

DNA-Based Diagnosis of Xeroderma Pigmentosum Group C by Whole-Genome Scan Using Single-Nucleotide Polymorphism Microarray

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In this study, we have established the molecular basis of xeroderma pigmentosum (XP) in two unrelated Chinese families. In the first patient with consanguineous parents, we mapped the disease-causing locus *XPC* using single-nucleotide polymorphism microarray. Mutational analysis of the *XPC* gene showed that the patient is homozygous for a nonsense mutation, E149X. After developing DNA-based diagnosis of *XPC*, we screened another XP patient for *XPC* mutations. We found that the second patient is a compound heterozygote of 1209delG and Q554X in this gene. These are the first *XPC*-causing mutations identified in Chinese patients.

Key words: homozygosity mapping/single-nucleotide polymorphism microarray/whole-genome scan/xeroderma pigmentosum

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To date, eight different disease-causing genes have been identified in xeroderma pigmentosum (XP) patients: seven involve defective nucleotide excision repair (NER) (*XPA*–*XPG*), and one, the *XP variant*, results in replication of damaged DNA on the leading strand (Cleaver, 2000; Moriwaki and Kraemer, 2001). According to the National Center for Biotechnology Information (NCBI) Build 34 human genome map, *XPA* is located on chromosome 9q22.33, *XPB* (*ERCC3*) is located on chromosome 2q14.3, *XPC* is located on chromosome 3p25.1, *XPD* (*ERCC2*) is located on chromosome 19q13.32, *XPE* (*DDB2*) is located on chromosome 11p11.2, *XPF* (*ERCC4*) is located on chromosome 16p13.12, *XPG* (*ERCC5*) is located on chromosome 13q33.1, and *XPV* (pol eta) is located on chromosome 6p21.1. In sum, eight of 22 autosomes have *XP* genes, and traditionally these *XP* subgroups have been determined by complementation assay. In Chinese populations, the complementation group assignment of *XP* patients has been limited (Kraemer *et al*, 1987). We report here on the molecular investigations of two Chinese families with *XP*.

Results

Using the whole-genome scan, we reduced the number of possible disease-causing *XP* loci in the first patient from eight to three, namely, *XPC*, *XPF*, and *XPV* (Table I). We next ranked the quality of the three homozygous chromosomal regions as follows: (1) the size of the homozygous chromosomal region (normalized with the size of the respective

chromosome); (2) the number of single-nucleotide polymorphisms (SNP) in the homozygous chromosomal region; and (3) the number of SNP on the centromeric and telomeric side of the *XP* locus in the homozygous chromosomal region. The best of each was scored as 3 and the worst was scored as 1. A high-quality homozygous chromosomal region should have the largest size, the highest number of SNP, and an equal number of SNP on both sides of the *XP* locus. The *XP* locus with the highest total score was selected for mutation screening.

Among the three loci, the *XPC* locus had the highest score (Table II). Therefore, we screened for the mutations of the *XPC* gene first. With direct DNA sequencing, the patient was found to be homozygous for a 445G>T mutation in exon 4, changing codon 149 from GAA to a premature stop codon TAA, i.e., E149X (Fig 1). The parents and the three sisters of the proband were all heterozygous for E149X. After developing DNA-based diagnosis of *XPC* for patient 1, we screened patient 2 for *XPC* mutations. In patient 2, two novel mutations were identified in exon 9. The first mutation is 1659C>T, changing codon 554 from CAG to a premature stop codon, TAG, i.e., Q554X (Fig 1). The second mutation is 1209delG causing a frameshift of the reading frame (Fig 1), and creating a premature stop codon downstream at codon 525. The mother was heterozygous for 1659C>T, whereas the father was heterozygous for 1209delG (data not shown).

