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ORIGINAL ARTICLE

Early-onset diabetes mellitus as a presenting feature of Werner's syndrome in an Indian family

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Abstract

Background: Diabetes mellitus (DM) in children and adolescents is typically caused by type 1 DM, followed by type 2 DM and maturity-onset diabetes of the young (MODY). We report an unusual Asian Indian family in which three members presented with DM at ages 15, 20, and 30, but not fitting the typical clinical picture of type 1 DM, type 2 DM, or MODY. The primary objective was to elucidate the molecular genetic basis of DM in this family.

Methods: The proband, a 22-year-old man, had short stature, gray hair, osteoporosis, and markedly reduced subcutaneous fat on the body, especially on the extremities along with acanthosis nigricans, and developed myxoid malignant peripheral nerve sheath tumor. Detailed family history revealed multiple loops of consanguinity. The proband underwent whole-genome sequencing, and seven relatives underwent whole-exome sequencing.

Results: The proband and three additional family members were found to have the homozygous c.561A>G nucleotide variant of WRN RecQ-like helicase (*WRN*) gene consistent with the diagnosis of Werner's syndrome. The c.561A>G variant induces a new splicing site on exon 6 resulting in a truncated WRN protein, p.Lys187Trpfs*13.

Fieke W. Hoff and Chao Xing contributed equally to the work.

Conclusion: Our report brings to attention the onset of DM during childhood or early adulthood in patients with Werner's syndrome who typically develop type 2 DM around the age of 30–40 years. Presence of consanguinity among parents, dysmorphic features, and malignancy should prompt consideration of diagnosis of Werner's syndrome.

KEYWORDS

diabetes mellitus, lipodystrophy, myxoid malignant peripheral nerve sheath tumor, Werner's syndrome, WRN

1 | INTRODUCTION

Diabetes mellitus (DM) in children and adolescents is typically caused by autoimmune damage to β-cells called type 1 DM. With rising prevalence of obesity among children and adolescents in the USA, we are also seeing more type 2 DM among these age groups. The recent prevalence estimates from the United States reveal that 215 per 100,000 children and adolescents have type 1 DM and 67 per 100,000 have type 2 DM, with prevalence of type 2 DM particularly increasing in Hispanics and African Americans (Lawrence et al., 2021). In addition to these two major subtypes of DM, some children develop maturity-onset diabetes of the young (MODY) due to pathogenic variants in MODY-associated genes, implying the existence of another type of insulin deficiency characterized by defective control of insulin secretion (Froguel et al., 1993; Hattersley et al., 2008; Macfarlane et al., 1999; Tuomi et al., 2014; Yamagata et al., 1996). Understanding the precise underlying cause of DM is important not only for prognostication but also for selecting proper therapeutic approach to prevent long-term complications of DM. Here, we report an unusual case of a 22-year-old Asian Indian man who presented with childhood onset of DM, who did not fit the typical picture of type 1 DM, type 2 DM, or MODY, and was discovered to have Werner's syndrome on whole-genome sequencing (WGS). Detailed investigations of the other family members revealed three other relatives with homozygous pathogenic variant in WRN RecQ-like helicase (*WRN*) gene (OMIM 277700), of whom two had early-onset DM.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

The protocol was approved by the Institutional Review Board (approval number: STU 082010–274) of UT Southwestern, Dallas, TX, USA. All subjects, including the patient and his family members, provided a written informed consent.

2.2 | Case descriptions

2.2.1 | WS 400.30

The proband, currently a 22-year-old Asian Indian man, presented to his primary care physician at the age of 15 years with symptoms of failure to thrive. At that time, his laboratory studies revealed a random blood glucose of 310 mg/dL (normal range 70–100 mg/dL) with a hemoglobin A1c of 11.4% (101 mmol/mol) (normal <5.6%) consistent with the diagnosis of DM. There was no evidence of ketoacidosis (blood pH was 7.34 (normal range 7.35–7.45), and serum beta-hydroxybutyrate level was 0.2 mmol/L (normal range 0.2–2.8 mmol/L)), and there were no ketones detected on urinalysis. Serum islet cell, insulin, and glutamic acid decarboxylase autoantibodies were all negative. Serum triglycerides were 268 mg/dL (normal <90 mg/dL), low-density lipoprotein cholesterol (LDL-C) was 78 mg/dL (normal <110 mg/dL), high-density lipoprotein cholesterol (HDL-C) was 27 mg/dL (normal >45 mg/dL), and total cholesterol was 140 mg/dL (normal <170 mg/dL). Serum alkaline phosphatase was 270 U/L (normal range 40–150 U/L), aspartate aminotransferase (AST) was 61 U/L (normal range 10–45 U/L), alanine aminotransferase (ALT) was 147 U/L (normal range 10–50 U/L), bilirubin was 0.65 mg/dL (normal range 0.1–1.3 mg/dL), amylase was 77 U/L (normal range 30–110 U/L), and lipase was 349 U/L (normal range 114–340 U/L). Serum thyroid-stimulating hormone (TSH) was 3.3 μU/mL (normal range 0.4–5.02 μU/mL) with a serum-free thyroxine (T4) level of 0.72 ng/dL (normal range 0.8–2.7 ng/dL) on levothyroxine 50 μg daily. An overview of clinical, metabolic, and other biomarkers is listed in Table S1.

