Lack of association of the WRN C1367T polymorphism with senile cataract in the Israeli population

M. Ehrenberg,1,2 O. Dratviman-Storobinsky,3 B.R. Avraham-Lubin,2,3 N. Goldenberg-Cohen2,3,4

1Department of Ophthalmology, Rabin Medical Center, Petach Tikva, Israel; 2Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 3The Krieger Eye Research Laboratory, Felsenstein Medical Research Center, Petach Tikva, Israel; 4Department of Ophthalmology, Pediatric Division, Schneider Children’s Medical Center of Israel, Petach Tikva, Israel

Purpose: Werner syndrome is an autosomal recessive disease of premature aging caused by a polymorphic C1367T mutation in the Werner (WRN) gene. Although there are differences between the pathobiology of normal aging and the phenotype of Werner syndrome, the clinical age-related changes are similar. The aim of the study was to investigate the incidence of the C1367T (rs1346044) polymorphism in patients with age-related cataract.

Methods: The study group consisted of 81 patients with senile cataract undergoing cataract extraction surgery. Data on age, sex, and medical history of microvascular disease and cancer were obtained from the medical files. Anterior lens capsule material was collected during surgery. DNA was extracted, amplified by polymerase chain reaction, and screened for the C1367T polymorphism in WRN using restriction enzymes followed by sequencing.

Results: There were 33 male and 48 female patients of mean age 74.3±9 years. Genotypic frequencies were 67% for TT and 33% for TC. None of the patients had the CC genotype. Ten patients had a history of myocardial infarct, 8 cerebrovascular accident, and 8 various tumors. The distribution of these morbidities was similar in the two genotype groups.

Conclusions: The distribution of the C1367T WRN polymorphism in patients with senile cataract is similar to that in the normal population. Cataract formation in the elderly is not linked to a WRN mutation.

Progeria is a rare group of diseases with striking features that resemble accelerated aging [1].

Werner syndrome is a less well known but more common form of progeria, with a frequency of 1×10⁻¹⁻¹×10⁻⁷ depending on the geographic area [2]. Also termed progeria of adults, Werner syndrome first becomes apparent in puberty (mean age) with growth arrest and thinning and graying of the hair. Other manifestations include skin wrinkling, wrinkled, osteoporosis, and premature arteriosclerotic disease that leads to heart attacks and strokes [3-5]. Werner syndrome has also been associated with various types of cancer [6]. Many laboratory abnormalities have been reported [7]. The syndrome is caused by a polymorphic C1367T mutation in the Werner gene (WRN) located on the short arm of chromosome 6.

WRN encodes a multifunctional nuclear protein of the RecQ family which functions as an exonuclease and endohelicase [8-12] with apparent involvement in transcriptional and chromosomal segregation and DNA repair/recombination [13]. Mutations in WRN lead to a loss of function of the protein and a breakdown in genome integrity [14]. Although the genetic basis of Werner syndrome is unknown, the inheritance pattern and paternal age effect, in addition to the absence of findings of consanguinity, point to a sporadic dominant mutation.

Diseases of premature aging have prompted interest among geneticists because the study of their underlying mechanisms can provide insights not only into these rare disorders themselves but also into the normal aging process [1,15]. While the phenotype of Werner syndrome differs from the pathobiology of normal aging, the clinical age-related changes are similar. To date, researchers have investigated the possible relationship of many mutations, deletions, and polymorphisms of the WRN gene to such age-related diseases as cardiovascular disease [6], hypertension, diabetes mellitus, dementia, osteoporosis [3], and some cancers [16-19]. In the eye, the literature has focused on complications after cataract surgery [20,21]; one case of a dislocated lens to the vitreous has been reported as well [22]. Although bilateral cataract develops early in patients with Werner syndrome [7], and other laminopathies have been linked with congenital cataract [23-26], to our knowledge there are no studies of possible protein changes in the lens as a result of progeroid mutations or a link between senile cataract and abnormalities in WRN.

The aim of the present study was to investigate a possible role of the C1367T (rs1346044) polymorphism in the WRN gene in age-related cataract in an Israeli population.

METHODS

Patients: The study group included 81 patients undergoing routine cataract surgery at a major tertiary center. The study
was approved by the institutional and national review boards, and all patients signed an informed consent form.

All patients were examined preoperatively by slit-lamp, and cataract type and grade were categorized. Background data were derived from the medical files. Anterior lens capsule material excised during surgery (one sample per patient) was analyzed for the C1367T polymorphism in the WRN gene.

