Supplemental Data document

For

Familial predisposition to TP53/complex karyotype MDS and leukemia in DNA repair-deficient xeroderma pigmentosum

By

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This supplemental document contains:

Supplemental Patient Data: clinical data and history

Patients and cohort

Patients were all seen and treated in hospital centers in France. Skin biopsies and blood samples were sent to the *Laboratory of DNA repair-deficient diseases* at Institut Gustave Roussy (Villejuif, France) between 1990 and 2018. Written informed consent was provided by the patients or their relatives in accordance with the Declaration of Helsinki and French law. This study was approved by the Institutional Review Board of the University Institute of Hematology (IUH; Saint-Louis Hospital, Paris, France).

Patient #1 (XP10VI) was a male from Morocco who was diagnosed as XP-C in our laboratory at the age of four. He had one XP-C brother (XP11VI, not included in this study) who died at the age of six for unknown reasons. Two of their cousins were XP-C brothers, including patient #2. Patient #1 developed AML-4 at the age of 27. No karyotype of leukemic cells was performed at this time. AML was treated with standard chemotherapy (daunorubicin and aracytine) but the patient died of major toxicity.

Patient #2 (XP82VI) was a male from Tunisia who was diagnosed as XP-C in our laboratory at the age of 1.5 years, at the same time as his XP brother was diagnosed at the age of four. They were cousins with patient #1, and their parents were cousins at the third degree. The older XP-C brother died at the age of 23 from metastatic melanoma (XP81VI, not included in this study). Patient #2 developed an AML-6 at the age of 16. Bone marrow karyotype detected a 5q deletion, a monosomy 7 and a rearrangement between chromosomes 9 and 20. Whole exome sequencing (WES) analysis showed a somatic heterozygous mutation in the *TP53* gene (Table 1; Supplemental Figure 4). Standard chemotherapy induced a complete remission. He subsequently received two cycles of aracytine and daunorubicin, followed by an allogeneic stem-cell transplantation (Supplemental Table 3), but he died rapidly (aged 18 years) from cardiac and hepatic toxicity.

Patient #3 (XP235VI) was a female originated from Tunisia who was diagnosed as XP-C in our laboratory at the age of nine. She had one XP brother who died of skin cancer at 18y and two non-affected sisters. Her first skin lesions appeared at the age

of two, and she developed hundreds of skin cancers on exposed sites (basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanomas). Since the age of 27.5, she received short cycles of Cetuximab but the poor tolerance of the drug led to treatment discontinuation at the age of 28. She was diagnosed with MDS (RAEB-1) at the age of 24, which evolved into RAEB-2 at 25 years (12% bone marrow blast cells, anemia and thrombocytopenia) with complex karyotype and aCGH profile including deletions 5q and 20p and monosomy 12. The complete karyotype referred as 45,XX,der(1)t(1;12),der(?2)t(2;12)(p25;?),der(5)del(5)(q12q3?4)?ins(5;12)(q13;q?21 q24),der(12)del(12)(q11),der(20)t(1;20)(p31;p11~12),-21inc[10]/46,XX[2]. WES analysis detected two somatic *TP53* mutations and several somatic *CSF3R* mutations. She received of 5-azacitidine cycles. From the fourth cycle, a partial response was observed with a decrease in blood cell transfusion requirements. Bone marrow blast cell fraction was decreased to 11% and 5% after 13 and 16 cycles, respectively. Relapse with overt acute leukemia occurred after 36 cycles with a strong dependency on blood cell transfusions. She died a few months later at the age of 29.

Patient #4 (XP309VI) was a female originated from Morocco, diagnosed as XP-C at the age of two. Her first basal cell carcinoma (BCC) was identified at the age of five, followed by numerous skin cancers. At the age of seven, she was diagnosed with B-cell acute lymphoblastic leukemia in a background of MDS with complex karyotype and monosomy 7. No material was available retrospectively for sequencing. She was treated with steroids, vincristine and low doses methotrexate. She achieved remission but relapsed one year later. At that time, she was locally irradiated with 12Gy (e^- 3MeV for two weeks) to treat a squamous cell carcinoma (SCC) on the right ear. She died a month later from acute leukemia at the age of 10.

