Whole Exome Analysis Identifies Frequent CNGA1 Mutations in Japanese Population with Autosomal Recessive Retinitis Pigmentosa

Satoshi Katagiri1,2, Masakazu Akahori1, Yuri Sergeev2, Kazutoshi Yoshitake4, Kazuho Ikeo4, Masaaki Furuno5, Takaaki Hayashi2, Mineo Kondo6, Shinji Ueno7, Kazushige Tsunoda8, Kei Shinoda9, Kazuki Kuniyoshi10, Yohinori Tsurusaki11, Naomichi Matsumoto11, Hiroshi Tsuneoka2, Takeshi Iwata1*

1 Division of Molecular and Cellular Biology, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan, 2 Department of Ophthalmology, The Jikei University School of Medicine, Tokyo, Japan, 3 National Eye Institute, National Institutes of Health, Bethesda, MD, United States of America, 4 Laboratory of DNA Data Analysis, National Institute of Genetics, Shizuoka, Japan, 5 RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Life Science Accelerator Technology Group, Transcriptome Technology Team, Yokohama, Japan, 6 Department of Ophthalmology, Mie University Graduate School of Medicine, Tsu, Japan, 7 Department of Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Japan, 8 Laboratory of Visual Physiology, National Institute of Sensory Organs, Tokyo, Japan, 9 Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan, 10 Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka, Japan, 11 Department of Human Genetics, Yokohama City University, Yokohama, Japan

Abstract

Objective: The purpose of this study was to investigate frequent disease-causing gene mutations in autosomal recessive retinitis pigmentosa (arRP) in the Japanese population.

Methods: In total, 99 Japanese patients with non-syndromic and unrelated arRP or sporadic RP (spRP) were recruited in this study and ophthalmic examinations were conducted for the diagnosis of RP. Among these patients, whole exome sequencing analysis of 30 RP patients and direct sequencing screening of all CNGA1 exons of the other 69 RP patients were performed.

Results: Whole exome sequencing of 30 arRP/spRP patients identified disease-causing gene mutations of CNGA1 (four patients), EYS (three patients) and SAG (one patient) in eight patients and potential disease-causing gene variants of USH2A (two patients), EYS (one patient), TULP1 (one patient) and C2orf71 (one patient) in five patients. Screening of an additional 69 arRP/spRP patients for the CNGA1 gene mutation revealed one patient with a homozygous mutation.

Conclusions: This is the first identification of CNGA1 mutations in arRP Japanese patients. The frequency of CNGA1 gene mutation was 5.1% (5/99 patients). CNGA1 mutations are one of the most frequent arRP-causing mutations in Japanese patients.

Citation: Katagiri S, Akahori M, Sergeev Y, Yoshitake K, Ikeo K, et al. (2014) Whole Exome Analysis Identifies Frequent CNGA1 Mutations in Japanese Population with Autosomal Recessive Retinitis Pigmentosa. PLoS ONE 9(9): e108721. doi:10.1371/journal.pone.0108721

Editor: Namik Kaya, King Faisal Specialist Hospital and Research center, Saudi Arabia

Received March 4, 2014; Accepted August 31, 2014; Published September 30, 2014

Copyright: © 2014 Katagiri et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All data are included within the manuscript and its Supporting Information files.

Funding: This study was supported by the grants to T.I. from the Ministry of Health, Labor, and Welfare of Japan (13803661); to M.A. and T.H. from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Scientific Research C, 25462744 and 25462738); to T.H. from the Vehicle Racing Commemorative Foundation; and to M.F. from the Research Grant for RIKEN Omics Science Center MEXT. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: iwatakeshi@kankakuki.go.jp

