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Variants of the xeroderma pigmentosum variant gene (*POLH*) are associated with melanoma risk

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ABSTRACT

Purpose: Xeroderma pigmentosum variant (XPV) is a rare recessive autosomal genodermatosis predisposing to multiple early onset skin cancers, including melanoma. XPV results from mutations of the *POLH* gene that encodes a DNA translesion polymerase. In this work, we tested the hypothesis that *POLH* variants could be associated with melanoma risk.
Experimental design: A common non-synonymous *POLH* variant, c.1783A>G p.M595V, was genotyped in 1075 melanoma patients and in 1091 ethnic-matched controls from France. In addition, we searched for rare *POLH* variants by sequencing the entire coding sequence in 201 patients having a familial history of melanoma ($n = 123$), sporadic multiple melanomas ($n = 65$) and a melanoma associated with a skin carcinoma ($n = 13$).
Results: Overall, the c.1783G, p.595V allele was statistically associated with melanoma (respective allelic frequencies, 0.040 versus 0.022, P-value $= 1.17 \times 10^{-3}$, odds ratio (OR) = 1.86 [1.27–2.71]), which was further confirmed by a meta-analysis including 274 patients and 174 matched controls from Italy (P-value $= 7.7 \times 10^{-4}$, OR = 1.84 [1.29–2.63]). Interestingly, three non-synonymous *POLH* variants were identified in three patients (c.295G>A p.V99M, c.815T>C p.I272T and c.1745C>T p.S582L) which were absent in 352 chromosome controls from healthy subjects.
Conclusions: Besides severe deficiencies in translesion synthesis which are major risks factors for skin carcinomas and melanomas, less deleterious *POLH* variants could act as low

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penetrance melanoma predisposing alleles. The ongoing identification of genetic markers implied in skin cancer predisposition could help to identify high-risk subjects as targets for clinical follow-up. Replication studies in other populations are awaited to assess these data.

1. Introduction

Cutaneous melanoma develops from the malignant transformation of melanocytes. Since the mid-1960s, melanoma incidence has increased by 3–8% per year in Caucasian populations[1–3] and hence has become a major public health problem in many countries. A positive family history of melanoma exists in 5–2% of cases and as in many diseases, melanoma can be linked to monogenic or multifactorial genetic predisposition. On the one hand, high penetrance genes (>50%), including *CDKN2A* and *CDK4*, are mutated in 20% of familial melanomas and in 10% of sporadic multiple melanomas.[4–7] On the other hand, polymorphisms in low penetrance genes interacting with environmental factors (mainly ultraviolet (UV) exposure) are associated with multifactorial susceptibility. In this area, variants in pigmentation genes have been shown to play an important role in melanoma risk.[8–11]

One of the best-studied genes is the melanocortin 1 receptor gene (*MC1R*), the stimulation of which by the alpha-melanocyte-stimulating hormone (α-MSH) results in the synthesis of the black photoprotective pigment eumelanin. Five loss-of-function *MC1R* variants alleles (p.D84E, p.R142H, p.R151C, p.R160 W and p.D294H), also known as the RHC variants (RHC, for Red Hair Colour), have been shown to be strongly associated with the risks of melanoma and non-melanoma skin cancers.[12,13] Very recently, other pigmentation genes have been shown to be associated with melanoma risk. One is the membrane-associated transporter protein gene (*MATP*), renamed solute carrier family 45 member 2 (*SLC45A2*) that encodes a melanosomal protein. Importantly, two recent studies that were conducted in Spain and France have shown that a *SLC45A2* variant, previously shown to be associated with dark pigmentation in Caucasians,[14] p.L374F, confers a protective effect from melanoma.[8,9] Furthermore, polymorphisms in *ASIP*, *TYR* and *TYRP1* that are associated with human pigmentation[15,16] were also shown to increase melanoma risk.[11] The *ASIP* gene product antagonises the interaction between MC1R and α-MSH, leading to pheomelanin synthesis, whereas TYR and TYRP1 are the key enzymes involved in melanin synthesis. Finally, two SNPs on 20q11.22 were found to be independently associated to melanoma by a genome-wide association study.[17] Because UV DNA damages can lead to skin cancer if not repaired, genes involved in DNA repair are attractive candidate genes. Melanoma incidence is 2000-fold higher in patients suffering from xeroderma pigmentosum (XP), an inherited disease with mutations in the nucleotide excision repair (NER) system.[18] A reduction in NER repair activity has also been observed in lymphocytes from melanoma patients.[19] Numerous studies have investigated the role of polymorphisms in DNA repair genes on melanoma risk across different populations [20,23,24–29]. However, variants in NER genes (*XPD/ERCC2*, *XPC* and *XPF/ERCC4*) have been associated with melanoma risk in some studies.[21,22,24,28–31]