Patient #5 (XP924VI) was a male originated from Morocco with known consanguinity. He had a younger brother with XP (not included in this study). He was diagnosed as XP-C at the age of 4.5. He developed a T-ALL at the age of 12, for which he was treated according to the FRALLE-2000T protocol with a limitation of cyclophosphamide doses. MDS was diagnosed on treatment, during maintenance therapy, at the age of 14. He was treated for the MDS with standard doses 5-azacitidine courses for one year, but AML-6 was later diagnosed at the age of 15, with

27% blast cells in bone marrow. MDS cell cytogenetics showed complex karyotype with 5q-, 7q-, while WES showed a *TP53* acquired mutation (Table 1 and Figure 1A, B). AML-6 was derived from the MDS clone with additional chromosomal abnormalities. By contrast, the T-ALL cells investigated by SNP-array and T-ALL panel genes showed an acquired neutral copy-number loss of heterozygosity at 6p and trisomy 20, with acquired *BCOR* and *PHF6* gene mutations but no *TP53* mutations. The patient died of erythroleukemia at the age of 15.

Patient #6 (XP185VI) is a female of Spanish origin, with parental consanguinity and no siblings, exhibiting the same mutation as the founder mutation from North Africa, suggesting that her genetic lineages are probably coming from North Africa toward Spain (Supplemental Figure 2). She was diagnosed as XP-C in our laboratory at the age of two and developed multiple skin cancers (BCC, SCC and melanoma) on exposed body parts. An anemia was detected at the age of 23, and was diagnosed at age 24 with RAEB-2, with 12% of bone marrow blasts and a highly complex karyotype and aCGH profile that included deletions in 5q, 7q, 12p, 20q, and subclonal del(4q) and trisomy 8q, as shown by aCGH, along with *TP53* and *TET2* gene mutations (Table 1). The complete karyotype referred as 43~44,XX,add(5)(q12),add(7)(q11),add(11)(q21),

der(12),-19,-20,21,+1~3mar[cp12]/91,idem x2[1].ish3q26(EVI+)[34].nucish(EVI1x2)

[197]. She was treated with 5-azacitidine. After four cures, while bone marrow examination revealed a persistent excess of blast cells (15%), a hematologic improvement was observed with less blood transfusion needs; after eight cures of azacitidine, she progressed to acute leukemia, with 49% medullar blasts at 25 years old. The final diagnosis was AML with myelodysplasia-related changes (AML-MRC). A tumoral mass in the left ovary was detected by MRI but was not analyzed further. She finally succumbed at age 25 from refractory leukemia.

Patient #7 (XP167VI) was a male originated from Algeria with known consanguinity. He had two siblings with XP, including his sister (patient #12, XPGAVI) and a brother who died at 18 years old for unknown reason. He had one XP cousin (patient #13, XPGMVI). He was diagnosed as XP-C at the age of three, and was also affected by Down syndrome (trisomy 21). He developed numerous skin cancers, an atypical fibroxanthoma and two *lentigo maligna* melanomas. He received

ocular radiotherapy at 11 years old. Anemia was first observed at 14, but he was diagnosed with RAEB-t at 25. Blood transfusions were used as a sole palliative treatment, and the patient died from AML at 26. His two relatives with XP (but no trisomy 21) also developed MDS (patients #12 and #13).

Patient #8 (**XPAHVI**) was a male from Tunisia, with two XP-C brothers and one XP-C sister without hematological malignancies (not included in this study). He developed numerous skin cancers on the face and a tumor on the tip of the tongue treated with surgery and curietherapy at 22 years old. At 24, radiotherapy of a tumor in the sinus started, but was rapidly stopped due to sudden deterioration of the general status. An AML was promptly diagnosed. No further treatment was given, and the patient died at 25 years old. Conventional cytogenetics and FISH analysis revealed numerous complex modifications of the leukemic cell genome, including trisomy 8 and chromosome 21 abnormalities, but no material was available for further analysis of somatic mutation.

Patient #9 (XP673VI) was a female originated from Morocco with an XP-C brother who developed a brain astrocytoma at the age of 14 and who is now 30 years old. She was detected as XP-C at the age of 12. She developed several SCC and BCC of the nose, lips and eyes between 11 and 20 years old. She was referred for anemia and adenopathy at 21. BM examination showed 42% leukemic blast cells. Immunophenotyping analysis revealed a pre-T ALL (EGIL T-II). The leukemic cell karyotype was normal but somatic mutations affecting *NOTCH1* (an activating mutation in the HD domain), *NRAS*, *DNMT3A* and *TET2* genes were found (Table1). An excess of blast cells was still detected in the BM after reduced-intensity induction therapy with steroids, vincristine and nelarabine for two months. She received a second line therapy with vincristine, asparaginase and daunorubicin that induced a complete remission, but succumbed to infection and liver failure at the age of 22.

