Genetics inMedicine | LETTER TO THE EDITOR

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 multisystem 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.

Raoul Heller[1] and Hanno J. Bolz[1,2]

[1]Institute of Human Genetics, University Hospital of Cologne, Cologne, Germany; [2]Bioscientia Center for Human Genetics, Ingelheim, Germany. Correspondence: Hanno J. Bolz (hanno.bolz@uk-koeln.de) or Raoul Heller (raoul.heller@uk-koeln.de)

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Response to Heller and Bolz

To the Editor: We appreciate the comments by Heller and Bolz[1] 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 study[3] and in the RetinoGenetics database[4] 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 MutationTaster[6] (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,

is predicted to be damaging by PolyPhen-2 (score 0.985) and Sorting Intolerant From Tolerant (SIFT) (score 0.002). The minor allele frequency for Y218C is less than 0.005 in all examined databases. Segregation testing also demonstrated that these two variants are inherited as a paternal allele and a maternal allele, respectively. (iii) The purpose of this study was to elucidate the mutational spectrum and genotype–phenotype correlations of inherited retinal dystrophy. *AHI1* was included in our sequencing panel because many previous studies have reported the genetic defects in *AHI1* as a cause for Joubert syndrome type 3, a syndromic retinal dystrophy.[8–12] Our study suggests that *AHI1* is a candidate gene for nonsyndromic retinitis pigmentosa. However, considering that this is the first study identifying *AHI1* mutations in patients with nonsyndromic retinitis pigmentosa, further studies are required to confirm our finding.

The crux of the doubt expressed by Heller and Bolz[1] about the pathogenicity of these two variants is based on (i) the high frequency of the variants in the ExAC database (Cambridge, MA; http://exac.broadinstitute.org, as of December 2014) and (ii) the SH3 domain of the AHI1 protein at which E1086G is located not being essential for AHI1 function.[13] First, the frequency of Y218C in the ExAC database is 5.6E−4 (64/114,524) and that of E1086G is 3.8E−3 (457/119,154), neither of which is considered high frequency in an autosomal-recessive pattern of inheritance. Of note, 12 individuals in ExAC are homozygous for E1086G. However, because the ExAC database was originally released in October 2014 and we submitted the manuscript in June 2014, we were unable to include this information in our report. Second, Heller and colleagues concluded in their study that the SH3 domain is not essential for AHI1 function based primarily on the finding that two homozygous truncating mutations, Arg1066* and Trp1088Leufs*16, are nonpenetrant in a zebrafish model. This conclusion is slightly overstated because (i) previous studies have reported a functional role of the SH3 domain,[14,15] and the frameshift Trp1088Leufs*16 mutation in the SH3 domain was identified in patients with Joubert syndrome;[8] (ii) the evidence from zebrafish is limited in Heller and colleagues' study[13] because the total number of fish examined is not given, creating a lack of statistical data, and the eyeball size seems to be decreased by e23i23MO injection; and (iii) previous studies have demonstrated that different mutations in the same gene may lead to drastically different retinal phenotypes in mice.[16] We believe that Heller and colleagues' viewpoint is extremely meaningful in this field; however, the exact role of the AHI1 protein domains in a mouse model remains unclear and warrants further investigation.

In brief, we agree with Heller and Bolz[1] that the pathogenicity of E1086G should be further examined because of the homozygosity reported in the ExAC database (as of January 2015). It is important to note that a rapid expansion of exome resources in recent years has increased the amount of information regarding the pathogenicity of variants, which might lead to inconsistent results when different database are analyzed. For the purpose of serving as a valuable reference for genetic disease studies and precisely defining the pathogenicity of variants, a comprehensive and integrative database is necessary.

DISCLOSURE

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Zi-Bing Jin, MD, PhD[1] and Xiu-Feng Huang, MB[1]

[1]Division of Ophthalmic Genetics, The Eye Hospital of Wenzhou Medical University, The State Key Laboratory Cultivation Base and Key Laboratory of Vision Science, Ministry of Health, Wenzhou, China. Correspondence: Zi-Bing Jin (jinzb@mail.eye.ac.cn)

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