Discussion

Of the 23 reported *XPC* families, most of the *XPC* mutations result in premature termination of the *XPC* protein (Legerski

Abbreviations: HuSNP, human SNP; XP, xeroderma pigmentosum

Table I. Homozygous chromosomal regions marked by HuSNP mapping

Chromosome	Marker/ gene	Genotype	Chromosome banding	cM
3	WIAF-4560	AA	3p26.3	0.33
	WIAF-685	AA	3p26.2	12.19
	WIAF-1793	BB	3p26.1	16.52
	WIAF-3235	BB	3p25.3	26.21
	WIAF-3008	BB	3p25.3	27.30
	WIAF-838	AA	3p25.3	29.01
	WIAF-2563	BB	3p25.2	32.79
	XPC		3p25.1	40.05
	WIAF-3865	AA	3p24.3	41.25
	WIAF-906	AA	3p24.3	41.25
	WIAF-1946	BB	3p24.2	47.84
	WIAF-1765	BB	3p24.1	49.81
	WIAF-721	AA	3p24.1	50.81
	WIAF-3380	BB	3p23	52.55
	WIAF-1789	BB	3p22.3	55.74
	WIAF-741	BB	3p22.3	55.77
6	WIAF-2103	AA	6p21.2	50.16
	WIAF-2810	AA	6p21.1	59.61
	XPV		6p21.1	66.47
	WIAF-538	AA	6p21.1	73.50
	WIAF-3166	BB	6p12.3	77.33
	WIAF-3444	BB	6p12.3	79.93
	WIAF-3784	BB	6p12.1	80.63
	WIAF-4584	AA	6p12.1	81.85
	WIAF-1670	BB	6q12.2	83.85
16	WIAF-3700	BB	6q12	86.29
	WIAF-2690	AA	6q14.1	88.01
	WIAF-506	BB	16p13.3	6.76
	WIAF-627	AA	16p13.3	7.01
	WIAF-924	BB	16p13.2	11.65
	WIAF-3828	BB	16p13.2	15.36
	WIAF-3324	AA	16p13.2	15.51
	XPF		16p13.12	28.30
	WIAF-2652	AA	16p12.3	37.48
	WIAF-1870	BB	16p12.1	44.43
	WIAF-2136	AA	16p12.1	56.86

AA, homozygous for the A marker; BB, homozygous for the B marker. We excluded chromosomal regions with XP loci marked by less than five homozygous SNP or with a size less than the median marker gap size 1.2 cM, because these regions provided insufficient data to determine homozygosity.

SNP, single-nucleotide polymorphisms; HuSNP, human SNP; XP, xeroderma pigmentosum.

and Peterson, 1992; Li *et al*, 1993; Khan *et al*, 1998; Cleaver *et al*, 1999; Chavanne *et al*, 2000; Slor *et al*, 2000; Gozukara *et al*, 2001; Khan *et al*, 2004), either because of nonsense or frameshift mutations. We present here the molecular detection and analysis of three novel XPC mutations, E149X, Q554X, and 1209delG, in a Chinese population. These mutations might be ethnic-specific mutations as they have not been reported in other populations.

