Zong et al. J Geriatr Med Gerontol 2020, 6:103
Volume 6 | Issue 4
DOI: 10.23937/2469-5858/1510103

Accepted: November 23, 2020; Published: November 25, 2020

Copyright: © 2020 Zong Y, 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.

CASE STUDY

Werner Syndrome Helicase (WRN) Gene Variants and Cancer in Japanese Elderly: An Autopsy Study
Yuan Zong1, Masashi Tanaka2, Masaaki Muramatsu1* and Tomio Arai2

1Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Japan
2Department of Neurology, Juntendo University Graduate School of Medicine, Japan

*Corresponding author: Masaaki Muramatsu, Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113 - 8510, Japan

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 = 2345, 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 ordinal 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 state may...
tering 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 SPSS. 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); 6.56%.

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.C1367R, with regard to their associations with total cancer presence. There were 1441 cancer positive cases and 901 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 to progeria symptoms. The molecular genetics surround-

### Table 1: WRN Variants and Total Cancer.

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

*TC: Total Cancer; **: p-value for Fisher’s exact probability test.
ng 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, there 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 glycophorin 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 carcinoma rather than carcinoma for unknown reason [11]. On the other hand, WS with diabetes appear to have carcinomas as well as carcinoma in WS patients in Japan [12]. We found moderately excess of cancer incidence in the heterozygotes but there was no carcinoma. 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 find any sign of association with total cancer presence in our cohort.

In conclusion, WRN p.R369X 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. 2009-19-4) 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.

**References**


