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Prevalence and genetic–phenotypic characteristics of patients with *USH2A* mutations in a large cohort of Chinese patients with inherited retinal disease

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ABSTRACT

Aims To investigate the frequency of *USH2A* mutation and the clinical and genetic differences between Usher syndrome type II (USH2) and retinitis pigmentosa (RP) in a large cohort of Chinese patients.

Methods A total of 1381 patients with inherited retinal disease (IRD) were recruited. The phenotypic and genotypic information of patients with *USH2A* mutations was evaluated.

Results The prevalence of patients with *USH2A* mutations was 15.75%, which was the most frequently detected gene in this cohort of patients. Hotspot of *USH2A* mutations was c.8559-2A >G and c.2802T >G. Patients with USH2 had an earlier and more serious decline of visual function and damage to retina structure than did patients with RP in the first 10 years ($p<0.05$), but there was no difference in the visual prognosis between the two groups when the course of disease exceeded 10 years ($p>0.05$). Missense variants had less severe consequences and were found more commonly in RP, whereas more deleterious genotypes were associated with an earlier onset of disease and were found more commonly in USH2.

Conclusions This study provides detailed clinical–genetic assessment of patients with *USH2A* mutations of Chinese origin, enabling precise genetic diagnoses, better management of these patients and putative therapeutic approaches.

INTRODUCTION

The *USH2A* gene (OMIM #608400), located on chromosome 1q41, consists of 72 exons, and encodes usherin, a transmembrane protein present in the basement membrane of many, but not all, tissues, including the photoreceptor layer of the retina and the hair cells in the cochlea. Usherin is important in the development and homeostasis of the inner ear and retina. Different mutations within this gene have been associated with a large heterogeneous group of diseases, including retinitis pigmentosa (RP), Usher syndrome type II (USH2), cone-rod dystrophy and deafness.[1 2] The different phenotypes associated with *USH2A* are thought to be due to an allelic hierarchy of *USH2A* mutations.[3]

To date, many efforts have been made to investigate the spectrum of mutations and genotype–phenotype correlations in patients with *USH2A* mutations. More than 1100 disease-causing variants in *USH2A* have been identified, including nonsense and missense mutations, splicing variants, small deletions and insertions, small indels and large rearrangements (Human Gene Mutation Database; professional version 2019.3). Previous studies confirmed that two truncating mutations in *USH2A* were associated mostly with USH2 in patients from Netherlands and Belgium, whereas other combinations can result in both RP and USH2.[4] Moreover, the presence of at least one truncating mutation was associated with earlier presentation of visual decline. However, the mutation frequency and genotypic–phenotypic characteristics vary widely between different ethnic groups.[5 6] For example, the most frequent mutations in *USH2A* were the p.Glu767Serfs*21 and p.Cys759Phe mutations in Madrid,[7] p.Glu767Serfs*21 of USH2 cases in Europe,[8] p.Cys759Phe in RP cases in Spanish patients.[9] Previous research found that p.Glu767Serfs*21 was associated mainly with USH2[10] and p.Cys759Phe with RP; however, Blanco-Kelly *et al* revealed that more than 60% of patients with p.Cys759Phe and 72.1% of patients with p.Glu767Serfs*21 had mild hearing loss.[7]

The genetic and clinical characteristics of patients with *USH2A* mutations have been reported in many studies; however, data for Chinese patients are limited. The exact genetic and phenotypic characteristics of patients with *USH2A* mutations in Chinese patients with inherited retinal disease (IRD) remain unknown. In the present study, we enrolled 1381 patients with IRD; patients underwent molecular analysis and all those with *USH2A* mutations were identified. Our aim was to investigate the genotypic–phenotypic characteristics and differences between RP and USH2 in a large series of patients with *USH2A* mutations in the Chinese population. These results would be useful for prognosis, clinical management and genetic counselling and should provide strong evidence-based data support for gene therapy studies.

METHODS

Subjects and ethical statement

This study was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University and adhered to the tenets of the Declaration of

Helsinki. A total of 1381 patients with IRD and their available family members (total participants: 3967) were recruited from our genetics department between January 2016 and June 2019. Written informed consent was obtained from all participants before peripheral blood samples were collected.