An alternative pathway is the one involving translesion synthesis (TLS), which is defective in XP variant (XPV). This pathway can bypass UV-induced mutations in the template strand and permits cells to synthesise an intact daughter DNA strand. XPV patients carry bi-allelic mutations in the *POLH* gene that encodes Pol, a specialised polymerase that is able to bypass UV lesions.[32–39] When *POLH* is defective, UV-induced DNA lesions are replicated by a more error-prone polymerase that produces more mutations,[40,41] therefore allowing skin cancers to occur.[42] Because XPV patients are also highly predisposed to skin cancers, notably melanoma, we considered that *POLH* variants could be involved in melanoma susceptibility.

Therefore, in this study, we investigated the role of a common *POLH* variant and searched for the presence of rare *POLH* variants with regard to melanoma risk.

2. Methods

2.1. Study population

The 1075 patients were of Caucasian origin and were prospectively recruited from the *Melan-Cohort*, between 2003 and 2007. This prospective cohort includes melanoma patients followed up in the Dermatology Departments of all the University-affiliated hospitals in Paris (Bichat, Ambroise Paré, Henri Mondor, Cochin, Tenon and Saint-Louis Hospitals). Inclusion criteria were cutaneous melanoma confirmed by pathological reports, free margins surgery calculated according to melanoma thickness (with at least 1 cm) and age ⩾ 18 - years. Exclusion criteria were non-melanoma lethal cancer, immunodeficiency (transplant or HIV), genodermatosis predisposing to skin cancer (albinism, Gorlin's disease and xeroderma pigmentosum) and African or Asian origin. For each patient, information was collected in a standard document containing: anatomo-clinical data (age at diagnosis, histological subtype, Breslow index and anatomical location) pigmentation characteristics (hair and eye colours and skin type, I–IV, according to Fitzpatrick classification), naevus count (<10, 10–50, 51–100 and >100), atypical naevi and sun exposure during childhood and adulthood.

Information regarding UV light exposure was obtained from a retrospective questionnaire. Intermittent UV exposure was evaluated by the presence of severe sunburns, both in childhood and after the age of 15 (yes/no). A score concerning the level of sun exposure during the holidays or during the hobbies (none/moderate/important) was also established. In

addition, the occurrence of therapeutic phototherapy (yes/no), or sunbed exposition (yes/no) was also investigated.

Melanoma was sporadic in 923 patients (86%) (including 53 patients who had multiple melanoma, 5%), and familial (at least two cases in first- or second-degree relatives, including the proband) in 150 cases (14%). Amongst the familial and multiple sporadic cases, 21 patients carried mutations in the *CDKN2A* gene and one patient carried mutations in the *CDK4* gene. Histological subtypes were superficial spread melanoma (SSM) in 59%, nodular melanoma in 15%, Dubreuilh melanoma in 7% and acrolentiginous melanoma in 5%. Melanoma was located on the limbs in 43%, trunk in 29%, head and neck in 13% and extremities in 5%.

The control group, matched by sex, age and geographic area to the case group, was recruited among healthy blood donors from the same Departments, and was composed of 1091 French subjects, mainly derived from a previous control group.[9] They were all of Caucasian origin and skin cancer free (as assessed by answering a preprinted questionnaire). Dermatologists collected, after examination, data on pigmentation characteristics for 220 of these subjects. For sequencing analysis, we selected a subgroup of 201 patients who met one of the following criteria: a familial history of melanoma ($n = 123$), sporadic multiple melanomas ($n = 65$) and a melanoma associated with skin carcinoma ($n = 13$). Median age and sex ratio (respectively, 43 years and 1.12 in patients) were not statistically different between patient and control groups. Pigmentation characteristics of all patients and 220 control subjects are summarised in a previous study.[9] Informed consent was obtained from all the patients and control subjects enrolled in the study. The local Medical Ethics Committee (CCPPRB) approved the study protocol. Genomic DNA was isolated from peripheral blood leucocytes using the QIAamp Blood Mini-Kit (QIAgen GmbH, Hilden, Germany).

