Frequency and phenotypic characteristics of RPE65 mutations in the Chinese population

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Abstract

Background: The retinoid isomerohydrolase RPE65 has received considerable attention worldwide since a successful clinical gene therapy was approved in 2017 as the first treatment for vision loss associated with RPE65-mediated inherited retinal disease. Identifying patients with RPE65 mutations is a prerequisite to assessing the patients’ eligibility to receive RPE65-targeted gene therapies, and it is necessary to identify individuals who are most likely to benefit from gene therapies. This study aimed to investigate the RPE65 mutations frequency in the Chinese population and to determine the genetic and clinical characteristics of these patients.

Results: Only 20 patients with RPE65 mutations were identified, and RPE65 mutations were determined to be the 14th most common among all patients with genetic diagnoses. Ten novel variants and two hotspots associated with FAP were identified. A literature review revealed that a total of 57 patients of Chinese origin were identified with pathogenic mutations in the RPE65 gene. The mean best Snellen corrected visual acuity was worse (mean 1.3 ± 1.3 LogMAR) in patients older than 20 years old than in those younger than 15 years old (0.68 ± 0.92 LogMAR). Bone spicule-like pigment deposits (BSLPs) were observed in six patients; they were older than those without BSLP and those with white-yellow dots. Genotype–phenotype analysis revealed that truncating variants seem to lead to a more severe clinical presentation, while best corrected visual acuity testing and fundus changes did not correlate with specific RPE65 variants or mutation types.

Conclusions: This study provides a detailed clinical-genetic assessment of patients with RPE65 mutations of Chinese origin. These results may help to elucidate RPE65 mutations in the Chinese population and may facilitate genetic counseling and the implementation of gene therapy in China.

Keywords: Inherited retinal dystrophy, RPE65 gene mutations, Next-generation sequencing, Chinese population, Genotype–phenotype correlations

Background

The retinoid isomerohydrolase RPE65 has received considerable attention worldwide since a successful clinical gene therapy was approved in 2017 as the first treatment for vision loss associated with RPE65-mediated inherited retinal disease (IRD) (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm589467.htm). Identifying patients with RPE65 mutations is a prerequisite to assessing the patients’ eligibility in receiving RPE65-targeted gene therapies, and it is necessary to identify individuals who are most likely to benefit from gene therapies.

To date, nearly 200 disease-causing mutations in the RPE65 gene have been reported (Human Gene Mutation Database (HGMD); professional version 2019.2), which are associated with a large heterogeneous group
of retinal dystrophies, including Leber congenital amaurosis (LCA) type 2, early onset severe retinal dystrophy, retinitis pigmentosa (RP) type 20 and fundus albipunctatus (FAP). Studies have shown that the mutation frequency and phenotypic variation of RPE65 varies notably between different ethnic groups. For example, RPE65 mutations are thought to affect approximately 1000 to 3000 people in the United States (Population clock. 2018. Available: https://www.census.gov/popclock/ [Accessed 14 Aug 2018]), while 6% of all LCA cases in Caucasians [1], 16% in the Danish LCA cohort [2], and only a few LCA cases were reported in Chinese populations [3, 4]. Generally, most studies associated with RPE65 mutations were performed in Western populations [2, 5, 6], and the exact frequency of RPE65 mutations in all forms of IRD and the variety of associated phenotypes in China has not been determined.

In the current study, we performed a comprehensive mutation analysis in 1434 IRD patients. Twenty patients with RPE65 mutations were identified, and their specific clinical phenotypes were presented. Moreover, we further reviewed the varied phenotypes and genotypes of all cases of RPE65 mutations of Chinese origin reported in the literature. These results provide a brief overview of the frequency and phenotypic characteristics of the RPE65 mutation in the Chinese population.

