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Investigating the disease association of *USH2A* p.C759F variant by leveraging large retinitis pigmentosa cohort data

Mariana DuPont[1], **Evan M. Jones**[2,3], **Mingchu Xu**[2,3], and **Rui Chen**[2,3]

[1]College of Arts and Sciences, Dillard University, New Orleans, LA, USA

[2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[3]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

Abstract

Retinitis pigmentosa (RP) is an inherited retinal disease with a prevalence of 1/4,000. RP is highly genetically heterogeneous and there are over 80 genes associated with RP to date. One particular *USH2A* variant, p.C759F, has long been reported in RP cases but its pathogenicity was questioned by a recent study. Here, by leveraging large scale next-generation sequencing data from 982 non-Asian RP probands, we used binomial tests to evaluate the enrichment of this allele in RP cohort. We observed significant enrichment of this allele both in homozygous state and in compound heterozygous state with another *USH2A* protein-truncating allele. The results highlighted the clinical significance of the *USH2A* p.C759F allele in RP cases, which is important for accurate molecular diagnosis.

Retinitis pigmentosa (RP) is a genetic disorder which affects the ability of retina to respond to light, leading to a progressive loss of vision. RP is estimated to have a prevalence of 1/4,000[1], with remarkable clinical and genetic heterogeneity. Currently, there are over 80 genes associated with RP and mutations in *USH2A* contributed to a significant proportion of cases (RetNet, https://sph.uth.edu/retnet/). According to HGMD database (http://www.hgmd.cf.ac.uk/), there are more than 400 disease-associated *USH2A* variants. However, a number of them are nonsynonymous variants and their pathogenicity remains doubtful without functional validation or convincing genetic data.

Among these *USH2A* nonsynonymous variants, one specific variant (NM_206933, c.G2276T; p.C759F) has long been reported to associate with autosomal recessive non-syndromic RP[2–4]. This C759F variant is highly conserved in vertebrates and predicted to be damaging by various *in silico* algorithms. However, the pathogenicity of this variant was questioned in a recent study since in one Spanish RP family, two asymptomatic individuals with homozygous C759F genotype were identified[5]. In this family, one *PDE6B* variant seems to be the disease-causing one due to the absence of this variant in control databases and the highly conserved amino acid it affects. According to previous literature, the *USH2A*

Contact Rui Chen, ruichen@bcm.edu, Baylor College of Medicine, N1519, 1 Baylor Plaza, Houston, TX, USA 77030.

DECLARATION OF INTEREST

The authors declared no conflicts of interest.

C759F variant occurs frequently in RP cases, both in homozygous state or in compound heterozygous state with another *USH2A* mutation[2,3]. Thus it is critical to further assess the clinical significance of this variant in order to achieve more accurate molecular diagnosis.

Here, we tried to re-evaluate this variant in a population genetics context by utilizing target capture next-generation sequencing (NGS) data in large RP cohorts. The capture panel design and NGS pipeline were described previously[6–11]. Based on gnomAD database (gnomad.broadinstitute.org), the C759F variant exclusively occurs in non-Asian controls. Thus, we collected the NGS data of 982 non-Asian RP probands who underwent molecular diagnosis in our lab for further analysis.

We evaluated the enrichment of C759F variant in RP cohorts by two ways. First, we tested if the homozygous C759F genotype is enriched in RP cohorts. Second, we tested if C759F variant is enriched given the RP proband has another *USH2A* protein-truncating (PTC) allele. The former test evaluated the clinical significance of homozygous C759F genotype in RP, while the latter one assessed the pathogenicity of C759F variant in compound heterozygous state with another *USH2A* PTC variant. In gnomAD database, the C759F variant frequency varies in different populations and we chose the highest one (0.0021, in Latino people) for our evaluation. The reason is if our tests already show significant enrichment based on this highest frequency, it can be inferred *a fortiori* that the statistical significance level would be higher if C759F frequency in general non-Asian population (probably lower than 0.0021) is used for our tests.

For the first test, we found six unrelated RP probands are homozygous for this variant in 982 non-Asian RP probands. For the second test, we identified 70 probands with at least one *USH2A* PTC allele, and among them, 16 probands also possess the C759F variant. Two-tailed binomial tests were used for the evaluation and both tests indicate extremely significant enrichment of the C759F allele (Table 1). This suggests that both C759F in homozygous state and in compound heterozygosity with another *USH2A* PTC allele have strong clinical significance.