**DNA Isolation:** The capsules containing single-layer lens epithelial cells were suspended in 5 ml of conservation medium until isolation of genomic DNA. DNA was extracted using standard sodium dodecyl sulfate (SDS)/proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation.

**C1367T polymorphism in the WRN gene:** A DNA sequence of 195 bp, which contains the polymorphic site, was amplified by polymerase chain reaction (PCR) using the following primers: 5′-GCC TAA TCA GAA TGT TAG TT-3′ and 5′-CCT CAG TAT TGA TGC CTA CTT C-3′. Amplification was performed in a 50-µl reaction volume containing 100 ng of sample DNA as a template. The PCR parameters were as follows: denaturation at 95 °C for 3 min, 35 cycles of 45 s at 95 °C, annealing at 58 °C for 45 s, and extension of 1 min at 72 °C with Taq polymerase. The PCR product was amplified on 2% agarose gel and visualized with ethidium bromide staining.

Direct sequencing of the PCR products was performed for selected samples, with Big Dye Terminator Cycle Sequencing reagents using the ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA; Figure 1).

The T→C alteration in WRN results in the loss of a BsaAI restriction site in the abnormal sequence. Therefore, upon digestion with BsaAI (R0531; New England Biolabs, Beverly, MA), the normal sequence yielded two fragments of 158 bp and 37 bp, whereas the C-altered sequence yielded three fragments of 93 bp, 65 bp, and 37 bp, which were separated on a 4% agarose gel (Figure 2).

**Statistical analysis:** The results were statistically analyzed with SPSS for Windows, version 15.0.1 (SPSS- Inc, Chicago, IL). A p value of less than 0.05 was considered statistically significant.

Between-group differences in cataract type and grade were analyzed by the χ² test; in sex and underlying diseases, by Fisher exact test; in age, by t-test. Correlations between variables were analyzed by Pearson correlation test.

**RESULTS**

Eighty-one patients participated in the study, 33 male (41%) and 48 female (59%), of mean (±SD) age 74.3±9.6 years (range: 52–93 years). Other morbidities included hypertension 66.7%, diabetes mellitus 36.1%, dyslipidemia 48.6%, s/p myocardial infarction 14.1%, s/p cerebrovascular accident 11.3%, and cancer 11.3% (colorectal carcinoma, 2.4%, including one patient also with prostate carcinoma; breast cancer, 2.4%, including one patient also with uterine carcinoma; transitional cell carcinoma of the bladder, 1.2%; synovial sarcoma, 1.2%).

Analysis of allele frequencies of the WRN polymorphism revealed the CT genotype in 33.3% of patients, and the TT genotype, in 66.67%. No homozygosity for CC was found.

There was no association of genotype polymorphism with the presence or stage of cataract. The distribution of cataract and other morbidities by genotype is shown in Table 1.

**DISCUSSION**

The present study investigated the C1367T WRN polymorphism and genotype frequencies in elderly patients with senile cataract. We found that the genotype distribution was similar to that reported in the general population [26-29], namely 2/3 TT and 1/3 CT. No association was found between the genotype and type or stage of cataract or other age-related morbidities.

Molecular studies strongly suggest that aging is triggered by two mechanisms: DNA damage and telomere shortening.
Figure 2. The C1367T polymorphism. Following the use of restriction enzymes, the gel shows: 1, DNA ladder; 2, PCR product well, demonstrating a 195 bp length sequence; 3, TT well, demonstrating two lengths of DNA of 158 bp and 37 bp; 4, CT well, demonstrating 4 DNA lengths of 158 bp, 93 bp, 65 bp, and 37 bp; 5, CC well, demonstrating 3 DNA lengths of 93 bp, 65 bp, and 37 bp (example taken as control).

Table 1. WRN Allele Polymorphism, Cataract Grading and Systemic Co-morbidities.

| Disorder                          | Gene polymorphism | Statistical significance |
|-----------------------------------|-------------------|-------------------------|
|                                   | C/T n=27 (33%)    | T/T n=54 (66.7%)        |                         |
| Nuclear cataract                  |                   |                         |                         |
| Stage 0                           | 1                 | 4                       | NS                      |
| Stage 1                           | 9                 | 28                      | NS                      |
| Stage 2                           | 14                | 24                      | NS                      |
| Stage 3                           | 8                 | 9                       | NS                      |
| Stage 4                           | 3                 | 3                       | NS                      |
| Hypertension                      | 70                | 65                      | NS                      |
| Diabetes mellitus type 1          | 39                | 35                      | NS                      |
| Dyslipidemia                      | 38                | 54                      | NS                      |
| Myocardial infarct                | 16                | 13                      | NS                      |
| Cerebro-vascular accident         | 4                 | 7                       | NS                      |
| Tumor                             | 12                | 11                      | NS                      |

In the Table, “NS” indicates not statistically significant.

