Six Genes Associated with the Clinical Phenotypes of Individuals with Deficient and Proficient DNA Repair

Tobias Gremmel¹, Susanne Wild¹, Winfried Schuller², Viola Kürten³, Klaus Dietz⁴, Jean Krutmann¹ and Mark Berneburg²

¹Institut für Umweltmedizinische Forschung at the Heinrich-Heine-University gGmbH, Auf’m Hennekamp 50, D-40225 Düsseldorf, Germany. ²Molecular Oncology and Aging, Department of Dermatology, Eberhard-Karls-University, Tübingen, Germany. ³Department of Dermatology, Heinrich-Heine-University, Moorenstr. 5, D-40225 Düsseldorf, Germany. ⁴Department of Medical Biometry, Eberhard-Karls-University, Tübingen, Germany.

Abstract: Xeroderma pigmentosum (XP) is a genetic disorder characterised by hypo-/hyperpigmentation, increased sensitivity to ultraviolet (UV)-radiation and an up to 2000-fold increased skin cancer risk. Cells from XP-patients are defective in nucleotide excision repair (NER) responsible for repair of UV-induced DNA damage. This defect accounts for their mutator phenotype but does not predict their increased skin cancer risk. Therefore, we carried out array analysis to measure the expression of more than 1000 genes after UVB-irradiation in XP cells from three complementation groups with different clinical severity (XP-A, XP-D, XP-F) as well as from patients with normal DNA repair but increased skin cancer risk (≥2 basal or squamous cell carcinoma at age <40yrs). Of 144 genes investigated, 20 showed differential expression with p < 0.05 after irradiation of cells with 100 mJ/cm² of UVB. A subset of six genes showed a direct association of expression levels with clinical severity of XP in genes affecting carcinogenesis relevant pathways. Genes identified in XP cells could be confirmed in cells from patients with no known DNA repair defects but increased skin cancer risk. Thus, it is possible to identify a small gene subset associated with clinical severity of XP patients also applicable to individuals with no known DNA repair defects.

Keywords: array analysis, basal cell carcinoma, DNA repair, squamous cell carcinoma, skin cancer risk, xeroderma pigmentosum

Introduction

Xeroderma pigmentosum (XP) is an autosomal recessive disorder with an estimated prevalence of 1:10⁶ worldwide. Clinically, it is characterised by hypo- and hyperpigmentation, telangiectasia and xerosis of the skin.¹⁻³ Furthermore, patients with XP show photosensitivity and a risk to develop skin cancer that is increased up to 2000-fold.⁴ Cells from patients with XP are defective in nucleotide excision repair (NER), a mechanism responsible for the repair of helix distorting DNA damage including damage induced by ultraviolet (UV) radiation.⁵⁻⁷ While it is clear that defective DNA repair of UV-induced photoproducts leads to mutagenic photoproducts, this alone does not account for the increased skin cancer risk in these patients.⁸ In line with this notion, it is known that carcinogenesis, including UV-induced skin carcinogenesis, involves several processes such as p53 signalling, apoptosis, cell cycle control, DNA repair and immunosurveillance⁹⁻¹⁰ and it has been shown that e.g. DNA repair, immunology and apoptosis are in bidirectional cross-talk.¹¹⁻¹² In support of the notion that photocarcinogenesis in XP patients may involve multiple factors, we have shown previously, that the absence of an increased skin cancer risk in two different DNA repair deficiency syndromes, namely Trichothiodystrophy (TTD) and Cockayne syndrome (CS) is not associated with the DNA repair defect of these syndromes.¹³

Different gene expression profiles for skin carcinogenesis have been published.¹⁴⁻¹⁶ Furthermore, an elegant study by da Costa et al. has differentiated the transcriptional profile in cells expressing the XPB/CS or XPB/TTD allele after UVC-irradiation.¹⁷ However, thus far results derived from gene expression profiling in DNA repair deficient cells after physiologically relevant doses of UVB-irradiation have not been attempted to be transferred to the DNA repair proficient background to identify a defined set of genes with functional relevance for skin carcinogenesis and possible prognostic value.
Investigation of differential gene expression in cells from patients with three different complementation groups of XP, characterized by distinct severities of their clinical phenotypes identified a subset of genes with functions in processes relevant for UV-induced skin carcinogenesis. Genes identified could also be detected in cells from patients with no known defects in DNA repair but increased skin cancer risk at a magnitude that continued tendencies discovered in DNA repair deficient cells. The detection of this gene-subset may allow further work to identify factors determining the lifetime skin cancer risk of an individual in the normal population.

