Detecting genetic variations in hereditary retinal dystrophies with next-generation sequencing technology

Xin Jin,1,2,3 Ling Hui Qu,1,3 Xiao Hong Meng,1,3 Hai Wei Xu,1,3 Zheng Qin Yin1,2,3

(The first two authors contributed equally to this work.)

1Southwest Hospital, Southwest Eye Hospital, Third Military Medical University, Chongqing, China; 2Department of Ophthalmology, Chinese PLA General Hospital, Beijing, China; 3Key Lab of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing, China

Purpose: To identify pathogenic mutations responsible for retinal dystrophies (RDs) in three unrelated Chinese families.

Methods: Three probands from unrelated families with RDs were recruited. Genomic DNA prepared from leukocytes was analyzed using gene chip–based next-generation sequencing (NGS) to capture and sequence all of the exons of 100 known RD-associated genes. Candidate variants were validated with PCR and Sanger sequencing in the respective families. Thorough ophthalmic examinations including best-corrected visual acuity, funduscopic examination, and full-field electroretinograms were performed in the affected individuals.

Results: We successfully identified causative mutations in patients from the Chinese families with RDs: the known mutation IMPDH1 c.942_944delGAA in a family with retinitis pigmentosa, the novel mutation ABCA4 c.1924T>A in a family with Stargardt disease, and the novel mutation NMNAT1 c.272A>G and known mutation NMNAT1 c.196C>T in a family with Leber congenital amaurosis. All variations segregated with the disease phenotypes in the respective families and were absent from ethnically matched control chromosomes. Prediction analysis demonstrated the two novel missense mutations might be damaging.

Conclusions: The results strongly suggested these mutations were responsible for different RD phenotypes in the Chinese families. NGS technology provides an accurate and economic method for identifying causative genes for RDs.

Hereditary retinal dystrophies (RDs) are a large group of clinically and genetically heterogeneous retinal diseases that constitute a major cause of vision handicap in the global population [1]. They are typically characterized by the progressive loss of rod and cone photoreceptor cells often leading to severe blindness. There are similar fundus appearances among different RDs, including retinal pigment epithelium (RPE) changes, attenuated retinal vessels, and waxy optic disc. To date, more than 100 genes associated with various RDs have been identified and partially characterized at the genetic and molecular level (RetNet). These gene functions involved diverse biologic pathways, including phototransduction, visual cycle, photoreceptor structure and morphogenesis, cell adhesion, cellular metabolism, vesicle and protein trafficking, synaptic function, cilium structure and transport, ion and small molecular transport, chaperones, RNA splicing, transcription factors associated with photoreceptor development, protein folding and subunit assembly, and posttranslational protein modification, among others. Some RDs associated with different pathogenic genes show the same phenotypes while patients with RDs with the same causative gene can present different phenotypes. Therefore, accurate and comprehensive molecular diagnosis is critical for confirming the clinical diagnosis, providing a disease prognosis, and in the long run providing the basis for novel therapeutic approaches and personalized medicine. However, molecular diagnosis of RDs is made challenging by the large number of disease genes. Recently, next-generation sequencing (NGS) technology has provided a high-throughput and cost-effective method for identifying causative genes for RDs that could sequence the candidate gene rapidly and accurately [2-4]. The causative mutations of approximately 50% to 80% of RD cases could be detected successfully using this method [5-7].

In this study, we identified four mutations in three unrelated Chinese pedigrees diagnosed with RDs: the known mutation IMPDH1 c.942_944delGAA in a family with autosomal dominant retinitis pigmentosa (adRP), the novel mutation ABCA4 c.1924T>A in a family with Stargardt disease (STGD), and the novel mutation NMNAT1 c.272A>G and the known mutation NMNAT1 c.196C>T in a family with Leber congenital amaurosis (LCA). These variants were identified with the combined approach of target region sequencing–based NGS and candidate mutations validation with Sanger
They segregated with the disease phenotype in the respective families and were not detected in the normal controls database.