In the first, the cumulative DNA damage accompanied by DNA repair deficiencies results in genomic instability and accelerated cellular senescence. Aging due to both mechanisms is strongly dependent on the TP53 (p53) status.

The WRN gene is considered the “caretaker” of the genome [31], with the protein serving as an important link between repair of defective DNA and processes related to aging. The gene is expressed within the central nervous system and throughout the brain, and is present in both neurons and glia. Analysis of WRN RNA levels throughout the life cycle revealed the highest levels in embryonic brain tissue and a biphasic pattern of expression from the early postnatal period into adulthood. Mutations in WRN are believed to result in the deleterious loss of normal WRN function.

The eye is part of the central nervous system. The transparency of the lens is maintained by a specific mechanism of nuclear differentiation and elimination of the lens fiber cells followed by apoptosis [10]. The purpose of the lens denucleation is to reduce light scatter. Studies of normal aging of the lens suggest that transcriptional shutdown precedes laminar reorganization and chromatin breakdown during lens fiber cell denucleation [15].

Senile cataract, which disrupts normal lens denucleation, is one of the most common age-related disorders. It manifests early in most patients with Werner syndrome, a classic progeroid premature-aging syndrome caused by a single-gene mutation. Although the function of WRN has been intensively investigated in primary fibroblast and fibroblast cell lines, little is known about the normal expression pattern of the protein in the eye. We speculated that a search for the C1367T WRN polymorphism responsible for Werner syndrome in elderly patients with cataract might shed light on the
molecular basis of both the disease and the normal aging process of the eye [32]. However, we did not identify any homozygosity of the CC alleles, and the distribution of the heterozygous polymorphism was similar to that found in the general Israeli population (~33%). Cataract, and other age-related diseases, were not linked to a mutation of the WRN gene.

These results are in line with the larger Elderly Brazilian Longitudinal Study [26], wherein no association was found between WRN C1367T and cardiovascular diseases, diabetes mellitus type 2, obesity, dementia, depression, and neoplasms. In addition, Bohr et al. [27], in the Baltimore Longitudinal Study of Aging, failed to show any influence of the WRN polymorphism on coronary artery disease. However, studies in Japanese populations found that patients homozygous for TT were at nearly threefold higher risk of myocardial infarct than the general population [33]; other researchers from countries other than Japan reported a similar risk for CT in their populations [34]. CC homozygosity posed a lower risk [34,35] and also protected against the development of type 2 diabetes mellitus [36]. In the present study, in which none of the patients had the CC genotype, the distribution of type 2 diabetes was similar in patients with TT and TC.

Studies of central nervous system diseases in this context yielded no association of the WRN polymorphism with increased risk of either Alzheimer disease [28] or gliomas [29]. Breast cancer was not associated with C1367T, but it was associated with another polymorphism of the WRN gene, A46729C [37].

The lack of an association of the WRN C1367T polymorphism and senile cataract in the present study could suggest that abnormalities in WRN in position C1367T do not lead to abnormal lens fiber cell denucleation in adults or that the influence of the protein on lens laminopathies is not crucial to the lens aging process. The lack of association with other age-related morbidities might distinguish Israelis from Japanese and other populations, or it might be explained by the limited number of participants in our sample. Additional studies are needed to further investigate possible links of WRN polymorphisms with age-related diseases, including cataract.

