Short Communication

Dominant \textit{PRPF31} Mutations Are Hypostatic to a Recessive \textit{CNOT3} Polymorphism in Retinitis Pigmentosa: A Novel Phenomenon of “Linked Trans-Acting Epistasis”

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Summary

Mutations in \textit{PRPF31} are responsible for autosomal dominant retinitis pigmentosa (adRP, RP11 form) and affected families show nonpenetration. Differential expression of the wildtype \textit{PRPF31} allele is responsible for this phenomenon: coinheritance of a mutation and a higher expressing wildtype allele provide protection against development of disease.

It has been suggested that a major modulating factor lies in close proximity to the wildtype \textit{PRPF31} gene on Chromosome 19, implying that a \textit{cis}-acting factor directly alters \textit{PRPF31} expression. Variable expression of \textit{CNOT3} is one determinant of \textit{PRPF31} expression. This study explored the relationship between \textit{CNOT3} (a \textit{trans}-acting factor) and its paradoxical \textit{cis}-acting nature in relation to RP11.

Linkage analysis on Chromosome 19 was performed in mutation-carrying families, and the inheritance of the wildtype \textit{PRPF31} allele in symptomatic–asymptomatic sibships was assessed—confirming that differential inheritance of wildtype chromosome 19q13 determines the clinical phenotype ($\textit{P} < 2.6 \times 10^{-7}$).

A theoretical model was constructed that explains the apparent conflict between the linkage data and the recent demonstration that a \textit{trans}-acting factor (\textit{CNOT3}) is a major nonpenetrance factor: we propose that this apparently \textit{cis}-acting effect arises due to the intimate linkage of \textit{CNOT3} and \textit{PRPF31} on Chromosome 19q13—a novel mechanism that we have termed “linked trans-acting epistasis.”

Keywords: \textit{PRPF31}, \textit{CNOT3}, retinitis pigmentosa, epistasis

Introduction

It has long been recognized that a disease-causing mutation can have different effects in different individuals and this may be due to environmental factors, stochastic molecular variation, epigenetic variation between individuals, or epistatic interactions (Nabholz & von Overbeck, 2004; Fraga et al., 2005; Raj & van Oudenaarden, 2008). Epistasis is the phenomenon whereby the phenotype caused by a mutation (or polymorphism) is masked by an allele at another locus, hence the alternative use of the terms gene–gene or genetic interaction. Although epistasis was first described over 100 years ago, it has received relatively little attention, in part due to our limited knowledge of epistatic mechanisms at a molecular level, and also due to our almost nonexistent ability to predict epistatic interactions \textit{de novo} (Bateson, 1909; Lehner, 2011). It is clear, however, that epistasis is a pervasive phenomenon, having been reported to determine the phenotypic outcome of genetic variations in all sorts of organisms, including plants, invertebrates, (e.g., flies) and vertebrates (e.g., birds, mammals) (Montooth et al., 2003; Kroymann & Mitchell-Olds, 2005; Carlborg et al., 2006; Shao et al., 2008). Several molecular mechanisms can underlie epistasis, including direct
interaction between the protein products of the genes, functional redundancy, involvement of the two genes in a common pathway or interaction between separate molecular pathways (Lehner, 2011). A further possibility is that epistatic interactions affect the regulatory network of genes. The multifaceted nature of gene expression regulation means that epistatic interactions are frequent and complex, although this idea has not been explored in great detail to date (Carter et al., 2007; Gertz et al., 2010).

Retinitis pigmentosa (RP) is a genetically heterogenous group of retinal degenerations, characterized by progressive cell death of the rod photoreceptors. Autosomal dominant retinitis pigmentosa (adRP) accounts for approximately 30–40% of cases and causative mutations have been identified in over 20 genes. Amongst these is an unusual class of causative genes—the splicing factors—six of which have been defined as causative of adRP (McKie et al., 2001; Vithana et al., 2001; Chakarova et al., 2002; Keen et al., 2002; Zhao et al., 2009; Tanackovic et al., 2011a).

Mutations in the ubiquitous splicing factor PRPF31 were found to underlie a major RP locus, termed RP11 (Al-Magheth et al., 1996; Vithana et al., 2001). PRPF31 protein plays a key role in the process of mRNA splicing, through its interaction with the U4/U6.U5 tri-snRNP—the key ribonucleoprotein complex of both major and minor spliceosomes. The PRPF31 protein contains a Nop domain that allows binding of the U4 snRNP within the U4/U6 di-snRNP (Liu et al., 2007). PRPF31 then links the U4/U6 di-snRNP with the U5 snRNP to form the U4/U6.U5 tri-snRNP; this process being mediated by the binding of PRPF6 by PRPF31 (Weidenhammer et al., 1997; Makarova et al., 2002; Liu et al., 2006). RNA interference mediated knockdown of PRPF31 expression inhibits formation of tri-snRNPs, with accumulation of U4/U6 di-snRNPs in the Cajal bodies (Schaffert et al., 2004).