At age 19 years, his blood glucose levels were relatively well-controlled (pre-prandial glucose measurements

in the range of 130–150 mg/dL) on 15 units of insulin glargine, 5 units of insulin aspart with each meal, and metformin 500 mg twice daily without any hypoglycemic episodes. On physical examination, he was 149.1 cm tall (−1.5 standard deviations (SD) of the mean, mid-parental height: 155.8 cm) with a body weight of 33.2 kg and a body mass index of 15 kg/m^2. He had lean extremities without much subcutaneous fat (Figure 1a–e). He had several gray hairs without evident hair loss; his eyes were prominent, but without proptosis; he had normally formed ears; no cataract or dorsocervical fat pad; a normal philtrum and tongue size; intact palate; and normal creasing patterns on the palms. He was noted to have a dry skin with mild acanthosis nigricans, but no striae or hyperextensible joints. A swollen lesion with normal function and range of motion of the right forearm was present on the wrist located at the same area where a lipoblastoma-like tumor was resected five months prior (Figure 1f). His reflexes and muscle tone were normal. He interacted appropriately for his age, and there was no history of developmental delays or

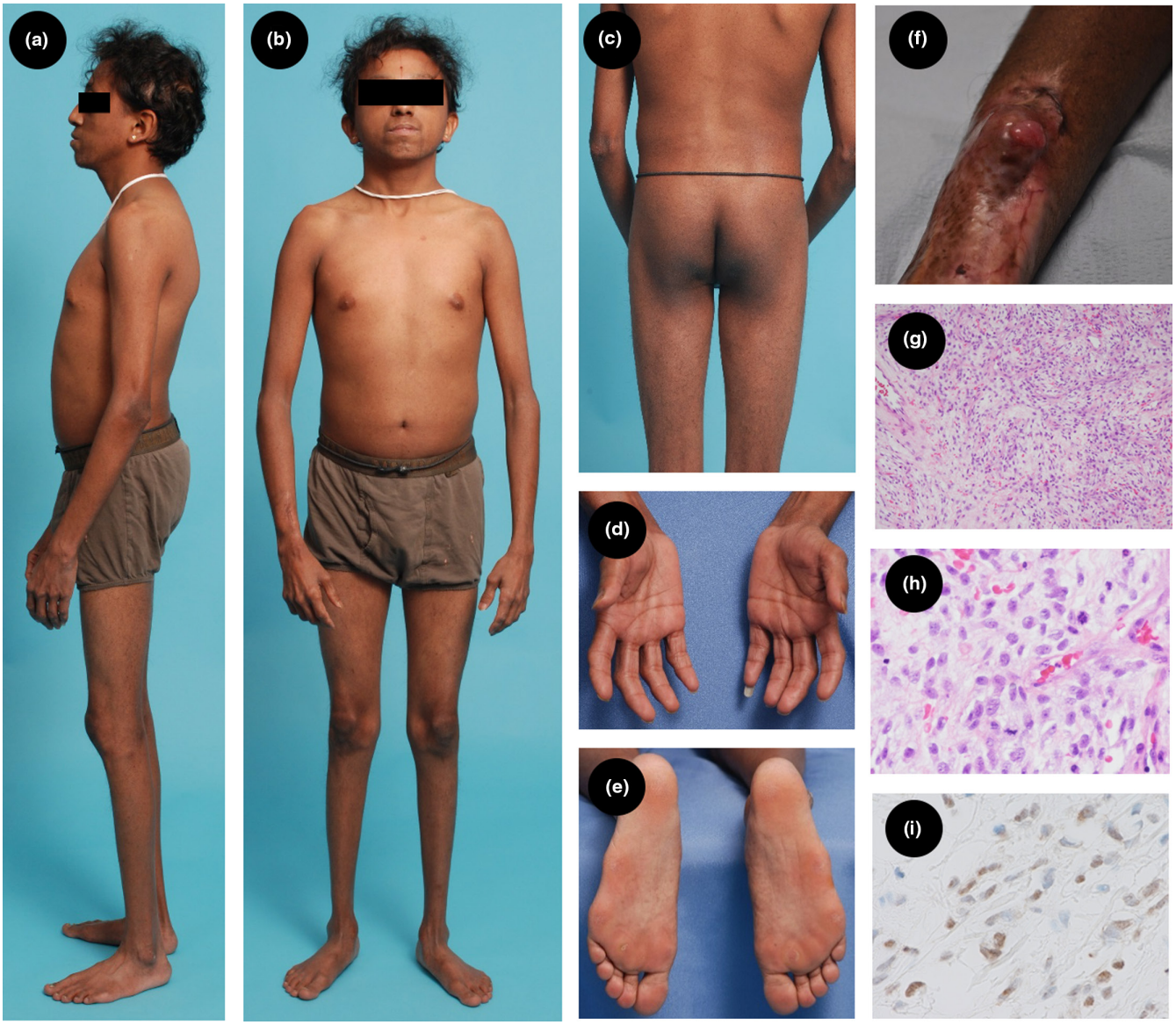

FIGURE 1 Clinical features of the proband at the age of 19 years. (a–c) Lateral, anterior, and posterior views of the patient showing scarce subcutaneous adipose tissue and muscle wasting of the upper and lower extremities with increased abdominal visceral adipose tissue. (d,e) Lipodystrophy of the hands and feet. (f) Right forearm lesion (recurrent) consistent with lipoblastoma, located distal to the area of his primary resection indicated by a well-healed scar. (g) Histopathology of the original biopsy of the peripheral myxoid malignant peripheral nerve sheath tumor, showing a cellular myxoid spindle cell tumor with mild nuclear pleomorphism and variably sized but mostly thin-walled blood vessels (100X). (h) Higher power view of that biopsy, demonstrating two mitotic figures, at 2 o'clock and 6 o'clock, with scant tumor cell cytoplasm and abundant intercellular myxoid material (400X). (i) Immunohistochemical stain for S-100 protein shows brown positive staining in more than half of the tumor cell nuclei (S-100, 600X).