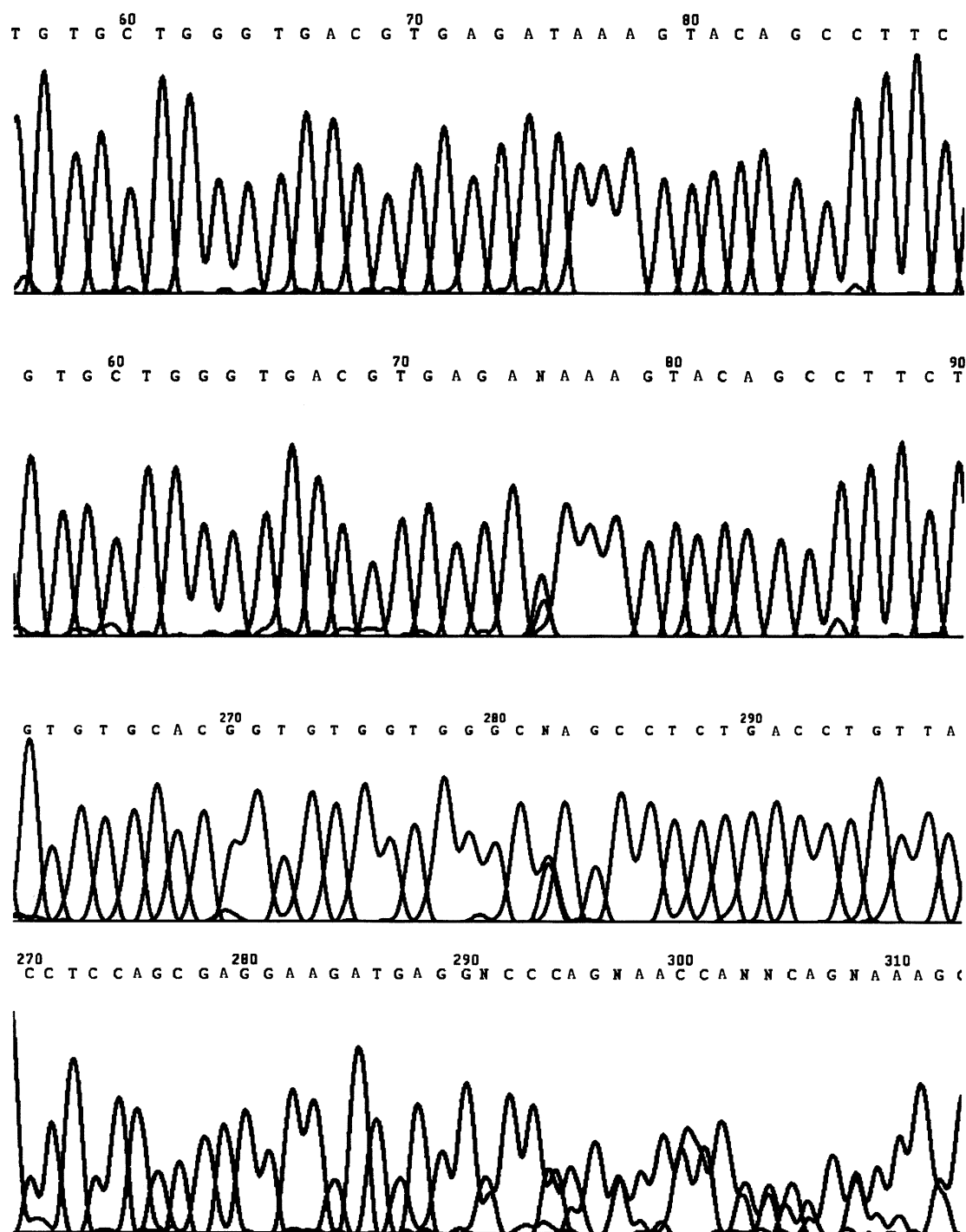
Although fibroblast cell lines of the two patients are not available for cell-based assays, nonsense or frameshift XPC mutations downstream of R149X, 1209delG, and Q554X have been fully characterized in previous studies and have shown defective NER activity (Li *et al*, 1993; Chavanne *et al*, 2000). Of these, 10 fully characterized nonsense or frameshift XPC mutations are located downstream of R149X and three fully characterized nonsense or frameshift mutations are located downstream of 1209delG and Q554X (<http://xpmutations.org>). Therefore, we predict that XPC cell lines having R149X, 1209delG, and Q554X will also have defects of NER and that these mutations are responsible for causing disease in our patients.

The mapping study was essential in preparing the ground for the mutation study and decreasing the burden of a complete sequencing of all possible loci for this disease. The results of the mapping study and the mutation study were consistent, validating this approach. We were able to prioritize the XPC loci for mutational analysis in the first patient based on the history of consanguinity, by performing a whole-genome scan for homozygous chromosomal regions, using SNP microarrays. Consanguinity of parents is common in patients with rare autosomal recessive diseases and has been reported in about 30% of the XP cases (Kraemer *et al*, 1987). In one study of XPC, 10 of 13 patients were homozygous for the mutated XPC alleles (Chavanne *et al*, 2000). The human SNP (HuSNP) genotyping assay is fully automated, and this parallel assay can be completed in 2 d. With future reductions in the cost of SNP genotyping, whole-genome scans should become more widely available. This is of particular advantage in finding disease loci in consanguineous families.

