Screening for SLC7A14 gene mutations in patients with autosomal recessive or sporadic retinitis pigmentosa

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ABSTRACT

Purpose: In this study, we aimed to detect mutations in the SLC7A14 cationic transporter gene, which has recently been reported as a causative gene for retinitis pigmentosa (RP), in Japanese patients with autosomal recessive (AR) or sporadic RP. Materials and Methods: We included 146 unrelated Japanese patients with AR or sporadic RP who lacked mutations in genes known to be associated with RP despite next-generation sequencing-based screening. We sequenced the seven SLC7A14 coding exons along with their flanking intronic DNA using the Sanger method. The detected polymorphisms were assessed for their pathogenicity with in silico prediction tools. For those who had heterozygous, nonsynonymous variants, we performed multiplex ligation-dependent probe amplification (MLPA) to search for additional deletion/duplication. Results: We detected four distinct SLC7A14 polymorphisms excluding synonymous polymorphisms. Two of these polymorphisms were assessed as detrimental by in silico prediction tools. However, all of the mutations were heterozygous. Neither homozygous polymorphisms nor compound heterozygous polymorphisms, which are considered detrimental variants, were detected. Neither deletion nor duplication was found with MLPA in patients with heterozygous variants. Conclusions: The four SLC7A14 mutations detected herein were unlikely to be pathogenic in this Japanese cohort. The frequency and pathogenicity of SLC7A14 mutations may vary depending on ethnicity, and these mutations may be rare in Japanese patients.

Introduction

Retinitis pigmentosa (RP) is a heterogeneous group of hereditary diseases that affect photoreceptors and induce night blindness and visual field defects. Inheritance traits of RP can be autosomal dominant, autosomal recessive (AR), or X-linked. Almost 3000 mutations occurring in more than 56 disease-causing genes are known to be associated with non-syndromic RP. Despite the identification of many genes, causative mutations still cannot be found in 50–60% of patients, even after screening of all known genes. Thus, much more work is required to obtain a complete genetic catalog of this disease.

In 2014, Jin and colleagues reported that a homozygous missense mutation in the cationic amino acid transporter gene, SLC7A14, resulted in AR RP in a Chinese individual. They performed screening on an additional 248 Chinese patients with AR RP and found an additional four missense mutations in 2% of patients with AR RP. The SLC7A14 gene is located on chromosome 3 and contains seven exons with a total length of open reading frame 2316 bp. While the specific function of SLC7A14 is not known, this protein is believed to either mediate the transport of cationic amino acids across the lysosomal membrane or act as a serine/threonine protein phosphatase-1 protein inhibitor. Jin and colleagues also demonstrated that knockdown of the gene leads to phenotypic changes in zebrafish and mice.

In this study, we investigated whether pathogenic SLC7A14 mutations could be detected in Japanese patients with AR RP.

Materials and methods

All procedures used in this study adhered to the tenets of the Declaration of Helsinki. The institutional review boards and the ethics committees of each institution involved approved the protocols of this study. All patients and their relatives were fully informed of the purpose and procedures of this study, and written consent was obtained from each participant.

Patients with non-syndromic RP in whom causative mutations were not found in previous screening with targeted-exome resequencing were included in this study. In brief, we screened exon/intron boundaries of all 193 known genes involved in inherited eye diseases using the Illumina HiSeq 2500 system on a custom capture panel. The diagnosis of RP was made based on the presence of night blindness, characteristic fundus appearance, and reduced or extinguished
electroretinogram response. As to the inheritance trait, probands who had family history of RP in more than two generations were considered as AD, who had either multiple affected siblings with unaffected parents or evidence of consanguinity were considered as AR, who had multiple affected families more than two generations but skipping female were considered as X-linked. After the data processing, we identified causative genes in 115 patients out of 317 screened RP patients. One patient with sporadic inheritance trait was in silico SLC7A14 prediction. In the remaining 201 patients, 50 had AD, 36 had AR, 5 had x-linked, and 110 had sporadic inheritance traits. Among them, those with AR or sporadic inheritance trait (n = 146) were subjected to this additional screening.