Mutations in PRPF31 are a relatively common cause of adRP, accounting for approximately 5–10% of cases (Waseem et al., 2007; Audo et al., 2010; Xu et al., 2012). Over 40 mutations have been identified to date, including nonsense, missense, and frameshift mutations, as well as large deletions (Audo et al., 2010; Utz et al., 2013). Most PRPF31 mutations result in null alleles, either through complete or partial absence of gene (large deletions) or through mRNA that quickly undergoes degradation (Rio Frio et al., 2008a; Audo et al., 2010). Carriers of such mutations can, therefore, be considered to be functional hemizygotesthe retina-specific isoforms of PRPF31 is not due to the existence of retina-specific isoforms of PRPF31 (Tanackovic & Rivolta, 2009). In patients carrying PRPF31 mutations, there is a generalized defect in spliceosome assembly and pre-mRNA processing and, as the retina has a particularly high requirement for pre-mRNA splicing, it was suggested that retina—as compared to other tissues—is relatively more affected by mutations of splicing factors (Tanackovic et al., 2011b).

Another consistent feature of PRPF31-associated adRP (RP11) is phenotypic nonpenetrance, whereby an asymptomatic individual carrying a mutant allele can have affected siblings or children. It is known that nonpenetrance is determined by the expression level of the wildtype PRPF31 allele, and that there is variable expression of the PRPF31 gene in the general population (Rio Frio et al., 2008a). It has been shown that asymptomatic mutation carriers have more than twofold higher expression levels of wildtype PRPF31 compared to symptomatic individuals (Vithana et al., 2003; Rivolta et al., 2006; Liu et al., 2008). Patients coinheriting a PRPF31 mutant and a higher expressing wildtype allele are asymptomatic because the residual level of protein is still sufficient for normal retinal function—the wildtype allele is able to compensate for the mutant allele.

McGee et al. (1997) looked at phenotypic discordance between mutation-carrying siblings and observed that the affected siblings and unaffected siblings consistently inherited different wildtype Chromosome 19q13 alleles from the nonmutation-carrying parent. It is generally thought, therefore, that a cis-acting factor that directly alters wildtype PRPF31 expression is responsible for the observed phenotypic nonpenetrance. Attempts to identify single nucleotide polymorphisms (SNPs) that might be responsible for altered gene expression have failed to identify any causative changes. One theory draws attention to the high-repeat content of Chromosome 19 and proposes that variability in repeat elements, such as Alu repeats, might play a role (Rose et al., 2011).

Another interesting aspect of this disease is that, prior to nonsense-mediated decay of the mutant PRPF31, there is increased expression of both PRPF31 alleles in mutation-carrying asymptomatic individuals (Rio Frio et al., 2008a). As there is some increased expression of both the wildtype and mutant alleles of PRPF31, there must be at least one factor that is trans-acting. One possible trans-acting factor was identified through the association of higher PRPF31 expression and an expression quantitative trait locus (eQTL) at 14q21–23 (Rio Frio et al., 2008b).

It has also been shown that PRPF31 expression level is influenced by CNOT3, with increased levels of CNOT3 protein causing transcriptional repression of PRPF31 (Venturini et al., 2012). CNOT3 is a component of the Ccr4-Not transcription complex, which is an evolutionarily conserved global regulator of RNA polymerase II-mediated transcription (Miller et al., 2012).
An inverse relationship has been shown between PRPF31 expression and CNOT3 mRNA levels, and siRNA-mediated silencing of CNOT3 provoked an increase in PRPF31 expression, these observations further confirm the repressive nature of the interaction (Venturini et al., 2012).

This paper explores an apparent paradox in the relationship between PRPF31 and CNOT3: This paradox arises when trying to reconcile (i) the current knowledge that a cis-acting factor in close proximity of PRPF31 is the major factor underlying nonpenetrance (McGee et al., 1997), whereas (ii) the trans-acting CNOT3 appears to be one of the major factors controlling PRPF31 expression (Venturini et al., 2012).

Methods

Two approaches were taken to address this paradox.

First, the conjecture of McGee et al. was reviewed—in which they suggest that inheritance of the wildtype allele of Chromosome 19q13.4 is responsible for determining phenotype in carriers of the PRPF31 mutation (McGee et al., 1997). This was tested with the null hypothesis that, for mutation-carrying sibships, the wildtype gene is randomly inherited from the nonmutation-carrying parent and, therefore, each haplotype from the unaffected parent is equally represented in the “symptomatic” and “asymptomatic” offspring within a given sibship.

To test the null hypothesis, previously collected microsatellite linkage data from six families (AD5, AD11, AD24, AD29, ADC1, RP1907) were reanalyzed to examine the inheritance of the wildtype Chromosome 19q13 (Al-Maghtheh et al., 1996; Vithana et al., 1998; Abu-Safieh, 2003; Rose et al., 2012) and by linkage analysis of microsatellite data for one further mutation-carrying family (ADB1) (Chakarova et al., 2006). The microsatellite markers covered the region surrounding PRPF31 and CNOT3 on Chromosome 19q13, this region including markers between D19S921 and D19S418 (Chromosome 19:53771240–55546128). Additional markers to north or south of this region were analyzed where data were available (Fig. 1).