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REFERENCES

1. Brown WT. Progeria: a human-disease model of accelerated aging. Am J Clin Nutr 1992; 55:1222S-4S. [PMID: 1590260]
2. Masala MV, Scapaticci S, Olivieri C, Pirodda C, Montesu MA, Cuccuru MA, Pruneddu S, Danesino C, Cerimele D. Epidemiology and clinical aspects of Werner's syndrome in North Sardinia: description of a cluster. Eur J Dermatol 2007; 17:213-6. [PMID: 17478382]
3. Okamoto N, Satomura K, Hatsuikawa Y, Hayashi M, Saijo K, Ohno T, Goto M. Premature aging syndrome with osteosarcoma, cataracts, diabetes mellitus, osteoporosis, erythroid macrocytosis, severe growth and developmental deficiency. Am J Med Genet 1997; 69:169-70. [PMID: 9056555]
4. Dominguez-Gerpe L, Arauju-Vilar D. Prematurely aged children: molecular alterations leading to Hutchinson-Gilford progeria and Werner syndromes. Curr Aging Sci 2008; 1:202-12. [PMID: 20021393]
5. Kamenisch Y, Berneburg M. Progeroid syndromes and UV-induced oxidative DNA damage. J Invest Dermatol Symp Proc 2009; 14:8-14. [PMID: 19675546]
6. Capell BC, Collins FS, Nabel EG. Mechanisms of cardiovascular disease in accelerated aging syndromes. Circ Res 2007; 101:13-26. [PMID: 17615378]
7. Duvic M, Lemak NA. Werner's syndrome. Dermatol Clin 1995; 13:163-8. [PMID: 7712642]
8. Li B, Jog S, Candelario J, Reddy S, Comai L. Altered nuclear functions in progeroid syndromes: a paradigm for aging research. ScientificWorldJournal 2009; 9:1449-62. [PMID: 20024518]
9. Martin GM. Somatic mutagenesis and antimutagenesis in aging research. Mutat Res 1996; 350:35-41. [PMID: 8657194]
10. Mounkes LC, Stewart CL. Aging and nuclear organization: lamins and progeria. Curr Opin Cell Biol 2004; 16:322-7. [PMID: 15145358]
11. Nakura J, Ye L, Morishima A, Kohara K, Miki T. Helicases and aging. Cell Mol Life Sci 2000; 57:716-30. [PMID: 10892338]
12. Opresko PL, Cheng WH, von Kobbe C, Harrigan JA, Bohr VA. Werner syndrome and the function of the Werner protein; what they can teach us about the molecular aging process. Carcinogenesis 2003; 24:791-802. [PMID: 12771022]
13. Huang S, Kennedy BK, Oshima J. LMNA mutations in progeroid syndromes. Novartis Found Symp 2005; 264:197-202. [PMID: 15773755]
14. Gee J, Ding Q, Keller JN. Analysis of Werner's expression within the brain and primary neuronal culture. Brain Res 2002; 940:44-8. [PMID: 12020873]
15. Dahm R, Gribbon C, Quinlan RA, Prescott AR. Changes in the nucleolar and coiled body compartments precede lamina and chromatin reorganization during fibre cell denucleation in the bovine lens. Eur J Cell Biol 1998; 75:237-46. [PMID: 9587055]
16. Opresko PL, Calvo JP, von Kobbe C. Role for the Werner syndrome protein in the promotion of tumor cell growth. Mech Ageing Dev 2007; 128:423-36. [PMID: 17624410]
17. Ozgenc A, Loeb LA. Werner Syndrome, aging and cancer. Genome Dyn 2006; 1:206-17. [PMID: 18724062]
18. Wang Z, Xu Y, Tang J, Ma H, Qin J, Lu C, Wang X, Hu Z, Wang X, Shen H. A polymorphism in Werner syndrome gene is associated with breast cancer susceptibility in Chinese women. Breast Cancer Res Treat 2009; 118:169-75. [PMID: 19205873]
19. Tao LC, Stecker E, Gardner HA. Werner's syndrome and acute myeloid leukemia. Can Med Assoc J 1971; 105:951. [PMID: 5290279]
20. Ruprecht KW. Ophthalmological aspects in patients with Werner's syndrome. Arch Gerontol Geriatr 1989; 9:263-70. [PMID: 2640084]

21. Jonas JB, Ruprecht KW, Schmitz-Valckenberg P, Brambring D, Platt D, Gebhart E, Schachtschabel DO, Naumann GO. Ophthalmic surgical complications in Werner's syndrome: report on 18 eyes of nine patients. Ophthalmic Surg 1987; 18:760-4. [PMID: 3431806]

22. Sharir M, Ragenbogen L. Bilateral spontaneous dislocated lenses, retinal vasculitis and progeria-like changes. Metab Pediatr Syst Ophthalmol 1990; 13:5-9. [PMID: 2370835]