Materials and Methods

Subjects

Family 1 The patient was an ethnic Chinese girl from southern China, whose parents were blood relatives (fifth degree). She was normal at birth, but at a very young age, many freckles were noted over her face, and her skin was rough and dry. The patient's elder brother had similar skin problems. Believing their daughter's skin problems were not serious, her parents failed to seek medical advice or to adopt stringent sun-protective measures. Similar to her peers, the patient attended public school with an average academic performance. In adolescence, the patient and her elder brother started to develop numerous non-melanoma skin cancers, mainly over their faces that were treated with multiple local excisions. The patient was referred to us at the age of 28 y for management of incessant skin cancers. By then, she had already had multiple excisions over her face and nose. Because of the mutilating nature of her surgeries, prosthesis was required to cover the nasal defect. In a therapeutic trial, she was put on systemic retinoid etretinate and, later, acitretin at a dose of 0.5 mg per kg per d. This was, however, discontinued after 2 y as new skin tumors continued

**Figure 1**

Upper half: electropherograms of exon 4 of the *XPC* gene of patient 1 with homozygous 445G>T (*top panel*) and her father with heterozygous 445G>T (*bottom panel*). *Lower half:* electropherograms of exon 9 of the *XPC* gene of patient 2 with heterozygous 1659C>T (*top panel*) and heterozygous 1209delG (*bottom panel*). The 1 bp deletion, 1209delG, causes a doubly heterozygous pattern: from 5'-CCTCCAGCGAGGAA-GATGAGGGCCCAAGACAAGCAGGAGAAG-3' to 5'-CCTCCAGCGAGGAAGATGA(G/G)(G/G)(G/C)(C/C)(C/C)(C/A)(A/G)(G/G)(G/A)(A/G)(A/C)(C/A)(A/A)(A/G)(G/C)(C/A)(A/G)(G/G)(G/A)(A/G)(G/A)(A/A)(A/G)-3'. The sequences are shown in the sense direction. A heterozygous site is denoted by the letter N. *Polymerase chain reaction (PCR):* The genomic sequence of the human *XPC* gene was obtained from the NCBI human genome sequence database. All the exons and intron-exon boundaries of the *XPC* gene were amplified by PCR. The primer sequences and the PCR conditions are available on the Journal website (<http://www.jidonline.org>) as supplementary material. *Mutational analysis:* The PCR products were purified by Microspin S-300 HR columns (GE Healthcare, Uppsala, Sweden), and both strands were sequenced using the amplification primers as sequencing primers and BigDyeDeoxy terminator cycle sequencing reagents according to the manufacturer's instructions (Applied Biosystems, Foster City, California). Products of the sequencing reactions were purified by Centri-Sep spin columns (Princeton Separations, Adelphia, New Jersey). Purified sequencing fragments were separated by capillary electrophoresis and detected via laser-induced fluorescence on an ABI Prism 3100 genetic analyzer (Applied Biosystems).

Table II. Scoring of homozygous chromosomal regions

XP genes	Size of homozygous chromosomal region (cM)	Portion of chromosome spanning the homozygous chromosomal region	No. of SNP in the homozygous chromosomal region	No. of SNP on the centromeric and telomeric sides of the XP gene	Total score
XPC	55.4	0.24	15	8,7	8
XPF	50.1	0.37	8	3,5	6
XPV	37.9	0.21	10	8,2	4

SNP, single-nucleotide polymorphisms; XP, xeroderma pigmentosum.

to develop. Her elder brother died, at the age of 32 y, of disseminated skin cancers; whereas our patient died at the age of 37 y, 2 y after she was diagnosed to have inoperable clear cell carcinoma of the ovary. Up to their death, no neurological deficits were noted.