METHODS

Pedigrees and controls: All three pedigrees (Family-012, Family-024, and Family-035) described in this study were from Sichuan province in China. A detailed medical history was obtained by interviewing family members. The patients from the respective families underwent comprehensive ophthalmological examinations at the Southwest Eye Hospital, including best-corrected visual acuity (BCVA), dilated fundoscopic indirect ophthalmoscopy, and full-field electroretinograms (ERGs). ERGs were also performed and recorded according to the standards of the International Society for Clinical Electrophysiology (ISCEV). The normal controls database was from BGI, including 840 unrelated individuals, who were more than 50 years old and had no symptoms of night blindness, vision reduction, and any personal or family history of known inheritance disease. This research followed the tenets of the Declaration of Helsinki and received approval from the Ethic Committee of Southwest Hospital. Informed consent was obtained from all participants included in this study.

Targeted region capture and next-generation sequencing: A custom-made capture array (Roche NimbleGen, Madison, WI), from Beijing Genomics Institute-ShenZhen, was designed to capture the coded exons of the 100 RD genes (Appendix 1). Genomic DNA of the probands (Family-012 III:4, Family-024II:6, and Family-035II:2) was extracted from peripheral blood according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). Then the genomic DNA was fragmented to 200–300 bp by Covaris S2 (Woburn, MA). The library was pooled and hybridized to the custom capture array for 72 h at 42 °C. After hybridization, the array was washed and eluted according to the manufacturer's instructions (Roche NimbleGen). The captured library was sequenced on Illumina HiSeq2000 analyzers for 90 cycles per read to generate paired-end reads (following the manufacturer's standard sequencing protocols). Image analysis and base calling were performed using the Illumina Pipeline to generate raw data.

Variant identification and validation: To detect the pathogenic variants of the probands, we applied filtering criteria to generate clean reads (with a length of 90 bp) for further analysis, and then aligned the clean reads against the human genome reference from the NCBI database (version hg19) using the BWA (Burrows Wheeler Aligner) Multi-Vision software package. Single-nucleotide variants (SNVs) and indels were identified using SOAPsnp software and the Samtools Indel Genotyper, respectively. All SNVs and indels were determined using the NCBI dbSNP, HapMap project, 1000 Genome Project, and the database of healthy Chinese adults from BGI.

The candidate variations in known causative RD genes were validated with PCR and Sanger sequencing. PCR primers were designed via Primer6.0 as shown in Table 1. The PCR reactions were performed as follows: denaturation at 94 °C for 3min, followed by 30 cycles of 94 °C for 30s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1min, ending with a final extension step of 72 °C for 5min. The PCR products were sequenced using a BigDye terminator v3.1 cycle sequencing kit (ABI, Foster City, CA) and analyzed on an ABI 3730XL Genetic Analyzer.

Prediction analysis: To predict the influence of these variants on protein function, we analyzed evolutionary conservation of these mutations using the ClustalW tool and examined the possible impact of the amino acid substitution with SIFT and PolyPhen tools available online.

RESULTS

Clinical findings: The pedigree Family-012 was consistent with autosomal dominant inheritance (Figure 1). There were six affected individuals out of 20 members in this four-generation Chinese family. All the patients in the family had visual disabilities but no history of systemic abnormalities. Night blindness was always the presenting symptom, and the age of onset was about 12 years old (mean age of onset=11.6 years). Affected members also reported palpable peripheral visual field loss and mild visual acuity decline in their 30s.

| Mutation         | Gene   | Forward primer (5'-3')                     | Reverse primer (5'-3')                     | Product length (bp) |
|------------------|--------|--------------------------------------------|--------------------------------------------|--------------------|
| c.942_944delGAA  | IMPDH1 | cacaagctgtctcttttcttc                      | gggtcctcataaacctccac                       | 310                |
| c.1924T>A        | ABCA4  | ccctcttgcttcctgt                            | aactccacacccctcatg                        | 261                |
| c.272A>G         | NMNAT1 | tgtgctgatataattttctgt                       | aagcggattgacatttaca                        | 308                |
| c.196C>T         | NMNAT1 | tgtgtgattatattttctgt                        | aagcggattgacatttaca                        | 308                |
Funduscopic examinations revealed similar clinical manifestations among affected individuals, including waxy-pale discs, attenuation of retinal arterioles, bone spicule-like pigmentation in the periphery, and atrophy of the RPE (Figure 2). ERGs showed non-recordable responses (Figure 3).