2.2. Genotyping

We selected non-synonymous *POLH* SNPs, the minor allele frequency of which was higher than 2% in Caucasian populations. Only one variant, c.1783A>G p.M595V (NCBI dbSNP, rs9333555, www.ncbi.nlm.nih.gov/SNP) corresponded to this exigency. It was genotyped in 1075 patients and 1091 controls. For a subset of these populations, genotypes from *MC1R* RHC variants (p.D84E, p.R142H, p.R151C, p.R160W and p.D294H) and from the p.F374L variant of *MATP/SLC45A2* were already available.[9] Genotyping was carried out with the KASPAR SNP Genotyping System KBiosciences® as previously described,[9] and was achieved in 986 patients and 1043 controls.

2.3. POLH sequencing

The *POLH* coding exons and exon-intron junctions (Genbank Accession, NM 123456) were amplified with 12 couples of primers selected by using Exon Primers USC Genome Browser primer (http://genome.ucsc.edu/cgi-bin/hgGateway/) (Table 1). A 60 °C hybridisation temperature was used for all PRCs. PCR mix comprised 25 ng genomic template DNA, 1.5 L 10× PCR buffer, 1.8 L 5 mM $MgCl_2$, 0.6 L dNTP 5 mM, 6 pmol each of PCR primer and 0.5 U Ampli Taqgold®. Samples were denatured for 7 min at 96 °C and were passed through 35 cycles of amplification: 30 s of denaturation at 96 °C, 30 s of primer annealing at 60 °C, 30 s of elongation at 72 °C and a final elongation for 10 min at 72 °C. The amplifications were carried out in 0.2-mL Eppendorf® tubes in an Eppendorf® thermocycler. PCR products were controlled on a 2% agar gel and were purified by EXOSAP-IT®. A sequencing reaction was performed, in one sense, on 8900 Fast thermal cycler Applied Biosystem®, using 10 ng PCR-purified products and a Big-dye terminator cycle kit Applied Biosystem®. Sequence analysis was performed on an ABI-Prism 3130 Applied Biosystem® and was read with Seqscape® Software v2.5 (Applied Biosystems). In patients, all coding exons were sequenced, whereas, in controls, we sequenced only exons in which rare variants were observed in patients. All sequence variants were confirmed by sequencing both DNA strands.

2.4. Statistical analysis

The main part of the statistical analysis was performed using the R computer package (version 2.7.1). Conformity of genotypes to Hardy–Weinberg equilibrium was tested in controls using the adjustment χ^2 test. The level of significance for all tests was set to a level corresponding to an alpha-error-rate of 5%. Odds ratios (ORs) were calculated with 95% confidence intervals. We first compared the genotypes of the *POLH* variant p.M595V in patients and controls with a Fisher's exact test. Statistical analysis was also performed to analyse clinical or genetic melanoma known risk factors (naevus count > 50, skin types I–II, dorsal lentigines, light hair colour (red-blond-light brown), light eye colour (blue-green-grey), *MC1R* RHC variants and *MATP* variant p.L374F). Finally, in order to confirm the association of the p.M595V variant with melanoma, we conducted a meta-analysis in both French and Italian populations via logistic regression analysis adjusted on the studied populations as previously described.[43]

2.5. Functional prediction of POLH polymorphisms

In order to evaluate the functional impact of *POLH* variants, we used different in silico prediction tools. Three tools were used to predict the functional impact of non-synonymous variants: SIFT (Sorting Intolerant From Tolerant, http://blocks.fhcrc.org/sift/SIFTseq_submit2.html); PolyPhen (POLYmorphism PHENotyping, http://www.bork.embl-heidelberg.de/PolyPhen/) and SNPs3D (http://www.snps3d.org/). In addition, we also used Pymol (http://pymol.sourceforge.net/), a free molecular graphics and modelling package, to predict the potential effect of *POLH* non-synonymous variants on Pol structure. PDB files were loaded into an RSCB Protein data bank (www.rcsb.org/pdb). The 628 to 662 C-terminal amino acids were identified in the 2i50 PDF file.[44] The complete crystal of the human Pol was not available because it remained difficult to purify. Polymerase domains of the Y-family DNA polymerases shared several homologies in humans and *Saccharomyces cerevisiae*. Therefore, we used the crystal of the yeast polymerase (2r8k and 1JIH).[45,46] This alignment allowed us to study variants localised in the first 511 amino acids. Finally, we used miRanda (miRanda www.microrna.org), an algorithm for the detection of potential microRNA (miRNA) target sites in genomic sequences, to study the effects of the SNPs situated in the 3′UTR

Table 1 – Primers sequences used for PCR-amplification.

