



CASE STUDY

Werner Syndrome Helicase (WRN) Gene Variants and Cancer in Japanese Elderly: An Autopsy Study

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Abstract

Werner Syndrome (WS) is a rare autosomal recessive disorder characterized by symptoms of premature aging, including elevated risk of malignancies. The causative WRN gene encodes a DNA helicase, which maintains the integrity of the human genome. While WS patients have functional null mutations in both alleles of the WRN gene, phenotypes of heterozygote carriers have not been described. Cellular assays showed that heterozygote carriers also have genetic instability to a lesser extent. To this end we searched for variants in the WRN gene among registered consecutive autopsy cases (n = 2343, mean age = 80) in the Japanese Geriatric SNP (JG-SNP) database, which includes detailed pathological documentation and Exome Bead-Chips analysis. The non-sense variant p.R369X (rs17847577), which is the second most prevalent mutation of WS in Japan, was found in 5 heterozygotes (3 men and 2 women); the minor allele frequency (MAF) was 0.11%. The mean age of p.R369X heterozygotes was 89.8-years-old, indicating that average length of life was attained. All three men had multiple cancers including lung cancer in common. One woman had thyroid cancer and another had no cancer. These results suggest that p.R369X heterozygotes can expect average length of life, although men might be cancer prone. We also determined other non-synonymous variants p.V114I (MAF=1.90%), p.E510D (0.68%), p.F1074L (33.38%) and p.C1376R (6.55%), with regard to cancer phenotypes in the database, and found that none of them were associated with presence of cancer in total. The information that p.R369X carriers can expect average lifespan, would be important to whom this variant was incidentally found after personal genome sequencing.

Keywords

Werner Syndrome, WRN gene, Mutation, Heterozygotes

Introduction

Werner Syndrome (WS; MIM#277700) is a rare recessive disorder characterized by an early onset of normal aging, which leads to geriatric symptoms such as cataract, diabetes, atherosclerosis, osteoporosis, and cancer [1,2]. WS is caused by mutations in the WRN gene which encodes a DNA helicase of the RECQ gene family [3]. WRN protein participates in DNA replication, recombination and repair, to maintain the integrity of the genome [3]. To date nearly one hundred different mutations in the WRN gene leading to nonfunctional allele have been identified in WS (according to HGMD professional as of Oct. 2020). The p.R369X missense variant (rs17847577) is the second most frequent WRN gene mutation in the Japanese population, which accounts for 15.9% of the WS cases [1]. A recent whole genome sequencing study in a large cohort of Japanese revealed that pathogenic or likely pathogenic WRN heterologous variants may be more prevalent than previously reported [4]. A large cohort study in the Tohoku district, Japan, identified p.R369X at the MAF of 0.02% in ordinary residents. While the phenotypes of WS patients who are homozygous for none functional alleles of the WRN gene are well characterized, those of heterozygotes are not well documented. Indeed, heterozygote carriers may have genetic instability according to *in vitro* assays such as fibroblast senescence assay [5] and glycophorin A (GPA) somatic cell mutation assay [6]. Importantly, more and more personal genome sequences are conducted, and individuals knowing their carrier



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state may increase. In such instance, more detailed information may be important.

Since WRN protein is involved in genome stability, a number of WRN missense mutations have been subjected to association studies with regard to aging phenotypes such as longevity, cardiovascular diseases, dyslipidemia, diabetes, osteoporosis, and malignancies (reviewed in 1, 2). To this end we employed a Japanese elderly cohort of consecutive autopsy cases in the JG-SNP study and determined the WRN non/missense mutations with various cancer phenotypes.

Material and Methods

Study population

We employed the subjects registered in the Internet Database of Japanese single nucleotide polymorphisms for Geriatric Research (JG-SNP) [7,8]. The study subjects comprised of consecutive autopsy cases collected at Tokyo Metropolitan Geriatric Hospital between 1995 and 2012. Autopsy procedures were performed on approximately 29% of patients who died in the hospital. There were a total of 2,343 subjects, where 1,298 were men and 1,045 were women; the mean age at the time of death was 80 years. The presence or absence of any disease was determined by a thorough examination on autopsy. The detail of JG-SNP database can be seen elsewhere [7]. Cancer-bearing subjects include those with any type of cancer, including occult cancer, found on autopsy. Smoking habit included both current smoking and ex-smoking. The distribution of any disease of the study group was not largely departed from the reports in a survey by the Ministry of Health, Labor, and Welfare of Japan [7].

Genotyping and statistical analysis

Genomic DNA was extracted from the renal cortex using a standard procedure. All samples were genotyped with Illumina Infinium Human Exome Bead-Chips Version 1.1 (Illumina, San Diego, CA) by iScan in accordance with the Illumina protocols. Genotype calling was performed for all samples as a single project using the Genotyping Module (version 1.9) of the Genome Studio data analysis software package. Initial genotype

clustering was performed using the default Illumina cluster file (Human Exome 12v1-1_A.egt) and manifest file (HumanExome-12v1-1_A.bpm) using the GenTrain2 clustering algorithm. We considered a per sample call rate of > 98% as eligible, and 15 samples were excluded. A total of 2,328 (99.4%) out of the initial 2,343 subjects were successfully genotyped. Association of the rare variants and cancer state of the patients were done by IBM SPSS Statistics software 25.0 (IBM; New York, USA). The pathological assessment, genotyping and statistical analysis were performed in different institutions in a double-blind fashion to minimize bias.