To perform linkage analysis in family ADB1, ABI PRISM Linkage Mapping Set (Applied Biosystems, Paisley, UK) was used, using only the primers for the region of interest on Chromosome 19q13 (Fig. 1). The following procedure was used: 5-µl Absolut qPCR mix (Thermoscientific, Loughborough, UK) was combined with 1-µl genomic DNA, 300-µM primer (forward/reverse), and distilled water to a total volume of 10 µl. The mixture was denatured at 94°C for 15 min, followed by 30 cycles of 94°C, 15 s; 60°C, 30 s; 72°C, 30 s. Finally, the mixture was held at 72°C for 5 min. The product was then diluted by combining 1-µl PCR product with 11-µl HiDi formamide (Applied Biosystems) and 0.1-µl GeneScan 500 Liz Size Standard (Invitrogen, Paisley, UK). The mixture was denatured for 5 min at 95°C, then incubated on ice for 2 min. The sample was genotyped on ABI 3730 and the results analyzed using GeneMarker v1.8 (Softgenetics, Pennsylvania, USA). The marker primers used were from ABI Prism Linkage mapping set v2.5 (Applied Biosystems). Where possible, previously collected data were validated, using the same methods as described in the original manuscripts.

Microsatellite Analysis

The inheritance of the wildtype chromosome 19q13 was analyzed in individuals from unrelated families carrying known mutations in PRPF31, each individual being required to have at least one mutation-carrying sibling. All families had evidence of nonpenetrance, with both symptomatic and asymptomatic mutation carriers within sibships. The inheritance of the wildtype Chromosome 19q13 was analyzed for six such families (AD5, AD11, AD24, AD29, ADC1, RP1907) by re-examination of previously collected microsatellite linkage data (Al-Maghtheh et al., 1996; Vithana et al., 1998; Abu-Safieh, 2003; Rose et al., 2012) and by linkage analysis of microsatellite data for one further mutation-carrying family (ADB1) (Chakarova et al., 2006). The microsatellite markers covered the region surrounding PRPF31 and CNOT3 on Chromosome 19q13, this region including markers between D19S921 and D19S418 (Chromosome 19:53771240–55546128). Additional markers to north or south of this region were analyzed where data were available (Fig. 1).

Statistical and Bioinformatic Analyses

The reference sibling was excluded from the dataset and the null hypotheses assessed using Fisher’s exact test.

Polymorphism data was obtained from Ensembl (http://www.ensembl.org, hg19 genome sequence) and dbSNP databases (http://www.ncbi.nlm.nih.gov/SNP). Pathogenicity of nonsynonymous SNPs was analyzed using standard parameters in Polyphen (http://genetics.bwh.harvard.edu/pph2/) and Sift (http://sift.bii.a-star.edu.sg/).
Results

Microsatellite data were derived for six members of family ADB1 (Fig. S1) and this, together with previously derived data from other unrelated families, provided data for 53 individuals—both symptomatic and asymptomatic (Fig. 2). The inheritance pattern shows a very highly significant deviation from the null hypothesis ($\chi^2 = 21.5; P = 2.6 \times 10^{-7}$) (Table 1).

Discussion

The highly concordant—rather than random—inheritance of the wildtype allele in mutation-carrying sibships (Table 1) indicates that the factor controlling the clinical manifestations of the PRPF31 mutation must be cis-acting. This is a necessary conclusion because, if it was trans-acting, the controlling allele could reside on either Chromosome 19 (or, indeed, any other chromosome) and thereby determine phenotype (Figs 3A and B); under these circumstances the inheritance of wildtype Chromosome 19 would be random and independent of phenotype—which is manifestly not the case (Table 1).

The simplest cis-acting factor would be one that directly affects the expression of the PRPF31 wildtype allele (Fig. 3C). Alternatively, there might be a cis-acting factor that affects another gene lying immediately upstream to PRPF31 and which cannot, therefore, be separated by recombination—an “indirect cis-acting” element.

CNOT3 and PRPF31: An Apparent Paradox?

The only known nonpenetration factor affecting the RP11 phenotype is transcriptional repression of PRPF31 by CNOT3, the latter being a trans-acting factor (Venturini et al., 2012). Furthermore, there is increased expression of both the mutant and wildtype PRPF31 alleles prior to nonsense-mediated decay of the mutant transcripts (Rio Frio et al., 2008a), and this necessitates at least one nonpenetrance factor which is trans-acting—again, this observation being compatible with a CNOT3 control mechanism. An apparent paradox appears to exist between this known, trans-acting control of PRPF31 expression by CNOT3, and the results of our study that implicate a mechanism which is cis-acting. This paradox can, however, be resolved if a trans-acting factor lies in close proximity to its target gene—in this case, PRPF31—and is therefore not separated by meiotic recombination (Fig. 3D). Such a factor, although working through a trans-acting mechanism, would appear to be cis-acting because of its linkage and proximity to the target gene—a mechanism that might usefully be termed “linked trans-acting epistasis.”