Family 2 The patient was born to parents who were clinically normal ethnic Chinese with no consanguinity. They had one daughter and one son; both were affected. Their daughter was born in 1976 and was healthy at birth. She had dry skin and developed freckles over the sun-exposed areas in early childhood. The patient attended normal school and had distinguishing academic performance. All along, she was under the care of a physician with the clinical diagnosis of XP. Though repeatedly advised on sunlight avoidance, both parents and the patient underestimated the damaging effect of sun exposure and failed to adhere to stringent sunlight protective measures. At the age of 8 y, she developed the first skin tumor at the tip of the nose. Thereafter, multiple skin tumors including basal cell carcinoma and squamous cell carcinoma developed over her face, nose, and scalp. She had been put on topical 5-fluorouracil (Efudix, ICN Pharmaceuticals, Costa Mesa, California), tretinoin and flurocytocine cream without success. Later, surgical excision and cryotherapy became routine procedures for her skin tumors. In 1988, at the age of 12 y, she was put on low-dose etretinate at 10 mg per d. Unfortunately, multiple skin tumors continued to come up over her face, nose, eyelid, and forehead. She finally died of disseminated carcinoma at the age of 21 y.

Her little brother was born in 1982. Multiple freckles were noted over the sun-exposed areas since early childhood. Having a bitter experience with their first child, the parents this time adopted vigorous measures to protect their second child. Long-sleeve shirt, broad beam hat, and high SPF (50) sunscreen were used. Over the years, only actinic keratosis developed over the patient's face and no skin tumor has ever been documented. The patient was also put on etretinate 20 mg daily at the age of 8 y and has continued with it since then. The patient had a normal academic performance and enjoyed his social life. No neurological deficit has been detected so far.

DNA extraction Genomic DNA of the patients, parents, and siblings was extracted from peripheral blood samples with a QIAamp blood kit (Qiagen, Hilden, Germany), according to the manufacturer's instruction. Participants gave their written informed consent. The medical ethical committee of the Chinese University of Hong Kong approved all described studies. The study was conducted according to Declaration of Helsinki Principles.

HuSNP probe array and HuSNP mapping assay In patient 1, we examined the homozygosity of SNP flanking all the known XP loci using a whole-genome scan by Affymetrix HuSNP probe arrays (Santa Clara, California). Because the parents are consanguineous, the disease-causing XP locus of patient 1 should fall in a chromosomal region marked by homozygous SNP, because the two disease-causing chromosomal regions, one from each parents, are identical-by-descent. SNP genotyping was performed according to the HuSNP protocol supplied by Affymetrix. HuSNP probe arrays are manufactured using technology that combines photolithographic methods and combinatorial chemistry. Tens to hundreds

of thousands of different oligonucleotide probes are synthesized in a 0.81 cm × 0.81 cm area on each HuSNP probe array. With a median marker gap size of 1.2 cM, 1308 fully mapped markers are evenly distributed across the human genome (Fan *et al*, 2000).

Briefly, starting with 120 ng of genomic DNA, a set of 24 simultaneously run multiplex PCR amplify the HuSNP represented in the GeneChip HuSNP mapping assay. The amplified SNP are further amplified and concomitantly labeled using biotinylated primers in a second set of 24 simultaneously run labeling PCR. The biotinylated PCR products are then pooled, concentrated, and prepared for hybridization. The biotinylated amplification products, which reflect the bi-allelic genotype (typed as A or B) in the sample DNA, are hybridized to the GeneChip HuSNP probe arrays during an overnight incubation at 44°C in the GeneChip hybridization oven. On the next day, the probe arrays are thoroughly washed and stained with a complex of streptavidin-phycoerythrin and biotinylated anti-streptavidin immunoglobulin G antibody. The automated wash and stain procedures are run on the GeneChip fluidics station 400, under the control of Affymetrix microarray suite software 5.0. The stained probe arrays are scanned in an Agilent GeneArray scanner, also under the control of Affymetrix microarray suite software 5.0. Each probe array is scanned twice to capture the light emitted at wavelengths of 530 and 570 nm, generating two scan image files. The Affymetrix microarray suite software 5.0 processes the two scan images to calculate the signal intensities on the probe array. Genotype calling is performed by Genotype Viewer 1.0 (Affymetrix).

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Supplementary Material

The following material are available from <http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23563/JID23563sm.htm>

Table S1. Primes for amplification of the coding exons of the XPC gene

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