Next-generation sequencing analysis

Molecular testing was performed by targeted next-generation sequencing as previously reported.[6] After sequencing, data analysis was performed as reported previously.[6 11] Previously reported variants were determined using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and Human Gene Mutation Database (professional 2019.3). The potential pathogenicity of the variants was interpreted according to the American College of Medical Genetics. Before confirmation by Sanger sequencing, the candidate variants were reviewed by clinical geneticists and ophthalmologists. Segregation analysis was performed within family members.

Clinical examination

Full ophthalmic examinations were performed on all patients with pathogenic mutations in *USH2A*, including the best Snellen-corrected visual acuity testing (BCVA), slit lamp biomicroscopy, fundus examination, visual field (VF, Humphrey Visual Field Analyzer, Carl Zeiss, Dublin, California, USA), swept-domain optical coherence tomography (Spectralis HRA +OCT, Heidelberg Engineering, Heidelberg, Germany) and full-field electroretinography (according to the standards of the International Society for Clinical Electrophysiology of Vision; www.iscev.org). VF was assessed by 30-2 Swedish Interactive Threshold Algorithm Fast Programs to measure 30° temporally and nasally and test 76 points. The VF data were excluded if fixation miss loss and false-positive and false-negative response rates were greater than 20%. Average depression of visual sensitivity was estimated by mean deviation (MD). The central foveal thickness (CFT, within the central 1 mm region) was defined as the distance between the internal limiting membrane and the inner border of the retinal pigment epithelium. To provide numeric values for low BCVAs, the following conversions were made: no light perception, 0; light perception, 0.0001; hand movements, 0.001; and counting fingers, 0.01. Clinical diagnosis of USH2 and RP was based on ocular examination and hearing tests. Patients with USH2 have typical RP fundus appearance, sensorineural hearing impairment and intact vestibular function, and RP referred to non-syndromic RP, which has typical RP fundus appearance without extraocular disorders in this study.

Statistical analysis

Measurement values of the groups were compared using the t-test and one-way analysis of variance test. Correlations were evaluated using the Pearson and partial correlation tests. Statistical analyses were performed using SPSS V.20.0 (SPSS/IBM Corp.) and Microsoft Excel (2010). $P<0.05$ was considered statistically significant.

Table 1 Distribution of clinical characteristics in patients with *USH2A* mutations

Characteristic	RP (n=75)	USH2 (n=88)	P value (t-test)
Female/male	35/40	35/53	0.374
Mean age ±SD (range), years	45.85±14.96 (2–78)	40.21±15.32 (6–80)	0.647
Mean onset age ±SD (range), years	23.37±15.72 (0–64)	15.34±12.62 (0–46)	0.01
Mean BCVA ±SD (range)	0.35±0.30 (0.0001–1)	0.38±0.32 (0.0001–1)	0.495
Age, ≤50 years	0.43±0.30*	0.46±0.31*	0.499
Age, >50 years	0.22±0.257	0.11±0.22	<0.05 (0.033)
Duration, ≤10 years	0.54±0.30†	0.56±0.29†	0.755
Duration, 10–20 years	0.44±0.26	0.47±0.36	0.705
Duration, >20 years	0.24±0.27	0.25±0.25	0.856
Mean duration ±SD (range), years	23.27±16.13 (3–78)	24.50±15.25 (2–70)	0.651
Mean MD ±SD (range), dB	−25.06±6.06 (−33.60 to −4.24)	−26.11±4.67 (−33.25 to −9.6)	0.362
Age, ≤50 years	−23.73±6.45*	−26.59±3.61	<0.01 (0.007)
Age, >50 years	−27.67±3.27	−27.29±7.46	0.835
Duration, ≤10 years	−20.71±7.45‡	−26.26±3.28	<0.01 (0.006)
Duration, 10–20 years	−25.55±3.95	−25.65±4.78	0.968
Duration, >20 years	−27.42±3.95	−27.34±4.61	0.910
Mean CFT (µm)	223.78±55.46	210.28±39.31	0.132
Age, ≤50 years	232.39±49.93*	210.75±39.63	<0.05 (0.015)
Age, >50 years	204.53±43.62	203.25±39.66	0.761
Duration, ≤10 years	256.67±52.76§	224.00±33.03	<0.05 (0.04)
Duration, 10–20 years	223.71±38.70	206.05±44.63	0.116
Duration, >20 years	220.95±51.83	204.60±35.07	0.268