Introduction

Retinitis pigmentosa (RP; OMIM #268000) is a heterogeneous group of inherited disorders characterized by visual field loss, night blindness, abnormal color vision and fundus degeneration. The prevalence of RP is approximately 1 per 4,000 persons and more than 1 million individuals are affected worldwide [1]. The inheritance of RP shows various patterns including autosomal recessive (arRP), autosomal dominant, X-linked, sporadic (spRP), mitochondrial [2] and digenic [3] inheritance. Among the various patterns of RP inheritance, arRP is the most frequent inheritance pattern and accounts for approximately 50% to 60% of all RP patients [1]. To date, 42 arRP-causing genes and three loci have been reported in the Retinal Information Network (RetNet; https://sph.uth.edu/retnet/). Among these arRP-causing genes, mutations in Usher syndrome 2A (USH2A) are the most frequent and account for approximately 17% cases including cases with additional hearing loss [1]. In non-syndromic arRP, the most frequent arRP genes are eyes shut homolog (EYS), USH2A and ATP-binding cassette sub-family A member 4 (ABCA4), which
### Table 1. Autosomal recessive retinitis pigmentosa (arRP)-causing mutations and potential arRP-causing variants found by exome sequencing.

| Family ID | Gene Name | GenBank ID | Exon | Nucleotide Change | Amino Acid Change | State | Frequency* | SNP ID | Reference | Pathogenicity |
|-----------|-----------|------------|------|-------------------|-------------------|-------|------------|-------|-----------|---------------|
| RP#002    | CNGA1     | NM_000087  | 5    | c.191delG         | p.G64VfsX29       | Homo  | 2          |       | HGVB      | Disease-causing |
| RP#004    | EYS       | NM_001142800 | 33   | c.6714deiT        | p.R2238PhX16      | Hetero| 0          |       | Collin et al. 2008 | Disease-causing |
| EYS       | NM_001142800 | 35       | c.C7002A | p.C2334X          | Homo  | 2          |       | This study | Potential disease-causing |
| RP#014    | EYS       | NM_001142800 | 4    | c.A141T           | p.E47D            | Hetero| 0          |       | This study | |
| EYS       | NM_001142800 | 26       | c.4957dupA       | p.S1653fsX2       | Hetero| 2          |       | Iwanami et al. 2012 | |
| RP#016    | TULP1     | NM_003322  | 1    | c.G3A             | p.M1I             | Hetero| 0          |       | This study | Potential disease-causing |
| TULP1     | NM_003322  | 13       | c.C1246T         | p.R416C           | Hetero| 0          | rs200769197 | dbSNP | |
| RP#017    | EYS       | NM_001142800 | 26   | c.4957dupA       | p.S1653fsX2       | Hetero| 2          |       | This study | Disease-causing |
| RP#019    | CNGA1     | NM_000087  | 6    | c.265delC         | p.L89FfsX4        | Hetero| 2          |       | Chen et al. 2013 | Disease-causing |
| CNGA1     | NM_000087  | 11       | c.1429delG       | p.V477YfsX17      | Hetero| 0          |       | This study | |
| RP#021    | CNGA1     | NM_000087  | 5    | c.191delG         | p.G64VfsX29       | Homo  | 2          |       | HGVB      | |
| RP#026    | USH2A     | NM_206933  | 49   | c.C9676T          | p.R3226X          | Hetero| 0          |       | This study | Potential disease-causing |
| USH2A     | NM_206933  | 55       | c.T10859C        | p.I3620T          | Hetero| 0          |       | HGVB      | |
| RP#027    | EYS       | NM_001142800 | 26   | c.4957dupA       | p.S1653fsX2       | Homo  | 2          |       | Iwanami et al. 2012 | Disease-causing |
| SAG       | NM_000541  | 11       | c.926delA        | p.T309TfsX12      | Homo  | 6          |       | Fuchs et al. 1995 | Disease-causing |
| RP#028    | USH2A     | NM_206933  | 41   | c.T71880C         | p.I2627T          | Hetero| 0          |       | This study | Potential disease-causing |
| USH2A     | NM_206933  | 55       | c.C10931T        | p.T3644M          | Homo  | 1          | rs185823130 | dbSNP | |
| USH2A     | NM_206933  | 70       | c.T15178C        | p.S5060P          | Hetero| 0          |       | This study | |
| RP#029    | CNGA1     | NM_000087  | 6    | c.265delC         | p.L89FfsX4        | Homo  | 2          |       | Chen et al. 2013 | Disease-causing |
| RP#030    | C2orf71   | NM_001029883 | 1    | c.C85T           | p.R29W            | Hetero| 4          | rs201706430 | dbSNP | Potential disease-causing |
| C2orf71   | NM_001029883 | 2        | c.C3748C         | p.R1250C          | Homo  | 2          |       | This study | |

HGVB = Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.html); dbSNP = (http://www.ncbi.nlm.nih.gov/SNP/); Frequency* show the number of mutations or variants found in 1150 alleles of 575 controls.

doi:10.1371/journal.pone.0108721.t001
account for approximately 10%, 8% and 5% to 6% of cases, respectively [1,4–10].