Patient #10 (XP538VI) was a male originated from Algeria from first cousin parents. He was diagnosed in our laboratory aged four at the same time as his sister, who died at the age of 12 with a severe anemia without additional information. The patient did not sun-protect himself well and developed numerous carcinomas on the face. At the age of 29, he was referred in a very bad condition with a severe anemia and thrombocytopenia, and 46% and 35% myeloid blasts in the blood and BM cells,

respectively. Bone marrow analysis identified an AML-2 with associated dysplasia. BM karyotype showed complex abnormalities referred as -4, -5, -7, -13, -15, -18, -19, -21,+5~ 8mar[cp27]/46, XY[3]. WES of leukemic cell and fibroblast DNA detected somatic deletions of 4q, 5q, 7q, 13q and a somatic region of copy neutral loss of heterozygosity at 17p that included a mutated *TP53*, and RAD21 mutation (Table 1). The patient died 10 days after the diagnosis.

Patient #11 (XP2006VI) is a female originated from Morocco with two XP-C brothers of 14 and 25 years old from a known consanguineous family. She was diagnosed XP at two years old in Morocco, and was followed by dermatologists in Italy and then France without any other particularity. A moderate thrombocytopenia was detected at the age of 28. She was referred at the age of 29 with gingival hemorrhages and numerous ecchymoses. Blood analysis revealed anemia and thrombocytopenia. BM aspirate failed to recover cells, and a BM biopsy showed AML-6 with myelofibrosis and a complex karyotype referred as 44,XX,t(1;19)(p13;p13),der(5;6)(p10;q10),+der(5;6)(5pter>5p10::hsr::6q12>6qter), der(21;22)(q10;q10)[23]/46,XX[2]. She received four cycles of 5-azacitidine, which decreased BM blast cell infiltrate from 31% to 7%, before receiving an allogeneic hematopoietic stem cell transplant from her healthy brother (19 years old) after a reduced-intensity conditioning regimen that was well tolerated (currently three months follow up, Supplemental Table 3).

Patients #12 (XPGAVI) and #13 (**XPGMVI**) were two cousins, and were the sister and cousin of patient #7, respectively. Their clinical history has been published.[1] They were both XP-C with consanguineous parents originated from Algeria. Patient #12 was diagnosed with XP at the age of five. She developed numerous actinic keratoses but had only one BCC. Following diffuse bleeding from the alimentary tract, she was diagnosed at the age of 24 with RAEB-2 (12% of blast cells), and she died at 25 from hemorrhages. Patient #13 was four years old at initial diagnosis of XP, with few skin cancers. He was diagnosed with a RAEB-t at the age of 27.[1] He received four weekly low doses of cytosine arabinoside, but he died quickly. At that time no cytogenetic analysis was performed in these two patients. Although we had no access to cells or DNA from both patients (who were reported in 1989), they should carry the homozygous founder *XPC* mutation as the other North African patients, since they

were directly (sibling) or indirectly (cousin) connected with patient #7, for whom we found deficient DNA repair and the causative delTG *XPC* mutation.

Supplemental Patient Data Reference

1. Berbis P, Beylot C, Noe C, Doutre MS, Broustet A, Privat Y. Xeroderma pigmentosum and refractory anaemia in two first cousins. *Br J Dermatol*. 1989;121(6):767-771.

Supplemental Table 1: Reported Xeroderma Pigmentosum (XP) patients with hematological disease

Total number of XP patients in the publication	Number of patients with blood malignancy (gender)	Country of origin	XP genotype	Type of blood disease (age at discovery)	Additional clinical information	References
25	1 (M)	Iraq	Likely *XPC*[a]	Myeloid leukemia (32y)	Death at 35y	1
5	1 (M)	UK	Likely *XPA or XPD*[b]	Acute lymphatic leukemia (3y)	Death at 6y	2
2	1 (F) Patient #12 in Table 1	Algeria	HMZ *XPC* delTG	RAEB-2 (24y)	Death at 25y of RAEB-t	3
2	1 (M) Patient #13 in Table 1	Algeria	HMZ *XPC* delTG	RAEB-t (27y)	Death at 27y	3
1	1 (F)	Pakistan	XP-C; unknown mutation[c]	Aplastic anemia (10y)	Gradual and late onset of pancytopenia	4
42	1 (M)	Libya	Likely *XPC*[a]	Lymphatic leukemia	Death of leukemia at 18y	5
3	1 (M)	Brazil	HMZ *XPC* delTG	T-cell lymphoma (3y)	Death at 13y	6
31 XP-C	1 (M) Patient #5 in Table 1	Morocco	HMZ *XPC* delTG	RAEB-t and AML-6 (12y)	Death at 15y	7
64 XP-C	1 (F)	Tunisia	HMZ *XPC* delTG	Leukemia (10y)	Death at 10y	8
2 XP	2 (F)	Morocco Morocco	Likely *XPC*[a] Likely *XPC*[a]	ALL (22y) RAEB/AML (25y)	Death at 25y Death at 28y	9