Exon	Primer	Amplicon size (bp)
2	XPV-F2-5′CTCCCATGCTCATGGTAACT3′	283
	XPV-R2-5′GACAGGGCCTTTCTCTGTTGTC3′	
3	XPV-F3-5′TTGAATGATGGCATCTGTGG3′	338
	XPV-R3-5′CTGGTGTTTTGA3′	
4	XPV-F4-5′TTATTGTTCATTATGGCAGGG3′	383
	XPV-R4-5′GGAAACTTAAACTACAGCCTGAATG3′	
5	XPV-F5-5′ACACTCTGAAGGAGAAATTGAGC3′	482
	XPV-R5-5′ACAGTGGGACATTGAAATTAT3′	
6	XPV-F6-5′CAGCTATTAAGCTCTGTGTGTGTG3′	322
	XPV-R6-5′GGGGAAATACATACCAGGCAG3′	
7	XPV-F7-5′TGAACCTTTTGGAGAGCTGT3′	281
	XPV-R7-5′CAAAACCATCTAGTAAAATCCAGACC3′	
8	XPV-F8-5′GCAGGGGTTTCGTCAGAG3′	419
	XPV-R8-5′TGTTCTGTCC TTGGGTTGA3′	
9	XPV-F9-5′TGGTGATCCTTTGATTATGGG3′	246
	XPV-F9-5′GCATAGGAAATATCCGTTTTCTTT3′	
10	XPV-F10-5′TTTCCAAACCATTGTCACCC3′	471
	XPV-R10-5′TTAGTCCTTCAATGAGGTAAAGGG3′	
11	XPV-F11.1-5′TGGATTAAATCTGTCCTATGGTG3′	599
	XPV-R11.1-5′GAGTATCCAGGGTGTGTCCC3′	
11	XPV-F11.2-5′GGAACTGAGCCCTTCTTTAAGC3′	514
	XPV-R11.2-5′CACTAATAAACGCCCCAGGCC3′	
11	XPV-F11.3-5′CAGCCCCACT CTTCAAACC3′	494
	XPV-R11.3-5′GGGCCTTGAACAAGTCATTT3′	

region of these targets sites. This effect is measured by the difference of scores among the alleles of the SNPs.

3. Results

3.1. *Univariate analysis of the POLH p.M595V variant and melanoma risk*

In the control group, genotype frequencies for the c.1783G>A p.M595V variant respected Hardy–Weinberg equilibrium. In the French population, the *POLH* variant c.1783A, p.595V was significantly more frequent in patients than in controls (*P*-value = 1.17×10^{-3}, OR = 1.86 [1.27–2.71]). Allelic frequency of 595V was 0.040 in patients versus 0.022 in controls (a frequency that corresponded to the reported data collection in the Europeans) (Table 2).

We also performed extensive stratified analyses in order to test for a genotype association of the p.M595V allele with genetic subgroups (familial, multiple, mutated for *CDKN2A* or *CDK4*), histological subtype {Superficial Spread Melanoma (SSM), Nodular, Acrolentiginous (ALM) and LMM (lentiginous malignant melanoma, i.e. Dubreuilh)}, melanoma location (head and neck, limbs and non-sun exposed sites), pigmentation characteristics (eye and hair colour, skin type, dorsal lentigines and naevus count), and the occurrence of severe sunburn in childhood. Only a moderate but significant statistical higher allelic frequency of the p.M595V variant was observed in patients who experienced either a high UV exposure during the hobbies before the age of 15, or phototherapy (*P*-value = 0.041, Table 3).