Family-024 had autosomal recessive macular dystrophy (Figure 1). Affected individuals typically experienced significant reduction in central visual acuity in their second decade of life. The patients also showed a delay in dark adaptation in their 40s. Fundus photographs showed extensive atrophic appearing RPE changes and pigmentation in the posterior pole of the retina (Figure 2). Abnormal cone function (assessed with light-adapted 30 Hz and light-adapted 3.0) and rod ERG abnormality (assessed using dark-adapted 0.01 to dark-adapted 10.0) were observed on ERG (Figure 3).

Family-035 was a small recessive inheritance family with only two affected individuals (Figure 1). They had horizontal nystagmus and poor vision but otherwise normal physical and mental health at 3 months of age. At 6–8 years of age, ophthalmological examinations showed that BCVA were about HM/30 cm in the both eyes, severe central atrophy with an appearance of macular coloboma. The remainder of the retina presented pigmented changes, attenuated retinal blood vessels, and optic disc pallor (Figure 2). All ERGs responses were nearly extinct (Figure 3).

Identification of candidate mutations: Approximately 43.3 kb of exons and adjacent intronic regions of the 100 RD genes for the respective proband were captured and sequenced in our study. For the 100 RD genes, the average coverage is approximately 98.1%, and the average median-depth is 479×. On average, 100% of base pairs with N200× coverage were successfully detected indicating high capabilities for identifying variants.

In the respective probands of the three pedigrees, we first identified SNVs and indels in the coding regions and introns that may affect splicing. Since RDs are rare disorders but have a clear phenotype, the probability of patients with RDs sharing casual mutations with the healthy population is low.

Figure 1. The pedigrees of the families with retinal dystrophies. Squares and circles indicate men and women, respectively. The dark symbols represent the affected members, and the slashed symbols denote that the subject is deceased. The patient above the arrow is the proband. WT or +: wild-type, M: mutation.

Figure 2. Bilateral fundus photography of the probands from the respective families with retinal dystrophies. Bilateral fundus photography shows attenuation of retinal arterioles, atrophy of the retinal pigment epithelium, and the waxy-pale disc of both eyes in all fundus photograph. Bone spicule-like pigmentation was found in the periphery retina of patient III:4 in Family-012, sheet pigmentation was found in the posterior pole retina of patient II:6 in Family-024, and macular coloboma was found in patient II:2 in Family-035.
Therefore, we compared these variants in the proband with that of dbSNP132, HapMap project, 1000 Genome Project, and the healthy Chinese adults database from BGI. After filtering against these databases, four variants that might be associated with RDs diseases were left: The first was a heterozygous nucleotide deletion variation c.942_944delGAA in IMPDH1, which was predicted to result in lysine deletion at position 314 (p.Lys314del p.K314del) in the proband of Family-012; the second was a homozygous single-nucleotide-polymorphic site c.1924T>A in ABCA4 leading to amino acid substitution of phenylalanine for isoleucine at position 642 (p.Phe642Ile p.F642I) in the proband of Family-024; the third and fourth were compound heterozygous mutations of c.272A>G and c.196C>T in NMNAT1 that caused glutamic acid substitution for glycine at position 91 (p.Glu91Gly p.E91G) and amino acid substitution of arginine for tryptophan at position 66 (p.Arg66Trp p.R66W) in the proband of Family-035. The mutations of ABCA4 c.1924T>A and NMNAT1 c.272A>G were novel and were first identified as associated with RDs, while IMPDH1 c.942_944delGAA and NMNAT1 c.196C>T have been previously reported to be pathogenic mutations that cause RP and LCA [5,8]. The Sanger sequencing results confirmed these variants (Figure 4), and they cosegregated with respective RDs phenotype in the families (Figure 1). The prediction analysis indicated that these variants were highly conserved across species (Figure 4). In addition, SIFT and PolyPhen2 were performed to predict the effect of the novel missense mutation: ABCA4 c.1924T>A was possibly damaging according to SIFT, and NMNAT1 c.272A>G was possibly damaging according to PolyPhen2 (Table 2).

