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RESEARCH NOTE

A novel *POLH* gene mutation in a xeroderma pigmentosum-V Tunisian patient: phenotype–genotype correlation

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Introduction

XP occurs at higher frequency in Tunisia (1:10,000) than in Japan (1:22,000) (Hirai *et al.* 2006) and the United States (1 per million) (Kleijer *et al.* 2008). XP-V cells are unable to synthesize intact daughter DNA strands on UV-irradiated templates resulting from an inability to carry out translesion synthesis (Lehmann *et al.* 1975; Masutani *et al.* 1999). Approximately 20% of XP patients belong to XP-V complementation group (Gratchev *et al.* 2003). In Tunisia, XP is classified into three clinical forms: severe, intermediate with or without neurological abnormalities, and moderate (Zghal *et al.* 2006). Previous molecular investigation showed homogeneity of mutational spectrum in *XPA* and *XPC* genes (Ben Rekaya *et al.* 2009; Messaoud *et al.* 2010a,b). The moderate clinical form of XP is characterized by mild dermatological manifestations, no neurological abnormalities and late onset of skin cancers. The median age of onset is 4 years. Mild skin symptoms and late onset of skin tumours have been already described in XP-V (Tanioka *et al.* 2007), XP-F (Matsumura *et al.* 1998) and XP-E patients (Rapić-Otrin *et al.* 2003). Post-UV cell survival in the presence or absence of caffeine (Itoh *et al.* 2000), unscheduled DNA synthesis (UDS) and detection of polymerase eta employing Western blot (Tanioka *et al.* 2007) cannot define exactly the molecular defects are in the polymerase eta. These laboratory assays are used to find out the UV sensitivities of the patients' cells and the DNA repair status of their cells as well as reduced levels or absence of expression of the protein predicts the defects in the polymerase eta. These parameters are very helpful in characterizing the XP patients' cells. The nucleotide sequencing confirms the defects in the XP genes. In the present study, we report the genetic and molecular analyses of *POLH* gene in a Tunisian patient with mild clinical phenotype suspected to be XP-V.

Materials and methods

The patient is a 75-year-old man who was hospitalized for an ulcerative tumour of the nose. He was born to consanguineous healthy parents and he originated from northwestern Tunisia. Family history revealed an XP affected brother and a paternal cousin who developed skin cancers after 55 years of age. The patient is a farmer by profession. He has no photophobia or ocular lesion. Although was often exposed to sunlight and had never used sunscreen, he did not sunburn easily. He developed two types of cancer separately: a pigmented basal cell carcinoma (BCC) of the left cheek at the age of 71; and a squamous cell carcinoma (SCC) of the labial commissural at the age of 72 (figure 1). Both were treated by surgery. On examination, the patient showed a poïkiloderma facial appearance with an ulcerative tumour on the left wing of the nose. Histological examination of skin biopsy showed a SCC. No ocular or neurological abnormalities were found. The patient had also hearing impairment and tooth decay. The hearing impairment is late onset according to the interview. No further audiometric test was possible.

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Keywords. xeroderma pigmentosum-V; late-onset phenotype; novel mutation; phenotype–genotype correlation.