neurocognitive impairments. Dual-energy X-ray absorptiometry (DEXA) showed a total body fat percentage of 22% (63rd age-matched percentile), with 26% visceral fat (80th percentile), lower extremity fat of ~17% (25th percentile), upper extremity fat of 17% (53rd percentile), and a trunk/limb fat mass ratio of 1.53 (99th percentile). Skinfold thickness measurements revealed reduced skinfold thickness below the 10th percentile of normal at the triceps, abdomen, suprailiac, and thigh regions (Figure S1). DEXA scan identified a total bone mass density below normal (Z-score −2.8; height-adjusted Z-score −3.34 (Zemel et al., 2011)). A hand X-ray was obtained during his initial presentation, which showed advanced bone age (>2 SD) older than the mean of his chronological age based on the method of Greulich and Pyle (1950).

His parents were consanguineous (second-degree cousins), and multiple layers of consanguinity were noted in the family (Figure 2a). His maternal uncle (WS 400.15) and first cousin (daughter of his maternal aunt) (WS 400.26) were also noted to have early-onset diabetes.

On follow-up, a new mass was noticed on the area of his previously resected lipoblastoma, and biopsies showed recurrence of an adipocytic neoplasm most consistent with lipoblastoma-like tumor with negative S-100 protein immunostain and negative sarcoma fusion next-generation sequencing panel (Figure 1f). He underwent resection of the lesion with a second recurrence from a deeper focus of disease within weeks, requiring re-excision with skin graft of the forearm after which he did well for a year. However, the lesion returned again, and this time pathology was consistent with a rare, intermediate-grade, myxoid malignant peripheral nerve sheath tumor (MPNST) with positive S-100 protein immunostain, loss of *Rb1*, and loss of trimethylation of histone H3K27me3 (Figure 1g–i). While he initially underwent radiation therapy with regression of the tumor, a second focus of the disease appeared. He was started on neoadjuvant chemotherapy with intravenous ifosfamide (10 g/m^2, 5 days per cycle) and doxorubicin (75 mg/m^2) with the plan to proceed with limb salvage surgery. Based on the aggressiveness of the tumor, depth,