*Best Snellen corrected visual acuity (BCVA), visual field (VF) and central foveal thickness (CFT) of patients younger than 50 years old had significant differences with that of patients older than 50 years ($p<0.001$, $p<0.01$, $p<0.05$).
†BCVA of patients with duration ≤10 years was better than that of patients with duration >20 years ($p<0.001$) but there was no difference from patients with duration 10–20 years ($p=0.079$).
‡VF of patients with duration ≤10 years was better than that of patients with duration >10 years ($p<0.05$), but there was no difference in mean deviation (MD) between patients with retinitis pigmentosa (RP) with duration 10–20 years and >20 years ($p>0.05$).
§CFT of patients with duration ≤10 years was thicker than that of patients with duration >10 years ($p<0.05$), but there was no difference in CFT between patients with RP with duration 10–20 years and >20 years ($p>0.05$).
BCVA, best Snellen corrected visual acuity; CFT, center foveal thickness; MD, mean deviation; RP, retinitis pigmentosa; SD, standard deviation; USH2, Usher syndrome type IIa.

Table 2 Correlations of best Snellen corrected visual acuity (BCVA), mean deviation (MD) and center foveal thickness (CFT) with age and disease duration in patients with *USH2A* mutations

	BCVA		MD		CFT	
	R value	P value	R value	P value	R value	P value
Age*	−0.323	<0.001	−0.015	0.434	−0.199	0.023
RP	−0.207	0.016	−0.149	0.129	−0.312	0.018
Ages						
≤50, years	−0.032	0.397	−0.294	0.035	−0.095	0.308
>50, years	−0.290	0.041	−0.605	0.003	−0.209	0.236
Durations						
≤10 years	−0.032	0.438	−0.120	0.312	−0.087	0.418
10–20 years	−0.433	0.041	−0.590	0.082	−0.688	0.003
>20 years	−0.285	0.013	−0.104	0.290	−0.437	0.024
USH2	−0.431	<0.001	−0.357	0.002	−0.182	0.092
Ages						
≤50	−0.277	0.003	−0.132	0.172	−0.228	0.062
>50	−0.387	0.019	−0.803	0.005	−0.246	0.297
Durations						
≤10 years	−0.376	0.032	−0.085	0.381	−0.542	0.028
10–20 years	−0.401	0.010	−0.471	0.052	−0.318	0.08
>20 years	−0.490	<0.001	−0.456	0.004	−0.527	0.01
Duration†	−0.182	0.002	−0.250	0.003	−0.036	0.362
RP	−0.272	0.002	−0.451	<0.001	−0.078	0.306
Ages						
≤50, years	−0.405	<0.001	−0.406	0.005	−0.007	0.485
>50, years	−0.030	0.431	−0.317	0.093	−0.147	0.308
Durations						
≤10 years	−0.105	0.301	−0.364	0.063	−0.377	0.179
10–20 years	−0.374	0.070	−0.867	0.006	−0.363	0.101
>20 years	−0.080	0.270	−0.088	0.319	−0.475	0.015
USH2	−0.062	0.243	−0.107	0.202	−0.007	0.480
Ages						
≤50, years	−0.038	0.353	−0.096	0.247	−0.056	0.353
>50, years	−0.146	0.225	−0.177	0.324	−0.288	0.265
Durations						
≤10 years	−0.357	0.040	−0.200	0.238	−0.663	0.007
10–20 years	−0.183	0.154	−0.392	0.092	−0.171	0.229
>20 years	−0.227	0.031	−0.211	0.119	−0.371	0.059

*Corrected for the time of disease duration.
†Corrected for age.
BCVA, best Snellen corrected visual acuity; CFT, center foveal thickness; MD, mean deviation; RP, retinitis pigmentosa; USH2, Usher syndrome type IIa.

