The challenge of defining pathogenicity: the example of *AHI1*

To the Editor: We have read with great interest the article “Genotype–Phenotype Correlation and Mutation Spectrum in a Large Cohort of Patients With Inherited Retinal Dystrophy Revealed by Next-Generation Sequencing,” by Huang et al.¹ The authors propose *AHI1* (MIM 608894) as a novel candidate gene for nonsyndromic retinitis pigmentosa (nsRP). Biallelic *AHI1* mutations have, so far, been described only in patients with Joubert syndrome type 3 (JBTS3, MIM 608629), a severe multi-system ciliopathy with frequent retinal degeneration.

We contest that the data shown suggest *AHI1* as a new candidate gene for nsRP. The authors identified two *AHI1* variants—c.653A>G (p.Y218C) and c.3257A>G (p.E1086G)—in an isolated patient with nsRP. They claim that “The mutations completely cosegregated with phenotype in all family members tested.” However, Supplementary Figure S3 reveals that only the parents were analyzed and that the variants were compound-heterozygous. From a formal genetic point of view, “cosegregation with the phenotype” implies presence of compound heterozygosity in several affecteds and hence appears overstated in a family with a single patient.

More importantly, we have severe doubts regarding the proposed pathogenicity of both variants. The p.E1086G variant affects the protein's SH3 domain, which has been shown to mediate interaction with other proteins. The authors consider it pathogenic because it has been reported previously, in the homozygous state in a Dutch patient with Joubert syndrome (JBTS).² However, p.E1086G, which has been annotated as rs148000791 in dbSNP, is present at high frequency in the ExAC database (457 of 119,154 alleles), and 12 individuals were even homozygous for this variant (as of January 2015). Moreover, we have recently shown that even two homozygous truncating mutations, p.Arg1066* and p.Trp1088Leufs*16, in the same gene region are nonpenetrant, indicating localized

loss-of-function tolerance and nonessentiality of the SH3 domain.³ The other allegedly RP-associated variant reported by Huang et al., p.Y218C, is described as being “located in a highly conserved region.” This claim is based on a peptide alignment with mammals only rather than with nonmammalian species and is therefore not convincing. The p.Y218C variant is re-presented with 64 alleles in the ExAC database, albeit not in a homozygous state. Although this does not exclude pathogenicity for a recessive allele, it is thus much more likely that p.Y218C, like p.E1086G, is a benign variant.

Assessing the pathogenicity of genetic variants requires the utmost caution because their inclusion in databases—and thus potentially in diagnostic, prenatal, and carrier testing—has far-reaching consequences. This may, as demonstrated by rare *AHI1* truncations without clinical consequences,³ pose a challenge. However, in the case of the two *AHI1* variants described by Huang et al., the evidence—particularly their high frequencies in the general population—calls their pathogenicity into question.

DISCLOSURE

H.J.B. is an employee of Bioscientia, which is part of a publicly traded diagnostic company. R.H. declares no conflict of interest.

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Response to Heller and Bolz

To the Editor: We appreciate the comments by Heller and Bolz¹ in their letter “The Challenge of Defining Pathogenicity: The Example of *AHI1*” and welcome discussion on the pathogenicity of the variants in *AHI1*. Our study was a large-scale screening investigation of patients with inherited retinal dystrophy.² We considered the two variants as damaging for the

following reasons. (i) The first variant, E1086G, was reported as a known disease mutation in a previous study³ and in the RetinoGenetics database⁴ as well as the Human Gene Mutation Database (CM080033), the major database for human mutation repositories.⁵ We also reexamined the pathogenicity using multiple other databases.² Our analyses indicate that this variant is predicted to be damaging by MutationTaster⁶ (score 0.999) and PolyPhen-2 (ref. 7) (score 0.994). In addition, the minor allele frequency for this variant is less than 0.01 in all four previously described databases.² (ii) Similarly, the second variant, Y218C,