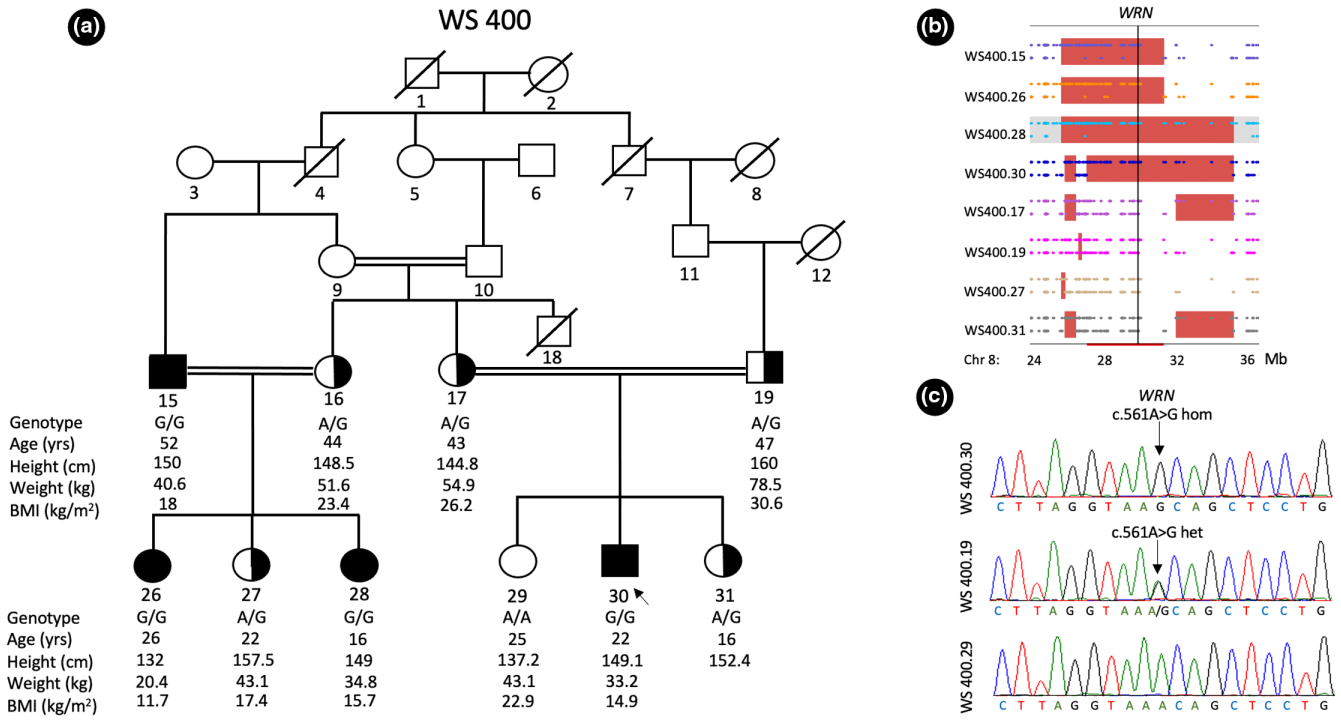

FIGURE 2 (a) Pedigree of the proband (arrow, WS 400.30) and his family members. Affected individuals with homozygous c.561A>G nucleotide variant of *WRN* are shown as filled black symbols. Heterozygous subjects are shown as half-filled symbols. Individual with wild type allele is shown as unfilled symbol. The pedigree WS 400 was consanguineous, and the parents of the affected subject were second cousins. Males are shown as *squares* and females as *circles*. Genotype, age, height, weight, and BMI are shown for each homozygous, heterozygous and wild type family member. (b) Schematic of segments on chromosome 8 of individuals that underwent next-generation sequencing, based on GRCh37/hg19 coordinates. For each individual, the top line displays markers with homozygous genotypes and the bottom line displays markers with heterozygous genotypes. The inferred homozygous regions are highlighted in color blocks: red for regions common to more than one individual and gray for regions unique to one individual. The *WRN* locus is indicated. (c) Sequence electropherogram of DNA for WS 400.30 (top) showing the homozygous (hom), WS 400.19 (middle) showing heterozygous (het) *WRN* c.561A>C variant and WS 400.29 (bottom) showing the wild-type *WRN* sequence.

and size (9 cm), as well as poor evidence in the literature that the tumor would be sensitive to chemotherapy, the decision was ultimately made to perform forearm amputation at the age of 21 years post two cycles of chemotherapy.

2.2.2 | WS 400.15

The proband's maternal uncle, currently a 52-year-old man, was diagnosed with DM in his thirties with a most recent HbA1c of 7.6% (60 mmol/mol) and a random blood glucose level of 99 mg/dL, treated on glimepiride and metformin. His fasting insulin was 12.3 μU/mL (normal 1.9–23 μU/mL), testosterone 284.06 ng/dL (adult male 86.49–788.22 ng/dL), and urinary protein 193.3 μg/mL with a urinary albumin-to-creatinine ratio of 189.5 μg albumin/mg of creatinine (normal <30). He appeared older than stated age and had a short stature and failure to thrive with a BMI of 16.7 kg/m^2. He walked with a limp and had a mottled and dry skin with decreased plantar fat and loss of foot arches. He also had loss of thenar muscles with inability to oppose right thumb and hypopigmentation over the elbow. He experienced premature graying with gradual loss of hair since the age of 35 years, and bilateral cataracts at the age of 35 years for which he underwent surgery. He also had non-healing ulcers with recurrent calluses over the feet and ankle.

2.2.3 | WS 400.26

This 26-year-old woman appeared much older than stated age with a thin habitus and short stature and a BMI of 11.7 kg/m^2. She had a mottled skin, marked reduction in muscle mass, and subcutaneous fat with thin extremities. There was decreased plantar fat and loss of foot arches. She developed bilateral cataracts for which she underwent surgery at age 14, began to experience thinning of the hair and graying at very early age, and was almost completely bald at the age of 14 years. She was diagnosed with primary amenorrhea till the age of 18 years, followed by irregular menses and premature ovarian failure as evidenced by serum follicle-stimulating hormone level of 75 mIU/mL (normal range 1.5–12.4 mIU/mL), luteinizing hormone level of 27 mIU/mL (post-menopause range 16–54 mIU/mL), and prolactin level of 1.68 ng/mL (normal 2.8–29.2 ng/mL), with no visible ovaries on ultrasound. She had thyromegaly with a right thyroid nodule which on fine-needle aspiration biopsy revealed benign pathology. She had a prominent nose, large ears, and a deep voice. She developed painless bumps growing on her right forearm and lower back due to presumed calcinosis. She was diagnosed with DM at the age of 20 years after being found to have severe hyperglycemia causing an episode of syncope. Her laboratory values at the age of 22 years were notable for a normal complete blood count, normal serum electrolytes, and a normal kidney function, with an elevated fasting glucose of 244 mg/dL (fasting, normal <100 mg/dL) and a HbA1c of 12.4% (112 mmol/mol) (normal <5.6%) on 18 units of short-acting insulin aspart twice a day. Serum C-peptide was 2.89 ng/mL (normal range 0.81–3.85 ng/mL). Her poorly controlled DM has resulted in blindness of the right eye due to retinopathy leading to hemorrhage. She also had albuminuria with urinary microalbumin 496 μg/mL (normal <25 μg/mL) and urinary albumin-to-creatinine ratio of 1116.1 μg albumin/mg of creatinine. Liver function tests were abnormal, and she had hypertriglyceridemia. Her parents are consanguineous (father, WS 400.15, is mother's maternal uncle, Figure 2a).