Large scale screening of selective exons in 30 RP-causing genes was previously performed in 193 Japanese RP families [11]. Although it only targeted exons with known mutations, the study failed to identify high frequency RP genes [11]. Another study of the Japanese RP population focused on the RP genes RHO [12–14] and EYS [15,16], and found that EYS was a frequent arRP gene with a prevalence rate of 9% to 16% [15,16]. However, almost all reported EYS gene mutations in these studies have not been reported in Western populations suggesting that Japanese individuals have a different genetic background [15,16]. These results suggest that the genetic background of RP in the Japanese population is different from that in the Western population.

The recent technological development of exon capture with 99% coverage of all exons and its combination with next generation sequencing enables effective genetic studies for hereditary diseases [17–20] and the investigation of novel mutations in multiple candidate genes [21].

The purpose of this study was to find frequent arRP genes in the Japanese population. In this study, we performed whole exome analysis of 30 Japanese arRP/spRP patients with confirmation in an additional 69 arRP/spRP patients. We found frequent arRP-causing mutations in the cyclic nucleotide gated channel alpha 1 (CNGA1) gene.

Materials and Methods

Informed consent

The protocol of this study was approved by the Institutional Review Board at the six participating institutions (National Hospital Organization Tokyo Medical Center, Jikei University School of Medicine, Mie University School of Medicine, Nagoya University Graduate School of Medicine, Teikyo University

Figure 1. Pedigrees identified with arRP-causing mutations or potential arRP-causing variants. The solid squares (male) and circles (female) represent affected patients. The proband of each family is indicated by a black arrow. Unaffected family members are represented by white icons. The slash symbol indicates deceased individuals. The doubled line indicates consanguineous marriage. The generation number is shown on the left.

doi:10.1371 journal.pone.0108721.g001
School of Medicine and Kinki University Faculty of Medicine). The protocol adhered to the tenets of the Declaration of Helsinki, and signed informed consent was obtained from all participants.

Clinical studies
In total, 99 unrelated arRP/spRP patients with no apparent syndrome were recruited from the National Hospital Organization Tokyo Medical Center, Jikei University School of Medicine, Mie University School of Medicine, Nagoya University Graduate School of Medicine, Teikyo University School of Medicine and Kinki University Faculty of Medicine. The patient history was taken and ophthalmic examinations were performed. Clinical diagnosis and evaluation for RP were based on the decimal best-corrected visual acuity (BCVA), slit-lamp examination, fundus examination, visual fields determined using kinetic perimetry (Goldmann perimeter [GP]; Haag Streit, Bern, Switzerland) and electroretinography (ERG) findings. Characteristic findings for diagnosis of RP include progressive visual field loss from peripheral, night blindness, abnormal color vision, fundus degeneration represented by bone spicule pigmentations and attenuation of retinal vessels, and the more or equally decreased rod responses compared with cone responses of ERG [1,22].

DNA preparation and exome sequencing analysis
We obtained venous blood samples from all participants and genomic DNA was extracted. Whole exome sequencing was performed for 30 arRP/spRP patients using a method previously described [23]. Briefly, construction of paired-end sequence libraries and exome capture were performed by using the Agilent Bravo automated liquid-handling platform with SureSelect XT Human All Exon kit V4 + UTRs kit (Agilent Technologies, Santa Clara, CA). Enriched libraries were sequenced by using an Illumina HiSeq2000 sequencer. Reads were mapped to the reference human genome (1000 genomes phase 2 reference, hs37d5) with Burrows–Wheeler Aligner software version 0.6.2 [24]. Duplicated reads were then removed by Picard Mark Duplicates module version 1.62, and mapped reads around insertion/deletion polymorphisms were realigned by using the Genome Analysis Toolkit (GATK) version 2.1–13 [25]. Base-quality scores were recalibrated by using GATK. To extract potentially RP-causing variants, we focused only on variants that could change the amino acid sequence, such as non-synonymous variants, splice acceptor and donor site variants, and insertion/deletion polymorphisms. The identified variants were filtered by a frequency of less than 1% in the 1000 Genomes project (http://www.1000genomes.org) and the Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.html). The remained variants were further screened within 212 genes registered as retinal disease-causing genes in the RetNet database updated on March 10, 2014. All remained variants of 30 arRP/spRP patients were summarized in Table S1 in File S1.