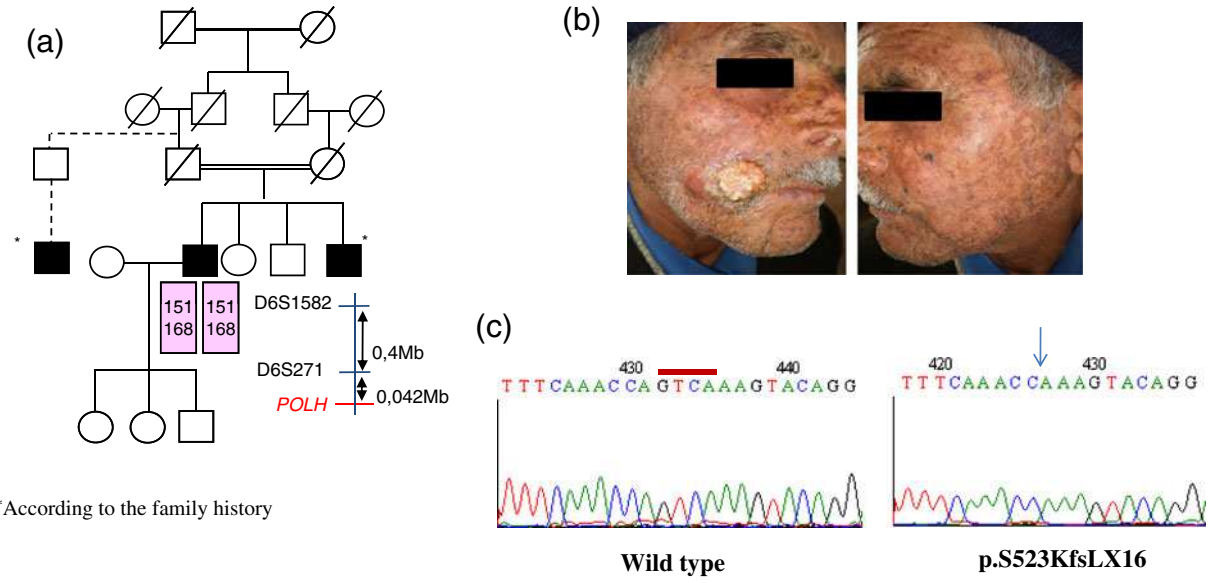

Figure 1. (a) The pedigree of the multiplex XP-V family and haplotype analysis with microsatellite markers close to the *POLH* gene. (b) Clinical photos of the affected patient, showing a poikiloderma facial and a SCC tumour lesion. (c) The sequence electropherogram of the sense strand in exon 11 showing the GTCA deletion which leads to the S523KfsLX16 mutation in comparison with the wildtype sequence of an unrelated control.

After obtaining patient's informed consent, genomic DNA was extracted from whole peripheral blood by salting out method. Linkage to *POLH* gene was performed using two informative microsatellite markers spanning a 0.4 Mb interval near to *POLH* locus (cen-D6S1582, D6S271, (*POLH*) - tel) (figure 1). Mutations were screened by direct sequencing of exons 10 and 11 of *POLH* gene.

Results and discussion

The patient developed his first skin cancer at the age of 71 years. This age is over the average age of onset of skin cancer in XP-V patients, estimated at 45 years (Johnson *et al.* 1999; Masutani *et al.* 1999; Itoh *et al.* 2000; Broughton *et al.* 2002; Tanioka *et al.* 2007; Inui *et al.* 2008; Masaki *et al.* 2008) and it is even higher than the average age of skin cancers in the general Tunisian population (55 years) (Mseddi *et al.* 2007). Hearing abnormalities were not previously described in XP-V patients; this particular association observed in the reported case may be due to his old age (presbycusis). Genetic study showed that the patient had a homozygous haplotype (151–168) for markers D6S271 and D6S1582 suggesting linkage to the *POLH* gene (figure 1). We first screened exon 10 where a previous mutation in a Tunisian XP-V patient was described (Broughton *et al.* 2002) but no mutation was identified. Considering similar genetic background with the Algerian population, we screened exon 11 in which a mutation has been reported in an Algerian patient (Broughton *et al.* 2002). Sequencing of this exon revealed a novel deletion of four bases in homozygous state c.1568_1571delGTCA (figure 1). This small deletion likely leads to a truncated protein; p.S523KfsX16, containing an intact polymerase activity domain located in the first 511 amino acids (Masutani *et al.* 1999) but lacking the terminal nuclear localization signal (C-NLS) situated between amino acids 682–698 and the PCNA (proliferating cell nuclear antigen) binding site located between amino acids 707 and 708 (Yang and Woodgate 2007). This suggests that the mutation found in our patient does not reduce the polymerase activity explaining his mild phenotype. Many mutations in the *POLH* gene have been described in Chinese, American, European and Japanese XP-V patients. Most of them lead to truncated proteins (Masutani *et al.* 1999; Tanioka *et al.* 2007; Itoh *et al.* 2000; Johnson *et al.* 1999; Broughton *et al.* 2002; Inui *et al.* 2008; Masaki *et al.* 2008). Only 10% of reported mutations are located in exon 11 although it corresponds to 42% of the coding region (table 1). This low number of mutations observed in this exon may be due to the under diagnosis of moderate clinical forms (Gratchev *et al.* 2003). No correlation between clinical features and the different *POLH* mutations has been found among previous XP-V reported cases (Tanioka *et al.* 2007; Inui *et al.* 2008). Generally, assessment of phenotype–genotype correlation in patients with XP is complicated because the clinical features are correlated with the degree of exposure to sunlight which is related among others to patient's age. We have previously described a complete phenotype–genotype correlation among Tunisian XP-A and XP-C patients (Ben Rekaya *et al.* 2009; Messaoud *et al.* 2010a,b). To investigate a possible phenotype–genotype correlation we reviewed all XP-V patients reported in the literature who had a mutation in

