3'</td>
<td>290-311</td>
</tr>
<tr>
<td>3</td>
<td>5' TGGTCTGATGCAGCTGACGCTGACG 3'</td>
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</tr>
<tr>
<td>3</td>
<td>5' TGGTCTGATGCAGCTGACGCTGACG 3'</td>
<td>290-311</td>
</tr>
<tr>
<td>3A</td>
<td>5' AGAATAAGCTCGAGCTCGAGCTCGAGCTCGAGCT 3'</td>
<td>417-436</td>
</tr>
<tr>
<td>3B</td>
<td>5' GCCACACGCTGACGCTGACGCTGACGCTGACG 3'</td>
<td>555-574</td>
</tr>
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<td>3C</td>
<td>5' GCCACACGCTGACGCTGACGCTGACGCTGACG 3'</td>
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<td>3D</td>
<td>5' GCCACACGCTGACGCTGACGCTGACGCTGACG 3'</td>
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<td>3E</td>
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<td>3F</td>
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<td>3G</td>
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<td>3H</td>
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<td>1005-1024</td>
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<td>1105-1124</td>
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<tr>
<td>3J</td>
<td>5' GCCACACGCTGACGCTGACGCTGACGCTGACG 3'</td>
<td>1205-1224</td>
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</table>

*Primers chosen from sequence data of Duthia and coworkers, nucleotide positions correspond to GenBank file X78078.*

**Table 2. Two-Point Linkage Data**

<table>
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<tr>
<th>Linked Alleles*</th>
<th>Lod Score (9)</th>
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<tr>
<td></td>
<td>Erosive Vitreoretinopathy</td>
</tr>
<tr>
<td>AT3F07</td>
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<tr>
<td>CRTL1</td>
<td>6/7</td>
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<tr>
<td>D5S426</td>
<td>2/5</td>
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<tr>
<td>D5S488</td>
<td>4/4</td>
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<td>2/3</td>
</tr>
<tr>
<td>GATN10115</td>
<td>2/3</td>
</tr>
</tbody>
</table>

*The first number of the pair is the allele that is most commonly linked to the disease (1-greatest number of repeats). The numbering system is consistent between the two families. The second number of the pair is the total number of alleles observed in the individual family.*

*\( \theta \) at which the highest lod score occurred is given in parentheses if not zero.*
In 1938, Wagner described a Swiss family with myopia, early cataract formation, liquefaction of the vitreous, retinal vascular changes, and RPE changes. He termed the disease "degeneratio hyaloido-retinalis hereditaria." This family has subsequently been studied by Böhringer et al., Ricci, and Maumenee et al. Prior to the recent reexamination of the Wagner pedigree (R.A.G. and E.P.M., unpublished data, 1994), only one patient with Wagner disease had ever been found to have a rhematogenous retinal detachment.

In 1993, Korkko and colleagues described a glycine-to-aspartate substitution in the COL2A1 gene in a family who they believed had a disease "similar to the original family described by Wagner." Their article describes only three patients, all of whom had retinal detachments and none of whom had the RPE changes found in erosive vitreoretinopathy and Wagner disease. In addition, radiographic examinations were not performed. We believe that the findings in this family are more compatible with Stickler syndrome than with Wagner disease.

In 1994, Brown et al. described a new entity known as erosive vitreoretinopathy. It has a much higher incidence of rheumatogenous retinal detachments and a poorer visual prognosis than Wagner disease. Both can be clinically distinguished from Stickler syndrome by the absence of systemic findings such as cleft lip or palate, joint pains, and radiographic abnormalities.

This study shows that both Wagner disease and erosive vitreoretinopathy are genetically distinct from Stickler syndrome and are caused by mutations in a 35-centimorgan region of the long arm of chromosome 5. The disparity between the alleles linked to the affected phenotypes in the two families with the majority of the linked causing Th...
linked markers (Table 2) suggests that the disease-causing mutation in each family arose independently. Thus, the different clinical features in these two diseases could stem from different mutations in a single gene or from mutations in two different but tightly linked genes.

Among the genes known to map to the long arm of chromosome 3 are two (CRTL1 and CSPG2) whose products (cartilage link protein and chordroitin sulfate proteoglycan core protein) contribute significantly to the structure of the extracellular matrices of cartilage and vascularized connective tissue, respectively. In the chicken, CRTL1 has been shown to be expressed in a variety of noncartilaginous tissues, including the eye. The expression of CRTL1 and CSPG2 in the human eye has not, to our knowledge, been investigated. CRTL1 and CSPG2 have some homologous domains and may have arisen from a common ancestral gene. It is therefore possible that other undiscovered members of this gene family also reside on 3q. The present study cannot exclude either CRTL1 or CSPG2 from potential involvement in erosive vitreoretinopathy or Wagner disease.

In summary, this study demonstrates that the mutations that cause Wagner disease and erosive vitreoretinopathy are linked to markers on the long arm of chromosome 5. In the future, the combination of mutation analysis of the COL2A1 gene and linkage analysis with chromosome 5 markers should allow the correct molecular cause of vitreoretinopathy to be determined in many families. The eventual identification of the specific disease-causing mutations in these families is likely to provide significant new insight into the structure of the vitreous and retina, and it is hoped that this information will also provide new insight into the pathogenesis of many forms of human retinal detachment.

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