

SHORT COMMUNICATION

High frequency of the V548A fs X572 *XPC* mutation in Tunisia: implication for molecular diagnosis

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Xeroderma pigmentosum (XP, OMIM 278700–278780) is a group of autosomal recessive diseases characterized by hypersensitivity to UV rays. There are seven complementation groups of XP (XPA to XPG) and XPV. Among them, the XP group C (XP-C) is the most prevalent type in Western Europe and in the United States. We report here on the clinical and genetic investigation of XP-C patients in 14 Tunisian families. As the *XPC* V548A fs X572 mutation has been identified in Algerian and Moroccan populations, Tunisian patients were first screened for this mutation by a direct sequencing of exon 9 of the *XPC* gene. All patients with a severe clinical form had this mutation, thus showing the homogeneity of the mutational spectrum of *XPC* in Tunisia. A potential founder effect was searched and confirmed by haplotype analysis. Taking into account the similarity of the genetic background, we propose a direct screening of this mutation as a rapid and cost-effective tool for the diagnosis of XP-C in North Africa.

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INTRODUCTION

Xeroderma pigmentosum (XP) is a rare genodermatosis, characterized by an extremely high sun sensitivity of the skin, by ocular abnormalities and by a high incidence of skin tumors.¹ XP patients have been identified throughout the world in all ethnic groups.² This disease is rare worldwide, but is relatively frequent in Tunisia, with an estimated prevalence of 1 patient per 10 000.³ This might be explained by the high rate of consanguineous marriage and endogamy.

XP is genetically heterogeneous: eight genes (*XPA* to *XPG* and *XPV*) have been identified so far. The XP complementation groups differ in frequency and geographical distribution. Among its seven complementation groups, the XP group C (XP-C) is the most prevalent type in Western Europe and in the United States.⁴ The *XPC* protein is involved in the first step of the nucleotide excision repair (NER); it is important in the recognition of target lesions and in the recruitment of the incision complex.⁵ This human protein forms a strong heterotrimeric complex with Rad23B and centrin 2, and is involved in the recognition of UV ray-induced DNA lesions during global genome repair but not during transcription-coupled repair.^{6–8} *In vitro* experiments have shown that the *XPC* complex might contribute toward cancer prevention by participating in the base excision repair of 8-OH-Gua and other oxidative DNA lesions.⁹

Among the studied populations, no hot-spot mutation in the *XPC* gene was identified. The V548A fsX572 mutation was previously

described in three unrelated families from Italy, Algeria and Morocco, and also with a compound heterozygous *XPC* mutation in Honduras and USA patients.^{10–13}

To elucidate the spectrum of *XPC* gene mutations, 20 Tunisian patients with an XP severe clinical form were analyzed. We report here the relatively homogeneous mutation spectrum of the *XPC* gene in Tunisian patients and discuss its implication for molecular diagnosis of XP-C in North Africa.

MATERIALS AND METHODS

Patients

Twenty XP-C patients (9 male and 11 female individuals) and their family members were examined at the Tunisian reference XP department (Dermatology Department of the Habib Thameur Hospital in Tunis). Classification of these patients as XP-C was on the basis of clinical features, such as early onset of the disease, which could start at the first month of birth, and severity. Chronic photophobia is the first sign, followed by photosensitivity marked by persistent erythema, pigmentosum macula and skin malignancy developed before 10 years of age. The diagnosis of skin cancer, suspected during clinical monitoring, was confirmed by dermoscopic and histological investigation.

Molecular investigation

After signing an informed consent form, all families were interviewed using a structured questionnaire, including a pedigree drawing, to collect information

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Table 1 Clinical characteristics of XP-C patients