Our data indicate two possibilities. First, this variant is *bona fide* disease-causing and the absence of RP symptom in the uncommon Spanish family[5] is just an instance of incomplete penetrance. To estimate the RP penetrance of this variant in homozygous state, we can estimate a series of numbers including the RP prevalence, the contribution of homozygous C759F to RP, as well as the C759F allele population frequency. Then, the penetrance could be estimated by applying the Hardy-Weinberg Equilibrium (HWE) on the numbers aforementioned. However, due to additional factors affecting HWE, like non-random mating (consanguinity), it is still difficult to achieve the accurate calculation of the penetrance.

As for the second possibility, the C759F variant *per se* is not disease-causing but closely linked with another pathogenic *USH2A* variant nearby that has not been detected by current sequencing approaches. Indeed, utilizing target capture sequencing data, we mapped the disease-associated haplotype of C759F variant in the six homozygous C759F RP patients and identified a 199kb-long shared haplotype (Table 2). This haplotype spans the exon14 to exon25 of *USH2A* mRNA and contains several highly conserved DNA regions in *USH2A*

introns, leaving the possibility that the true disease-causing variant resides in these non-coding regions. In this scenario, the exception observed in the Spanish family[5] suggests that this haplotype has undergone recombination and C759F is no longer linked with the true disease-causing variant in this family.

Collectively, though without functional validation, our data strongly support a clinical significance of *USH2A* p.C759F variant in RP patients due to the statistically significant enrichment of this allele in disease individuals, and this should be particularly noticed in future RP molecular diagnostics.

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Table 1

Binomial tests for C759F variant enrichment

Test	n	k	h.p.	p-value	e.p.	CI
Homo	982	6	4.41×10^{-6}	$<2.2\times10^{-16}$	0.0061	(0.0022, 0.0133)
Comp Het	70	16	0.0021	$<2.2\times10^{-16}$	0.2286	(0.137, 0.344)

Homo, test for C759F homozygous genotype enrichment; Comp Het, test for C759F enrichment given the RP proband has another *USH2A* PTC allele; n, number of trials; k, number of stipulated outcomes; h.p., the hypothesized probability of successes (there is no enrichment of C759F variant); e.p., the estimated probability of successes; CI, the 95% confidence interval of estimated probability of successes. Two-sided exact binomial test.

Table 2

USH2A p.C759F associated haplotypes of six individuals.

Chr1 position	Distance (bp)	ID	Freq	REF	ALT	NEI_227	MEP_109	BLL_124	FBP_461	RKK_75	RKK_87
216219781	+200679	rs6657250	0.6716	A	G	AA	AA	AA	AA	AA	AB
216221721	+198739	rs10864227	0.4636	C	T	AA	AA	AB	BB	AA	AA
216258213	+162247	rs56222536	0.1420	A	G	BB	BB	BB	BB	BB	BB
216270618	+149842	rs7540411	0.1313	G	A	BB	BB	BB	BB	BB	BB
216348449	+72011	rs55761862	0.0651	A	C	BB	BB	BB	BB	BB	BB
216373804	+46656	rs523689	0.9591	T	G	BB	BB	BB	BB	BB	BB
216390475	+29985	rs419767	0.9590	C	T	BB	BB	BB	BB	BB	BB
216420460	0 (C759F)	rs80338902	0.0014	C	A	BB	BB	BB	BB	BB	BB
216420480	−20	rs111033281	0.0010	A	G	AA	AB	BB	BB	BB	BB
216592003	−171543	rs4253963	0.6486	T	C	BB	BB	BB	BB	BB	BB
216595306	−174846	rs10779261	0.7063	C	T	BB	BB	BB	BB	BB	BB

Original variant calling format (VCF) files of target capture sequencing data were used for identifying the haplotypes of six individuals with homozygous C759F genotype. Distance, the genomic distance from the C759F variant; ID, dbSNP ID; Freq, the frequency of the alternative allele; REF, reference allele; ALT, alternative allele; AA, homozygous reference; AB, heterozygous; BB, homozygous alternative. The results show that all six individuals share a 199kb haplotype between Chr1:216221721 to Chr1: 216420480. The genomic coordinates are based on hg19.