Selection of disease-causing mutations was restricted to three genetic criteria: first, homozygosity or compound heterozygosity of known arRP-causing mutations; second, compound heterozygosity of known arRP-causing mutations; and third, homozygosity of known arRP-causing mutations.
Table 2. Identification of patients with CNGA1 sequence mutations and variants in this study.

| Family ID | Exon | Nucleotide Change | Amino Acid Change | Pathogenicity | Polyphen-2 (score) | SIFT (score) | SNP ID | Reference | State Frequency | PolyPhen-2 (score) | SIFT (score) | SNP ID | Reference | Pathogenicity |
|-----------|------|-------------------|-------------------|--------------|-------------------|--------------|---------|-----------|----------------|-------------------|--------------|---------|-----------|--------------|
| RP#002    | 5    | c.191delG         | p.G64Vfs29X       | Homo         | 2                 | HGVB         | Disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#019    | 6    | c.265delC         | p.L89FfsX4        | Hetero       | 2                 | Chen et al. 2013 | Disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#021    | 6    | c.1429delG        | p.V477YfsX17      | Hetero       | 0                 | This study   | Disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#029    | 5    | c.191delG         | p.G64Vfs29X       | Homo         | 2                 | HGVB         | Disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#040    | 11   | c.G1271A          | p.R424Q           | Hetero       | 7                 | Benign (0.266) | Not disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#063    | 11   | c.G2042C          | p.G681A           | Homo         | 1                 | Benign (0.001) | Not disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |
| RP#094    | 6    | c.G1060A          | p.R354C           | Hetero       | 5                 | Benign (0.101) | Not disease-causing |
|           |      |                   |                   |              |                   |              |         |           |                |                   |              |         |           |              |

Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/); SIFT (http://sift.jcvi.org); HGVB = Human Genetic Variation Browser (http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.html); Frequency* show the number of mutations or variants found in 1150 alleles of 575 controls.

doi:10.1371/journal.pone.0108721.t002

Direct sequencing of the CNGA1 gene

The CNGA1 mutations identified by whole exome sequencing were further confirmed by direct sequencing. An additional 69 arRP/spRP patients were analyzed by direct sequencing for all coding exons (4 to 11) of CNGA1. The targeted exons (4 to 11) of the CNGA1 gene were amplified by PCR using the primer pairs given in Table S2 in File S1. The PCR products were purified using Agencourt APMure XP (Beckman Coulter, Brea, CA) and used as a template for sequencing. Both DNA strands were sequenced by an automated sequencer (3730xl DNA Analyzer; Life Technologies Corporation, Carlsbad, CA) using the BigDye Terminator kit V3.1 (Life Technologies Corporation).

Assessment of found mutations or variants in this study

Novel mutations and variants were defined as those not present in the literature, dbsNP database (http://www.ncbi.nlm.nih.gov/SNP/), Human Genetic Variation Browser, 1000 Genome project database or the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk). In addition, the frequency of identified mutations or variants in this study was investigated using in-house exome sequencing data from 575 unaffected Japanese controls at Yokohama City University. Segregation was confirmed for both the arRP-causing mutations and potential arRP-causing variants by direct sequencing when parent samples were available.

Results

Whole exome sequencing analysis and identification of frequent arRP gene mutations