RESULTS

Cohort characteristics

Of the 1381 patients with IRD, 1035 received a genetic diagnosis. The prevalence of patients with *USH2A* mutations was 15.75% (n=163), making *USH2A* the most frequently mutated gene in this cohort of patients with IRD. The mean age was 42.85±15.37 years (range, 2–80 years; median, 43 years) in our cohort of 93 men and 70 women. Table 1 shows the demographic and clinical characteristics. Eighty-eight patients (53.99%, 88/163) from 77 families were diagnosed with USH2 (40.19±15.32 years), and 75 patients (46.01%, 75/163) from 70 families were diagnosed with RP (45.85±14.96 years).

Phenotypic studies

The median age of onset of patients with USH2 was 15.34±12.62 years (range 0–46), which was younger than that of patients with RP (23.37±15.72 years; range 0–64; p<0.05). There was no difference in mean BCVA or CFT between RP and USH2 groups overall, but when dividing the cohort into patients younger and older than 50 years, the difference became statistically significant (p<0.05). The VF and CFT were impaired more severely in patients with USH2 (−26.59±3.61 dB, 210.75±39.63 µm) than in patients with RP (−23.73±6.45 dB, 232.39±49.93 µm) younger than 50 years (p<0.01 and p<0.05, respectively), whereas BCVA was impaired more severely in patients with USH2 (0.11±0.22) older than 50 years (p<0.05, table 1). In the first decade from disease onset, BCVA, VF and CFT declined in both groups, but VF and CFT declined more severely in patients with USH2 (−26.26±3.28 dB, 224.00±33.03 µm) than in patients with RP (−20.71±7.45 dB, 256.67±52.76 µm; p<0.05); nevertheless, the differences became insignificant as the disease progressed (p>0.05). Moreover, BCVA in both groups and VF and CFT in the RP group declined with disease progression (p<0.05), whereas progression of VF and CFT in patients with USH2 did not differ significantly between patients with a disease course >10 years and that <10 years (p>0.05).

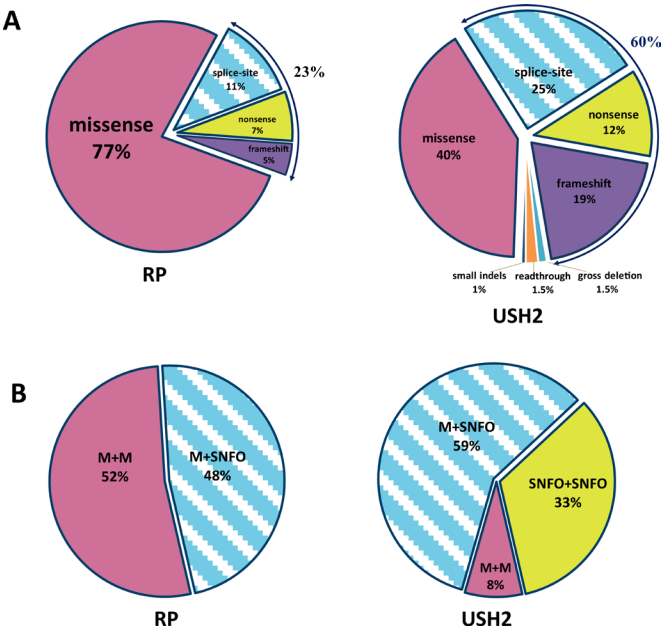

Figure 1 Proportions of *USH2A* gene mutations in patients with retinitis pigmentosa (RP) and Usher syndrome type II (USH2) in this study. (A) The proportion of different mutations types in patients with RP and USH2. (B) The proportion of different combinations of mutations types in patients with RP and USH2. M, missense; SNFO, splice site, nonsense, frameshift, or other (readthrough, gross deletions and small indels).

Legal blindness due to VF loss occurred within 10 years after disease onset in patients with USH2, whereas VF loss in patients with RP was relatively comparable to that in patients with USH2 only 10 years after disease onset ($p>0.05$). All of these results indicate that patients with USH2 have an earlier and more serious decline of visual function and damage to retinal structure than patients with RP in the first 10 years after onset, but that visual prognosis between the two groups does not differ when the course of disease exceeds 10 years.