23. Szeverenyi I, Cassidy AJ, Chung CW, Lee BT, Common JE, Ogg SC, Chen H, Sim SY, Goh WL, Ng KW, Simpson JA, Chee LL, Eng GH, Li B, Luney DP, Chuon D, Venkatesh A, Khoo KH, McLean WH, Lim YP, Lane EB. The Human Intermediate Filament Database: comprehensive information on a gene family involved in many human diseases. Hum Mutat 2008; 29:351-60. [PMID: 18033728]

24. Van Esch H, Agarwal AK, Debeer P, Fryns JP, Garg A. A homozygous mutation in the lamin A/C gene associated with a novel syndrome of arthropathy, tendinous calcinosis, and progeroid features. J Clin Endocrinol Metab 2006; 91:517-21. [PMID: 16278265]

25. Zhang LY, Yam GH, Fan DS, Tam PO, Lam DS, Pang CP. A novel deletion variant of gammaD-crystallin responsible for congenital nuclear cataract. Mol Vis 2007; 13:2096-104. [PMID: 18079686]

26. Smith MA, Silva MD, Araujo LQ, Ramos LR, Labio RW, Burbano RR, Peres CA, Andreoli SB, Payao SL, Cendoroglo MS. Frequency of Werner helicase 1367 polymorphism and age-related morbidity in an elderly Brazilian population. Braz J Med Biol Res 2005; 38:1053-9. [PMID: 16007276]

27. Bohr VA, Metter EJ, Harrigan JA, von Kobbe C, Liu J, Gray MD, Majumdar A, Wilson DM 3rd, Seidman MM. Werner syndrome protein 1367 variants and disposition towards coronary artery disease in Caucasian patients. Mech Ageing Dev 2004; 125:491-6. [PMID: 15246744]

28. Payão SL, de Labio RW, Gatti LL, Rigolin VO, Bertolucci PH, Smith Mde A. Werner helicase polymorphism is not associated with Alzheimer's disease. J Alzheimers Dis 2004; 6:591-4. [PMID: 15665399]

29. Pinto GR, Yoshioka FK, Clara CA, Santos MJ, Almeida JR, Burbano RR, Rey JA, Casartelli C. WRN Cys1367Arg SNP is not associated with risk and prognosis of gliomas in Southeast Brazil. J Neurooncol 2008; 90:253-8. [PMID: 18670736]

30. Ding SL, Shen CY. Model of human aging: recent findings on Werner's and Hutchinson-Gilford progeria syndromes. Clin Interv Aging 2008; 3:431-44. [PMID: 18982914]

31. Opresko PL. Telomere ResQue and preservation--roles for the Werner syndrome protein and other RecQ helicases. Mech Ageing Dev 2008; 129:79-90. [PMID: 18054793]

32. Beauregard S, Gilchrest BA. Syndromes of premature aging. Dermatol Clin 1987; 5:109-21. [PMID: 3549072]

33. Ye L, Miki T, Nakura J, Oshima J, Kamino K, Rakugi H, Ikegami H, Higaki J, Edland SD, Martin GM, Ogihara T. Association of a polymorphic variant of the Werner helicase gene with myocardial infarction in a Japanese population. Am J Med Genet 1997; 68:494-8. [PMID: 9021029]

34. Castro E, Edland SD, Lee L, Ogburn CE, Deeb SS, Brown G, Panduro A, Riestra R, Tilvis R, Lohija J, Penttinen R, Erkkola R, Wang L, Martin GM, Oshima J. Polymorphisms at the Werner locus: II. 1074Leu/Phe, 1367Cys/Arg, longevity, and atherosclerosis. Am J Med Genet 2000; 95:374-80. [PMID: 11186893]

35. Yamada H, Yamada Y, Fukatsu A, Miura N, Aoki T, Futemma A, Kakumoto S. Polymorphism of Werner helicase-associated gene in long-term hemodialysis patients. Nephron 2000; 86:543. [PMID: 11124623]

36. Hirai M, Suzuki S, Hinokio Y, Yamada T, Yoshizumi S, Suzuki C, Satoh J, Oka Y. WRN gene 1367 Arg allele protects against development of type 2 diabetes mellitus. Diabetes Res Clin Pract 2005; 69:287-92. [PMID: 16098926]

37. Ding SL, Yu JC, Chen ST, Hsu GC, Shen CY. Genetic variation in the premature aging gene WRN: a case-control study on breast cancer susceptibility. Cancer Epidemiol Biomarkers Prev 2007; 16:263-9. [PMID: 17301258]