Table notes, abbreviations and references

F, female; M, male; RAEB, Refractory Anemia with Excess of Blasts; AML, acute myeloid leukemia; ALL, acute lymphoid leukemia

Publications since 1958 that reported tumors other than skin in XP patients were analyzed. Among 1,510 published XP individuals, 11 were reported with a hematological malignancy. Five had the homozygous *XPC* founder mutation from North Africa[10] c.1643_1644 delTG; p. Val548AlafsX572 (HMZ *XPC* delTG), and another patient is XP-C by complementation without knowing the precise mutation.[4] Others are likely to be XP-C, except one likely XP-A or XP-D.

[a] XP-C group is likely based on Mediterranean basin origin and the patient clinical features, i.e. very early skin cancers on exposed body parts without associated neurological abnormalities.

[b] This patient was reported with the De Sanctis-Cacchione syndrome, which is mainly associated with *XPA* mutation but can be mistaken as XP-D patients.[11]

[c] This patient was reported as XP-C by complementation without the precise mutation.[4]

Supplemental Table 1 References

1. Berlin C, Tager A. Xeroderma pigmentosum; report of eight cases of mild to moderate type and course: a study of response to various irradiations. *Dermatologica*. 1958;116(1):27-35.

2. Reed WB, Landing B, Sugarman G, Cleaver JE, Melnyk J. Xeroderma pigmentosum. Clinical and laboratory investigation of its basic defect. *JAMA.* 1969;207(11):2073-2079.

3. Berbis P, Beylot C, Noe C, Doutre MS, Broustet A, Privat Y. Xeroderma pigmentosum and refractory anaemia in two first cousins. *Br J Dermatol.* 1989;121(6):767-771.

4. Salob SP, Webb DK, Atherton DJ. A child with xeroderma pigmentosum and bone marrow failure. *Br J Dermatol.* 1992;126(4):372-374.

5. Khatri ML, Bemghazil M, Shafi M, Machina A. Xeroderma pigmentosum in Libya. *Int. J. Dermatol.* 1999;38(7):520-524.

6. Leite RA, Marchetto MC, Muotri AR et al. Identification of XP complementation groups by recombinant adenovirus carrying DNA repair genes. *J Invest Dermatol.* 2009;129(2):502-506.

7. Hadj-Rabia S, Oriot D, Soufir N, et al. Unexpected extradermatological findings in 31 patients with xeroderma pigmentosum type C. *Br J Dermatol.* 2013;168(5):1109-1113.

8. Jerbi M, Ben Rekaya M, Naouali C, et al. Clinical, genealogical and molecular investigation of the xeroderma pigmentosum type C complementation group in Tunisia. *Br J Dermatol.* 2016;174(2):439-443.

9. Pintens S, Pierret L, Keymolen K, Gutermuth J, De Raeve L. Xeroderma pigmentosum and leukaemia in two sisters. *J Eur Acad Dermatol Venereol.* 2016;30(10):e42-e43.

10. Soufir N, Ged C, Bourillon A, et al. A prevalent mutation with founder effect in xeroderma pigmentosum group C from north Africa. *J Invest Dermatol.* 2010;130(6):1537-1542.

11. Kraemer KH, Sarasin A. Xeroderma pigmentosum. In: Elder DE, Massi D, Scolyer RA, Willemze R, eds. WHO Classification of Skin Tumours. 4th ed. Lyon, France: IARC Press; 2018:386-387.