In the case of RP11, it is known that the trans-acting CNOT3 gene is located only a short distance from PRPF31 and the two genes are effectively linked during meiotic recombination. This linkage renders their epistatic interaction equivalent to a cis-acting mechanism (Fig. 3D) and we consider it likely that differential CNOT3 expression is the trans-acting factor that modulates expression of both PRPF31 alleles, but with degradation of the mutant PRPF31 mRNA (Rio Frio et al., 2008a).

A New Conjectural Model for “Linked Trans-Acting Epistatic” Inheritance

In the context of CNOT3 control and PRPF31 mutations, this novel “linked trans-acting epistatic” model of inheritance leads to some further theoretical consequences: First, it is known that lower expression of CNOT3 leads to higher expression of PRPF31, and vice-versa (Venturini et al., 2012).
Figure 2 Inheritance of the wildtype allele of Chromosome 19 in seven families carrying a mutation in PRPF31. In each group of siblings, the oldest mutation-carrying sibling (asterisked) acts as reference wildtype and was denoted orange if symptomatic or green if asymptomatic. The other mutation-carrying siblings were then assigned a concordant color triangle if they inherited the same wildtype allele as the reference sibling, or a discordant color triangle if they inherited the different wildtype allele to the reference sibling. “A” represents family AD5–1; B = AD5–2; C = AD11; D = AD24; E = AD29; F = RP1907; G = ADB1; H = ADC1.

Table 1 The Nonreference Siblings were Counted and Classified as Asymptomatic or Symptomatic and Concordant or Nonconcordant—The Latter with Respect to the Reference Sibling (the Oldest Mutation Carrier). The Expected Values were Calculated Assuming Random Inheritance of Chromosome 19q—in which Case, there would be Equal Numbers of Concordant and Nonconcordant for both Asymptomatic and Symptomatic Individuals.

| Concordance of genotype with that of reference sibling | Observed concordance | Expected concordance |
|--------------------------------------------------------|-----------------------|-----------------------|
| | Concordant | Nonconcordant | Concordant | Nonconcordant |
| Symptomatic | 2 | 16 | 9 | 9 |
| Asymptomatic | 14 | 0 | 7 | 7 |
Epistasis between PRPF31 and CNOT3

In any mutation-carrying individual, there will be a higher or lower expressing CNOT3 allele syntenic to the PRPF31 mutation, and a higher or lower expressing one syntenic to the wildtype PRPF31 (Fig. 4). In reality, as polygenic factors act to determine expression of the two genes, there will be a continuum of expression level at a population level. There will, however, be a threshold—above which the level is considered “high” and below which the level is considered “low.” With this in mind, it is possible to construct a simplified model.

Linkage analysis has shown that the CNOT3 and PRPF31 loci are not separated by recombination, and so the mutation-carrying chromosome will always carry the same syntenic CNOT3 allele; the PRPF31 mutation will be linked either to a “lower expressivity” or to a “higher expressivity” CNOT3 allele (Fig. 4). A necessary consequence is that, for any given sibship with two parents, individuals can only fall into one of two groupings—either “A” and “B,” or else “C” and “D” (Fig. 4); for example, it is not possible for siblings to have genotypes “A” and “D.” Further to this inference, the phenotypic difference between symptomatic and asymptomatic siblings can only be attributed to a difference in the CNOT3 allele linked to the wildtype PRPF31 allele.

Considering the four possible genotypes (Fig. 4), expression of CNOT3 is the same for individuals “B” and “C” and, as CNOT3 is trans-acting, this would result in similar PRPF31 expression and, therefore, the same phenotype. It needs to be considered what the phenotype of “B + C” individuals is: asymptomatic or symptomatic? It is clear that “B + C” individuals cannot be symptomatic, as this would lead symptomatic “C + D” siblings pairs who inherit different wildtype alleles—which we have demonstrated does not occur. It must be the case, therefore, that “B + C” individuals are asymptomatic.

Further to the deduction of all affected families being in the “C+D” group, it is therefore necessary for symptomatic individuals to have two copies of a higher expressing CNOT3 allele (that is, a homozygous recessive inheritance).