Methods
Subjects, ethics statement and NGS analysis
A total of 1434 Chinese patients with IRDs and their available family members (total participants: 3576) were recruited from the eye genetic disease clinic of the Eye and ENT Hospital of Fudan University between January 2017 and June 2019. Of these, 956 patients had been mentioned in our previous report [Gao and others 2019]. Written informed consent in accordance with the tenets of the Declaration of Helsinki was obtained from all participants or their guardians. This study was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University. DNA was isolated from peripheral blood using the FlexiGene DNA Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer’s protocol. NGS analysis and bioinformatics analysis were performed as previously reported [7]. We designed a high-throughput targeted enrichment approach to exon-capture regions of 586 genes that are involved in common inherited eye diseases. The probe length of the panel is 90 nt, the total target area obtained is 2.3 M. On average, the mean coverage depth was more than 400X, and the coverage of target region was ~99.9% using BGISeq-2000. Then the sequence data obtained were analyzed as previously reported [7]. Previous reported variants were determined using Human Gene Mutation Database (HGMD, professional version 2019.2). For variants that passed the initial filtration, Sanger sequencing was carried out for segregation analysis and variants validation.

Clinical evaluations
All patients with pathogenic mutations in RPE65 underwent a full ophthalmic examination, including best Snellen corrected visual acuity testing (BCVA), they were converted to equivalent value of logarithm of minimal angle of resolution (logMAR) unit, slit lamp biomicroscopy, tonometry, fundus examination, wide-field fundus imaging (Optos PLC, Dunfermline, United Kingdom), swept-domain optical coherence tomography (SD-OCT, Spectralis HRA + OCT, Heidelberg, Engineering Inc., Heidelberg, Germany), visual field (Humphrey Visual Field Analyzer, Carl Zeiss Inc., CA, USA), and full-field electroretinography (ERG, according to the standards of the International Society for Clinical Electrophysiology of Vision; available at www.iscev.org).

Results
Genetic analyses
Of the 1434 patients with IRD, 74.55% of patients (n=1069) received a genetic diagnosis, and a total of 1516 variants involved 87 genes were identified. Only 41 alleles representing 26 distinct variants in 17 families (20 patients: 10 males, 10 females) were identified in the RPE65 gene (NM_000329.2, Table 1 and Additional file 5: Table S1), accounting for 2.83% of all the variants, and the gene was ranked as the seventh most common gene detected in this cohort of patients with IRD (Additional file 1: Figure S1A and S1B). However, the number of patients with RPE65 mutations only accounted for 1.87% (20/1069) of all patients with genetic diagnoses and was the 14th most common among all the patients (Additional file 1: Fig. 1c, d). Pedigrees and mutations of the 17 families are available in Additional file 2: Figure S2. Of the 26 distinct variants identified in this study, 10 (c.1039C>T p.Arg347Cys, c.1255C>T p.Pro419Ser, c.1444G>A p.Asp482Asn, c.334T>A p.Cys112Ser, c.354-2A>G, c.376del p.Val126*fs1, c.806_809delinsTGG AGCCATGAAG p.SerLeu269MetGluProTer, c.837del p.Phe279Leufs46, c.886del p.Arg296*fs1, c.94+2T>A) were novel (Additional file 3: Figure S3), including seven likely pathogenic variants and three missense variants of uncertain significance (p.Pro419Ser, p.Cys112Ser and p.Arg347Cys), which are localized in highly conserved residues (Additional file 4: Figure S4). Bioinformatics analysis results of the novel variants are shown in Additional file 6: Table S2. Of the 16 variants reported previously, p.His68Tyr and c.998+1G>A were firstly reported to be associated with LCA.
To date, a total of 39 patients from 27 unrelated Chinese families have been reported with pathogenic mutations in RPE65 (Additional file 5: Table S1). Together with the 20 patients (two have been previously reported: F4-1 and F5-1) in this study [8], 57 patients of Chinese origin were diagnosed. Of the 115 variants identified in these patients, the majority of pathogenic defects (71.3%, n = 82) were missense variants, and 28.7% (n = 33) were nonsense, frameshift, or splice-site mutations that severely affected protein function (Fig. 1a). Variants were distributed from exon 2 to exon 14 (Fig. 1b).