3.2. *Meta-analysis*

In order to confirm the association of the p.M595V variant with melanoma, we further genotyped this variant in Italian melanoma patients and controls. This study concerned 274 patients enrolled between 2000 and 2007 at the Department of Dermatology, University of L'Aquila, Italy, and 174 healthy controls matched by sex, age and geographic area to the cases group. In the Italian population, the allelic frequency of p.M595V was also higher in melanoma patients than in controls (allelic frequency, 0.024 versus 0.014), although the dif-

Table 2 – Univariate analysis of the p.M595V POLH variant and melanoma risk. Genotyping was performed in 1075 patients and 1043 controls, but it was inconclusive in 89 patients and 48 controls.

SNP	Genotype	Patients (986)	Controls (1043)	P-value	OR [IC95%]
POLH p.M595V	AA	908 (92.1%)	997 (95.6%)	–	Reference
	GA	77 (7.8%)	46 (4.4%)	1.17×10^{-3}	1.86 [1.28–2.71]
	GG	1 (0.1%)	0 (0%)		
	MAF[a]	0.040	0.022		

a MAF, minor allele frequency.

Table 3 – Univariate analyses of the p.M595V POLH variant with anatomo-clinical parameters. In bold, statistical significant associations.

Anatomo-clinical parameter	*P*-value
Age at diagnosis (<35 year versus >50 year)	0.24
Naevi count (⩽50 versus >50)	0.62
Skin type (I–II versus II–IV)	0.74
Ephelides	0.49
Dorsal lentigines	0.26
Tumour location (limbs, head and neck, trunk and NPE[a] sites)	0.87
Tumour type (SSM, nodular, LMM, ALM)	0.20
Breslow index (<1 mm versus >2 mm)	0.38
Melanoma + non-melanoma skin cancer	1
Multiple sporadic melanoma	0.28
Familial history of skin cancer	0.12
Familial history of visceral cancer	0.65
UV exposition	
Phototherapy[b]	**0.041**
Sunbeds[b]	0.063
Sunburns before the age of 15[b]	0.32
Sunburns after the age of 15[b]	0.61
UV exposure during holidays before the age of 15[c]	0.95
UV exposure during holidays after the age of 15[c]	0.11
UV exposure during the hobbies before the age of 15[c]	**0.041**
UV exposure during the hobbies after the age of 15[c]	0.58

a *Abbreviation*: NPE, non-photo-exposed sites.
b Yes versus No.
c Important versus none or moderate.

ference was not significant (Fig. 1). We then conducted a meta-analysis via logistic regression analysis adjusted to the studied populations as previously described.[47] The meta-analysis, which comprised 1349 melanoma patients and 1265 controls, clearly confirmed that the *POLH* variant p.M595V was significantly associated with melanoma risk in both populations (P-value = 7.7×10^{-4}, OR = 1.84 [1.29–2.63]) (Fig. 1).

3.3. *In silico analysis of the p.M595V variant*

In silico analyses did not predict any functional impact of the p.M595V variant on POLH. Unfortunately, it was localised in a part of the protein that was not crystallised and could not be studied by Pymol. Conversely, the c.1783A was predicted to create a miRNA-binding site for the miRNA hsa-miR-769-3p. This would therefore potentially alter *POLH* mRNA stabilisation.

3.4. *POLH sequencing analysis*

We identified three rare non-synonymous variants in three patients, which have not been previously described as SNPs or *POLH* mutations (Figs. 1–3 and Table 4).

The c.295G>A p.V99M variant was characterised in a patient harbouring a *CDKN2A* mutation (c.49-54 del p.N17-T18 del), who developed two melanomas at the ages of 28 and 49, and whose brother was diagnosed with the same tumour. The change of the valine for a methionine is predicted to have a functional impact on Pol (Table 4). The Val99 residue lies in a α-helix of the polymerase palm subunit (Fig. 2a), and the substitution by a methionine residue could trigger lengthening of the lateral chain, bring a sulphured residue in a narrow area and could cause interaction with the lateral chain of Arg111, an important residue of domain III. Interestingly, this domain is the catalytic epicentre of the protein (residues Asp115 and Glu116), which catalyses the nucleotide transfer reaction. The c.812T>C p.I272T variant was found in a patient who developed three melanomas at the ages of 54 and 55. It is localised near the Vth domain of the polymerase region, and is also predicted to have a functional impact on POLH (Table 4). This amino acid change shortens the lateral chain,