To identify frequent arRP-causing genes, we performed whole exome sequencing in non-syndromic 30 arRP/spRP patients. We focused on 212 retinal disease-causing genes registered in RetNet database updated on March 10, 2014. The average of mean depth for all 30 samples reached 71.11±7.68-fold and the average of coverage at 4- and 12-fold for all 30 samples reached 98.1% and 92.5% respectively. The analysis of arRP-causing mutations and potential arRP-causing variants was conducted according to the criteria described in Materials and Methods. Segregation of identified arRP-causing mutations and potential arRP-causing variants were conducted in five families: RP#002, RP#004, RP#011, RP#016 and RP#019. Although the results of segregation in RP#002, RP#004, RP#016 and RP#019 matched the inheritance pattern, two USH2A variants in RP#011 (Table S1 in File S1) did not match the inheritance pattern because the father of RP#011 carried two identical USH2A variants. Therefore, we concluded that the two USH2A variants in RP#011 were not arRP-causing. Consequently, the eXome analysis identified eight arRP-causing mutations including three novel mutations and five known mutations in eight arRP/spRP patients [6,16,26,27] and identified potential arRP-causing mutations of known and predicted arRP-causing mutations; and third, homozygosity or compound heterozygosity of predicted arRP-causing mutations. Mutations were defined as disease causing only if these criteria were fulfilled. Mutations causing exon truncation through frameshift, splicing and termination were considered to be more severe than missense mutations with unknown pathogenic relevance. In addition, to investigate the potential disease-causing variants, we added three genetic criteria: first, compound heterozygosity of known arRP-causing mutation and missense potential arRP-causing variant; second, compound heterozygosity of predicted arRP-causing mutation and potential arRP-causing variant; and third, homozygosity or compound heterozygosity of potential arRP-causing variants.

Frequent CNGA1 Mutations in Japanese RP
variants including six novel variants and five known variants in five arRP/spRP patients (Table 1). The arRP-causing mutations were found in CNGA1 (four patients), EYS (three patients) and S-antigen retina and pineal gland (SAG) (one patient). Potential arRP-causing variants were found in USH2A (two patients), EYS (one patient), tubby like protein 1 (TULP1) (one patient) and chromosome 2 open reading frame 71 (C2orf71) (one patient).

Figure 3. Fundus photographs of the patients with heterozygous or homozygous CNGA1 mutations. Funduscopy indicates retinal degeneration with pigmentation and attenuation of retinal vessels in all patients. Macular edema does not existed in any patient, although retinal degeneration in the macular region is observed in RP#002, RP#021 and RP#094 (A, C and E).

doi:10.1371/journal.pone.0108721.g003
In particular, pedigree RP#002 with a homozygous c.191delG (p.G64VfsX29) mutation, RP#019 with compound heterozygous c.265delC (p.L89FfsX14) and c.1429delG (p.V477YfsX17) mutations, RP#021 with a homozygous c.191delG mutation and RP#029 with a homozygous c.265delC mutation were identified. Further direct sequencing confirmed that the parents in pedigree RP#019 had c.265delC or c.1429delG respectively. The CNGA1 sequence was compared with the NCBI reference sequence for the CNGA1 transcript (GenBank ID: NM_000087.3).

Screening of all CNGA1 exons in 69 additional arRP/spRP Japanese patients

Direct sequencing of the coding region of the CNGA1 gene in 69 arRP/spRP patients identified homozygous c.265delC mutation in pedigree RP#094 and three heterozygous variants c.G860A (p.R287K), c.G1271A (p.R424Q) and c.G2042C (p.G681A) in pedigrees RP#040, RP#063 and RP#087 respectively. All pedigrees identified to have arRP-causing mutations or potential arRP-causing variants are shown in Figure 1.

Identified CNGA1 mutations and variants

Among the three arRP-causing mutations and three variants found in this study, two (c.265delC and c.G1271A) were previously reported as arRP-causing or potential arRP-causing [11,26] and four were not reported as arRP-causing or potential arRP-causing (c.191delG, c.265delC, c.G860A and c.G2042C). The polyphen-2 program predicted that all three missense variants in p.R287K (c.G860A), p.R424Q (c.G1271A) and p.G681A (c.G2042C) were benign. In contrast, the SIFT program predicted that p.R424Q (c.G1271A) potentially could cause severe damage to the protein, whereas p.G681A (c.G2042C) and p.R287K (c.G860A) potentially could cause mild damage. All identified mutations and variants in CNGA1 gene are summarized in Table 2 and sequence data are given in Figure 2.