Results

The Exome Chip contained 43 SNPs of the WRN gene. 38 of them were monomorphic and 5 were polymorphic in the JG-SNP database. The MAF of each variant was V114I (rs2230009); 1.9%, R369X (rs17847577); 0.11%, E510D (rs113811718); 0.68%, F1074L (rs1801195); 33.38%, and C1376R (rs1346044); 6.55%.

Among these variations, p.R369X was the WS pathogenic mutation. We found 5 heterozygotes (3 men and 2 female) in the database. The mean age of the heterozygotes was 89.8-years-old. All three men carried multiple cancers, including a common lung cancer. Other cancers were gastric, prostate and bile duct cancers. One woman had thyroid cancer, and another had no cancer. We sought for other WS symptoms of diabetes, osteoporosis, coronary artery disease and cataract, and found among these five carriers, one diabetes, one osteoporosis, two coronary artery diseases and none cataract.

We also determined other non-synonymous variants p.V114I, p.E510D, p.F1074L and p.C1376R, with regard to their associations with total cancer presence. There were 1446 cancer positive cases and 897 cancer negative cases. None of these SNPs gave a positive sign of association with the presence of cancer in total (Table 1). As for independent cancers nominal association signs were detected but these were more likely a by chance association (data not shown).

Discussion

WS is an autosomal recessive disorder, which leads

Table 1: WRN Variants and Total Cancer.

SNV		TC(+)*	TC(-)	p-value**	OR	95% CI
p.V114I	VI + II	49 (3.39%)	40 (4.46%)	0.221	0.752	0.491-1.152
	VV	1396 (96.60%)	857 (95.54%)			
p.E510D	ED	23 (1.60%)	9 (1.00%)	0.274	1.611	0.742-3.498
	EE	1418 (98.40%)	894 (99.00%)			
p.F1074L	LL + FL	971 (67.48%)	593 (65.67%)	0.368	1.085	0.909-1.294
	FF	468 (32.52%)	310 (34.33%)			
p.C1367R	RR + CR	196 (13.61%)	111 (12.29%)	0.379	1.124	0.876-1.442
	CC	1244 (86.39%)	792 (87.71%)			

TC: Total Cancer; **: p-value for Fisher's exact probability test.

to progeria symptoms. The molecular genetics surrounding the causative WRN gene mutations are well studied. From the early studies, the heterozygotes of WRN mutation in Japanese was estimated to be around 0.06% suggesting that there may be millions of heterozygote carriers [9]. However, few documents are available on the phenotypes of the heterozygotes. In consecutive autopsy cases of elderly Japanese (n = 2343), we found 5 heterozygotes of the nonsense WRN mutation R369X (MAF=0.11%). The same allele was found in a cohort of ordinary residents of 3552 participants in the Tohoku district at MAF of 0.02% [10]. The similar order of digits suggests that p.R369X heterozygotes generally do not have a survival disadvantage, and can attain full life span of 80 to 90-years-old. Typical phenotypes diabetes, osteoporosis, coronary artery diseases were not apparently accumulated in these cases.

Phenotypic description of heterozygote carriers is scarce. Fibroblast senescence assay [5] and glycoprotein A (GPA) somatic cell mutation [6] assay have shown that cells derived from heterozygote carriers have genetic instability. This may be in the same line with our observation that there were multiple cancers in the heterozygotes. All three men had lung cancer, together with either gastric cancer, prostate cancer or cancer of the bile duct. WS have been known to have a tendency to develop sarcoma rather than carcinoma for unknown reason [11]. On the other hand, WS with diabetes appear to have carcinomas as well as sarcoma in WS patients in Japan [12]. We found moderately excess of cancer incidence in the heterozygotes but there was no sarcoma. Since the sample size is small, cautious interpretation is needed, but it appears that heterozygotes may have prone to carcinoma. Whether or not p.R369X heterozygote carriers confer cancer risk and the types of malignancies prone in the heterozygotes needs further investigation in a larger cohort.

Since WRN gene is implicated in genome integrity, it was considered as candidate gene, and non-synonymous variants were subjected to study association with various geriatric phenotypes (reviewed in 1, 2). In a previous study using the same JG-SNP database, we have also shown that p.V114L associate with osteoporosis [6]. p.V114L was also reported to associate with high breast cancer risk after ionizing radiation exposure [13]. In our study p.V114L was not associated with total cancer, but only nominally associated with adenocarcinoma, which is likely a spurious association (data not shown). p.C1367R has also been shown to associate with various cancers [14-18], but we did not find any sign of association with total cancer presence in our cohort.

In conclusion, WRN pR369X carriers can attain average life span, albeit men may be prone to multiple cancer risk including lung cancer. WRN gene variants warrant further follow up in a larger size cohort.

Ethical Statement

This study was approved by the Tokyo Medical and Dental University Ethics Committee (approval no. 2016-011-02) and the Tokyo Metropolitan Geriatric Hospital Ethics Committee (approval no. 230405). Written informed consent was obtained from a family member of all participants involved in this study before autopsy.

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