### Phenotypic characterization

Of the 20 patients in the 17 families, four were diagnosed with RP, one with FAP, and 15 with LCA (Table 2). The mean age at visit was 16.4 ± 12.59 years (range 3–49 years; median, 10 years). All accepted patients (LCA and RP) experienced poor vision at an early age. The mean BCVA with LCA patients was 0.82 ± 0.92 (range 3.00–0.40) LogMAR, and 90% (27/30) of eyes had a BCVA worse than 0.52 LogMAR. Of patients younger than 15 years, the mean BCVA was 0.68 ± 0.92 (range 1.30–0.40) LogMAR, while for patients older than 20 years, the mean BCVA was worse (p < 0.001, mean 1.30 ± 1.30 (range 3.00–1.00) LogMAR. Of the four patients with RP, the mean BCVA was 0.37 ± 1.05 (range 0.52–2.22) LogMAR, while the FAP patient maintained better vision (0/0.05 LogMAR). Bone spicule-like pigment deposits (BSLPs) were observed in six patients from five families (30%, 6/20, Fig. 2a), and no typical deposits were seen in 70% of patients (n = 14, Fig. 2b).

### Table 1: RPE65 variants identified in this cohort of patients

| Nucleotide change | Amino acid change | Mutation type | Exon/Intron | Patients | ACMG category | References |
|-------------------|-------------------|---------------|-------------|----------|---------------|------------|
| c.1399C>G         | p.Pro467Ala       | Missense      | E13 F1-F3   | P         | [8, 13]       |
| c.272G>A          | p.Arg91Gln       | Missense      | E4 F2-F16   | P         | [14–16]      |
| c.271C>T          | p.Arg91Trp       | Missense      | E4 F2-F16   | P         | [17, 18]      |
| c.1338G>T         | p.Arg446Ser      | Missense      | E12 F5-F7   | P         | [4, 19]       |
| c.1543C>T         | p.Arg515Trp      | Missense      | E14 F6-F2   | P         | [20, 21]      |
| c.1444G>A         | p.Asp482Asn      | Missense      | E13 F6-F17  | LP Novel  |
| c.1255C>T         | p.Pro149Ser      | Missense      | E12 F8     | VUS Novel  |
| c.202C>T          | p.His68Tyr       | Missense      | E3 F8      | P         | [22, 23]      |
| c.1590C>A         | p.Phe530Leu      | Missense      | E14 F9     | P         | [4, 24]       |
| c.997G>C          | p.Gly333Arg      | Missense      | E9 F10     | P         | [25]          |
| c.334T>A          | p.Cys112Ser      | Missense      | E4 F10     | VUS Novel  |
| c.131G>A          | p.Arg44Gln       | Missense      | E3 F12     | P         | [14, 26]      |
| c.200T>G          | p.Leu67Arg       | Missense      | E3 F13     | P         | [27, 28]      |
| c.130A>G          | p.Tyr43Cys       | Missense      | E12 F13    | P         | [29]          |
| c.1039C>T         | p.Arg347Cys      | Missense      | E10 F14    | VUS Novel  |
| c.1076G>C         | p.Ala360Pro      | Missense      | E10 F14    | LP Novel  |
| c.493C>T          | p.Gln165*        | Nonsense      | E5 F4-F4   | P         | [4]           |
| c.1380G>A         | p.Trp460*        | Nonsense      | E13 F15    | P         | [31]          |
| c.944+2T>A        | -                 | Splicing      | I2 F1      | LP Novel  |
| c.998+1G>A        | -                 | Splicing      | I10 F3     | P         | [13]          |
| c.354-2A>G        | -                 | Splicing      | I5 F7-F2   | LP Novel  |
| c.858+1del        | -                 | Splicing      | I9 F9-F12  | P         | [8]           |
| c.376del          | p.Val26fs*1      | Frameshift    | EX5 F16    | LP Novel  |
| c.806_809delInsTGG| p.SerLeu269MetGluProTer| Frameshift    | EX8 F17    | P         | Novel        |
| c.837del          | p.Phe279Leufs*46 | Frameshift    | EX8 F11    | LP Novel  |
| c.866del          | p.Arg296fs       | Frameshift    | EX9 F15    | LP Novel  |

F: family; E: Exon; I: Intron; P: Pathogenic; LP: Likely pathogenic; VUS: variants of uncertain significance
were nonrecordable in 14 of the 15 LCA patients and notably attenuated in the four RP patients.