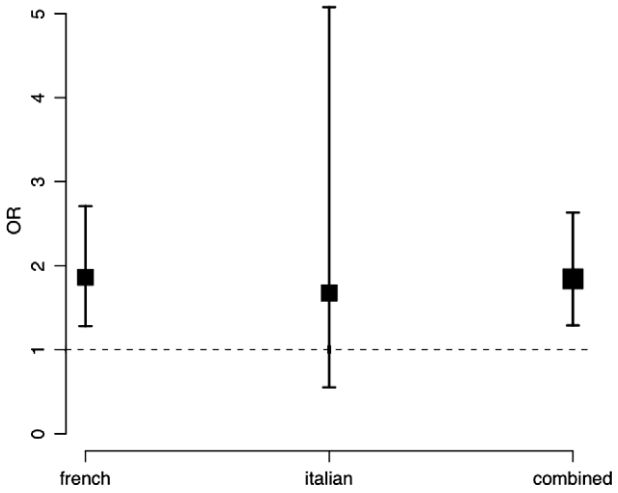

Fig. 1 – Forest Plot showing the association of the M595V variant with melanoma in the French and Italian populations and the meta-analysis on both populations.

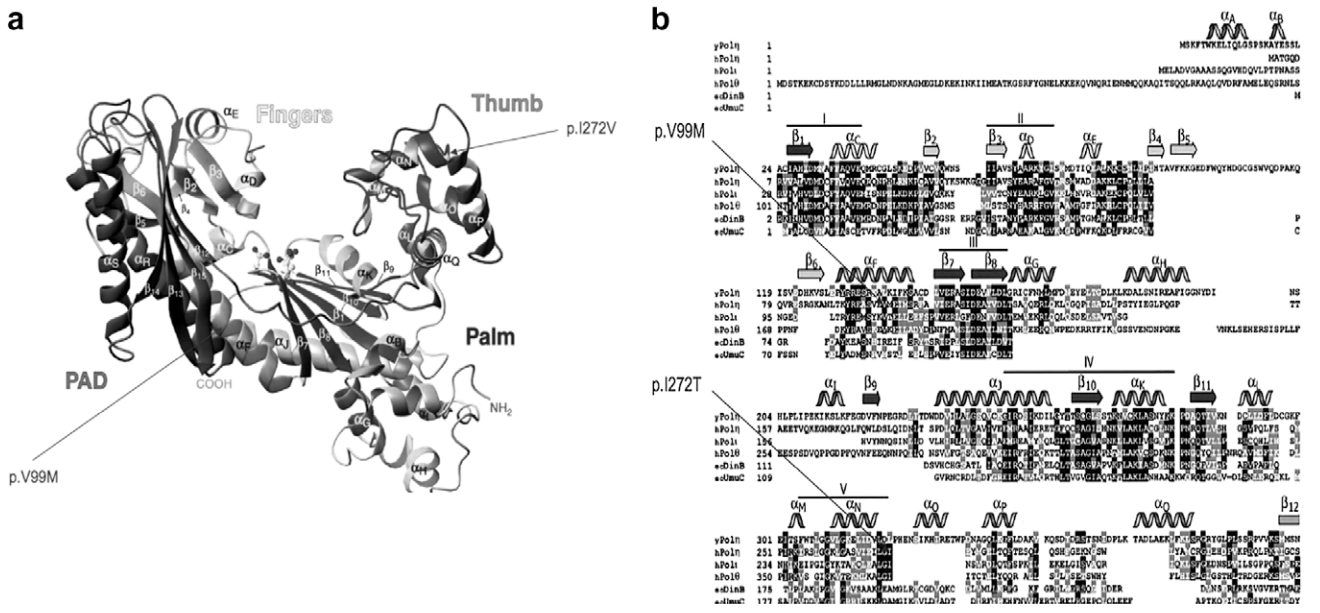

Fig. 2 – Pol structure and sequence alignments of different TLS polymerases (adapted from Ref. [46]). (a) Structure of the Pol g polymerase region and localisation of the two rare non-synonymous variants observed in melanoma patients. Pol g has the shape of a polydactyl right hand, in which a polymerase-associated domain (PAD) mimics an extra set of fingers. Four domains thus define the DNA-binding groove: palm, fingers and thumb domains characteristic of all known polymerases, and the PAD that packs alongside the fingers. The palm domain is the most similar to that in other polymerases and carries the active site residues that catalyse the nucleotidyl transfer reaction. The fingers and thumb domains are unusually small and stubby and are radically different from those in other DNA polymerases. The overall structure suggests that openness of the active site is the critical feature, which enables DNA polymerase to replicate the thymine–thymine dimer. (b) Comparison of sequences within the TLS DNA polymerase family. *S. cerevisiae* Pol (yPol), human Pol (hPol), human Pol (h Pol), human Pol (h Pol), *Escherichia coli* DinB (ecDinB) and *E. coli* UmuC (ecUmuC).