Family code	Number of patients/family	Sex	Age (years)	Age at first consultation (months)	City geographic origin	Age of onset of the first tumor (years)			
						BCC	SCC	MM	Photophobia
XPC1AR	1	M	9	5	TNE	7	9	–	+++
XPC2DJ	1	M	14	72	TME	13	6	–	++
XPC5NA	2	M	13	96	TNE	9	–	12	+++
		F	11	1		6	7	–	++
XPC7MED	1	M	4	24	TSE	–	3	–	+++
XPC8MED	1	F	10	28	TSE	–	6	–	+++
XPC11MO	2	M	24	50	TME	8	6	–	++
		M	22	36		8	7	–	++
XPC12KA	3	F	11	48	TMW	8	–	–	++
		M	7	24		7	–	–	++
		F	18	48		7	+	–	++
XPC23MO	1	M	2	18	TME	–	–	–	+
XPC26B	1	F	8	60	TNE	6	–	–	+++
XPC29KER	1	F	3	10	TME	–	–	–	++
XPC30MO	2	F	15	36	TME	11	9	–	++
		M	2	20		–	–	–	++
XPC32DJ	2	F	24	60	TME	11	9	14	++
		F	15	72		5	7	10	++
XPC35SFA	1	F	4	36	TME	–	–	–	+
XPC36NA	1	F	12	72	TNE	8	9	10	+
XPC46DJ	1	M	13	24	TNE	8	11	–	++

Abbreviations: BCC, basal-cell carcinoma; MM, melanomas; SCC, squamous-cell carcinoma; TME, Tunisian Middle East; TMW, Tunisian Middle West; TNE, Tunisian Northern East; TSE, Tunisian Southern East.

about consanguinity, affected members, associated diseases and family history. DNA was extracted from peripheral blood leucocytes by the classical salting-out method.¹⁴ To screen for the V548A fsX572 XPC mutation, a region of 609 bp length of exon 9 of the XPC gene was amplified from genomic DNA using the primers F: 5'-CCAGGGTGTCTTATAAAGAGG-3' and R: 5'-CAAGGCCTTACCTCCAAG-3' (3'-UTR). The PCR amplification reaction was carried out in a volume of 25 µl containing 10 ng of DNA, 10× PCR buffer, 0.2 mM of each dNTP, 1.5 mM MgCl₂, 0.5 µM of one of each primer and 0.5 U Amplitaq DNA polymerase (Invitrogen, Foster City, CA, USA). DNA was denatured for 5 min at 96°C, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 35 s, elongation at 72°C for 40 s and terminal elongation for 10 min at 72°C. The PCR products were purified by using the QIA quick gel extraction purification kit (Qiagen, Hilden, Germany) and sequenced with the Big Dye terminator kit (Applied Biosystems, Foster City, CA, USA) using one of the PCR primers on an ABI prism 3130 DNA sequencer (Applied Biosystems) in accordance with the manufacturer's recommendations.

To seek for a potential founder effect, available family members were genotyped using three microsatellite markers spanning a 1.4 Mb interval flanking the XPC locus (cen-D3S3602, D3S1585 (XPC) and D3S3542-tel). Microsatellite markers were selected from the genetic maps available on NCBI (<http://www.ncbi.nih.gov/>) and Ensembl (<http://www.ensembl.org/>) genome browsers on the basis of their heterozygosity percentage and closeness to the XPC gene. Genotyping was performed as described elsewhere.¹⁵

RESULTS

In this study, we report on 20 XPC cases belonging to 14 families who originated from various geographical locations in Tunisia, mainly from coastal regions. Twelve of the 14 families (85%) were consanguineous and the most common form is first-cousin marriages (57%). The average age of the patients is 11 years, with an allocation from 2 to 24 years. Clinical features are summarized in Table 1. For patients who were referred to our reference XP department before the age of 1 year, the diagnosis was based on the presence of the specific pigmentosum