Genotype–phenotype correlations
All RPE65 variants identified in the Chinese population are shown in Fig. 3 and Additional file 5: Table S1. To date, 39 variants have been reported to be associated with LCA, 23 with RP, and 8 with FAP. As no clinical features of the FAP patient with one frameshift were provided by Guoxing Yang et al. [9], the FAP diagnosis requires further confirmation, and this patient was not included in the statistical analysis. Of all the variants associated with LCA (n = 73), 71.2% (n = 52) were missense variants, and 28.8% (n = 21) were nonsense (n = 5), frameshift (n = 9), or splice-site mutations (n = 7) that severely affected protein function, while in the FAP group (n = 16), 93.75% (n = 15) variants were missense, and only one splice-site mutation was identified (Fig. 1c). In the RP group, 58.3% (n = 14) of variants were missense variants, 20.8% (n = 5) were truncating stops, and 20.8% were frameshift (n = 2) or splicing defects (n = 3). Therefore, of all the variants (n = 97) associated with LCA and RP, which have a severe early-onset clinical presentation, only 68% (n = 66) were missense mutations, and the remaining 32% (n = 31) of
variants were truncating mutations. Nevertheless, in the FAP group, which showed relatively mild symptoms, 93.8% (n = 15) of all the variants were missense. Of all the LCA and RP patients, 50% had missense + missense mutations, 35.4% had missense + nonsense/frameshift/splice-site mutations, and 14.6% did not have any missense mutations. However, of the eight FAP patients, 7 were missense + missense, and only one was a missense + splice-site mutation.

Only one variant, c.1399C>G (p. Pro467Ala), was associated with LCA, RP and FAP (Fig. 1d). This mutation was located in a highly evolutionarily conserved region (Additional file 4: Figure S4A) and altered the corresponding amino acid from proline to alanine. The 3D structural model of these amino changes is portrayed in Additional file 4: Figure S4B. Of the eight FAP patients, five had the p. Pro467Ala mutation, and the other three had the p.Arg515Trp mutation. It is likely that the two variants were hotspots of FAP. c.131G>A p.Arg44Gln was associated with both LCA and FAP; when the second mutated allele is a truncating mutation (c.858+1del), the patients were likely to present an LCA diagnosis, while if the second mutated allele is a missense mutation (p.Arg515Trp), the patients were likely to present an FAP diagnosis. p.Arg515Trp, p.Ala145Asp and p.Ala214Serfs*20 were associated with both RP and FAP, while p.Ala434Glu, p.Ser238Cys, c.94+2T>A, and p. Leu328Phe were only associated with FAP (Fig. 1d). Further analysis of the relationship between BCVA and fundus changes with specific mutations revealed that there was no correlation between them. Different individuals, even those with the same mutation, were found to show different changes. For example, patient F6-2 showed white-yellow dots scattered in the periphery of the retina, while patient F6-1, who had the same mutations, did not display these dots. However, we can’t rule out the possibility that these dots would disappear with age.