Table 1. Clinical features of reported XP-V patients with mutations in exon 11 of *POLH* gene.

Patient	Geographic origin	Age (years)	Age of onset of the 1st skin cancer (years)	Number of skin tumours /symptoms	Mutation	State	Reference
XPV86VI	Algeria	32	ND	< 10	c.C1561T→ p.Q521X	Mutations at the homozygous state	Johnson *et al.* (1999)
XP37BR	Scotland	69	ND	< 10	c. Ins C 1668→p. fs556		
XPV12HM	Japan	45	43	Multiple BCC	c.del1661 A →p. 554 fsX30		Tanioka *et al.* (2007)
XPV14KO	Japan	71	71	SCC			Masaki *et al.* (2008)
XPV17KO	Japan	61	No skin cancer	0			
XPV9KO	Japan	13	No skin cancer	0			
XPV11KO	Japan	79	77	SCC			
XPV13KO	Japan	67	36	BCC			
XPV34KE	Tunisia	75	71	2 SCC and 1BCC	c.1568_1571 delGTCA, p.S523KfsX16		Present article
XP1AB	Scotland	51	ND	> 50	c.C1543A/ Del224–226 →p.T548-X/L75	Compound heterozygous mutations	Broughton *et al.* (2002)
Ops4	Japan	8	No skin cancer	Freckles, slight pigmented	c.A1603G/ A1766C →p.K535E/K589T		Itoh *et al.* (2000)
XPV8HM	Japan	39	25	SCC	c.A1766C/C725G→ p.S242X/K589T		Tanioka *et al.* (2007)
XPV16KO	Japan	35	35	BCC			Masaki *et al.* (2008)
XP139DC	USA	19	ND	BCC	p.N359VfsX32/p.T569RfsX10X		Inui *et al.* (2008)

BCC, basal cell carcinoma; SCC, squamous cell carcinoma; ND, not determined.

exon 11 (table 1) (Itoh *et al.* 2000; Broughton *et al.* 2002; Tanioka *et al.* 2007; Inui *et al.* 2008; Masaki *et al.* 2008). Nine patients had mutation at homozygous state in exon 11, while the other five were heterozygous with one mutation in exon 11 and the second located elsewhere. Among the nine homozygous patients three (XPV14KO, XPV11KO and XPV34KE) developed their first skin cancer after the age of 71 years and one 61-years-old patient (XPV17KO) did not have any cancer. In contrast, among the five compound heterozygous patients, two (XPV8HM and XPV16KO) developed their first skin cancer at the age of 25 and 35 years respectively; one patient XP1AB (51 years old) developed more than 50 skin cancers and the patient XP139DC had BCC at 19 years. For Ops4, who has two mis-sense mutations in the catalytic domain, as he was 8 years old, he is too young to develop skin cancers (table 1). It seems that patients having a mutation at the homozygous state in exon 11 shared a milder phenotype than patients being compound heterozygous (only one allele mutated in exon 11 and the other affecting upstream of exon 11 thus likely affecting the catalytic domain) (figure 2). A previous study revealed that healthy individuals who are heterozygous for *POLH* mutation showed reduced levels of recovery of replicative DNA synthesis in the presence of caffeine after UV irradiation (Itoh *et al.* 2000). A phenotype–genotype correlation could be established in XP-V patients when a distinction is made between homozygous and compound heterozygous patients. Based on all cases published analysis, we hypothesize that mutations outside the catalytic domain of pol eta is always associated with a very mild phenotype regardless of the type of mutation. Generally many factors might be related to the development of skin cancers such as the localization of the mutation, accumulation of sun exposure, lifestyle of a patient and other genetic determinants like protective polymorphisms (Masaki *et al.* 2008).