Haplotype analysis

The haplotypes of CNGA1 and the surrounding sequences were determined for four arRP patients, RP#002, RP#019, RP#021 and RP#029. Single-nucleotide polymorphisms (SNPs) with a frequency higher than 5% (1000 Genomes project database) were determined within 1 kb upstream and downstream of CNGA1 (chromosome 4, positions 47,937,994–48,014,961) as shown in Table S3 in File S1. The haplotype analysis determined an identical haplotype for four alleles in patients RP#002 and RP#021 suggesting a common ancestor for the c.191delG mutation. Moreover, identical haplotypes for one allele in patient RP#019 and for both alleles in patient RP#029 were detected suggesting a common ancestor for the c.265delC mutation.

Clinical features of CNGA1 mutations

To characterize the clinical features of patients with CNGA1 mutations, we additionally investigated the clinical data of five patients with compound heterozygous or homozygous CNGA1 mutations (Table 3). All five patients reported that they noticed night blindness from childhood. Funduscopy showed retinal degeneration with pigmentation and attenuation of retinal vessels in all patients (Fig. 3). Macular edema was not observed in any patients, although retinal degeneration in macular regions was detected in RP#002, RP#021 and RP#094 (Fig. 3A, 3C and 3E). The BCVA of RP#019 and RP#029 remained at 1.0, whereas that of RP#002, RP#021 and RP#094 was reduced. ERG showed no recordable pattern in four patients and could not

Table 3. Ophthalmic findings in five patients with retinitis pigmentosa with compound heterozygous or homozygous CNGA1 mutations.

| Patient | Diagnosed Age | Examined Age | Sex | Onset of night blindness | BCVA | ERG | Visual field |
|---------|---------------|--------------|-----|--------------------------|------|-----|-------------|
|         | Right | Left |         |       |     |     |             |
| RP#002  | 42,31 | M | Childhood | 0.5  | 0.7 | Non-recordable | Severely constricted |
| RP#019  | 26,35 | F | Childhood | 1.0  | 1.0 | Non-recordable | Ring scotoma |
| RP#021  | 60,65 | M | Childhood | 0.2  | 0.1 | Non-recordable | Severely constricted |
| RP#029  | 15,51 | F | Childhood | 1.0  | 1.0 | Non-recordable | Severely constricted |
| RP#094  | 16,46 | M | Childhood | 0.4  | 0.3 | Non-recordable | Severely constricted |

BCVA = decimal corrective visual acuity; ERG = electroretinography; M = male; F = female.

Frequent CNGA1 Mutations in Japanese RP
be conducted in one patient. The GP of RP#022 showed ring scotoma with a preserved peripheral visual field, whereas that of another four patients was severely constricted.

Discussion

Mutations in the CNGA1 gene were identified for the first time in a Japanese population with a high frequency of 5.1% for homozygous or compound heterozygous mutations. The exome analysis of arRP/spRP patients revealed that 43.3% carried arRP-causing mutations or potential arRP-causing variants in CNGA1 (13.3%), EYS (13.3%), USH2A (6.7%), C2orf71 (3.3%), SAG (3.3%) and TULP1 (3.3%). Although the prevalence of other five gene mutations was consistent with that in previous studies [1,4–8,28–30], the prevalence of CNGA1 was clearly higher than in population of European descent [31,32]. We screened for mutation in all the coding exons of CNGA1 in an additional 69 arRP/spRP Japanese patients to further investigate the prevalence of CNGA1 mutations in the Japanese population. We identified an arRP-causing homozygous CNGA1 mutation in one patient. Consequently, three CNGA1 frameshift mutations (c.191delG, c.265delC and c.1429delG) were identified as arRP-causing mutations in five patients (Table 2).

Rod cyclic nucleotide-gated ion channels contain CNGA1 and CNGB1 protein at a ratio of 3 CNGA1:1 CNGB1 [33]. Each molecule of CNGA1 protein has at least three functional domains as described in the UniProtKB, Cross-References, ProteinModelPortal; these domains function as a cation-transporter domain (residues 202–396, the Pfam ion_trans_motif, http://pfam.sanger.ac.uk), cGMP-binding domain (residues 404–596, SWISSMODEL structure based on the PDB file: 4hbn_A, http://www.rcsb.org/pdb/home/home.do) and carboxy-terminal leucine zipper (CLZ) domain (residues 623–690, experimental structure based on the PDB file: 3wdf). The p.G64VfsX29 (c.191delG) and p.L99fsX4 (c.263delC) protein had no transmembrane lesions, and most of the protein structure including all three functional domains was abolished. In contrast, the p.V477YfsX17 (c.1429delG) mutant protein had the correct structure up to 5th transmembrane domain helix, but lacked the 6th transmembrane domain helix, the cGMP-binding site, and the coiled-coil CLZ domain. The cGMP-binding site is important for the function of CNGA1 as a cation channel. Loss of the cGMP-binding site is likely to influence the final stage of the photo transduction pathway [31]. In addition, the absence of the coiled-coil CLZ domain completely disrupts the 3:1 stoichiometry in CNG channels [33]. Although the p.V477YfsX17 (c.1429delG) mutant may retain part of its structure, the protein function is predicted to be completely lost.

