Xeroderma pigmentosum group A with mutational hot spot (c.390-1G>C in *XPA*) in South Korea

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Purpose: Xeroderma pigmentosum (XP) is rare autosomal recessive genetic disorder of DNA repair in which the ability to repair damage caused by ultraviolet light is deficient. We reported the first molecularly confirmed Korean patient of XP by targeted exome sequencing. The prevalence of XP included all subtype and carrier frequency of XP-A the using public data were estimated for the first time in South Korea.

Materials and Methods: We described a 4-year-old Korean girl with clinical diagnosis of XP. We performed targeted exome sequencing in the patient for genetic confirmation considering disease genetic heterogeneity and for differential diagnosis. We verified a carrier frequency of c.390-1G>C in *XPA* gene known as mutational hot spot using Korean Reference Genome Data Base. We estimated the period prevalence of all subtypes of XP based on claims data of the Health Insurance Review and Assessment Service in South Korea.

Results: We identified homozygous splicing mutation of *XPA* (c.390-1G>C) in the patient. The carrier frequency of risk for *XPA* (c.390-1G>C) was relatively high 1.608 e-03 (allele count 2/1244). The prevalence of XP in South Korea was 0.3 per million people.

Conclusion: We expect that c.390-1G>C is hot spot for the mutation of *XPA* and possible founder variant in South Korea. However, the prevalence in South Korea was extremely low compared with Western countries and Japan.

Key words: Xeroderma pigmentosum, complementation group A, *XPA* gene, Hereditary neurodegenerative disease, High throughput nucleotide sequencing.

Introduction

Xeroderma pigmentosum (XP) is a rare multigenic, multiallelic and autosomal recessive disease involving a defect in DNA repair caused by exposure to ultraviolet (UV) light [1]. XP is characterized by UV light sensitivity associated with cancer and sometimes by a progressive neurological degeneration. XP is classified into seven genetic complementation groups (XP-A to XP-G) and one XP variant (XP-V, POLH) [2-4]. Of these

groups, most patients with XP-A, B, D, F and G have neurological complications, whereas those in groups C, E and V are free from neurodegeneration [5,6]. The *XPA* (MIM 278700) patients have most severe progressive neurological degeneration [6,7]. The *XPA* gene (MIM 611153) is located on chromosome 9q22.3 and a mutation that is homozygous for the G-C transversion mutation at the splice site of intron 3 (IV3: -1 G to C) occurs most frequently. More than 90% of the mutant alleles in Japanese *XPA* patients have the same G to C base change mutation at this

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site [8]. Here, we reported the case of a 4-year-old Korean girl with homozygous splicing mutation of *XPA* gene (c.390-1G>C), identified by targeted exome sequencing. This pathologic splicing variant in exon 3 of *XPA*: c.390-1G>C is known as mutational hot spot and results in severe progressive neurologic degeneration. We estimated the prevalence of XP contained all subtype and carrier frequency of the mutation hot spot in c.390-1G>C in *XPA* using public data. These estimates are reported for the first time in South Korea.

Materials and Methods

1. Human subjects

A 4-year-old girl first visited the pediatric neurology clinic of Eulji General Hospital with developmental delay and short status. The most favored clinical diagnosis was XP.

The use of human clinical materials and blood in this study was approved by the Ethical Committee of the Eulji General Hospital (IRB No. #2013-08-001-001). We received the written informed consent from patient's parent.

2. Targeted exome sequencing

We performed targeted exome sequencing in patient for genetic confirmation considering disease genetic heterogeneity and for differential diagnosis. Genomic DNA was enriched by using the TruSight One Sequencing Panel (Illumina, Inc., San Diego, CA, USA), which includes 125,395 probes targeting a 12-Mb region spanning 4,813 genes, and sequenced on the Illumina MiSeq platform. Raw sequence reads were processed and aligned to the hg19 human reference sequence.

3. Estimation of XP prevalence including all subtype in South Korea

We calculated the prevalence of XP from 2010 to 2014 using claims data of Health Insurance Review & Assessment Service (HIRA) [9]. South Korea has a universal health coverage system in which the National Health Insurance covers approximately 98% of the overall Korean population. HIRA claims data is an important source of information for healthcare service research; data includes diagnosis, treatment, procedures, surgical history and prescription drugs.