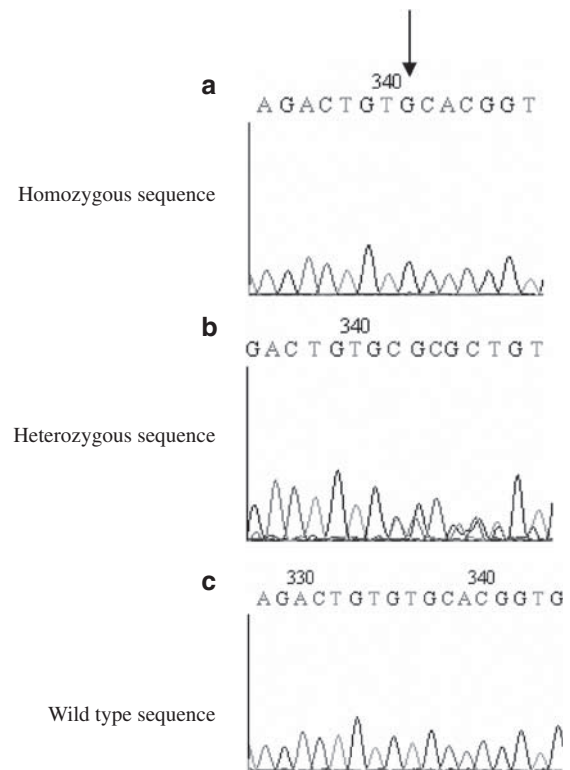


Figure 1 Genomic DNA sequence showing the TG deletion V548A fsX572 in exon 9 of the XPC gene in a homozygous state (a) or in a heterozygous parent (b), which is compared with the wild-type sequence of an unrelated control (c).