To better assess disease progression and the difference between the two groups, we assessed correlations among disease duration, age, BCVA, MD and CFT using correlation analysis (table 2). Results showed that BCVA decreased significantly with disease progression and age in both patients with RP and USH2 ($p<0.01$), but MD and CFT were not correlated with disease progression or age ($p>0.05$). Thus, the rate of disease progression may depend on the initial diagnosis or the course of the disease and on other key factors.

Genetic studies

A total of 344 mutations were identified, of which 197 (57.27%) were missense, 33 were nonsense, 43 were frameshift, 64 were splice-site, 3 were readthrough, 3 were gross deletions and 1 a small indel. The most common mutations were c.2802T >G (11.88%) and c.8559-2A >G (9.28%), accounting for 21.16% of all mutations. It is likely that these two variants represent a hotspot of *USH2A* in the Chinese population. Six mutations, c.99_100insT, c.11156G >A, c.15178T >C, c.4821G >C, c.8232G >C and c.9469C >T, accounted for another 15.94% of the total. Of the 156 distinct variants identified in this study, 84 were novel, including 13 pathogenic variants, 32 likely pathogenic variants and 39 variants of uncertain significance (see online supplementary table 1). Of the 163 patients with pathogenic mutations on both alleles, 14 (8.59%) were homozygous and 149 (91.41%) were compound heterozygous. Only eight distinct pathogenic homozygous mutations were detected, of which four (c.15575_15579delAGGAA, c.8232G >C, c.8559-2A >G, c.99_100insT) were associated with USH2 and four (c.11156G >A, c.13465G >A, c.2802T >G, c.4616C >T) with RP. These variants are presumed to be USH2 or RP specific. Additionally, one de novo mutation (c.2802T >G) was identified in the 54 trios (1.85%).

Genotype–phenotype correlations

Of all the *USH2A* mutations associated with RP (n=158), 77.22% (n=122) were missense variants and 22.78% (n=21) were nonsense (n=11), frameshift (n=7), or splice-site mutations (n=18) that severely affected protein function. In the USH2 group (n=187), which had a severe early-onset clinical presentation, only 40.64% (n=76) mutations were missense and 59.36% were non-missense, including splice-site (n=46), frameshift (n=36), nonsense (n=22), readthrough (n=3), gross deletions (n=3) and small indels (n=1) (figure 1). Moreover, of all the patients with RP, 39 (52%) had missense +missense (M+M) mutations and 36 (48%) had missense +splice-site/frameshift/nonsense mutations (M+SFNO); no patients with SFNO+SFNO mutations were identified. However, in patients with USH2, only 7 (8%) patients had M+M mutations, 52 (59%) had M+SFNO and 29 (33%) had SFNO+SFNO mutations. These results suggested that USH2 was associated with a more severe genotype.

To further understand the relationship between genotype and clinical features, we stratified all patients into three groups: group I comprised patients with M+M mutations, group II had patients with M+SFNO mutations and group III had patients with SFNO +SFNO mutations. Then, BCVA, MD and CFT of the three groups were compared and analysed (tables 3 and 4). Overall, 84.78% (39/46) of the patients in group I and 40.91% (36/88) in group II had RP, whereas all patients in group III had USH2 (no patients with RP were identified in group III). Moreover, the median age of onset of group III (12.59±11.37 years) was earlier than that of patients in group I (22.74±14.86 years) or group II (19.32±14.92 years; $p<0.001$). These results suggested that SFNO mutations in *USH2A* were associated mostly with USH2 ($p<0.001$) and resulted in an earlier onset of disease. We found no significant differences in mean duration, BCVA, MD or CFT among the three groups, although BCVA decreased significantly with disease progression in all groups ($p<0.001$). We found no significant correlations between disease duration and MD or CFT in the three groups ($p>0.05$), indicating that MD and CFT progression were not directly related to mutation type.

