沃納氏徵候群

—報告一典型病例及其家族基因突變之研究—

陳冠宇[1,2] 許漢銘[1] 趙曉秋[1]

國立成功大學醫學院附設醫院 皮膚部[1] 國軍台中總醫院 皮膚科[2]

Werner's Syndrome

—A Case Report and Mutation Analysis—

Guan-Yu Chen[1,2] Hamm-Ming Sheu[1] Sheau-Chiou Chao[1]

Werner's syndrome is an autosomal recessive disorder causing premature aging and cancers. A 33-year-old male suffered from unhealing wound on right foot for 2 years. He looked older than his age, with short stature, light body weight, thin hair, balding of beard, high-pitched voice, bird-like face, atrophy of the subcutaneous fat tissue, calluses on the sole, toe nails dystrophy, cataract, diabetes mellitus, and hypogonadism. Linkage analysis revealed that the *WS* gene (*WRN*) was localized to the short arm of chromosome 8 (8p11-21) and was cloned in 1996. Mutational analysis of the proband showed a novel homozygous mutation of C-to-T transition at nucleotide IVS 25+6 within the intron 25. The mother and brother showed heterozygous of C-to-T transition in the same position. The estimated prevalence was 1:300000 for the Japanese. The incidence of neoplasia has been associated with an excess of non-epithelial malignant tumors , especially sarcomas. Clinicians must keep in mind their high risk for cancers. (Dermatol Sinica 20：186-191, 2002)

Key words: Werner's syndrome, Autosomal recessive, Mutation analysis, *WS* gene

沃納氏徵候群爲自體隱性遺傳疾病，合併有早熟性老化及癌症。患者爲33歲男性，右腳出現無法癒合的傷口已二年，外觀比實際年齡老，長不高，體重輕，頭髮稀疏，長不出鬍子，高頻的音調，鳥樣臉，皮下脂肪萎縮，足底有胼胝，指甲萎縮，白內障，糖尿病和性腺機能不足。此症之連環遺傳分析於1996年發現*WS* gene(*WRN*)位於第八對染色體(8p11-21)的短臂上。我們經由核酸定序做基因突變分析，發現在患者intron 25內核甘酸IVS 25+6的地方發生C-to-T移位的homozygous的新突變。病人的媽媽及哥哥在同樣的位置表現出C-to-T移位的heterozygous突變。此症日本人的盛行率大約爲三十萬分之一。相關的腫瘤大多

From the Department of Dermatology, National Cheng-Kung University Hospital[1] and Armed Forces Taichung General Hospital[2]

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Reprint requests: Sheau-Chiou Chao, M.D., Department of Dermatology, National Cheng-Kung University Hospital, 138 Sheng-Li Road, Tainan, Taiwan TEL: 886-6-2353535-5417 FAX: 886-6-2004326 E-mail: joly@mail.ncku.edu.tw

爲非上皮性惡性腫瘤，特別是肉瘤。臨床醫師必須牢記這些患者爲癌症的高危險群。(中華皮誌20：186-191, 2002)

INTRODUCTION

Werner's syndrome (*WS*) is an autosomal recessive disorder causing symptoms of premature aging accompanied by rare cancers. This syndrome is frequent found in Japan because of the previously high rate of inbreeding. The disorder is known to have an increased incidence of neoplasia and has been associated with an excess of non-epithelial malignant tumors, especially sarcomas.[1] We described a Taiwanese patient with *WS* and a novel splicing mutation in *WS* gene.

CASE REPORT

A 33-year-old male suffered from unhealing wound on right foot for 2 years and consulted our hospital in March 2001. He was the youngest of the seven children; none of his six siblings were diagnosed as having WS (Fig. 1). The marriage of his parents was nonconsanguineous. He looked older than expected for his age, with short stature (149.5cm), light body weight (40 kg), thin hair, balding of beard, high-pitched voice, bird-like face, atrophy of the subcutaneous fat tissue, calluses on his sole and toe nails dystrophy (Fig 2). The patient had both eyes treated for cataract by extracapsular extraction when he was 22. He had diabetes mellitus (fasting blood sugar:140 mg/dl) that had been treated with oral hypoglycemic agents since 2 years ago.