| Table 2 Clinical characteristics of the 20 patients identified in this study |
|---------------------|----------------|----------------|----------------|----------------|
| Patients | Age (years) /sex | BCVA LogMAR R/L | Refraction R/L | Age at disease presentation (years) | ERG | Fundus | Others | Diagnosis |
| F1-1   | 12/F | 0/0.05 | +0.5/+0.75 | Congenital | Undetectable rod ERG, subnormal cone ERG | a | No | FAP |
| F2-1   | 6/M | 0.60/0.82 | −0.5/−2.0 | Congenital | Extinct | a, b | Nystagmus | LCA 2 |
| F3-1   | 7/M | 0.40/0.40 | −4.0/−3.75 | Congenital | Extinct | N | Nystagmus | LCA 2 |
| F4-1   | 15/M | 0.52/0.52 | −3.5/−3.0 | Congenital | Extinct | a, b | Nystagmus | LCA 2 |
| F4-2   | 3/F | 1.00/1.00 | +0.5/+0.75 | Congenital | Extinct | N | Nystagmus | LCA 2 |
| F5-1   | 9/F | 1.30/0.70 | +4.5/+4.5 | Congenital | Extinct | N | Nystagmus | LCA 2 |
| F6-1   | 10/M | 0.22/0.40 | −/+0.5 | 3 | Profoundly attenuated rod and cone ERGs | N | No | RP 20 |
| F6-2   | 9/F | 0.40/0.40 | −/+0.25 | 3 | Profoundly attenuated rod and cone ERGs | a | No | RP 20 |
| F7-1   | 29/M | 1.00/3.00 | − | Congenital | Extinct | b | Nystagmus | LCA 2 |
| F7-2   | 31/M | 1.3/1.3 | − | Congenital | Extinct | b | Nystagmus | LCA 2 |
| F8-1   | 7/F | 1.00/0.13 | +1.5/+2.25 | Congenital | Extinct | a | Nystagmus | LCA 2 |
| F9-1   | 11/M | 0.52/0.40 | −0.5/−0.5 | Congenital | Profoundly attenuated rod and cone ERGs | a | No | RP 20 |
| F10-1  | 49/F | 3.00/3.00 | −2.25/−1.75 | Congenital | Extinct | b | Nystagmus | LCA 2 |
| F11-1  | 20/M | 1.00/1.00 | −1.0/−1.25 | Congenital | Profoundly attenuated rod and cone ERGs | N | Nystagmus | LCA 2 |
| F12-1  | 31/F | 1.00/1.00 | −1.0/−1.25 | Congenital | Extinct | b | Nystagmus | LCA 2 |
| F13-1  | 9/F | 0.52/0.40 | +2.5/+2.25 | Congenital | Extinct | N | Nystagmus, oculo-digital sign | LCA 2 |
| F14-1  | 5/F | 0.60/0.70 | +2.25/+3.0 | Congenital | Extinct | N | Nystagmus, esotropia | LCA 2 |
| F15-1  | 5/M | 0.82/1.00 | +2.0/+2.25 | Congenital | Extinct | N | Nystagmus | LCA 2 |
| F16-1  | 30/M | 2.00/2.00 | −/+ | Congenital | Extinct | N | Nystagmus | LCA 2 |
| F17-1  | 30/F | 0.30/0.40 | −4.0/−4.5 | Congenital | Profoundly attenuated rod and cone ERGs | N | No | RP 20 |

F: family; R: right; L: left; LP = 3 LogMAR; HM = 2 LogMAR; a. white or white-yellow dots; b. Bone-spicule-like pigment; deposits; N: no a or b; LCA: Leber congenital amaurosis; RP: retinitis pigmentosa; FAP: fundus albipunctatus
Discussion

*RPE65* mutation-associated IRD usually occurs at an early age, and the majority of patients become fully blind in childhood or adulthood [10, 11]. Recent research has indicated that certain forms of *RPE65*-mediated IRDs are amenable to gene therapy. Achieving an in-depth understanding of *RPE65* mutations and related phenotypic characteristics in the Chinese population is a prerequisite for developing *RPE65*-targeted gene therapies in China.

Only 20 patients with associated *RPE65* mutations were identified from January 2017 to June 2019 in our hospital, accounting for 1.87% of all IRD patients and making *RPE65* mutations the 14th most common among all patients. Together with other patients who have been reported, only 57 patients with *RPE65* mutations have been identified in China [3, 8–16]. These results suggest that *RPE65* mutations are rare in Chinese populations. As these mutations appear to be a low-probability, high-cost event, our data may provide strong clinical-based evidence for gene therapy researchers, economists, government policy makers, and ophthalmologists to make decisions in their corresponding work. The mean age of patients with *RPE65* mutations was 16.4 ± 12.59 years (median, 10 years), and the mean BCVA was 0.82 ± 0.92 LogMAR with LCA patients and 0.37 ± 1.05 LogMAR with RP patients. Of patients younger than 15 years, the mean BCVA was 0.68 ± 0.92 LogMAR, while for patients older than 20 years, the mean BCVA was worse (mean 1.3 ± 1.3 LogMAR). These data indicate that the optimal intervention window for subretinal gene therapy is within the first 2 decades of life.

El Matri, L. et al. reported that white dot deposits occurred in earlier stages, and clumped pigment occurred in later stages, in patients with *RPE65* mutations [12]. A similar result was obtained in the current study: patients with *RPE65* mutations are more likely to exhibit WYD in the first decade but show BSLP after the second decade of life. However, some patients (40%) could not have BSLP or WYD, and these changes had no correlation with specific mutations or types or locations of mutations.