2.2.4 | WS 400.28

This 16-year-old girl has a BMI of 15.7 kg/m^2 (height 149 cm, weight 34.8 kg) but has no evidence of premature aging. Laboratory blood values at age 12 years were notable for a normal complete blood count and a normal metabolic panel, with a low normal creatinine adjusted for her age of 0.5 mg/dL (normal range 0.51–0.95 mg/dL), and normal liver function tests, lipid panel, and thyroid function. Her HbA1c was mildly elevated at 5.7% (39 mmol/mol), consistent with the diagnosis of prediabetes. She had menarche at age 11 years and normal pubertal growth spurt. At age 16 years, her fasting and post-prandial blood glucose values were 84 mg/dL and 98 mg/dL, respectively.

2.3 | DNA sequencing

Genomic DNA was isolated from peripheral blood samples using the Easy-DNA Kit (Invitrogen, Carlsbad, CA). The proband (WS 400.30) underwent whole genome sequencing (WGS), and his seven relatives (WS 400.15, 400.17, 400.19, 400.26, 400.27, 400.28, and 400.31) underwent whole-exome sequencing (WES) using the IDT xGen Exome capture kit on the Illumina platform. For both experiments, the sequencing read length was paired-end 2 ×150 base pairs (bp). Sequences were aligned to the human reference genome b37. The WGS mean coverage was 33-fold, and the mean coverage of the targeted regions for WES ranged between 48-fold and 106-fold. Genetic variations were called using the Genome Analysis Toolkit (McKenna et al., 2010) and annotated using SnpEff (Cingolani et al., 2012). Given this is a consanguineous family, we hypothesized an autosomal recessive inheritance, and we therefore mapped the disease gene by a combination of two approaches. First, we searched for runs of homozygosity (ROH) greater than

1 Mb and shared by the three affected individuals (WS 400.15, 400.26, and 400.30), but not by the proband's unaffected parents (WS 400.17 and 400.19) using BCFtools/ROH (Narasimhan et al., 2016). Second, we filtered for rare conserved homozygous variants in the affected individuals, but not in the unaffected. The criteria include minor allele frequency less than 0.01 in the Genome Aggregation Database (v2.1.1, http://gnomad.broadinstitute) and the GERP score (Davydov et al., 2010) greater than 2. These eight individuals and two additional individuals (WS 400.16 and 400.29) underwent Sanger sequencing to confirm segregation of the candidate variants within the pedigree.

2.4 | RNA sequencing

Total RNA was extracted from whole blood samples using the RiboPure RNA Purification Kit (Catalog number #AM 1928, Invitrogen by Thermo Fisher Scientific) following the manufacturer's suggested protocol. A total of 1–20 μg RNA was treated with *DNase I* from the DNase-free kit from Ambion (catalog number # AM1906, Grand Island, NY). Complementary DNA (cDNA) was made using 1–2 μg *DNase I*-treated RNA using the Reverse Transcription Kit from Applied Biosystems Inc (catalog number # N8080234, Carlsbad, CA). RT-PCR was carried out using 20 ng complementary DNA and 2.5 mM primer pair (F 5′-tttccccagggattaaaaatg-3′ and R 5′-gcaaacctttgcacagtatca-3) located in exons 5 and 7 to capture any possible alternate splicing. Conventional touchdown PCR protocol was used for the amplification. The amplified PCR product was analyzed in an agarose gel and sequenced. Primers used were synthesized by Integrated DNA Technologies (Coralville, IA). The strategy for cDNA (obtained from total RNA) amplification, detection on an agarose gel, Sanger sequencing of the homozygous truncated PCR product, and its alignment at nucleotide and protein levels is shown in Figure 3.