With this assumption in mind, it is possible to predict an approximate frequency of CNOT3 alleles within the

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Figure 3  Four mechanisms for modulation of the expression of PRPF31. (A) Nonsyntenic modulation: there is a trans-acting factor (purple diamond) being transcribed from another chromosome (i), this binding to an element on Chromosome 19 (ii), and thereby modulating the expression of PRPF31 (iii). This mechanism is not compatible with the observed nonrandom inheritance of wildtype alleles. (B) Distant modulation on Chromosome 19: a mechanism similar to (A) applies to a hypothetical trans-acting factor transcribed from a remote part of Chromosome 19. As the remote, controlling gene is liable to be separated from the PRPF31 mutation during meiotic recombination, inheritance of the wildtype Chromosome 19 and the trans-acting factor are, effectively, independent. This mechanism is not compatible with the observed nonrandom inheritance of wildtype alleles. (C) “Direct cis-effect”: there is a cis-element (pink) that lies in close proximity to PRPF31 (i), this then being bound by a transcription factor (pink diamond), and the interaction directly affects PRPF31 expression level (ii). This mechanism is compatible with the observed nonrandom inheritance of wildtype alleles. (D) “Linked trans-effect”: there is a cis-element (turquoise) linked in close proximity to the PRPF31 gene, such that the two are not separated by recombination (i). The cis-acting element causes differential expression of CNOT3 (blue diamonds; larger size indicating transcription from a higher expressed allele) (ii), this thereby modulating the expression of both the mutant and wild-type PRPF31 alleles (iii). This situation is compatible with the observed inheritance pattern of wildtype alleles, as long as the cis-element (turquoise) and PRPF31 are not separated during meiotic recombination. The hypothetical trans-acting factor would bind and activate both PRPF31 copies, but only the wildtype allele is functionally expressed—as PRPF31 mutations generally result in null alleles (that is, large deletions or nonsense alleles that are immediately degraded; Rio Frio et al., 2008a).
Table 2  Ensembl and dbSNP Report Three Nonsynonymous SNPs as Rare Normal Variants within the Coding Region of the PRPF31 Gene, these being Predicted to be Pathogenic by both Sift and Polyphen. This Raises the Issue that these Rare Changes might be Pathogenic on a Different Genetic Background.

| SNP          | Exon | Change      | Sift | Polyphen | Heterozygote frequency |
|--------------|------|-------------|------|----------|------------------------|
| rs150280707  | 6    | p.Val169Ile | 0.1  | 0.946    | <0.0001                |
| rs119475042  | 7    | p.Ala216Thr | 0    | 1        | <0.0001                |
| rs151337240  | 13   | p.Ala453Asp | 0    | 0.999    | 0.001                  |

Figure 4  Combinations of CNOT3 genotype in patients carrying the PRPF31 mutation. The L indicates a low-expressing CNOT3 allele—which, due to the repressive nature of the interaction, leads to high level of PRPF31 expression. The H indicates a high-expressing CNOT3, which leads to lower level of PRPF31. Sibling pair “A + B” carries a PRPF31 mutation with a syntenic low-expressing CNOT3 (L), whereas sibling pair “C + D” carries a syntenic high-expressing CNOT3 (H). Because of the intimate linkage of CNOT3 and PRPF31, these alleles are effectively locked together during meiotic recombination—this dictating that all individuals from a given sibship will be either “A + B,” or else “C + D.” The level of CNOT3 protein in individuals “B” and “C” is identical, and they would therefore be expected to have the same asymptomatic phenotype. They cannot both be symptomatic, as the “C + D” sibling pair would be incompatible with the observed nonrandom inheritance of the wildtype allele. Individual “A” should also be unaffected, having the lowest levels of CNOT3 (with correspondingly highest level of PRPF31). The most important conclusion of this inheritance model is that the only symptomatic individual (“D”) inherits two high-expressing CNOT3 alleles—there is homozygous, recessive inheritance of the CNOT3 trait.

population, given that approximately 30% of mutation carriers are asymptomatic. Using standard Hardy–Weinberg equilibrium, we can predict that 83.7% of CNOT3 haplotypes lead to higher expressing alleles (which when inherited recessively lead to disease) and the remaining 16.3% of haplotypes lead to lower expressing alleles, with a level of CNOT3 above our theoretical threshold. This is, of course, a gross oversimplification, as it assumes that CNOT3 is the only factor affecting PRPF31.

A further necessary deduction from this hypothesis is that mutation–carrying families can occur with absolutely no clinically affected individuals, despite harboring PRPF31 mutations (entirely “A and B” sibling pairs). As such families are clinically normal, they will never present to medical attention unless a meiotic recombination affecting the CNOT3–PRPF31 interzone occurs on the mutation–carrying chromosome—this being a remotely improbable event given the tight linkage between CNOT3 and PRPF31. To prove the existence of such “A and B” families would be arduous: RP affects 0.03% people, of which adRP accounts for 60%, and PRPF31 mutations account for less than 10% of adRP cases—the population prevalence therefore being about 0.002%. To find a completely asymptomatic mutation–carrying (“A + B”) family would require screening of a vast number of control individuals. It is possible, however, that some of the rare changes reported in the reference databases are mutations—but on a genetic background that masks the clinical phenotype: For example, there are three nonsynonymous coding region SNPs within the PRPF31 coding sequence that both Sift and Polyphen predict to be deleterious (Table 2).