DISCUSSION

In this study, we provide a brief overview of *USH2A* mutation frequency in a large cohort of Chinese patients. Of the 1035 patients with IRD, 163 had mutations in *USH2A*; this prevalence (15.75%) being the highest of patients with IRD in this study population. Additionally, 53.99% were diagnosed with USH2 and 46.01% were diagnosed with RP, indicating a relatively balanced clinical profile of *USH2A*-associated diseases in this population. These data provide valuable information for researchers in gene therapy as well as economists and government policy-makers.

Pierrache *et al* have described genotype–phenotype correlations and compared visual prognosis in USH2 and RP in a large

Table 3 Clinical characteristics of patients with different types of *USH2A* mutations

	M+M (n=46)	M+SNFO (n=88)	SNFO+SNFO (n=20)	Significant (P value)
Patients (RP/USH2)	39/7	36/52	0/29	1:<0.001 2:<0.001 3:<0.001
Onset age, yrs	22.74±14.86	19.32±14.92	12.59±11.37	1:0.108 2:<0.001 3:<0.001
Duration, yrs	23.18±16.13	25.28±15.98	21.54±14.00	1:0.360 2:0.539 3:0.119
BCVA*	0.35±0.31	0.34±0.30	0.40±0.33	1:0.932 2:0.407 3:0.336
Duration, ≤10 years	0.57±0.27	0.50±0.32	0.66±0.18	1:0.408 2:0.329 3:0.080
Duration, 10–20 years	0.46±0.29	0.41±0.33	0.54±0.37	1:0.617 2:0.569 3:0.273
Duration, >20 years	0.22±0.26	0.26±0.25	0.20±0.19	1:0.403 2:0.670 3:0.209
MD*, dB	−24.83±5.62	−25.43±4.32	−25.62±4.66	1:0.511 2:0.245 3:0.444
Duration, ≤10 years	−22.32±7.05	−22.58±6.04	−23.96±2.99	1:0.387 2:0.196 3:0.343
Duration, 10–20 years	−24.11±3.03	−25.75±4.03	−25.00±4.51	1:0.596 2:0.998 3:0.367
Duration, >20 years	−25.60±4.68	−26.96±2.57	−26.63±4.72	1:0.331 2:0.365 3:0.480
CFT*, µm	250.43±68.66	229.34±67.86	230.38±76.44	1:0.098 2:0.126 3:0.585
Duration, ≤10 years	282.10±105.39	281.72±86.18	250.70±99.36	1:0.826 2:0.157 3:0.276
Duration, 10–20 years	256.24±52.26	238.22±33.58	232.25±22.25	1:0.170 2:0.456 3:0.961
Duration, >20 years	218.00±31.57	199.50±45.98	197.75±65.60	1:0.488 2:0.873 3:0.137

1:M+M versus M+SNFO; 2: M+M versus SNFO+SNFO; 3: M+SNFO versus SNFO+SNFO.
*Corrected for the time of disease duration.
M, missense; SNFO, splice-site, nonsense, frameshift, or others (readthrough, gross deletions and small indels).
RP, retinitis pigmentosa; USH2, Usher syndrome type IIa; BCVA, best Snellen corrected visual acuity; MD, mean deviation; CFT, centre foveal thickness.

Table 4 Correlations of best Snellen corrected visual acuity (BCVA), mean deviation (MD) and central foveal thickness (CFT) with disease duration in patients with different types of *USH2A* mutations

	M+M		M+SNFO		SNFO+SNFO	
	r	P value	r	P value	r	P value
BCVA versus duration	−0.574	<0.001	−0.284	0.001	−0.528	<0.001
−≤10 years	−0.433	0.025	−0.444	0.012	−0.684	0.031
−10 to 20 years	−0.152	0.338	−0.339	0.045	−0.132	0.327
−>20 years	−0.384	0.005	−0.002	0.493	−0.038	0.436
MD versus duration	−0.315	0.027	−0.245	0.024	−0.239	0.155
−≤10 years	−0.300	0.171	−0.030	0.454	−0.120	0.411
−10 to 20 years	−0.894	0.053	−0.352	0.108	−0.894	0.053
−>20 years	−0.525	0.006	−0.220	0.106	−0.303	0.197
CFT versus duration	−0.177	0.154	−0.455	<0.001	−0.01	0.481
−≤10 years	−0.773	−0.004	−0.04	0.437	−0.894	0.053
−10 to 20 years	−0.051	0.452	−0.013	0.480	−0.049	0.446
−>20 years	−0.673	0.002	−0.540	0.007	−0.278	0.219