The following laboratory values were abnormal: serum cholesterol 224 mg/dl (normal 0-200 mg/dl), triglyceride 427 mg/dl (normal 0-150 mg/dl), uric acid 9.7 mg/dl (normal 4-7 mg/dl), fasting sugar 132 mg/dl (normal 60-120 mg/dl), E2 49.48 pg/ml (normal 0-44 pg/ml) and prolactin 38 ng/ml (normal 3.1-16.5 ng/ml). His semen count was 18 M/ml (normal 60-150 M/ml), motility was 12% (normal >60%), deformity was 68% (normal <40%) and testis was small, so hypogonadism was impressed. Cytogenetic anylasis resulted 46,XY, normal male karyotype. Abdominal echo revealed hemangioma on right lobe of liver and the X-ray of wrist showed calcification of cartilage.

PCR AMPLIFICATION AND HETERODUPLEX ANALYSES

Genomic DNA was extracted from peripheral blood of the proband and other members of the families. DNA samples were then subjected to mutation screening by amplification of segments of *WS* gene spanning all 35 exons of the gene using primers synthesized on the basis of intronic sequences.[2]

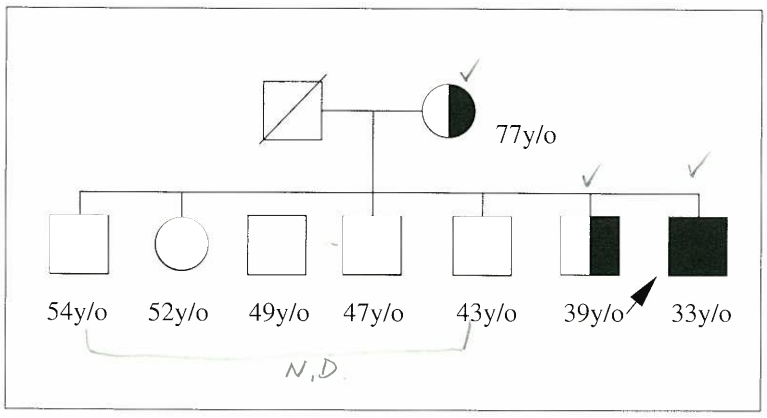

Fig. 1
Pedigree of the Werner's syndrome patient. Arrow denotes patient.

For PCR amplification, approximately 200 ng of genomic DNA, 12.8 pmol of each primer, 10 μ mole dNTP and 1.25 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, New Jersey, USA) were used in a total volume of 50 μℓ. The amplification conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 45 seconds, annealing temperature (AT) for 45 seconds and 72°C for 45 seconds, and extension at 72°C for 10 min. The PCR product were examined on 2% agarose gel and 3-10 μ l of the samples were prepared for heteroduplex analysis using conformation-sensitive gel electrophoresis (CSGE) as described by Ganguly *et al.*[3] The PCR products demonstrating shifted bands were subjected to directed automated sequencing (377 ABI Advanced Biotechnologies, Columbia, Md.). The oligonucleotide primers were designed to amplify the mutations in intron 25 of the *WS* gene is listed in Table I.

RESULTS

Direct DNA sequencing of the PCR products from the proband showed a homozygous mutation of C-to-T transition at nucleotide IVS 25+6 within the intron 25 (Fig. 3A, B, C). The mother and brother showed heterozygous of C-to-T transition in the same position. This mutation may be influent of splicing with the generation of a frameshift and termination. The mother and brother also showed heterozygous of A-to-G transition in the IVS 25+7. Digestion of PCR products amplified from patient's, mother and his brother DNA, and from 50 normal controls DNA by restriction endonucleases TspRI, showed that the IVS 25+7 is a polymorphism only (Fig. 3D)

DISCUSSION

WS is an autosomal recessive disorder characterized by the precocious appearance of many alterations that otherwise would appear only in old age.[4] Goto *et al.*[5] proposed diagnostic criteria for *WS* consisting of four major signs and symptoms: (1) characteristic habitus and stature (short stature, light body weight, slender extremities with stocky trunk, and beak-shaped nose); (2) premature senescence (bird-like appearance, alopecia, skin hyperpigmentation, hoarseness, diffuse arteriosclerosis, juvenile bilateral cataracts and osteoporosis); (3) scleroderma-like skin changes (atrophic skin and muscle, circumscribed hyperkeratosis, telangiectasia, tight skin over bones of feet, skin ulcers and localized calcification); and (4) endocrine abnormalities (diabetes mellitus and hypogonadism). Fulfillment of at least three of these four major signs and symptoms are required for diagnosis. Our patient fulfilled the Goto's criteria of *WS*.[5]