Conclusions
Coinheritance of a heterozygous PRPF31 mutation and a homozygous higher expressivity variant of CNOT3 appears to determine the clinical presentation of RP11, this being a classic example of epistasis, where a disease mutation is masked by genetic interaction with a second locus. In this case, a mutation in PRPF31 is hypostatic to a trait acting on CNOT3, with the RP11 phenotype only being observed when there is homozygous (recessive) inheritance of the higher expressivity CNOT3 (“symptomatic” or risk) allele.

Traditionally, genetic disease has been split into two groups—“Mendelian” and “complex”—but this is a gross oversimplification. PRPF31-associated adRP can usefully
be thought of as a “hybrid” genetic disease, with features of Mendelian and complex disorders: primarily, there is a PRPF31 gene mutation (the monogenic “Mendelian” component)—and, superimposed on this, there is polygenic control of expression of the remaining, genetically active wildtype PRPF31 gene—the “complex” component of the disorder.

Nonpenetrant traits have previously been largely regarded as monogenic, this greatly hindering research as most investigations have sought “all-or-nothing” phenomena. As shown in this study, it appears more appropriate to investigate phenotypic nonpenetrance as a polygenic trait, with interaction of major risk alleles (such as repression by CNOT3) and a number of minor alleles to determine the overall level of PRPF31 expression and, consequentially, the clinical manifestation of the monogenic mutation. It should also be noted that given the continuous nature of PRPF31 expression in the population, some mutation carriers will be “nearer” the threshold for normal retinal function and hence will have milder disease presentation. One might foresee the ability to quantify the odds ratios for such risk factors and—based upon the genome—produce a predictive model for development of the disease.

Although control of CNOT3 expression has been discussed as if a single entity, it should be emphasized that this, too, is multifactorial with cis-acting factors on Chromosome 19q13 affecting CNOT3 expression; such factors being within core promoter, intronic regulatory, or long-range regulatory elements. These factors will produce a haplotype that has a cumulative effect on CNOT3, seen as a continuous distribution of CNOT3 expression level in the population.

Based on the evidence that approximately 30% of mutation carriers are asymptomatic, we predicted that approximately 16% of haplotypes result in CNOT3 level that is lower than the theoretical threshold. It should be noted, however, that in some populations the frequency of asymptomatic mutation carriers is significantly lower than the average—for example, based on recent data in Chinese families, approximately 5% of mutation-carrying family members were asymptomatic (Xu et al., 2012; Yang et al., 2013). In these populations, the lower expressing CNOT3 haplotypes would be very rare.

Several SNPs—such as rs4806718—lie close to, or within, the CNOT3–PRPF31 region and correlate with the phenotype of some, but not all, sibling groups (unpublished data; Venturini et al., 2012); the importance of SNPs should, however, be viewed with caution until their functional effect has been demonstrated. Functional polymorphisms within the regulatory elements of both PRPF31 and CNOT3 might have a combined effect to determine the overall expression level of PRPF31. Likewise, trans-acting factors from other chromosomes—such as the eQTL described on Chromosome 14 (Rio Frio et al., 2008b)—might modulate PRPF31 and CNOT3 expression.

Identification of all polymorphisms with a role in the CNOT3–PRPF31 regulatory network is daunting: It would be necessary to sequence the 19q13 region in a large cohort of control individuals with predetermined expression levels of CNOT3 and PRPF31. Sequencing this region is, however, very complex as it is highly enriched with repetitive elements—especially Alu repeats, that may act as long-range regulators of gene expression (Tomilin, 1999). At present, high-repeat content presents an insurmountable barrier to sequencing, and currently available commercial sequencing technologies are not able to read through repeat elements of greater than 500 bp. After identification of SNPs, it would be necessary to statistically evaluate the individual risk contributed by each allele, followed by experimental validation of their functional effect on transcription—a mammoth task!

In summary, linkage data from seven unrelated families from different ethnic populations have unequivocally shown that inheritance of the wildtype Chromosome 19 is the major determinant for the PRPF31 adRP mutation of RP11. When considered alongside recent work into CNOT3 expression and function, it can be concluded that the RP11 phenotype is hypostatic to normal variant alleles of CNOT3, and that recessive coinheritance of the CNOT3 “high-expressing” allele is one of the major determinants of the disease phenotype in PRPF31 mutations—a classic example of epistasis affecting a Mendelian disorder.

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References

Abu Safih, L. (2003) Molecular genetic study of autosomal dominant retinitis pigmentosa on chromosome 19q13.4. Doctoral thesis, University College London.

Al-Maghtheh, M., Vithana, E., Tarttelin, E., Jay, M., Evans, K., Moore, T., Bhattacharya, S., & Inglehearn, C. F. (1996) Evidence for a major retinitis pigmentosa locus on 19q13.4 (RP11) and association with a unique bimodal expressivity phenotype. Am J Hum Genet 59, 864–871.

Audo, I., Bujakowska, K., Mohand-Saïd, S., Lancelot, M. E., Moskova-Doumanova, V., Wasem, N. H., Antonio, A., Sahel, J. A., Bhattacharya, S. S., & Zeitz, C. (2010) Prevalence and novelty of PKP31 mutations in French autosomal dominant rod-cone dystrophy patients and a review of published reports. BMC Med Genet 11, 145.
Liu, S., Rauhut, R., Vorlocher, H. P., & Lehner, B. (2011) Molecular mechanisms of epistasis within and between genes. Trends Genet 27, 1–4.