![Fig. 2](image_url)

**Fig. 2** a Color fundus photographs and spectral domain optical coherence tomography (SD-OCT) of patients showed bone spicule-like pigment deposits (BSLP). The image in the upper right is the corresponding enlarged figure showing BSLP in the fundus (black arrow). b Color fundus photograph and SD-OCT of patients without BDL or white-yellow dots (WYD). The image in the upper right is the corresponding enlarged figure showing pigment dispersion in the mid-periphery. c Color fundus photograph and SD-OCT of patients with WYD (black arrow). The image in the upper right is the corresponding enlarged figure showing WYD scattered in the periphery of the retina. d Number of patients (black font) and their corresponding mean ages (blue font) in different groups. Group 1: patients with WYD. Group 2: patients without WYD. Group 3: patients with BDL. Group 4: patients without BDL. Group 5: patients without WYD and BDL. Group 6: patients with WYD and BDL. *p < 0.05; **p < 0.01
To date, genotype–phenotype correlations of patients with RPE65 mutations have not been highly distinct. It has not been determined why some mutations in RPE65 lead to LCA, while others lead to RP or FAP. In addition, the fact that most patients are compound heterozygotes hinders efforts to assess the effect of each mutation on the phenotypes and to evaluate possible allelic hierarchy. In this study, we found four notable cases of possible
discordancy between clinical and molecular diagnosis. First, truncating variants seem to lead to a more severe clinical presentation (LCA or RP), while almost all FAP patients were caused by missense mutations. Second, two hotspots (p.Pro467Ala and p.Arg151Trp) associated with FAP were identified, helping to further elucidate the mutational spectrum of RPE65 in the Chinese population. Third, p.Pro467Ala was associated with LCA, RP and FAP, suggesting that this mutation may have a mild effect on protein function, and the phenotype is primarily affected by the second allele. Fourth, BCVA and fundus changes did not correlate with specific RPE65 variants or mutation types.

Conclusions

In the current study, we performed a comprehensive analysis of the phenotypes and genotypes of the 20 patients with RPE65 mutations identified in this study and all the RPE65 mutation cases of Chinese origin reported in the literature. Our data provide a brief overview of the frequency and phenotypic characteristics of RPE65 mutations in the Chinese population, help to characterize RPE65 mutations in China, and represent a possible reference for genetic counseling and the selection of eligible patients for gene augmentation.

Abbreviations

ACMG: American College of Medical Genetics; BCVA: Best corrected visual acuity testing; BSLP: Bone-spicule-like pigment deposits; CFT: Central foveal thickness; CME: Cystoid macular edema; ERG: Full-field electroretinography; FAP: Fundus albipunctatus; HGMD: Human Gene Mutation Database; IRD: Inherited retinal disease; LCA: Leber congenital amaurosis; NGS: Next-generation sequencing; OMIM: Online Mendelian Inheritance in Man; RP: Retinitis pigmentosa; SD-OCT: Spectral domain optical coherence tomography; WYD: White-yellow dots.

Supplementary Information

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Authors’ contributions

Ji-Hong Wu conceived and designed the experiments. Wei Liu, Qing Chang, Ping Xu, Ji-Hong Wu, Feng-Juan Gao, Dan-Dan Wang and Yu-He Qi collected the clinical samples. Feng-Juan Gao, Ji-Hong Wu, Fang-Yuan Hu, Jiang-Kang Li, Fang Chen, Wei Li and Dan-Dan Wang analyzed sequencing data. Ge-Zhi Xu, Wei Liu, Feng-Juan Gao and Yu-He Qi recruited patients, performed clinical examination of patients and clinical interpretation. Feng-Juan Gao and Ji-Hong Wu drafted and revised the manuscript. All authors read and approved the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

Please contact authors for data requests.

Declarations

Ethics approval and consent to participate

The study was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University.

Consent for publication

Written informed consent was obtained from all the subjects or their guardians.

Competing interests

None of the authors has any conflicting interests to disclose. There are no financial disclosures in this article.

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