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GENETIC FACTORS MODIFYING CLINICAL EXPRESSION OF AUTOSOMAL DOMINANT RP

Stephen P. Daiger¹, Suma P. Shankar¹, Alice B. Schindler¹, Lori S. Sullivan¹, Sara J. Bowne¹, Terri M. King², E. Warick Daw³, Edwin M. Stone⁴, and John R. Heckenlively⁵

¹Human Genetics Ctr and Dept. of Ophthalmology, Univ. of Texas, Houston, TX, USA

²Dept. of Internal Medicine, Univ. of Texas, Houston, TX, USA

³Dept. of Epidemiology, Univ. of Texas MD Anderson Cancer Center, Houston, TX, USA

⁴Dept. of Ophthalmology, Univ. of Iowa, IA, USA

⁵Kellogg Eye Ctr, Univ. of Michigan, Ann Arbor, MI, USA

1. INTRODUCTION

Factors modifying clinical expression of inherited diseases are likely to be complex, involving genetic factors, environmental factors and stochastic effects. One way to reduce the complexity is to focus on individuals who share a dominant mutation identical by descent, thus eliminating variability in the underlying mutation and variation in *cis* to the mutation. A further simplification is to limit analysis to a single, extended family, which may reduce, though not eliminate, environmental effects.

Autosomal dominant retinitis pigmentosa (adRP) offers a number of opportunities for such studies. We are investigating factors which modify clinical features consequent to an Arg677ter stop mutation in the RP1 gene, a mutation which causes adRP with variable age-of-onset, progression and end stage consequences, that is, variable severity (Berson et al., 2002; Jacobson et al., 2000; Sullivan et al., 1999).

The RP1 locus was mapped by linkage testing to 8q13 in a large, extended adRP family, RP01, largely situated in southeastern Kentucky (Blanton et al., 1991; Field et al., 1982). Subsequently, the RP1 gene was identified by positional cloning and mutation screening in RP01 and additional adRP families (Guillonnet al., 1999; Pierce et al., 1999; Sullivan et al., 1999). Mutations in the RP1 gene account for approximately 4% of adRP families in the United States. The RP1 Arg677ter mutation, found in RP01 and other families, accounts for approximately 1/2 of the total (Bowne et al., 1999).

The RP1 gene codes for a novel protein, 2,156 amino acids in length, with high sequence similarity over the first 10% of the amino terminus to doublecortin, a protein involved in cortical folding, but with very little sequence similarity elsewhere. The protein is photoreceptor-specific; localizes to the rod interconnecting cilium; stabilizes disc architecture in outer segments; and is most likely involved in ciliary matrix assembly and function (Gao et al., 2002; Liu et al., 2002; Liu et al., 2003; Liu et al., 2004; Pierce et al., 2004).

The Arg677ter mutation is in the final, 4th exon of the gene and probably escapes nonsense-mediated decay. If so, the product of the mutant message would be a severely truncated protein,

retaining the doublecortin domain, but lacking most of the remaining sequence. Thus the pathophysiology probably involves a dominant negative or gain of function effect, rather than haploinsufficiency.

Factors modifying simple mendelian diseases, such as retinitis pigmentosa, may be just as complex as factors contributing to multifactorial diseases such as age-related macular degeneration (Haider et al., 2002; Nadeau, 2003). However, for dominant-acting diseases one factor is particularly likely: the “wild type” allele in *trans* to the mutation. For example, alleles in *trans* to alpha-spectrin mutations modify the severity of elliptocytosis (Gratzer, 1994; Randon et al., 1994). Also, of direct relevance to retinitis pigmentosa, variability in expression of the allele in *trans* to disease-causing mutations in PRPF31 modify penetrance of the RP11 form of adRP (McGee et al., 1997; Vithana et al., 2003). This is particularly relevant to RP1 because several polymorphic amino acid substitutions exist at the locus which result in at least 6 distinct amino acid haplotypes, i.e., distinct proteins, in Caucasian populations (Bowne et al., 1999). These protein haplotypes in *trans* are potential modifiers of clinical expression of the RP1 Arg677ter mutation.

2. CLINICAL AND MOLECULAR CHARACTERIZATION OF RP01

A published genealogy traces retinitis pigmentosa in the RP01 family to a single individual born in 1803 (Breeding, 1982). Over 100 living, affected family members have been identified by pedigree reconstruction (Blanton et al., 1991). The family has been the subject of clinical and genetic studies for more than 30 years, including field studies in Kentucky and ophthalmic evaluations at the Jules Stein Eye Institute, UCLA (Field et al., 1982; Heckenlively et al., 1982; Lehmer et al., 1992).

RP01 has classical type 2 adRP with late onset of night blindness, usually by the third decade of life, and slow progression. Characteristic findings include diffuse retinal pigmentation, progressive decrease in recordable ERGs, and concentric visual field loss. Fundoscopic findings include retinal atrophy, bone spicule-like pigment deposits, and vascular attenuation.