Supplemental Table 2: Genome-wide inbreeding coefficient f and mating types inferred for six XP-C patients

Patient ID	Mutation status	*f*	1C	2C	2x1C
XP10VI	Homozygous *XPC* delTG	0.0510	0.05	0.9497	2.00E-04
XP82VI	Homozygous *XPC* delTG	0.0940	0.2486	0.0011	0.7503
XP185VI	Homozygous *XPC* delTG	0.1110	0.0943	0	0.9057
XP235VI	Homozygous *XPC* delTG	0.1010	0.0459	0	0.9541
XP309VI	Homozygous *XPC* delTG	0.0720	0.8828	0.0075	0.1097
XP924VI	Homozygous *XPC* delTG	0.0740	0.6939	0.0163	0.2897

We estimated the genomic inbreeding coefficient of each individual. FSuite Software[1] was used to estimate and detect inbreeding on individuals without known genealogy, and to estimate the individual probability to be offspring of different mating types. We found as expected a high level of inbreeding in all affected patients.

f: Inbreeding coefficient; 1C: first-cousin offspring; 2C: second-cousin offspring; 2×1C: double first-cousin offspring.

Supplemental Table 2 Reference

1. Gazal S, Sahbatou M, Babron MC et al., FSuite: exploiting inbreeding in dense SNP chip and exome data. *Bioinformatics*. 2014;30(13):1940-1.

Supplemental Table 3: Summary of the allogeneic hematopoietic stem cell transplant medical history in two XP patients

Patient	Donor	Graft source	Age and status at transplant	Conditioning regimen & GVHD prophylaxis	Engraftment	GVHD	Hematological outcome	Evolution	Cause of death
Patient #2 (XP82VI)	Father Haploidentical (8/10)	Peripheral HSC with CD34 selection	17y Blastic, no pre-transplant antileukemic treatment	Bu 3 mg/kg/d from D-8 to D-7; Cy: 10 mg/kg/d from D-6 to D-3; ATG 2.5 mg/kg/d from D-6 to D-3; Flu 30 mg/m² from D-6 to D-4 No GVHD prophylaxis (T-depletion with CD34 selection)	No	NA	D28 post HSCT: 18% of blasts on bone marrow aspiration	Death 18y, at D60 post HSCT	Toxicity with persistent leukemia
Patient #11 (XP2006VI)	Brother Matched related	Bone marrow	29y Partial remission post treatment with azacitidine: 7% blasts	Flu 30 mg/m²/d from D-4 to D-2; Cy 10 mg/kg/d from D-5 to D-2 GVHD prophylaxis, CSA + MMF	Yes Mix chimerism: D26 post HSCT, 18% donor cells on whole blood; D67 post HSCT, 43% donor cells on whole blood	No	BM not evaluated	Last Follow Up: alive at D85 post HSCT	