Genomic DNA was collected from blood samples using a DNA extraction kit (Qiagen QIAamp Blood Kit; Qiagen, Valencia, CA, USA). Polymerase chain reaction (PCR) was performed using 100 ng of genomic DNA. All seven exons of SLC7A14 (NM_020949) were amplified using a Gene Amp 9600 PCR system (Applied Biosystems, Foster City, CA, USA). Primers were designed according to a previous study.3 The primers were designed such that they contained 20 nucleotides and were located in an intronic position next to an exon. Each PCR mixture contained 20 ng of genomic DNA, 10 µL of 2 × GC1 buffer (TaKaRa, Japan), 0.2 mM of each dNTP, 1 µM of each primer, and 0.5 units of Taq polymerase in a total volume of 20 µL. Denaturation was performed at 94°C for 15 s. The annealing step of the amplification cycle was performed at 58°C for 15 s, and polymerization was performed at 72°C for 90 s.

PCR products were used as templates in direct sequencing reactions using fluorescent dideoxynucleotides (BigDye Terminator Ready Reaction Mix; Applied Biosystems) on an ABI 3730xl automated sequencer (Applied Biosystems). The sense direction primer was used to sequence exons 1–7, and the antisense primer was used to sequence exons 6 and 7. All sequence chromatograms were visually confirmed and in cases where the signal to noise ratio was deemed too low, the sequencing reaction was repeated.

The frequencies of variants were analyzed by the Human Genetic Variation Database (HGVB), a database constructed at Kyoto University, where allele frequencies have been analyzed in 3248 healthy Japanese individuals (accessed November 21, 2014 from http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html).8 Two additional allele frequency databases were also used: Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (accessed November 21, 2014 from http://evs.gs.washington.edu/EVS/) and dbSNP (accessed November 21, 2014 from http://www.ncbi.nlm.nih.gov/SNP/index.html). These databases contained allele frequency data from healthy patients of various races.

We also employed in silico prediction tools (SIFT, Polyphen2, and Provean programs from dbNSFP9) to evaluate the effects of the variants.

For those who had one possible pathogenic variant, we performed multiplex ligation-dependent probe amplification (MLPA) to search for additional deletion/duplication. The analysis was performed according to the manufacturer's instruction (MRC-Holland, Amsterdam, the Netherlands). In brief, 100 ng of genomic DNA were analyzed with seven pairs of custom-designed probes (Supplemental Table 1–available online) using MLPA EK1-FAM P200 custom kit. GeneAmp PCR System 9700 (Thermo Fisher Scientific, Waltham, MA) were used for reaction, and ABI 3730 GeneScan (Thermo Fisher) with ROX 500 (Thermo Fisher) were a set of peak detection. The zygosity was checked with the patterns of peaks.

### Results

We detected four distinct SLC7A14 polymorphisms in 146 patients in addition to 13 synonymous polymorphisms. The polymorphisms found in the study are shown in Tables 1 and 2. None of the polymorphisms were located in splice site, nor caused stop gain or frameshift. Some of the variants were assessed as detrimental with in silico prediction tools; however, all variants were heterozygous. Neither homozygous variants nor compound heterozygous variants were detected. While we detected two previously reported