4. Carrier frequency of pathologic mutational hot spot in *XPA*: c.390-1G>C in South Korea

We estimated Korean ethnicity-specific recessive allele frequencies using public data. The Korea Center for Disease Control and Prevention's genome center recently released a Korean Reference Genome Data Base (KRGDB) [10]. From 2012 until now, reference whole genome sequencing of genomes of 622 South Koreans have been compiled using the Illumina HiSeq2000 platform (Illumina, Inc.). This database is the world's largest for a single ethnic group. To ensure sequencing accuracy, the average sequencing depth of coverage was at least 30X per person.

Results

1. Clinical description

She was born vaginally and was small for gestational age at term with a weight of 2,340 g (<10th centile). Her mother and father were non-consanguineous Koreans 33 and 32 years of age, respectively. She had two healthy older siblings (Fig. 1). There was no family history of neurological disease or

Fig. 1. Pedigree of the family with xeroderma pigmentosum group A. The darkened symbol indicated by the black arrow indicates the patient "III-6". Marked symbols with dots are definite carriers. Asterisks indicate sampled subjects.

developmental delays. During infancy, she was treated with Pavlik harness due to left developmental dysplasia of the hip. In a physical examination at her first visit, she showed a dull face and had freckles on her face (Fig. 2). The freckles were first evident at 5 months of age. We did not think the freckles were critical because they were not dark and because her mother had similar freckles on her face. We performed G-band karyotyping for the detection of chromosomal aberrations and an array comparative genomic hybridization analysis for the detection of copy-number variations using the CGX-3 135K Whole-Genome Array (Roche NimbleGen, Inc., Madison, WI, USA). We did not find any abnormalities using these approaches.

She revisited at 5 years, 8 months of age for progressive intellectual disability. She was short for her age. Her body height was 103.6 cm (−2.02 standard deviation [SD], 3 percentile), body weight was 20 kg (0.21 SD, 50–75 percentile), and body mass index was 18.63 kg/m² (1.66 SD). Head circumference was 48.2 cm (−1.57 SD, 5–10 percentile). Freckles were darker on sun-exposed skin including face, neck and arms (Fig. 2). She wore long sleeves during the summer due to photosensitive freckles. She showed an ataxic gait. Intellectual functioning was measured by Korean-Wechsler Preschool and Primary Scale of Intelligence. She had a moderate intellectual disability with Full Scale Intelligence Quotient of 48, Verbal Intelligence Quotient of 59, and Protection Intelligence Quotient of 46. Her social maturation quotient of 48.60 and social age of 2.77 years also indicated moderate trainable intellectual disability. Brain

magnetic resonance imaging revealed mild ventriculomegaly with no other structural abnormalities (Fig. 2). Her hearing tests were still in the normal range: speech recognition threshold of the right and left ear was 35 dB and 40 dB, respectively. The radiographic bone age of the left wrist corresponded to a chronological age of 5 years according to the Greulich and Pyle standard (Fig. 2). Laboratory tests, including a complete blood count, chemistry panel (including creatine phosphokinase), lipid profile, thyroid function test, and somatomedin-C were all in the normal range.

2. Mutation analysis in the patient

We identified a homozygous splicing mutation of *XPA* gene (c.390-1G>C). There were no pathologic mutations in other known XP genes including *ERCC3*, *XPC*, *ERCC2*, *DDB2*, *ERCC4*, *ERCC5*, *ERCC1*, and *POLH*. The homozygous mutation was confirmed by Sanger sequencing in the patient. We also checked carrier testing for at-risk relatives of all available family members depicted in Fig. 3. Her mother, father, and two siblings harbored the heterozygote *XPA*: c.390-1G>C mutation. Of carriers, only her mother had freckle-like pigmentation on her face, which had become apparent in her third decade of life.

3. Estimation of XP prevalence including all subtypes and carrier frequency of mutation hot spot in *XPA*: c.390-1G>C in South Korea

Using the public data resource, the prevalence including all

Fig. 2. (A) At 4 years of age, she showed dull face and had facial freckles. (B) At 5 years and 8 months of age, freckles were darker on sun-exposed skin including face and neck. (C, D) Brain magnetic resonance imaging at 5 years and 8 months of age revealed mild ventriculomegaly with no other structural abnormalities.

Fig. 3. Identification of a homozygous splicing mutation of *XPA* gene (c.390-1G>C) using targeted exome sequencing. (A) *XPA* located on chromosome 9q22.33. It comprises six exons and five introns. (B) Integrative Genomics Viewer reads were aligned to chr9:100449544 C/G. (C) Sanger sequencing confirmed mutation in proband (III-6), heterozygous mutations in four carriers (II-3, II-4, III-4, and III-5), and wild-type in unaffected family member (II-6).

subtypes of XP from 2010 to 2014 was estimated as 0.3 per million people. The prevalence was higher in women than in men (1:1.49). There are eight genetically different complementation groups, comprising subgroups *XPA* through *XP-V*. Unfortunately, the HIRA data lacked subgroup information. Based on KREGDB, the frequency of risk for *XPA* (c.390-1G>C) is 1.608 e-03 (allele count 2/1244).