Taking into account the geographic and demographic features in Tunisia, i.e a sunny weather exposing to UV radiation and the high rate of endogamy that contributes to an increase in genetic diseases, it would be of great interest to perform mutation screening of *POLH* gene in patients showing photosensitivity in an effort to prevent skin cancer.

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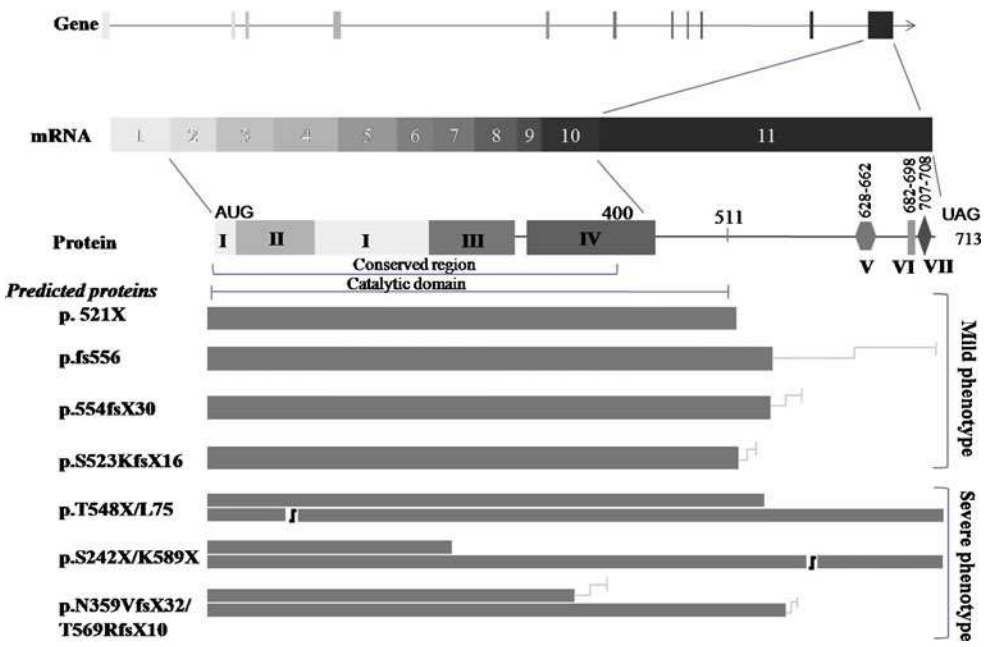

Figure 2. Mutation spectrum in exon 11 of *POLH* gene and predicted proteins in XP-V cells. The top line shows the 11 exons of *POLH* gene. The second line shows mRNA with the ATG initiation codon in exon 2 and the TAG stop codon in exon 11. The 713 amino-acid pol η protein is shown in the third line: the 400 amino acid N terminal domain is highly conserved in Y-family polymerases and contains the catalytic domain of the polymerase: The polymerase domain I (palm), II (finger), III (thumb), IV (LF, little finger). The C-terminal region from amino acids 628–662 (UBZ, domain V) contains a C2H2 zinc finger that is involved in DNA-binding ubiquitin. There is a nuclear localization signal (NLS, domain VI) located at amino acids 682–698. A PCNA, binding site (B/PIP, domain VII) is located at the extreme C terminus of the protein (707–708). The bottom portion of the figure shows the different predicted protein sizes associated to each allele described in the reviewed XP-V patients. UBZ, ubiquitin binding zing domain; NLS, nuclear localization signal; B/PIP, β clamp/ PCNA interacting peptide; NLS, nuclear localization signal; B/PIP, β clamp/ PCNA interacting peptide.

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