Kroymann, J. & Mitchell-Olds, T. (2005) Epistasis and balanced polymorphism influencing complex trait variation. Nature 435, 95–8.

Lehner, B. (2011) Molecular mechanisms of epistasis within and between genes. Trends Genet 27, 323–331.

Liu, S., Rauhut, R., Vorlocher, H. P., & Lührmann, R. (2006) The network of protein-protein interactions within the human U4/U6.U5 tri-snRNP. RNA 12, 1418–1430.

Liu, S., Li, P., Dybkov, O., Nottrott, S., Hartmuth, K., Lührmann, R., Carlomagno, T., & Wahl, M. C. (2007) Binding of the human Prp31 Nop domain to a composite RNA-protein platform in U4 snRNP. Science 316, 115–120.

Liu, J. Y., Dai, X., Sheng, J., Cui, X., Wang, X., Jiang, X., Tu, X., Tang, Z., Bai, Y., Liu, M., & Wang, Q. K. (2008) Identification and functional characterization of a novel splicing mutation in RP gene PRPF31. Biochem Biophys Res Commun 367, 420–426.

Makarova, O. V., Makarov, E. M., Liu, S., Vorlocher, H. P., & Lührmann, R. (2002) Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6.U5 tri-snRNP formation and pre-mRNA splicing. EMBO J 21, 1148–1157.

McGee, T. L., Devoto, M., Ott, J., Berson, E. L., & Dryja, T. P. (1997) Evidence that the penetrance of mutations at the RP11 locus causing dominant retinitis pigmentosa is influenced by a gene linked to the homologous RP11 allele. Am J Hum Genet 61, 1059–1066.

McKie, A. B., McHale, J. C., Keen, T. J., Tarttelin, E. E., Goliath, R., van Lith-Veerkhooven, J. J., Greenberg, J., Kamesar, R. S., Hoyng, C. B., Cremers, F. P., Mackey, D. A., Bhattacharya, S. S., Bird, A. C., Markham, A. F., & Inglehearn, C. F. (2001) Mutations in the pre-mRNA splicing factor gene PRP8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet 10, 1555–1562.

Miller, J. E. & Reese, J. C. (2012) Ctf4+Not complex: the control of eukaryotic cells. Crit Rev Biochem Mol Biol 47, 315–333.

Monteith, K. L., Marden, J. H., & Clark, A. G. (2003) Mapping determinants of variation in energy metabolism, respiration and flight in Drosophila. Genetics 165, 623–635.

Nabholz, C. E. & von Overbeck, J. (2004) Gene-environment interactions and the complexity of human genetic diseases. J Insur Med 36, 47–53.

Raj, A. & van Oudenaarden, A. (2008) Nature, nurture, or chance: stochastic gene expression and its consequences. Cell 135, 216–226.

Rio, F., Wade, N. M., Ransijn, A., Berson, E. L., Beckmann, J. S., & Rivolta, C. (2008a) Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. J Clin Invest 118, 1519–1531.

Rio, F., Covic, N., Ransijn, A., Beckmann, J. S., & Rivolta, C. (2008b) Two trans-acting eQTLs modulate the penetrance of PRPF31 mutations. Hum Mol Genet 17, 3154–3165.

Rivolta, C., McGee, T. L., Rio Frio, T., Jensen, R. V., Berson, E. L., & Dryja, T. P. (2006) Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations. Hum Mutat 27, 644–653.

Rose, A. M., Mukhopadhyay, R., Webster, A. R., Bhattacharya, S. S. & Waseem, N. (2011) A 112 kb deletion in chromosome 19q13.42 leads to retinitis pigmentosa. Invest Ophthalmol Vis Sci 52, 6597–6603.

Rose, A. M., Shah, A. Z., Waseem, N. H., Chakarova, C. E., Alfano, G., Coussa, R. G., Ablan, R., Koenekoop, R. K., & Bhattacharya, S. S. (2012) Expression of PRPF31 and TFPT: regulation in health and retinal disease. Hum Mol Genet 21, 4126–4137.

Schaffert, N., Hossbach, M., Heintzmann, R., Achtel, T., & Lührmann, R. (2004) RNAi knockdown of hPrp31 leads to an impairment of pre-mRNA splicing and autosomal-dominant retinitis pigmentosa. Proc Natl Acad Sci U S A 101, 6010–6014.

Gertz, J., Gerke, J. P. & Cohen B. A. (2010) Epistasis in a quantitative trait captured by a molecular model of transcription factor interactions. Theor Popul Biol 77, 1–5.

Keen, T. J., Hims, M. M., Welch, M. A., Doran, R. M., Mackey, D. A., Mansfield, D. C., Mueller, R. F., Bhattacharya, S. S., Bird, A. C., Markham, A. F., & Inglehearn, C. F. (2002) Mutations in a protein target of the Pim-1 kinase associated with retinitis pigmentosa. Europ J Hum Genet 10, 245–249.