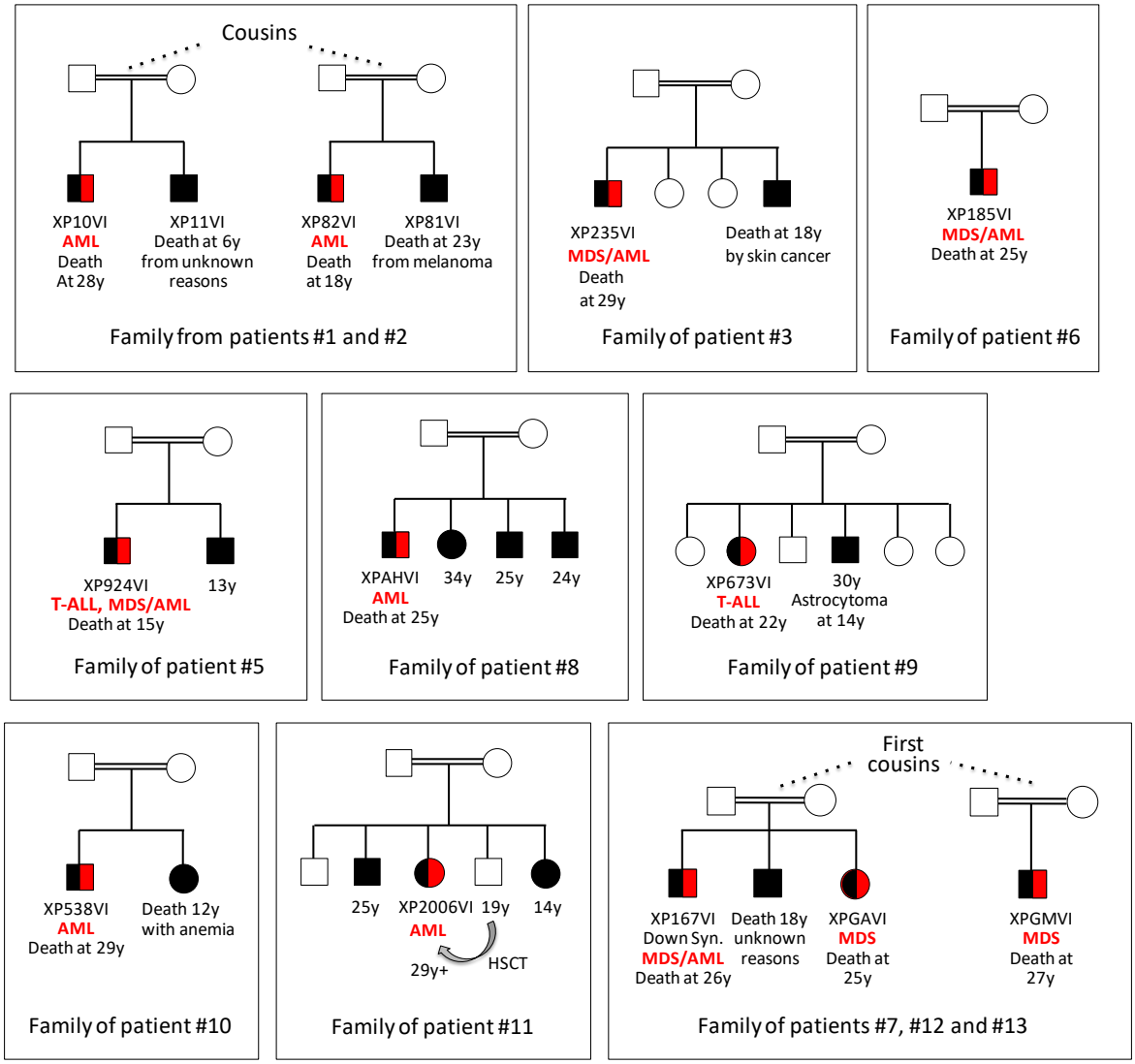
Table abbreviations: HSC, hematopoietic stem cells; HSCT, hematopoietic stem cell transplant; GVHD, graft versus host disease; D, day; Bu, busulfan; Cy, cytoxan; Flu, fludarabine; ATG, anti-thymocyte globulin; CSA, cyclosporine A; MMF, mycophenolate mofetyl; NA, not applicable.

Supplemental Table 4: Acquired TP53 mutations in MDS/AML in XP-C patients.

Patient ID	Mutation location	Mutation effect	Genomic position	Exon number	c.DNA changes	AA changes	Nucl. changes	Trinucl. context
XP82VI	exonic	Non synonymous	7577088	exon 8	c.A850C	p.T284P	A>C	CAC>CCC
XP185VI	exonic	Non synonymous	7578206	exon 6	c.A643C	p.S215R	A>C	TAG>TCG
XP185VI	exonic	Non synonymous	7578469	exon 5	c.G461T	p.G154V	G>T	GGC>GTC
XP235VI	exonic	Stop	7573991	exon 10	c.G1036T	p.E346X	G>T	TGA>TTA
XP235VI	splicing	-	7578176	exon 6	c.672+1G>T	-	G>T	GGT>GTT
XP538VI	exonic	Non synonymous	7577124	exon 8	c.G814A	p.V272M	G>A	GGT>GAT
XP924VI	exonic	Stop	7577100	exon 8	c.A838T	p.R280X	A>T	GAG>GTG

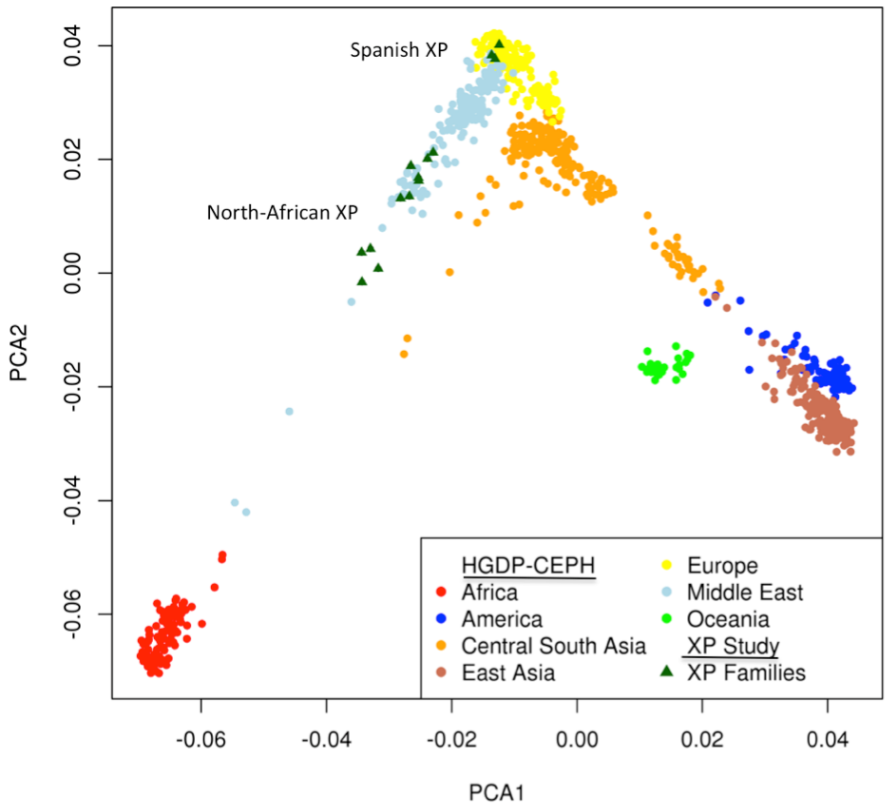
TP53 gene transcript reference number NM_001126112.