DISCUSSION

We reported four mutations associated with different RDs in three Chinese families: IMPDH1 c.942_944delGAA with adRP, ABCA4 c.1924T>A with STGD, and NMNAT1 c.272A>G and c.196C>T with LCA. To the best of our knowledge, our study is the first to describe the mutations of ABCA4 c.1924T>A and NMNAT1 c.272A>G are associated with RDs. Target regions of known RD gene sequencing–based NGS was used to identify these variations, and Sanger sequencing of candidate mutations verified these mutations cosegregated with the disease phenotypes in the respective families, which were absent in the BGI normal controls database. Prediction analysis of evolutionary conservation and human nsSNPs functional effects suggested that this mutation might be highly deleterious. IMPDH1 encoding inosine monophosphate dehydrogenase 1 widely expressed rate-limiting enzyme of the de novo pathway of guanine nucleotide biosynthesis and was
Figure 4. Pathogenic mutations and orthologs. The arrows indicate the mutations in the respective pathogenic genes: heterozygous nucleotides deletion variation c.942_944delGAA in IMPDH1, homozygous single-nucleotide-polymorphic site c.1924T>A in ABCA4, and compound heterozygous single-nucleotide-polymorphic sites c.272A>G and c.196C>T in NMNAT1. The amino acids p.K314, p.F642, p.E91, and p.R66 located in IMPDH1 ABCA4 and NMNAT1 are marked with the column and are highly conserved among all species shown.
identified in 2002 as associated with RP by linkage mapping [9]. IMPDH1 might account for 5% to 10% of adRP cases among Americans and Europeans [10,11]. Patients with IMPDH1 mutations presented severe autosomal dominant degenerative retinopathy, while it was interesting that Impdh1(−/−) null mice displayed a slowly progressive form of retinal degeneration [11,12]. Therefore, IMPDH1 misfolding and aggregation, rather than reduced enzyme activity, were the likely cause of the severe phenotype in the human form. IMPDH1 c.942_944delGAA was first identified as an adRP mutation in a European family in 2013 [5]. All affected members in Family-012 carried this mutation and presented relatively earlier onset of symptoms and similar disease severity compared with American and European patients with IMPDH1 mutations. We analyzed the RP phenotype associated with IMPDH1 c.942_944delGAA and confirmed the pathogenicity of the mutation.

The ABCA4 gene produces a photoreceptor-specific ATP-binding cassette transporter involved in clearance from photoreceptor cells of the byproduct of the retinoid cycle of vision [13]. The spectrum of retinal dystrophies caused by mutations in the ABCA4 gene is broad, and includes severe RP, STGD, cone-rod dystrophy, age-related macular degeneration, and so on. Diverse phenotypes related to ABCA4 might perplex the clinical diagnosis sometimes. Fundus manifestations of patients in Family-024 seemed similar to RP, and all patients had complained of night blindness; however, atrophic-appearing RPE concentrating in the region around the macular and delayed and reduced ERG responses were in accordance with STGD Group 3, which had macular and generalized cone and rod system dysfunction [14,15]. Therefore, the diagnosis of Family-024 was corrected for STD, and ABCA4 c.1924T>A was identified as the causative mutation for this family.

LCA is the most severe human form of inherited photoreceptor-neuron degeneration resulting in congenital blindness, with an incidence of approximately 1 in 80,000 [16]. Seventeen genes have been implicated in the LCA. NMNAT1 was first mapped to 1p36 through linkage analysis in a large consanguineous Pakistani family with LCA in 2003; however, it was not accurately identified until 2012 [8]. NMNAT1 encodes the nuclear isoform of nicotinamide mononucleotide adenylyltransferase, a rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD+) biosynthesis [17,18]. It was suggested that NMNAT1 mutations could cause severe and rapid macular degeneration, in addition to the typical LCA phenotype of nystagmus, severe loss of vision, and abnormal ERGs. The phenotype associated with NMNAT1 mutations was classified in LCA9. Patients with LCA9 presented a peculiar, prominent retinal feature termed macular coloboma that consists of an atrophic lesion in the central retina with a pigmented border, and the remainder of the retina was abnormal as well, with pigmented changes, attenuated retinal blood vessels, and optic disc pallor [19]. The symptoms and fundus manifestations of the two affected individuals in Family-035 were exceedingly compatible with LCA9, including early onset of nystagmus, severe loss of vision, macular coloboma, and so on. The compound heterozygous variants in NMNAT1, c.196C>T and c.272A>G, cosegregated with the phenotypes of Family-035. NMNAT1 c.196C>T has been detected in a Pakistani LCA9 pedigree and was proved to decrease NMNAT1 enzyme activity [20]. NMNAT1 c.272A>G is a novel mutation, which was absent in previous reports of the pathogenic mutations spectrum causing LCA9.