We additionally identified the heterozygous CNGA1 missense variants c.G860A (p.R287K), c.G1271A (p.R424Q) and c.G2042C (p.G681A) (Table 2). Heterozygous c.G1271A variant has been previously reported [11]. Based on the mild score given by the polyphen-2 program and the severe score given by the SIFT program, we also predicted that this variant is potentially disease causing. In contrast, the two novel missense variants c.G860A and c.G2042C were predicted to cause mild damage by both the polyphen-2 and SIFT programs suggesting that it is non-pathogenic. Overall, all three missense CNGA1 variants (c.G860A, c.G1271A and c.G2042C) were found in only one allele of CNGA1. We conclude that these three CNGA1 variants were not disease causing in nature, at least from the phenotypic observation.

The clinical course of the five patients with compound heterozygous or homozygous CNGA1 mutations included night blindness from childhood, visual field loss in middle age, non-recordable ERG and characteristic retinal degeneration pattern of RP, which were consistent with previously reported phenotypes of CNGA1 mutations [32,34]. Retinal degeneration in the macular region and severely decreased BCVA occurred in 3/5 patients suggesting that the advanced stage of CNGA1 mutations included degeneration of the entire retina with both rod and cone photoreceptors. Although the genotype-phenotype correlation for CNGA1 mutations was not clear in this study, all five patients with CNGA1 mutations showed typical phenotypes of RP.

Previous reports have shown a strong association of CNGA1 with arRP [11,26,31,32,34,35]. Dryja et al. estimated the prevalence of CNGA1 mutations in arRP patients to be between 1.7 and 2.3% (3 or 4 of 173 patients) [31]. The prevalence of CNGA1 mutations in a Spanish arRP population was 2.1% (1 of 46 patients) [32], whereas that in a Chinese population with hereditary retinal dystrophy was 4.0% (1 of 25 patients) [26]. The average prevalence of CNGA1 mutations in arRP/spRP patients was 7.6% (1 of 13 patients) [26]. These findings suggest that the prevalence of CNGA1 mutations is higher in Asian population than in populations of European descent. The prevalence of CNGA1 mutations in Chinese populations requires further study because only one Chinese patient has been reported to have a homozygous mutation in this gene [26]. Jin et al. investigated CNGA1 exons 6, 8 and partial 11 in 193 Japanese RP families and found a single heterozygous CNGA1 variant (c.1271G>A) [11]. In our study, all coding exons of CNGA1 were screened and the estimate prevalence of CNGA1 mutations reached at 5.1% (5 of 99 patients) including four homozygous and one compound heterozygous patients. Our findings suggest that the prevalence of CNGA1 mutations is higher in Asian populations than in European populations. Moreover, c.191delG mutation has only been reported in Human Genetic Variation Browser (the database of genetic variations in Japanese population, http://www.genome.med.kyoto-u.ac.jp/SnpDB/), c.265delC mutation only reported in Chinese population [26] and c.1429delG mutation identified as novel. The CNGA1 mutations found in this study only overlapped with mutations identified in studies of Asian individuals indicating that the founder is specific to Asian populations. Lastly, the haplotypes for the CNGA1 mutations found in this study were individually unique (Table S3 in File S1). Further investigation of haplotypes is required to clarify the origin of these CNGA1 mutations.