Supplemental Figure 1: Available family trees of patients from the cohort with XP and a hematological disorder



Available family trees for patients included in this study are shown. Black squares (males) or circles (females) show patients with XP. Age at last follow up or at death is indicated. More information is available in Table 1 and in Supplemental Patient Data. Family information for patient #4 was not available.

Supplemental Figure 2: Distribution of variants determined by the Principal Component Analysis (PCA) of the XP-C individuals and families and the HGDP-CEPH panel individuals



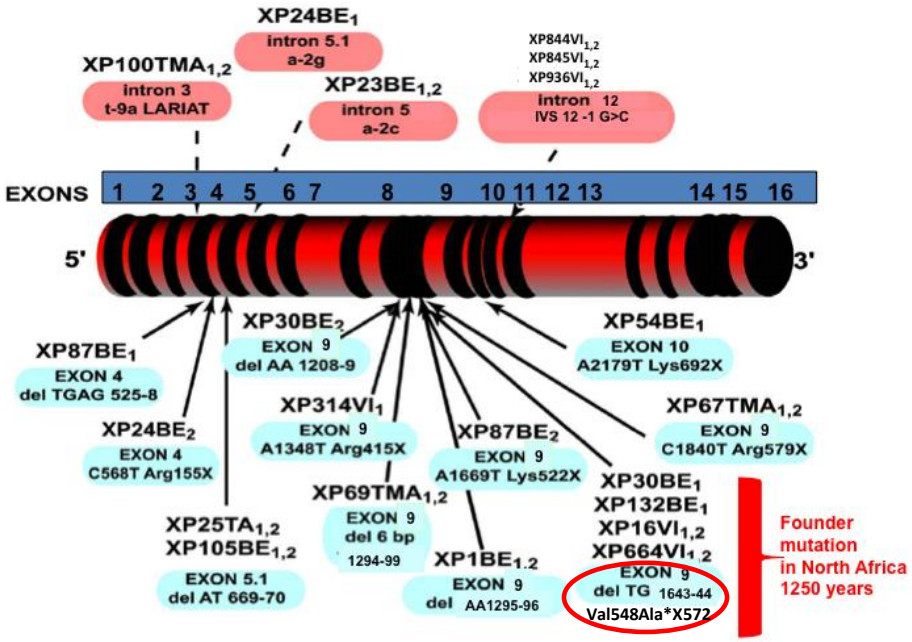
We exome-sequenced germinal DNA from 14 individuals, including three XP-C families (Families of patient #1 and #2: two parents and two XP individuals; patient #6 and her two parents) and three XP-C individuals (patient #3, #4 and #5). In order to explore the population stratification of DNA variants, we performed a principal component analysis (PCA) using PLINK 1.9 software.[1] The HGDP-CEPH Diversity Panel is a resource of 1,063 lymphoblastoid cell lines (LCLs) from 1,050 individuals in 52 world populations.[2] Single nucleotide polymorphisms (SNPs) shared by the two datasets (HGDP-CEPH and XP families), after reducing the linkage disequilibrium (LD), are used to perform the PCA analysis. We based the principal components on a subset of seven HGDP population groups, and the XP individuals have been projected onto those PCAs. We extracted the top two principal components of the variance-standardized relationship matrix (PCA1 and PCA2).

Supplemental Figure 1 References

1 Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience.* 2015;4:7.