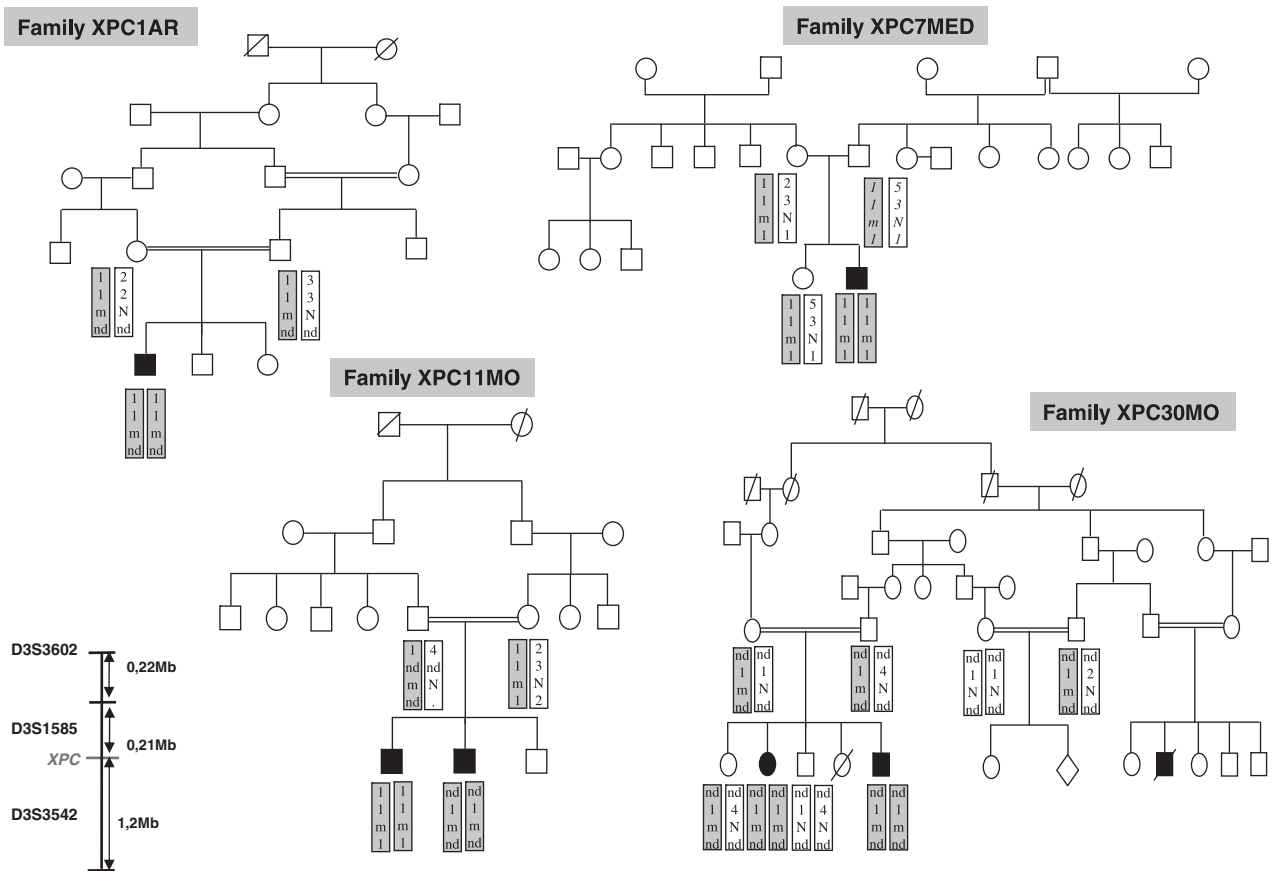


Figure 2 Pedigrees and haplotype analysis for four investigated XP-C Tunisian families, with microsatellite markers flanking the *XPC* gene. The disease haplotype is indicated by shading. m, mutant allele; N, normal allele; nd, not determined.

macula (association between achromia spots and badly limited multiple pigmented macula). No patient had neurological abnormalities.

Mutation screening showed that all studied patients presented the homozygous deletion of two bases TG in exon 9 at position 1744–1745 (NC_000003.10). This deletion leads to a frameshift mutation and a premature termination of the encoded protein, V548A fsX572 *XPC* (Figure 1). To investigate whether a common ancestral chromosome accounted for the recurrence of the identified mutation, patients and their family, whenever available, were genotyped with three selected microsatellite markers flanking the *XPC* gene, and the mutation-associated haplotypes were constructed by visual inspection. Six XP-C patients from four unrelated families were homozygous for the same haplotype, thus suggesting a founder effect (Figure 2).

DISCUSSION

XP is among the most severe and even life-threatening genodermatosis. It is a highly heterogeneous disease at the clinical and genetic level. In Tunisia, a preliminary investigation showed that approximately 40% of collected cases had a severe clinical form and they are suspected to be XP-C.³ We reported here on the clinical and mutational investigation of XP-C in Tunisian patients to introduce carrier detection, genetic counseling and prenatal diagnosis.

Clinical investigation showed that all studied patients correspond to a severe clinical form. Ninety percent of them developed pre-cancerous lesions or cancer: basal-cell carcinoma (BCC) (70%, the average age of onset of the first BCC is 8.2 years) or squamous-cell carcinoma (SCC) (55%, the average age of onset of the first SCC is 7.3 years), and

some patients developed several melanomas (MM) (20%, the average age of onset of the first MM is 11.6 years). The severity of this disease among Tunisian patients is similar to that described previously in North African, Italian, Turkish and Ashkenazi-Jewish Israeli patients.^{16–18}

The V548A fs X572 mutation affects the C-ter of the *XPC* protein residues 492–940. These residues had critical interactions with DNA, RAD23B, CETN2 and TFIIH. The *XPC*–RAD23B–CETN2 complex participates in the first fundamental step of GG-NER, DNA damage recognition, and sets the stage for the following two steps: DNA damage excision and gap-filling synthesis and ligation.¹⁹ Therefore, it is expected that the *XPC* V548A fs X572 mutation reduces the ligand-binding capacity, explaining the severe phenotype in the affected patients.

As previous studies of XP-C showed that Moroccan, Algerian and Italian patients had the V548A fsX572 *XPC* mutation,^{10–13,18} patients were screened for this mutation. All investigated patients bear this mutation. The identification of the prevalent mutation opened the way for molecular diagnosis. Indeed, premarital counseling was proposed to one couple (family XPC30MO) and the results showed that the father was heterozygous for the mutation, whereas the mother did not carry this mutation (homozygous normal). Consequently, this couple has the same risk of having an affected child as the general population. To identify a potential founder effect, haplotype analysis was performed. All available patients from unrelated families shared the same haplotype, thus suggesting a common ancestor. A similar figure should be found in other North African countries as they share

a common ethnic background and consequently the same mutational spectrum as already shown for other genodermatoses.^{20,21}

In our study, clinical similarity correlates with genetic homogeneity; in fact, all patients with a severe clinical form of XP bear the V548A fsX572 XPC mutation. The recurrence of this mutation will greatly simplify the molecular diagnosis of XPC to propose genetic counseling, prenatal diagnosis and to allow an early management of affected children.

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