3 | RESULTS

The ROH analysis revealed 3 genomic regions of >1 Mb shared by the three affected but not by the two unaffected family members: chr8:27764627–32505633, chr16:64980264–66413195, and chr18:47320560–49867223, and there was only one variant in the *WRN* gene, located in the chromosome 8 ROH region (Figure 2b), passing the filtering criteria. The affected carried a homozygous variant NC_000008.10:g.30924605A>G (rs775802030, NM_000553.6:c.561A>G, p.Lys187=) in *WRN* (Figure 2c). The homozygous variant was previously reported to cause Werner's syndrome by creating a cryptic splice site that results in frameshift deletion (Saha et al., 2013). Note that individual WS 400.28 also shares this ROH region and carries the homozygous c.561A>G variant, though she has almost no signs and symptoms of Werner's syndrome. To confirm this variant is causal in the current pedigree, we first performed in silico analysis using SpliceAI (Jaganathan et al., 2019). It predicted that the probability of the position 8:30924602 (=30924605-3) to be used as a splice donor increases by 0.78, which is consistent with the previous study (Saha et al., 2013). We followed the in silico prediction and confirmed the predicted alternate cryptic donor splice site three bases upstream of the putative variant upon Sanger sequencing of the patient's cDNA (Figure 3). The alternative splice site deletes 98 bases, r.557_654del98, which results in a frameshift and a truncated protein p.Lys187Trp*fs**13.

4 | DISCUSSION

At initial presentation, the precise etiology of DM was unclear in the proband. Because of reduced subcutaneous fat on the extremities and acanthosis nigricans, a presumptive diagnosis of lipodystrophy was considered. However, his clinical features were not consistent with either congenital generalized lipodystrophy or familial partial lipodystrophy (Garg, 2004). Therefore, we conducted WGS/WES in the family, which resulted in establishing the diagnosis of Werner's syndrome. As more details of the affected relatives in India emerged, the affected subjects revealed many of the typical clinical manifestations of Werner's syndrome, including short stature, low body weight, endocrinopathies, graying and loss of hair, bilateral cataracts, advanced bone age with osteoporosis, and scleroderma-like skin changes (Huang et al., 2006; Takemoto et al., 2013). Werner's syndrome is an autosomal recessive disorder characterized by an accelerated intrinsic aging process first described by Dr. Werner at the Kiel University in 1904 (Werner, 1985). It is caused by biallelic pathogenic variants of the *WRN* gene, resulting in a loss of function of WRN protein, which has dual exonuclease and helicase activities (Oshima et al., 1996; Yu et al., 1996). Werner's syndrome is a rare disease estimated to occur in 1:1,000,000 to 1:10,000,000 live births with a relatively high prevalence reported in Japan, Sardinia, India, and Pakistan (Saha et al., 2013).

Werner's syndrome is also associated with metabolic disorders at a high rate, and various endocrinopathies have been reported including type 2 DM, dyslipidemia, and hypogonadism in both males and females, as well as atherosclerosis and osteoporosis (Goto et al., 1996; Lauper et al., 2013; Okabe et al., 2012; Oshima et al., 2016; Takemoto et al., 2021). It was already reported in 1966 by Epstein et al. (1966) that 50%–70% of the Werner syndrome patients will develop type 2 DM. DM associated

with Werner's syndrome is often characterized by insulin resistance and hyperinsulinemia associated with a low BMI with accumulated visceral fat. DM in Werner's syndrome patients presents at an average age of onset between 30 and 40 years, and it is unclear what contributed to much earlier onset of DM in two of our patients (i.e., additional genetic or environmental factors) (Epstein et al., 1966; Goto et al., 1996; Takemoto et al., 2013). Only two previous cases of genetically confirmed Werner's syndrome have been reported to develop DM during childhood. The first one is an 18-year-old girl with compound heterozygous variants of *WRN* (c.1270-2A>T and c.3020delG), who was diagnosed with type 2 DM with normal levels of glutamic acid decarboxylase and insulin autoantibodies (Wang et al., 2022). Another 28-year-old woman with compound heterozygous *WRN* variants (c.1290_1293del and c.2732+5G>A) presented with partial lipodystrophy, fibrosing steatohepatitis, and insulin-resistant DM at the age of ten years in the absence of progeroid features (Atallah et al., 2022). Our report