2 Cann HM, de Toma C, Cazes L et al: A human genome diversity cell line panel. *Science.* 2002;296(5566):261–262.

Supplemental Figure 3: Location of the reported XPC gene mutations



The founding mutation found in the described XP-C patients is the homozygous delTG in exon 9, as indicated with red circle and bracket (adapted from Khan et al., 2006). The *XPC* gene has 16 exons. Mutations in introns are boxed in red above the gene diagram, those located in exons are indicated under the gene. The types of mutations are boxed in blue and names of the cells bearing the corresponding mutation are in black letters. The founding *XPCdelTG* mutation leads to the complete absence of XPC protein, as most known pathogenic *XPC* mutations (Khan et al., 2006).

Supplemental Figure 2 Reference

Khan SG, Oh KS, Shahlavi T, et al. Reduced XPC DNA repair gene mRNA levels in clinically normal parents of xeroderma pigmentosum patients. *Carcinogenesis*. 2006;27(1):84-94.

Supplemental Methods: Whole Exome Sequencing (WES) and analysis

Genomic DNA was captured using Agilent in-solution enrichment methodology with their biotinylated oligonucleotides probes library (SureSelect All Exon V5, or SureSelect Clinical Research Exome, Agilent), followed by 75-base paired-end massively parallel sequencing on Illumina HiSeq2500, HiSeq4000, or NextSeq500. Sequence capture, enrichment, and elution were performed according to the manufacturer's instruction and protocols (SureSelect, Agilent). Briefly, 600 ng of each genomic DNA was fragmented by sonication and purified to yield fragments of 150–200 bp. Paired-end adaptor oligonucleotides from Illumina were ligated on repaired, A-tailed fragments and then purified and enriched by 4–6 PCR cycles. Five hundred ng of these purified libraries was then hybridized to the SureSelect oligo probe capture library for 24 h. After hybridization, washing, and elution, the eluted fraction was PCR amplified for 10–12 cycles, purified, and quantified by qPCR to obtain sufficient DNA template for downstream applications. Each eluted-enriched DNA sample was then sequenced on an Illumina HiSeq2500/4000 or NextSeq500 as paired-end 75 b reads. Image analysis and base calling were performed using Illumina Real Time Analysis Pipeline version 1.12.4.2 with default parameters. Mean coverage was 83X +/− 18X for normal blood samples and 122X +/− 15X for tumor samples, with respectively 87% (77%–93%) and 90% (85%–95%) of the targeted regions covered at 20X or more.

Fastq files were aligned to the reference genome hg19 with the BWA-MEM algorithm (v0.7.12).[1] After alignment, the BAM files were treated for PCR duplicate removal and then sorted and indexed with Picard (v1.121) for further analyses (http://broadinstitute.github.io/picard). Base recalibration and local realignment around indels were done with GATK (v3.1-1).[2]

For defining **somatic mutations**, we used the MuTect (version 1.1.7)[3] algorithm for identifying substitutions. Variants were also called using VarScan2 (v2.3.7)[4] instead of MuTect in order to call other variants with less specificity. Results from VarScan2 were filtered based on somatic p-value (< 0.05) and annotations (coding regions only). The resulting somatic mutations were annotated with the snpEff and snpSift algorithms.[5] We removed variants that were also annotated as known polymorphisms (reported by 1000 Genomes or the ESP databases) unless the variant was also reported in Catalogue of Somatic Mutations in Cancer (COSMIC)[6] or ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).

For defining **germline variants,** nucleotide variants of coding exons and affecting the amino acid composition of proteins, and variants of non-coding RNA splice sites of coding and non-coding genes, were selected. Only variants with a depth >10 reads and a Variant Allelic Frequency (VAF) > 4 % were selected, and variants with a total depth of variant-supporting bases > 5 were kept. Variants with a strand bias, i.e. a depth of variant supporting bases on forward or reverse strand equal to 0, were filtered out. Then, variants with a maximum frequency > 1% in the overall population were filtered out using Pop Freq Max database of ANNOVAR that contains databases from 1000 Genomes Project (2015 Aug) annotations (http://www.internationalgenome.org), ESP6500 (http://evs.gs.washington.edu/EVS), ExAC (http://exac.broadinstitute.org), and CG46. Residual variants were eventually manually cured by comparison with our in-house WES database. This WES data analysis resulted to only one shared rare variant, namely XPCdelTG.

Supplemental Methods References

1. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2010;26(5):589–595.

2. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20(9):1297–1303.

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