Discussion

XP-A is a very rare hereditary neurodegenerative disease in peripheral and central nervous system that is caused by a defect in the DNA repair system, due to biallelic pathogenic variants in the nucleotide excision repair gene *XPA*. The primary progressive neuronal degeneration of XP-A is clinically characterized by

cognitive impairment, ataxia, choreoathetosis, progressive sensorineural hearing loss, spasticity, seizures, peripheral neuropathy with diminished or absent deep tendon reflexes and sometimes acquired microcephaly [6,7]. Skin and eye sensitivity to the sun can be minimized by protecting body surfaces from UV radiation by wearing protective clothing, applying broad-spectrum, high sun-protective factor sunscreens, UV-absorbing glasses, having long hair and supplementing the diet with vitamin D. However, once established, neurological deterioration cannot be prevented and no treatments are available. The median age at death in persons with XP with neurodegeneration is younger than that in persons with XP without neurodegeneration [11]. *XPA* mutations within the DNA binding site of exons 3-5 manifest as more severe neurologic disorders and less residual DNA repair function than

mutations near the C-terminal region of exon 6 (Fig. 2) [12]. To date, progressive neurodegeneration in XP-A is suspected due to specific gene roles during postnatal developing brain or a consequence of repair deficiencies in specific brain tissues. The exact pathophysiology of progressive neuron loss in XP-A remains unknown.

XP is very rare but appears to be present globally and in every ethnic group. Prevalence rate for all groups of XP is estimated as 1:1,000,000 in the USA and Europe [13]. The prevalence is increased in communities in which consanguinity is common, such as the Middle East and North Africa [14]. In Japan, the frequency is more than 10 times higher than in Western countries. Consanguinity is allowed in Japan, likely explaining the high prevalence of 1:22,000 [15]. Interestingly, the severest type, XP-A, predominates in Japan, affecting about 60% of Japanese XP patients, whereas XP-A is rare in the USA and Europe, with a prevalence of about 9% [15]. Until now, the prevalence of XP in South Korea has been unknown. Presently, we estimated the prevalence of XP in South Korea as 0.3 per million people using public HIRA data. Prior to this study, the prevalence in South Korea was unknown. Even though we have no information about the sub-groups of XP, the present finding shows that, like Western countries, XP is very rare in South Korea.

To date, more than 29 *XPA* mutation sites have been identified worldwide based on the Human Gene Mutation Database. These comprise 10 missense/nonsense, 9 splicing, and 10 small insertion/deletion mutations. Most of the mutations are sporadic single case reports. More than 90% of the mutant alleles XP-A patients have the same G>C transversion at a splice site in last nucleotide intron 3, the so-called "founder mutation" [15,16]. An XP-A founder variant consisting of a splicing variant in exon 3, c.390-1G>C, of XP-A is present as a heterozygous variant in about 1% of the general population of Japan [17]. Our patient harbored c.390-1G>C in *XPA*. At least four carrier family members were identified. We also checked frequency of risk allele (or haplotype) for *XPA* (c.390-1G>C) mutation using KRDB. Allele frequency of c.390-1G>C was 0.16% of the South Korean general population. This risk allele frequency is low compared to Japan, but is very high compared to the global frequency based on ExAC database as 2.644×10^{-5} (1/37823). We expect that c.390-1G>C is strong hot spot for the mutation of *XPA* and possible founder variant in South Korea.

Heterozygotic XP variants are usually clinically asymptomatic. Especially, individuals with a heterozygous variant (about 1% of the general population of Japan) of XP-A are clinically normal [18]. In this study, the patient's mother had mild freckle-like

photosensitive pigmentation on her face, which began to show up during her third decade of life. We suggest that mid-late onset skin photosensitivity, skin cancer, internal organ cancer, or neurodegeneration should be monitored due to reduced dosage effect in carriers.

In summary, we describe a 4-year-old Korean girl with homozygous splicing mutation of *XPA* (c.390-1G>C) using targeted exome sequencing. Relative high carrier frequency (0.16%) of c.390-1G>C in *XPA* was identified in South Korea using public genome data. The estimated prevalence of XP including all subtype in South Korea was 0.3 per million people with female predominance. This prevalence of XP was extremely low compared with Western countries and Japan.

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