Thus, in total, we have identified four mutants in three Chinese families associated with adRP, STGD, and LCA, including a deletion mutation encoding p.K314del in IMPDH1 and three missense mutations encoding p.F642I in ABCA4, p.E91G, and p.R66W in NMNAT1 with gene panel–based NGS. All mutations were validated with directed PCR and Sanger sequencing. Identification of these mutations reaffirms the clinical and genetic heterogeneity of RDs, and further expands the mutation spectrum of RDs.
APPENDIX 1. KNOWN CAUSATIVE GENES RESPONSIBLE FOR RDS.

To access the data, click or select the words “Appendix 1.”

ACKNOWLEDGMENTS

The authors are grateful to all family members for their participation in this study. This work was supported by the National Basic Research Program of China (No. 2013CB967002) and the National Natural Science Foundation of China (No. 81130017).

REFERENCES

1. Inglehearn CF. Molecular genetics of human retinal dystrophies. Eye (Lond) 1998; 12:Pt 3b571-9. [PMID: 9775219].
2. Liu T, Jin X, Zhang X, Yuan H, Cheng J, Lee J, Zhang B, Zhang M, Wu J, Wang L, Tian G, Wang W. A novel missense SNRNP200 mutation associated with autosomal dominant retinitis pigmentosa in a Chinese family. PLoS ONE 2012; 7:e45464. [PMID: 23029027].
3. Shanks ME, Downes SM, Copley RR, Lise S, Broxholme J, Hudspith KA, Kwasniewska A, Davies WI, Hankins MW, Packham ER, Clouston P, Seller A, Wilkie AO, Taylor JC, Ragoussis J, Nemeth AH. Next-generation sequencing (NGS) as a diagnostic tool for retinal degeneration reveals a much higher detection rate in early-onset disease. Eur J Hum Genet 2013; 21:274-80. [PMID: 22968130].
4. Fu Q, Wang F, Wang H, Xu F, Zaneveld JE, Ren H, Keser V, Lopez I, Tuan HF, Salvo JS, Wang X, Zhao L, Wang K, Li Y, Koenekoop RK, Chen R, Sui R. Next-generation sequencing-based molecular diagnosis of a Chinese patient cohort with autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 2013; 54:4158-66. [PMID: 23661369].
5. Glöckle N, Kohl S, Mohr J, Scheurenbrand T, Sprecher A, Weisschuah N, Anmerg M, Schubach M, Poloschek C, Zrenner E3, Biskup S, Berger W, Wissinger B, Neidhardt J. Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet 2014; 22:99-104. [PMID: 23591405].
6. Schorderet DF, Iouranova A, Favez T, Tiab L, Escher P. IROme: a new high-throughput molecular tool for the diagnosis of inherited retinal dystrophies. Biomed Res Int. 2013; 2013:198089.
7. Corton M, Nishiguchi KM, Avila-Fernandez A, Nikopoulos K, Rivero-Alvarez R, Tatu SD, Ayuso C, Rivolta C. Exome sequencing of index patients with retinal dystrophies as a tool for molecular diagnosis. PLoS ONE 2013; 8:e55574. [PMID: 23940504].
8. Koenekoop RK, Wang H, Majewski J, Wang X, Lopez I, Ren H, Chen Y, Li Y, Fishman GA, Genead M, Schwartzzentuber J, Solanki N, Traboulsi EI, Cheng J, Logan CV, McKibbin M, Hayward BE, Parry DA, Johnson CA, Nageeb M, Poulter JA, Mohamed MD, Jafri H, Rashid Y, Taylor GR, Keser V, Mardon G, Xu H, Inglehearn CF, Fu Q, Toomes C, Chen R. Mutations in NMMAT1 cause Leber congenital amaurosis and identify a new disease pathway for retinal degeneration. Nat Genet 2012; 44:1035-9. [PMID: 22842230].
9. Bowne SJ, Sullivan LS, Blanton SH, Cepko CL, Blackshaw S, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Daiger SP. Mutations in the inosine monophosphate dehydrogenase 1 gene (IMPDH1) cause the RP10 form of autosomal dominant retinitis pigmentosa. Hum Mol Genet 2002; 11:559-68. [PMID: 11875050].