Kroymann & Mitchell-Olds, T. (2005) Epistasis and balanced polymorphism influencing complex trait variation. Nature 435, 95–8.

Lehner, B. (2011) Molecular mechanisms of epistasis within and between genes. Trends Genet 27, 323–331.

Liu, S., Rauhut, R., Vorlocher, H. P., & Lührmann, R. (2006) The network of protein–protein interactions within the human U4/U6.U5 tri-snRNP. RNA 12, 1418–1430.

Liu, S., Li, P., Dybkov, O., Nottrott, S., Hartmuth, K., Lührmann, R., Carlomagno, T., & Wahl, M. C. (2007) Binding of the human Prp31 Nop domain to a composite RNA-protein platform in U4 snRNP. Science 316, 115–120.

Liu, J. Y., Dai, X., Sheng, J., Cui, X., Wang, X., Jiang, X., Tu, X., Tang, Z., Bai, Y., Liu, M., & Wang, Q. K. (2008) Identification and functional characterization of a novel splicing mutation in RP gene PRPF31. Biochem Biophys Res Commun 367, 420–426.

Makarova, O. V., Makarov, E. M., Liu, S., Vorlocher, H. P., & Lührmann, R. (2002) Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6.U5 tri-snRNP formation and pre-mRNA splicing. EMBO J 21, 1148–1157.

McGee, T. L., Devoto, M., Ott, J., Berson, E. L., & Dryja, T. P. (1997) Evidence that the penetrance of mutations at the RP11 locus causing dominant retinitis pigmentosa is influenced by a gene linked to the homologous RP11 allele. Am J Hum Genet 61, 1059–1066.
Epistasis between PRPF31 and CNOT3

Epistasis between Pre-mRNA splicing-factor gene PRPF31 (RP11): Review of Disease Mechanism and Report of a Family with a Novel 3-Base Pair Insertion. *Ophthalmic Genet* 34, 183–188.

Venturini, G., Rose, A. M., Shah, A. Z., Bhattacharya, S. S., & Rivolta, C. (2012) CNOT3 is a modifier of PRPF31 mutations in retinitis pigmentosa with incomplete penetrance. *PLoS Genet* 8, e1003040.

Venturini, G., Al-Maghtheh, M., Bhattacharya, S. S., & Inglehearn, C. F. (1998) RP11 is the second most common locus for dominat retinitis pigmentosa. *J Med Genet* 35, 174–175.

Vithana, E. N., Abu-Safieh, L., Allen M. J., Carey A., Papaoannou M., Chakarova C., Al-Maghtheh M., Ebenezer N. D., Willis C., Moore A. T., Bird A. C., Hunt D. M., & Bhattacharya S. S. (2001) A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). *Mol Cell* 8, 375–381.

Vithana, E. N., Abu-Safieh, L., Pelosini, L., Winchester, E., Hornan, D., Bird, A. C., Hunt, D. M., Bustin, S. A., & Bhattacharya, S. S. (2003) Expression of PRPF31 mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? *Invest Ophthalmol Vis Sci* 44, 4204–4209.

Waseem, N. H., Vaclavik, V., Webster, A., Jenkins, S. A., Bird, A. C., & Bhattacharya, S. S. (2007) Mutations in the gene coding for the pre-mRNA splicing factor, PRPF31, in patients with autosomal dominant retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 48, 1330–1334.

Weidenhammer, E. M., Ruiz-Noriega, M., & Woolford, J. L. Jr. (1997) Prp31p promotes the association of the U4/U6 x U5 tri-snRNP with prespliceosomes to form spliceosomes in Saccharomyces cerevisiae. *Mol Cell Biol* 17, 3580–3588.

Xu, F., Sui, R., Liang, X., Li, H., Jiang, R., & Dong, F. (2012) Novel PRPF31 mutations associated with Chinese autosomal dominant retinitis pigmentosa patients. *Mol Vis* 18, 3021.

Yang, Y., Tian, D., Lee, J., Zeng, J., Zhang, H., Chen, S., Guo, H., Xiong, Z., Xia, K., Hu, Z., & Luo, J. (2013) Clinical and genetic identification of a large chinese family with autosomal dominant retinitis pigmentosa. *Ophthalmic Genet*, in press.

Zhao, C., Bellur, D. L., Lu, S., Zhao, E., Grassi, M. A., Bowne, S. J., Sullivan, L. S., Daiger, S. P., Chen, L. J., Pang, C. P., Zhao, K., Staley, J. P., & Larsson, C. (2009) Autosomal-dominant retinitis pigmentosa caused by a mutation in SNRNP200, a gene required for unwinding of U4/U6 snRNAs. *Am J Hum Genet* 85, 617–627.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1** Genotype data for family ADB1, covering a large region on Chromosome 19q13 (D19s571 – D19s210).

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