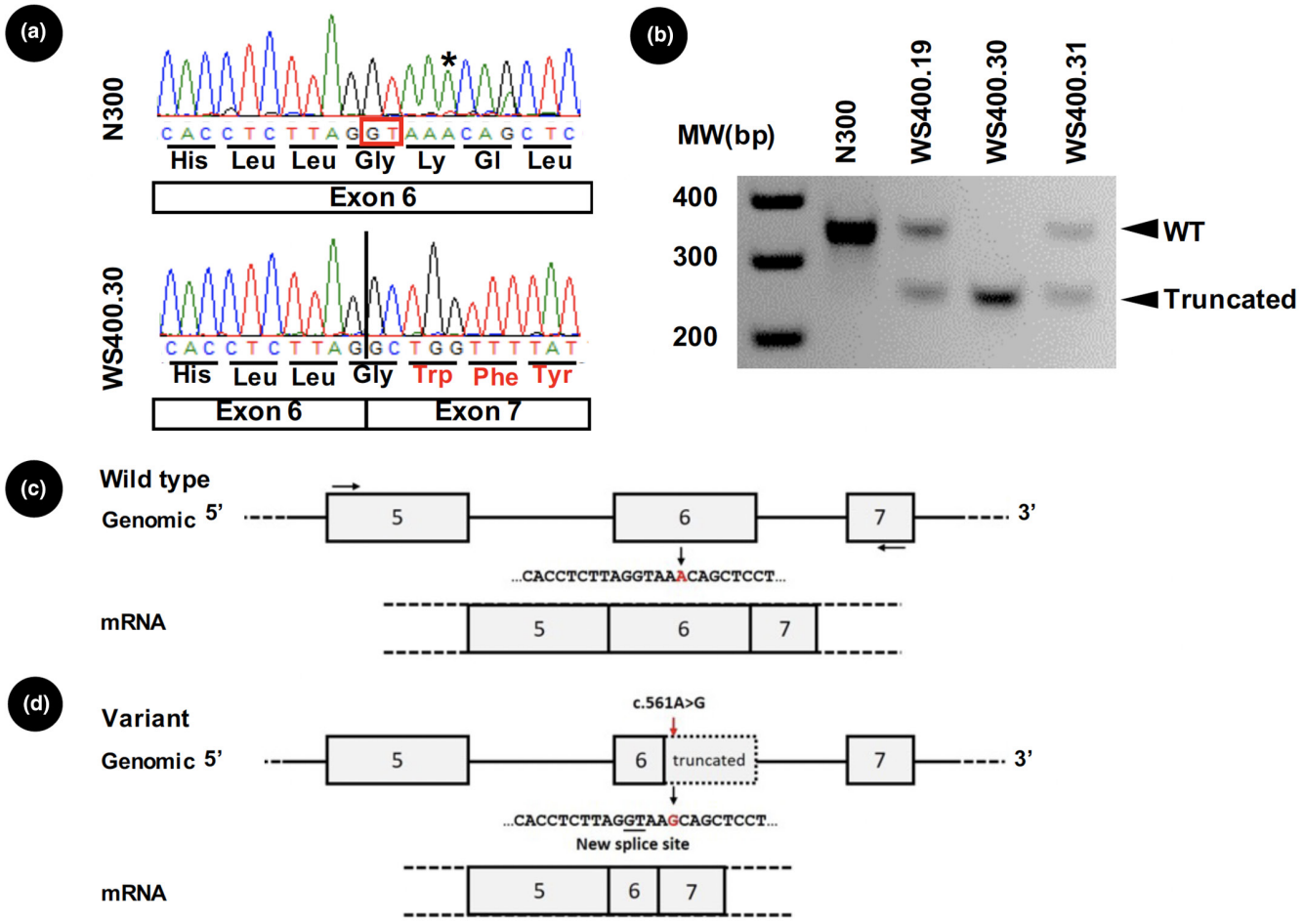

FIGURE 3 (a) Partial electropherogram of cDNA derived from peripheral blood RNA showing the nucleotide sequences for unaffected (N300) and affected (WS 400.30) patient with the corresponding amino acids shown below the nucleotides. The altered nucleotide is marked with an asterisk. The cryptic dinucleotide splice donor is boxed in red. The vertical black line in WS 400.30 indicates the resulting alternatively spliced mRNA. Because of the frameshift, the translation of the nucleotides in exon 7 will result in an aberrant protein, as shown in red text. (b) The cDNA was amplified with primer pair: F 5′-tttccccagggattaaaaatg-3′ and R 5′-gcaaacctttgcacagtatca-3′. The location of the primer pair used is in panel c. The PCR product from WS 400.30, who was homozygous for the variant, yielded one truncated PCR product (258 bp), which was sequenced. Shown also are the PCR products amplified from WS 400.19 and WS 400.31, who were heterozygous for the variant, and showed both the expected wild type (WT) (356 bp) and the truncated (258 bp) PCR product. Unaffected fibroblast (N300) cDNA was used as a positive control. (c) Illustration of WT splicing process. Shown is the partial gene structure of human *WRN* exons 5–7. Exons are shown as boxes and are drawn to scale; introns are denoted by lines but not to scale. The WT sequence where the variant occurs is also shown below exon 6 in red. Also shown is the partial splicing of the exons for mRNA. (d) Illustration of aberrant splicing process resulting from the variant. Shown is the partial gene structure of the *WRN* and the variant c.561A>G in exon 6. The GT dinucleotide [splice site acceptor] is underlined, and the mutated base is in red. Because of this variant, the cryptic splice site is activated, resulting in a frameshift and truncated protein. Also shown is the altered mRNA.

TABLE 1 Clinical features of the proband and his three additional family members diagnosed with Werner's syndrome and two previously reported patients with identified homozygous c.561A>G *WRN* variant.