10. Wada Y, Sandberg MA, McGee TL, Stillberger MA, Berson EL, Dryja TP. Screen of the IMPDH1 gene among patients with dominant retinitis pigmentosa and clinical features associated with the most common mutation, Asp226Asn. Invest Ophthalmol Vis Sci 2005; 46:1735-41. [PMID: 15851576].
11. Bowne SJ, Sullivan LS, Mortimer SE, Hedstrom L, Zhu J, Spellcy CJ, Gire AI, Hughbanks-Wheaton D, Birch DG, Lewis RA, Heckenlively JR, Daiger SP. Spectrum and frequency of mutations in IMPDH1 associated with autosomal dominant retinitis pigmentosa and leber congenital amaurosis. Invest Ophthalmol Vis Sci 2006; 47:34-42. [PMID: 16384941].
12. Aherne A, Kennan A, Kenna PF, McNally N, Lloyd DG, Alberts IL, Kiang AS, Humphries MM, Ayuso C, Engel PC, Gu JJ, Mitchell BS, Farrar GJ, Humphries P. On the molecular pathology of neurodegeneration in IMPDH1-based retinitis pigmentosa. Hum Mol Genet 2004; 13:641-50. [PMID: 14981049].
13. Cideciyan AV, Swider M, Aleman TS, Tsybovsky Y, Schwartz SB, Windsor EA, Roman AJ, Sumaroka A, Steinberg JD, Jacobson SG, Stone EM, Palczewski K. ABCA4 disease progression and a proposed strategy for gene therapy. Hum Mol Genet 2009; 18:931-41. [PMID: 19074458].
14. Testa F, Rossi S, Sodi A, Passerini I, Di Iorio V, Della Corte M, Banfi S, Surace EM, Menchini U, Auricchio A, Simonelli F. Correlation between photoreceptor layer integrity and visual function in patients with Stargardt disease: implications for gene therapy. Invest Ophthalmol Vis Sci 2012; 53:4409-15. [PMID: 22661472].
15. Fujinami K, Lois N, Davidson AE, Mackay DS, Hogg CR, Stone EM, Tsunoda K, Tsubota K, Bunce C, Robson AG, Moore AT, Webster AR, Holder GE, Michaelides M. A longitudinal study of stargardt disease: clinical and electrophysiologic assessment, progression, and genotype correlations. Am J Ophthalmol 2013; 155:1075-88. [PMID: 23499370].
16. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog Retin Eye Res 2008; 27:391-419. [PMID: 18632300].
17. Keen TJ, Mohamed MD, McKibbin M, Rashid Y, Jafri H, Maumenee IH, Inglehearn CF. Identification of a locus (LCA9) for Leber’s congenital amaurosis on chromosome lp36. Eur J Hum Genet 2003; 11:420-3. [PMID: 12734549].
18. Lau C, Niere M, Ziegler M. The NMN/NaMN adenylyltransferase (NMNAT) protein family. Front Biosci (Landmark Ed) 2009; 14:410-31. [PMID: 19273075].

19. Perrault I, Hanein S, Zanlonghi X, Serre V, Nicouleau M, Defoort-Delhemmes S, Delphin N, Fares-Taie L, Gerber S, Xerri O, Edelson C, Goldenberg A, Duncombe A, Le Meur G, Hamel C, Silva E, Nitschke P, Calvas P, Munnich A, Roche O, Dollfus H, Kaplan J, Rozet JM. Mutations in NMNAT1 cause Leber congenital amaurosis with early-onset severe macular and optic atrophy. Nat Genet 2012; 44:975-7. [PMID: 22842229].

20. Falk MJ, Zhang Q, Nakamaru-Ogiso E, Kannabiran C, Fonseca-Kelly Z, Chakarova C, Audo I, Mackay DS, Zeitz C, Borman AD, Staniszewska M, Shukla R, Palavalli L, Mohand-Said S, Waseem NH, Jalali S, Perin JC, Place E, Ostrovsky J, Xiao R, Bhattacharya SS, Consugar M, Webster AR, Sahel JA, Moore AT, Berson EL, Liu Q, Gai X, Pierce EA. NMNAT1 mutations cause Leber congenital amaurosis. Nat Genet 2012; 44:1040-5. [PMID: 22842227].