BCVA, best Snellen corrected visual acuity; CFT, center foveal thickness; M, missense; MD, mean deviation; RP, retinitis pigmentosa; SNFO, splice-site, nonsense, frameshift, or others (readthrough, gross deletions and small indels); USH2, Usher syndrome type IIa.

cohort of patients with *USH2A* mutations,[4] but all their participants were of European origin. It is uncertain whether the same conclusions would be reached in a Chinese population and, to date, the underlying genetic and phenotypic characteristics of Chinese patients with *USH2A* mutations have not been comprehensively explored. In this study, we found that patients with USH2 had an earlier and more severe decline of visual function and damage to the retinal structure than did patients with RP in the first decade from disease onset, which is consistent with a previous study.[4] However, our study confirmed that the progression of RP accelerated in the second decade of the disease, and that there was no difference in visual prognosis between patients with USH2 and RP when the disease duration exceeded 10 years. These data indicate that the optimal intervention window for subretinal gene therapy is within the first decade from disease onset.

Consistent with previous studies, the hotspot of *USH2A* was c.8559-2A >G in Chinese and Japanese patients,[12 13] but hotspot of *USH2A* in European patients (p.Glu767Serfs*21 and p.Cys759Phe) was not detected in this study.[7 10] Besides, we found that c.2802T>G was a *USH2A* mutation hotspot in our cohort of Chinese patients. We also identified 84 novel variants, confirming that the mutation spectrum of *USH2A* in Chinese patients differs from that of other populations. Thus far, it is unknown why some mutations in *USH2A* lead to USH2 and others to RP. As most patients are compound heterozygotes, it is difficult to assess the effect of individual mutations on the phenotype. Nevertheless, variants in the homozygous state represent an ideal model by which to search for disease-specific alleles. Previous studies found that five variants (c.2802T >G, c.10073G>A, c.11156G>A, c.12295–3T>A and c.12575G>A) are RP specific, and three (c.12295–3T>A, c.9056-2A>G, and c.5776+1G>A) are USH2 specific.[3] Further data from our cohort showed that c.13465G>A, c.4616C>T, and two reported variants, c.2802T>G and c.11156G>A, were specific to RP, whereas c.15575_15579delAGGAA, c.8232G>C, c.8559-2A>G and c.99_100insT were specific to USH2. A known genotype would allow for better correlation of clinical signs and counselling of patients.

In patients with RP, 77.22% of *USH2A* mutations were missense, whereas in patients with USH2, only 40.64% of mutations were missense; all other mutations were nonsense, frameshift or splice-site mutations that severely affected protein function. This may imply that missense variants in *USH2A* have less severe consequences and are therefore found more commonly in the milder RP than in the more severe USH2. Each RP genotype documented included at least one missense variant, whereas the two deleterious mutations were restricted to the USH2 phenotype, consistent with previous reports.[4] We hypothesise that RP results from genotypes that include milder hypomorphic alleles; further experimental verification of this hypothesis is needed. We further explored this observation by analysing the relationship between genotype and clinical features of *USH2A* mutations, which confirmed that more deleterious genotypes, including frameshift, nonsense, gross deletions and small indels, were associated with an earlier onset of disease, regardless of the phenotype. The disease progression rate was

not directly related to the genotype; thus, additional genetic or environmental modifiers may play a role in disease progression.

In summary, we presented the overall frequency of *USH2A*-associated IRD and a detailed clinical and genetic characterisation of patients of Chinese origin with *USH2A* mutations. We found that missense variants had less severe consequences and were found more commonly in the milder RP; the more deleterious genotypes were associated with an earlier onset of disease. Although the rate of disease progression was not directly related to genotype, we found no difference in visual prognosis among patients with *USH2A* mutations when the course of disease exceeded 10 years. Our data provide a deeper understanding of *USH2A* mutations in China and enable precise genetic diagnoses and better management of these patients, and serve as a well-founded reference for genetic counselling and development of potential therapeutic approaches.

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