brings a hydroxyl group in a hydrophobic area and could cause interaction with the Leu270 of the Vth polymerase domain, a highly conserved residue (Fig. 2b).

The c.1745C>T p.S582L variant concerned a patient who developed, at the age of 63, a melanoma and a skin squamous cell carcinoma, and whose daughter was also suffering from melanoma. This variant is localised between the PAD and UBZ domains. Because this protein region does not exist in yeast and because its human crystal was not available, the study on the 3D structure could not be performed. Yet, a serine change for a leucine clearly modifies the hydrophobicity of the protein. One synonymous variant, c.2007A>G p.S669S, was also observed in a woman who suffered from familial melanoma and basal cell carcinomas. No impact on splicing was predicted for any of these variants. Sequencing analysis of 176 DNA control samples from healthy individuals showed that these rare *POLH* variants were not present in 352 healthy chromosomes, indicating that they were not common polymorphisms.

4. Discussion

Although much less severe than in classical xeroderma pigmentosum, the phenotype of XP variant is characterised by the development of early multiple skin cancers, notably melanoma, which might be present in up to 25% of patients.[38] Hence, we hypothesised that variants in the *POLH* gene could be involved in predisposition to melanoma, and report the first association study between *POLH* and risk of melanoma. *POLH* is highly polymorphic, but most of the coding polymorphisms exhibit a very low frequency in Caucasians. Thus, only the c.1783G>A p.M595V variant was genotyped in this association study. Due to a combined sequencing approach, we also investigated the role of rare *POLH* variants in genetic predisposition to melanoma. Firstly, in this large study we showed a clear association between the c.1783G allele (p.595V) of *POLH* and melanoma risk in the French population which was statistically confirmed by a meta-analysis on Italian and French populations. This variant is localised between the specific TLS polymerase domain (PAD) and the ubiquitin-binding zinc finger (UBZ) domain of the protein (Fig. 3). To date, the function of this specific region has not been clearly identified. However, this residue is localised in the 120 last C-terminal amino acids, which are required for Pol localisation into UV-induced damage foci and which are indispensable for complementation of UV with caffeine sensitivity.[41] In addition, the G allele is predicted to induce a miRNA-binding site for the human miRNA hsa-miR-769-3p, which would diminish *POLH* mRNA stability. Consequently, this common *POLH* variant could affect translesion synthesis activity. On the other hand, this variant, if not causal by itself, might be in linkage disequilibrium with a functional variant localised outside the *POLH* coding sequence.

We also observed a moderate association of the M595V variant with UV exposure (phototherapy and to high UV exposure during the hobbies before the age of 15), suggesting an interaction between UV light and *POLH* (Table 3). However, as the esti-