The incidence of *WS* is low and estimated prevalence was 1:300000 for the Japanese.[6] There were approximately 400 cases have been reported since Werner's original publication.[7] The average life span of patients is 47 years.[8] The principal causes of death are vascular disease (cardiovascular or cerebrovascular) and malignancy.[9]

Chronic ulcers of the feet and legs may bring patients to hospital like our case. If these fail to heal or become gangrene, amputation was suggested. Fleischmajer and Nedwich[10] analyzed scleroderma-like tissue from an amputated foot of a patient with *WS*. The histologic changes were (1) replacement of subcutaneous tissue by hyalinized connective tissue, (2) marked hyalinization of the collagen at the lower levels of the dermis, and (3) hyalinization and

Table I. Oligonucleotide primers used for *WRN* gene amplification.[2]

	Forward primer (5' →3')	Backward primer (3'→5')	AT
Exon 25,26	TGT TCA GAA TGA GCA CGA TGG G	GGT AAA CAG TGT AGG AGT CTG C	53°C

AT: annealing temperature

formation of saccular aneurysms in the dermal blood vessels.

Goto[11] assessed the risk factors for atherosclerosis in *WS*, coagulation/fibrinolytic system parameters and lipid levels were investigated in 9 non-smoker patients with *WS* and compared with normal control values. The levels of thrombin antithrombin III complex, D-dimer, tissue plasminogen activator and PA inhibitor 1 were significantly increased, while the level of thrombomodulin in the fasting plasma was significantly decreased in the *WS* cases compared with normal lipid profiles confirmed that 8 of the 9 patients were of hyperlipidemia type IIb, 7 had hyperinsulinemia and 5 had diabetes mellitus. The hypercoagulable condition suggested the existence of multiple risk factors for atherosclerosis in *WS* in addition to the previously reported hyperinsulinemia and hyperlipidemia.[11]

Previous study showed that *WS* was associated with a remarkable excess of rare tumors, included bone and soft tissue sarcomas, myeloid disorders,[4] benign meningiomas, acral lentiginous melanomas and thyroid carcinomas (Table II).[12,13] It is noteworthy that carcinomas that often affect the elderly, such as those of the skin, lung, colon, or prostate, seldom occurred. The ratio of epithelial to nonepithelial cancer was 1:1 instead of the usual 10:1 in populations older than 20 years.[14] "Variegated translocation mosaicism" is the designation proposed by Nichols[15] for a phenomenon he and others[16,17] have observed in *WS*: skin fibroblast cell lines from such patients are usually composed of one or several clones, each marked by a distinctive, apparently balanced translocation. Martin *et al.*[18] first reported that cultured skin fibroblast-like cells from a patient with *WS* had a shortened life span. Whether the chromosome abnormalities induce a series of biological disorder (such as inactivation of tumor-suppressor genes or activation of oncogenes) that lead to malignancy has not been determined.[19]

Linkage analysis revealed that the *WS* gene (*WRN*) was localized to the short arm of chromosome 8 (8p11-21)[5] and was cloned in 1996.[20] In this case, a novel mutation, C→T substitution, change gtaaacggt to gtaaatggt at 6 bp downstream in the intron from the 3' end of

Table II. Known malignancies in patients with Werner's syndrome

Type of Tumor	No. of Cases
Mesenchymal	
Fibrosarcoma	6
Fibrous histiocytoma	6
Leiomyosarcoma	6
Osteosarcoma	5
Other sarcoma	5
Epithelial	
Thyroid cancer	10
Squamous cell carcinoma of skin	4
Basal cell carcinoma of skin[21]	1
Bladder cancer	4
Hepatocellular carcinoma	3
Gastric cancer	3
Breast cancer	3
Ovarian cancer	2
Cholangiocarcinoma	2
Melanoma	12
Leukemia	6
Myelofibrosis[4]	1

*All cases from Duvic *et al.*[12] except Basal cell carcinoma reported by Morita *et al.*[21] and Myelofibrosis reported by Cottoni *et al.*[4]