| Patient ID | Age (years) | Onset stage (years old) | Sex | BCVA (OD/OS) | SLC7A14 mutation Exon | rs number | In-silico prediction | Allele frequency in normal database |
|------------|-------------|-------------------------|-----|--------------|------------------------|-----------|----------------------|-------------------------------------|
| K6461      | 47          | 37                      | F   | 1.5/1.5      | c.725C>T              | rs756436577 | Benign               | HGVD 0.2 ESP6500 0.007 dbSNP 0.28 |
| K6091      | 20          | Early childhood         | M   | 0.2/0.4      | c.988G>A              | rs2276717 | Probably damaging    | HGVD 1.4 ESP6500 0.007 dbSNP 0.007 |
| K6147      | 44          | Early childhood         | F   | 1.2/1.2      | c.330R                | rs13910464 | Deleterious          | HGVD 1.1 ESP6500 0.28 dbSNP 0.28  |
| K6259      | 47          | Early childhood         | M   | 0.1/0.1      | c.2083C>T             | rs18101174 | Neutral              | HGVD 0.2 ESP6500 0.007 dbSNP 0.04  |
| K6350      | 65          | Early childhood         | F   | 0.03/0.03    |                        |           |                      | HGVD 0.2 ESP6500 0.007 dbSNP 0.04  |
| K6206      | 76          | Early childhood         | M   | 0.8/0.6      | c.1391G>T             | rs79668755 |                        | HGVD 1.1 ESP6500 0.28 dbSNP 0.28  |
| K6394      | 71          | Early childhood         | M   | 0.4/0.4      | c.330R                | rs18101174 |                       | HGVD 0.2 ESP6500 0.007 dbSNP 0.04  |

BCVA: best corrected visual acuity; NA: not available.
mutations (c.988G>A and c.1391G>T) in six individuals, these mutations were also heterozygous. We also performed MLPA for those with heterozygous variants but did not find deletion/duplication. In addition, all variants were not infrequent in healthy Japanese controls; the allele frequency was 0.2–1.4% in the HGVD database.

Discussion

Causative genes for AR RP are diverse, and most genes account for only a small proportion of patients, with the exception of a few genes, such as USH2A and EYS in a specific ethnicity. Thus, if mutations in SLC7A14 account for 2% of individuals with AR RP in various ethnicities, there would be important implications for management of the disease.

Based on the frequency of SLC7A14 mutations in Chinese patients with AR RP (2%), we estimated that we can find 2.9 patients (2% of 146 patients) with such mutations in our cohort. However, in the present study, we did not find any patients with SLC7A14-associated AR RP, despite screening 146 patients without causative mutations in known genes. There are several potential explanations for this result. First, the sample size may have been too small. It is possible that the population coincidentally lacked a patient with the gene mutation since the probability was 0.98. Even if we considered the original population, which was screened with targeted-exome resequencing (243 patients with AR or sporadic RP, of whom 97 had causative mutations in other genes), the probability would still be 0.98, which is rare but not impossible. Thus, if we included more patients, we may have identified some patients with SLC7A14 mutations.

Another possible explanation is the limitations based on our technique; the PCR-based mutation detection method used in this study may be unable to detect large deletions in heterozygotes. Although such deletions were not found by MLPA analysis in patients with heterozygous variants, there still is a chance that we missed homozygous or compound heterozygous large SLC7A14 deletions. Alternatively, it is possible that the previous study overestimated the frequency of SLC7A14-associated AR RP. We found that two of the mutations reported in the previous study are relatively common variants in healthy Japanese individuals. The allele frequencies of c.988G>A (1.4%) and c.1391G>T (1.1%) were slightly too high for rare diseases, such as RP, even considering that the inheritance trait was AR. In addition, these variants were also observed in another normal database Exome Aggregation Consortium (ExAC), Cambridge, MA (accessed September 3, 2014 from http://exac.broadinstitute.org). Although the allele frequency was 0.19% and 0.02%, respectively, five normal controls had the homozygous variant of c.988G>A. The pathogenicity of the variants should be confirmed in further studies.

In conclusion, SLC7A14-associated AR RP is rare in the Japanese population. The frequency and pathogenicity of SLC7A14 mutations in AR RP patients may vary depending on ethnicity. Further studies with more patients of different racial backgrounds are required to clarify these issues.

Acknowledgments

The authors would like to thank the Exome Aggregation Consortium and the groups that provided exome variant data for comparison. A full list of contributing groups can be found at http://exac.broadinstitute.org/about. The authors also thank Professor Fumihiko Matsuda for his technical advice and support.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding

This study was supported in part by the Japan Ministry of Health, Labor and Welfare (No. 12103069), JSPS KAKENHI (No. 26861445), and the Japanese Retinitis Pigmentosa Society. The funding organizations had no role in the design or execution of this research.

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