The family also shows substantial within-family variability. For example, three members, examined in their 50s, had only small areas of regional pigmentation while two of these had children with typical RP. Early changes in the children included focal depigmented spot atrophy with pigmentation of the edge and abnormal RPE granularity. By contrast, typical late changes in adults vary from diffuse atrophy with pavingstone-type changes to typical bone spicule pigmentation. Further, at least two instances of a “skipped generation” have been documented in RP01.

We have DNA samples, and in many cases, lymphoblastoid cell lines, from 96 members of the family. Of these, 66 have the Arg677ter mutation. Of the 66, we have at least minimal clinical data on 44 and detailed clinical records, including fundus photographs, on 35. For linkage and segregation analysis we use a pedigree structure with 150 individuals, including two inbreeding loops (Blanton et al., 1992).

3. DO GENETIC FACTORS PLAY A ROLE?

To answer this question we assigned an age-adjusted affection status to the 44 individuals with at least minimal records and tested for segregation of severity using the non-parametric pedigree analysis program Loki (Daw et al., 1999; Daw et al., 1999a; Heath, 1997). Loki uses an iterated, Monte Carlo Markoff-chain approach to estimate the extent to which genetic differences play a role in variation of a trait (severity in this case) and the potential number of contributing genes. Based on this evaluation, 36% of the differences in severity in RP01 can be explained by variation at the RP1 locus (other than the mutation itself) and 18% can be

explained by unlinked loci. For the unlinked loci, Loki estimated that there is an 80% probability that 6 or fewer loci are involved, and that one of these loci may account for at least 9% of the total variation (Figure 1; unpublished). These results are provisional, given the “soft” nature of the clinical phenotype, but they support the likelihood that genetic differences play a role in the variability of adRP caused by the Arg677ter mutation.

4. POLYMORPHIC AMINO ACID HAPLOTYPES AT THE RP1 LOCUS

Numerous non-pathogenic amino acid substitutions have been reported at the RP1 locus (Berson et al., 2002; Bowne et al., 1999; Sohocki et al., 2001; Sullivan et al., 1999). Of these, at least 5 have high heterozygosity, that is, the lesser allele has a frequency of 10% or greater (Table 1). Although there is considerable linkage disequilibrium between these alleles, 6 distinct amino acid haplotypes were found in 100 unrelated CEPH parents, using offspring genotypes to reconstruct haplotypes, as shown in Table 1 (Sohocki et al., 2001; and unpublished). These polymorphic protein haplotypes are potential modifiers, in *trans*, of the Arg677ter mutation.

5. DO THE HAPLOTYPES IN *TRANS* PLAY A ROLE?

To test this possibility, we determined the protein haplotype in *trans* in each of the 66 individuals carrying the Arg677ter mutation. Since a sequence of approximately 3 megabases on 8q13 is tracking with disease in the family, i.e., without recombination, there is effectively no variation in *cis* to the mutation (Blanton et al., 1992). (Not all Arg677ter mutations descend from a common ancestor [Bowne et al., 2002; Schwartz et al., 2003]). The haplotype in *cis* to the mutation in RP01 is haplotype 2 in Table 1; thus the allele in *trans* can be determined deductively from each individual's genotype.

For this analysis we evaluated fundus photographs and clinical findings for the 33 individuals with detailed records. As dependent variables we considered age-of onset, other age-based landmarks, professionally graded fundus photographs and a composite, age-adjusted severity score. The independent variables in this case were the polymorphic amino acid alleles in *trans*, tested individually, and the protein haplotypes. Analytic methods included parametric significance testing and analysis of variance, and Kaplan-Meier survival modeling, implemented using the SAS statistical package (Allen, 1995).

In summary, a few combinations of dependent variables with independent variables show significant association, but are not significant if corrected for multiple comparisons. However, one of the significant associations, based on survival analysis, shows a protective effect of haplotype 3 in females (Figure 2). Because of the preliminary nature of the analysis, this should be taken as simply suggestive, but it is consistent with the Loki analysis and justifies further research.

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Figure 1. Number of genes estimated to contribute to RP1 Arg677ter severity based on Loki analysis in RP01.

Figure 2.
Kaplan-Meier survival analysis, RP1 protein haplotype 3 versus severity in males and females.

Table 1

Polymorphic amino acid substitutions and haplotypes in RP1

Substitution	Frequencies
Arg872His (CGT → CAT)	75%, 25%
Asn985Tyr (AAT → TAT)	54%, 46%
Ala1670Thr (GCA → ACA)	78%, 22%
Ser1691Pro (TCT → CCT)	77%, 23%
Cys2033Tyr (TGT → TAT)	46%, 54%
Haplotype	Frequencies
1: Arg - Tyr - Ala - Ser - Tyr	41%
2: Arg - Asn - Ala - Ser - Cys	30%
3: His - Asn - Thr - Pro - Cys	26%
4: Arg - Tyr - Ala - Ser - Cys	1%
5: His - Tyr - Ala - Ser - Tyr	1%
6: His - Asn - Ala - Pro - Cys	1%