	WS 400.30	WS 400.15	WS 400.26	WS 400.28	KERA1010 (Saha et al., 2013)	YOSI1010 (Saha et al., 2013)
Ancestry	India	India	India	India	India	Pakistan
Age	22	52	26	18	23	32
Sex	M	M	F	F	M	F
Height (cm)	149	150	132	149	148	Unknown
Weight (kg)	33.2	40.6	20.4	34.8	29	Unknown
BMI (kg/m^2)	14.9	18	11.7	15.7	13.2	Unknown
Short stature	+	+	+	+	+	+
DM (age of onset)	15	30	20	−	26	Unknown
Cataract	−	+	+	−	+	Unknown
Gray hair	+	+	+	−	+	+
Endocrine disorders	Osteoporosis, hypothyroidism, and lipodystrophy	−	Primary amenorrhea, premature ovarian failure, thyromegaly, and calcinosis	Prediabetes	Gynecomastia and testicular atrophy	Osteoporosis and osteomalacia
Metabolic disorders		Hypertriglyceridemia, hypercholesterolemia, and hyperlipidemia			Hypertriglyceridemia and hepatic steatosis	
Malignancy	Myxoid malignant peripheral nerve sheath tumor					Stage IV malignant melanoma
Others		Non-healing ulcers with recurrence calluses over the feet and ankle, mottled and dry skin, decreased plantar fat with loss of foot arches, loss of thenar muscles.	Prominent nose, large ears, and deep voice		Palmoplantar hyperkeratosis	Pinched facial features and miscarriages

Abbreviations: BMI, body mass index; DM, diabetes mellitus.

strongly suggests consideration of Werner's syndrome in differential diagnosis of lean patients with childhood-onset DM who do not have evidence of autoimmunity.

Our proband and two of his maternal cousins and uncle were found to have the homozygous c.561A>G single-nucleotide variant in *WRN*. While this *WRN* variant is not predicted to result in a change of the corresponding amino acid (p.Lys187=), it induces a new splicing site on exon 6 resulting in a 98 bp deletion and an alternatively spliced truncated protein causing a loss of function of the WRN protein. This gene variant was previously reported in two other patients of South Asian ancestry (Saha et al., 2013) (Table 1). Moreover, it resides on the same haplotype as that in the previous two patients (Saha et al., 2013), which suggests it is a founder mutation in South Asians.

The mechanism of DM in Werner's syndrome is still largely unknown. Hyperinsulinemia and insulin resistance are well documented in those with DM, and our proband also had acanthosis nigricans indicating the presence of insulin resistance. Like our proband, some patients with Werner's syndrome have been reported to have partial lipodystrophy with lack of subcutaneous fat from the extremities but increased visceral fat (Atallah et al., 2022). It is therefore likely that limited triglyceride storage in peripheral adipose tissue may predispose patients to DM and hepatic steatosis. Autopsy reports have not revealed any β-cell or islet atrophy or islet amyloidosis (Ishii, 1976; Ishii et al., 1985).

As stated above, WRN protein is a member of the RecQ DNA helicase family involved in homologous recombination and non-homologous end-joining DNA repair mechanisms. Mutations of other members of this family are seen in Bloom's syndrome and Rothmund–Thomson's syndrome, all of which are characterized by increased genomic instability and increased cancer predisposition (Sharma et al., 2020). While the association of Werner's syndrome with elevated risk of malignant tumors is widely reported in the literature, it is often associated with thyroid neoplasms, melanoma, and bone and soft tissue sarcomas, and only four cases of MPNST have been reported in the literature so far (Goto et al., 1996; Rosa et al., 2003). MPNST is extremely rare, but devastating tumor that originates in the nerve sheath with a 5-year survival of 35%–60% (Ducatman et al., 1986; Stucky et al., 2011). The only curative treatment option is resection with a sufficient wide margin, often requiring amputation of the limb as was done in our proband.

Finally, so far, WS 400.28 has minimal signs and symptoms of Werner's syndrome as compared to the proband and her elder sister who are severely affected. Given the current young age, future follow-up of this girl will reveal the severity of the phenotype of Werner's syndrome.

In conclusion, we report an Indian man with Werner's syndrome who presented with childhood-onset DM and developed an aggressive malignancy requiring amputation at a young age. Clinicians should pay specific attention to a family history of consanguinity in a patient presenting with early onset of DM in the presence of other dysmorphic clinical features as these could all be seen in the context of a genetic syndrome. Although it is still unclear why endocrinopathies are associated with Werner's syndrome at such a high rate, we recommend early routine surveillance for DM and other age-related metabolic derangements in patients diagnosed with Werner's syndrome. Given the elevated risk of aggressive cancer, all individuals with Werner's syndrome should be screened indefinitely for cancer starting at a young age.

AUTHOR CONTRIBUTIONS

Fieke W. Hoff: writing – original draft; writing – review and editing. **Chao Xing**: formal analysis and methodology. **Vinaya Simha**: data curation; writing – review and editing. **Anil K. Agarwal**: formal analysis and methodology. **Xunzhi Zhang**: formal analysis and methodology. **Leena Lekkala**: data curation; writing – review and editing. **Madhumati S. Vaishnav**: data curation; writing – review and editing. **Frank Vuitch**: data curation; writing – review and editing. **Abhimanyu Garg**: conceptualization; methodology; writing – original draft; and writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest relevant to this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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