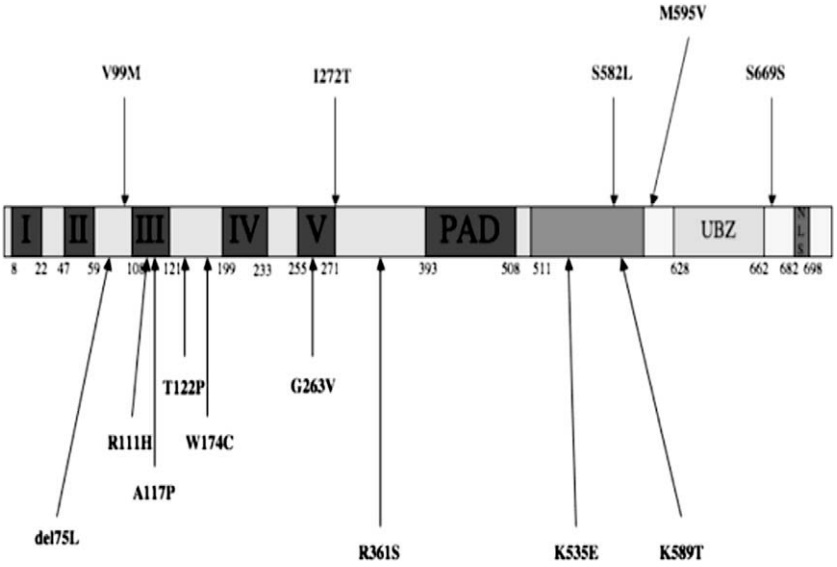

Fig. 3 – Localisation of *POLH* mutations according to the different Pol g domains. Dark grey regions indicate conserved DNA polymerase domains, whereas the N terminal region (particularly the UBZ domain and NLS nuclear localisation signal) comprises amino acids involved in Pol g relocalisation. Rare non-synonymous *POLH* variants identified in melanoma patients and p.M595V are shown at the top of the bar. *POLH* missense mutations previously identified in XPV patients are noted below the bar.

Table 4 – In silico predictions of the effects of non-synonymous POLH variants on the POLH protein.

Patients	Melanoma characteristics	*POLH* variant	SIFT prediction	POLYPHEN prediction	SNPS3D prediction	Pymol analysis
P629	Familial, two first degrees	c.295G>A p.V99M	Deleterious	Deleterious	–1.47	Functional
P3	Multiple sporadic	c.815T>C p.I272T	Tolerated	Benign	–0.54	Functional
P126	Familial, two first degrees	c.1745C>T p.S582L	Tolerated	Benign	+0.94	NA[a]

a NA, not applicable.

mation of the UV exposure level during infancy often suffers from a recall bias, this result should be taken cautiously.

In addition, three rare non-synonymous *POLH* variants were identified in 3/201 patients and these were absent in 352 control chromosomes. Two of them, p.V99M and p.I272T, are located in the Pol polymerase domain (Fig. 2a) and seem to have a functional impact. Firstly, these changes involve conserved amino acids among human TLS polymerases, Pol, Pol and Pol (Fig. 2b).[46] Secondly, in silico prediction tools and 3D structure analysis strongly suggest direct deleterious consequences on the protein's function (Table 4). Thirdly, both mutations localise within a cluster comprising the majority of missense *POLH* mutations identified in XPV patients (Fig. 3).[37] It should also be noted that the third non-synonymous variant, p.S582L, although not predicted to have a functional impact, is localised in the C-terminal part of the protein in which two missense mutations (K535E and K589Y) have previously been identified in XPV patients (Fig. 3). Interestingly, the latter mutations were identified in heterozygotes and yet the mutated cells showed a reduced level of recovery DNA synthesis (RDS) in the presence of caffeine with UV.[33] Finally, it has been demonstrated that heterozygous *POLH*-deficient mice are also susceptible to UV-induced skin tumours,[48] suggesting that heterozygous *POLH* mutations might also confer susceptibility to skin cancer in humans.

Two of three *POLH* variants were identified in patients having familial melanomas, but unfortunately, segregation analyses could not be performed because DNAs of the affected relatives were not available. One of these patients (P629, Table 4) who had three distinct melanomas (three SSMs localised on the arm, back and foot; at the ages of 28, 49 and 60) also harboured a *CDKN2A* mutation. It has been shown previously that *MC1R* variants modulate risk of melanoma in families segregating CDKN2A mutations.[49] Whether this could be also the case for *POLH* variants remains hypothetical at the present time, although it should be noted that this patient did not carry any *MC1R* variant.

In conclusion, these results strongly suggest that genetic variations in the *POLH* gene are associated with an increased melanoma risk. Although severe deficiencies in DNA repair

pathways (NER and translesion synthesis) are major risks factors for skin carcinomas and melanomas, less deleterious *POLH* variants could act as low penetrance melanoma predisposing alleles. Replication studies, in different populations and/or in larger populations of patients and controls are necessary to confirm the higher frequency of rare *POLH* variants in patients. In addition, functional studies are awaited to assess the impact of these variants on Pol protein. Furthermore, whether *POLH* variants interact with pigmentation genes (*MC1R*, *TYR*, *TYP1* and *ASIP*) in predisposition to melanoma requires further work. The ongoing identification of genetic markers implied in skin cancer predisposition could help to identify high-risk subjects as targets for clinical follow-up.

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Conflict of interest statement

None declared.

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