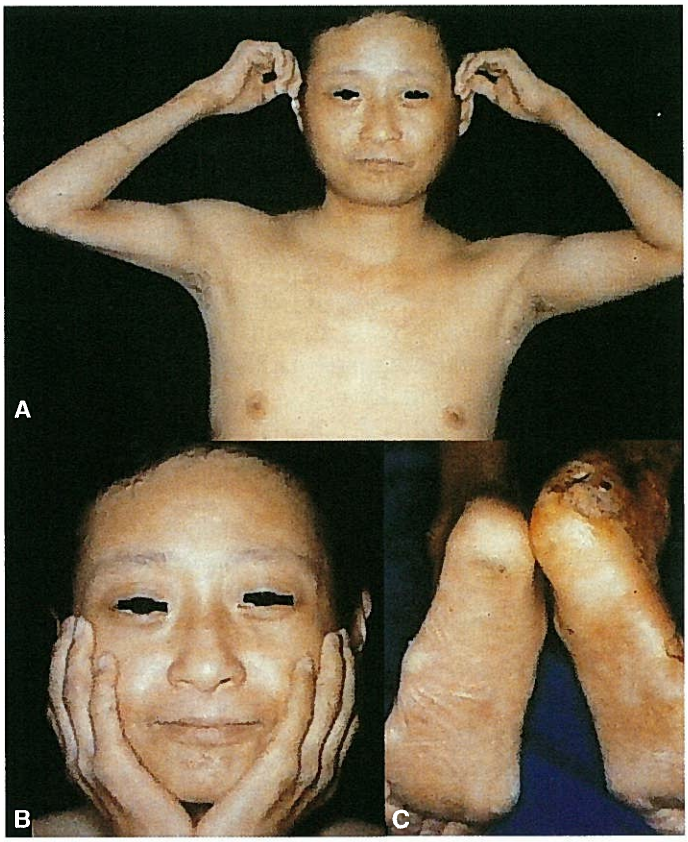

Fig. 2
Werner's syndrome is typified by (A) atrophy of the subcutaneous fat tissue. (B) scleroderma-like features of face and hands. (C) hyperkeratotic calluses and ulcers over feet.

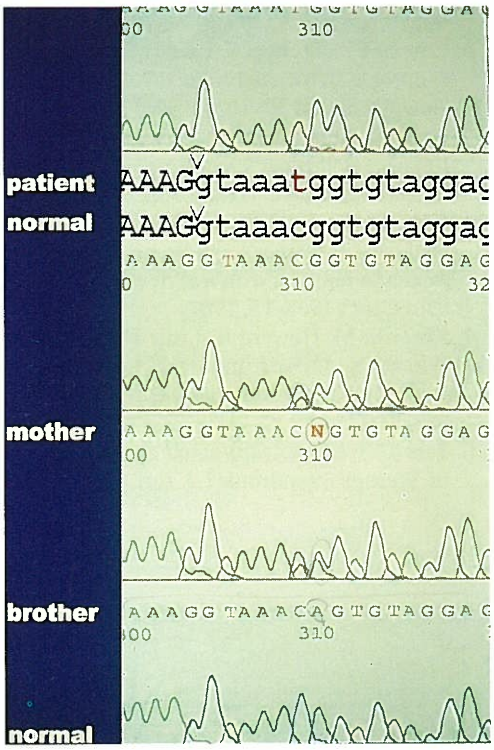

Fig. 3
A, DNA sequencing of the PCR product showed a homozygous mutation of C-to-T transition at nucleotide IVS 25+6 within the intron 25; B&C, DNA sequencing of mother and proband's brother showed a heterozygous mutation of C-to-T transition at nucleotide IVS 25+6 within the intron 25; D, A sequence from a normal subject showed that G to A transition at nucleotide IVS 25+7 is a polymorphism.
∨ : the exon and intron boundary of exon 25

exon 25 (IVS+6). This mutation may be influent of splicing with the generation of a frameshift and termination. Unfortunately, we didn't get the RNA to confirm this novel splicing mutation.

WS is often mistaken for scleroderma. The dermatologist was consulted due to scleroderma-like skin, lower-extremity ulcers or calluses, thinning and graying of hair or baldness, nail dystrophy, wrinkling and aging of the face or skin malignancies. Patients should have a thorough clinical and laboratory work-up and clinicians must keep in mind their high risk for cancers.

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