Supporting Information

File S1 Supporting Tables. Table S1, All rare variants of 30 arRP/spRP patients of this study, focusing on 212 retinal disease-causing genes registered in the Retinal Information Network (https://sph.uth.edu/retnet/). Table S2, CNGA1 primers and PCR conditions. Table S3, Haplotype analysis of four retinitis pigmentosa patients with CNGA1 mutations. (DOC)

Acknowledgments

We thank the patients and their families for participation in this study. The authors wish to acknowledge RIKEN GeNAS for the sequencing of the exome-enriched libraries using the Illumina HiSeq2000.

Author Contributions

Conceived and designed the experiments: TI SK. Performed the experiments: SK MA TI. Analyzed the data: SK MA TI. Contributed reagents/materials/analysis tools: HT. Wrote the paper: SK TI.
References

1. Hartong DT, Berson EL, Dryja TP (2000) Retinitis pigmentosa. Lancet 368: 1795–1809.
2. Mankovich FC, Millington-Ward S, Keenan A, Kiang AS, Humphries M, et al. (1999) Retinitis pigmentosa and progressive sensorineural hearing loss caused by a C12258A mutation in the mitochondrial MTTS2 gene. Am J Hum Genet 66: 971–985.
3. Kajiwara K, Berson EL, Dryja TP (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264: 1604–1608.
4. Rivolta C, Swelko EA, Berson EL, Dryja TP (2000) Missense mutation in the USH2A gene: association with recessive retinitis pigmentosa without hearing loss. Am J Hum Genet 66: 1975–1978.
5. Abd El-Aziz MM, Barragan I, O’Driscoll CA, Goodstadt L, Prigmore E, et al. (2010) Identification of novel mutations in the ortholog of Drosophila eyes shut/spacemaker that is mutated in patients with retinitis pigmentosa. Am J Hum Genet 83: 594–603.
6. Jin ZB, Mandai M, Yokota T, Higuchi K, Ohmori K, et al. (2008) Identification of a novel mutation in the rhodopsin gene (C2ORF71) in patients with recessive retinitis pigmentos. Jpn J Hum Genet 40: 271–277.
7. Fuchs S, Nakazawa M, Wada Y, Tamai M, Oguchi Y, et al. (1995) A homozygous l-base pair deletion in the arrestin gene is a frequent cause of autosomal recessive retinitis pigmentosa in the Israeli population. Invest Ophthalmol Vis Sci 36: 4387–4394.
8. Aluado I, Sahel JA, Mohand-Said S, Lancelot ME, Antoñ 2007) 4266–4272.
9. Katagiri S, Yoshitake K, Akahori M, Hayashi T, Furuno M, et al. (2013) Whole-exome sequencing identifies a novel ALMS1 mutation (p.Q2051X) in two Japanese brothers with Alström syndrome. Molecular Vision 19: 2393–2406.
10. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.
11. Abd El-Aziz MM, O’Driscoll CA, Kaye RS, Barragan I, El-Ashry MF, et al. (2010) Identification of novel mutations in the ortholog of Drosophila eyes shut gene (EYS) causing autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 51: 4266–4272.
12. Aluado I, Sahel JA, Mohand-Said S, Lancelot ME, Antoñ 2007) 4266–4272.
13. Fujiki K, Hotta Y, Hayakawa M, Shiono T, Shiono T, et al. (1995) Missense mutation of rhodopsin gene found in Japanese families with autosomal dominant retinitis pigmentosa (ADRP). Jpn J Hum Genet 37: 125–132.
14. Fujiki K, Hotta Y, Murakami A, Yoshii M, Hayakawa M, et al. (1995) Missense mutation of rhodopsin gene is a frequent cause of autosomal recessive retinitis pigmentosa. Jpn J Hum Genet 37: 125–132.
15. Saga M, Mashima Y, Ako K, Uchida K, Uchida K, et al. (1994) Autosomal dominant retinitis pigmentos. A mutation in codon 101 (Glu→Lys) of the rhodopsin gene in a Japanese family. Ophthalmic Genet 13: 61–67.
16. Fujiki K, Hotta Y, Hayakawa M, Shiono T, Shimizu K, et al. (1992) Point mutations of rhodopsin gene found in Japanese families with autosomal dominant retinitis pigmentos. Jpn J Hum Genet 37: 125–132.
17. Shunktarenko Y, Ilyina T, Ilyina T, et al. (2000) Candidate genes are possible major causes of autosomal recessive retinitis pigmentos. Jpn J Hum Genet 83: 594–603.