**Materials and Methods**

**Cell culture**

Fibroblast cultures were established from skin biopsies taken from patients with informed consent as approved by the ethics committee of the University of Düsseldorf, Germany (Nr.1480). Normal fibroblasts were derived from non-sunexposed skin of normal individuals of skin phototype II-III and estimated lifetime sun exposure of a caucasian individual in the northern hemisphere without outdoor occupation, and these were matched to the ages of the investigated patients. Xeroderma pigmentosum cell lines were XP19BR (XP-A), XP15BR (XP-A, complex splice mutation (personal communication A. Lehmann)), XP16BR (XP-D, amino acid change: R683W), XP2DF (XP-D), XP24BR (XP-F) and XP4DF (XP-F). These cells were kindly provided by Alan Lehmann from the Genome Stability Center of the University of Sussex Falmer Brighton, Great Britain with the exception of XP2DF and XP4DF which were generated and characterized by our own group. Details of the cell lines employed are given in Table 1. Patients suffering from complementation group XP-D did not show any features of trichothiodystrophy or Cockayne syndrome and patients with XP-F clinically showed the mildest phenotype as well as one XP-F patient showing the highest age, 66 years. Patients with ≥2 basal or squamous cell carcinoma were included in the study. Six of these patients were female and one male. Ages at enrolment ranged between 18 and 39. Numbers of tumours prior to enrolment ranged between 2 and 10. Primary fibroblasts were cultured in MEM (PAA Laboratories, Pasching, Austria) supplemented with 15% FCS (Peribo Science, Bonn, Germany) and kept at 37 °C in a humidified atmosphere with 5% CO₂. For culture and irradiation cells were kept in 10-cm culture dishes and grown to confluency to avoid cell cycle influence.

**Unscheduled DNA synthesis (UDS)**

Measurement of DNA repair capacity was carried out similarly to the method described by Lehmann et al. in our experiments radioactive measurement of 3-H-thymidine incorporation was replaced by peroxidase based fluorescence measurement of

| Patient | Group | Age | Biopsy skin site | Phototype | Sun exposure | Passage | Tumour |
|---------|-------|-----|-----------------|-----------|--------------|---------|--------|
| 1       |       | 18 y | Non-sunexposed  | III       | Intermediate | 8       | BCC    |
| 2       |       | 21 y | Non-sunexposed  | III       | Intermediate | 7       | BCC    |
| 3       |       | 39 y | Non-sunexposed  | II        | Intermediate | 2       | BCC    |
| 4       |       | 29 y | Non-sunexposed  | III       | High         | 7       | SCC    |
| 5       |       | 33 y | Non-sunexposed  | II        | Intermediate | 6       | BCC    |
| 6       |       | 36 y | Non-sunexposed  | II        | Intermediate | 4       | BCC    |
| 7       |       | 38 y | Sun-exposed     | III       | Intermediate | 6       | BCC    |
| XP19BR  | XP-A  | 15 y | Non-sunexposed  | II-III    | Low          | 2       |       |
| XP15BR  | XP-A  | 0.3 y| Non-sunexposed  | II-III    | Low          | 6       |       |
| XP16BR  | XP-D  | 1 y  | Non-sunexposed  | II-III    | Low          | 6       |       |
| XP2DF   | XP-D  | 21 y | Non-sunexposed  | III       | Intermediate | 7       |       |
| XP24BR  | XP-F  | 30 y | Non-sunexposed  | I-II      | Intermediate | 4       |       |
| XP4DF   | XP-F  | 66 y | Non-sunexposed  | II        | Intermediate | 8       |       |

Complementation group, age, body site of skin biopsy for fibroblast generation, skin phototype according to Fitzpatrick, estimated lifetime sun exposure, passage level of fibroblasts as well as the types of tumours are given where applicable. For estimated lifetime sun exposure low indicates close to no sun exposure at all due to XP-adequate protection, intermediate indicates sun exposure comparable to a caucasian individual in the northern hemisphere without occupational outdoor activity (includes XP patients applying no XP-specific sun protection) and high indicates sun exposure comparable to a caucasian individual in the northern hemisphere with occupational outdoor activity.
5-Bromo-2'-deoxy-uridine (BrdU) incorporation and the experimental procedure was carried out employing the Cell Proliferation Biotrak ELISA kit, version 2 (Amersham, Freiburg, Germany), according to the protocol. Briefly, fibroblasts were plated at $1 \times 10^5$ in triplicates overnight followed by three days of serum starvation with medium containing 0.5% FCS. Subsequently, cells were exposed to 10 mM hydroxyurea for 30 min, washed, and then irradiated with different doses of UVC. Immediately after irradiation, cells were incubated with labelling solution including 10 µM BrdU for 3 hours. Labelling solution was removed followed by fixation (room temperature, 30 min), staining with peroxidase-labelled monoclonal anti-BrdU antibody and fluorescence detection by an Emax ELISA reader (Molecular Devices, Workingham, Great Britain).

**UVB-Irradiation**

Irradiation was carried out as described previously. Briefly, lids were removed from tissue culture dishes, cells were washed with PBS once and irradiated with FS20 tubes (Westinghouse Electric, Pittsburgh, PA), which are known to emit primarily in the UVB range, with a tube-to-target distance of 22 cm and a UVB-dose of 100 mJ/cm². The UVB output was monitored by means of an IL1700 research radiometer and SED 240 UVB photodetector (International Light, Newburyport, MA, USA).

**Array analysis**

For array analysis, cells were harvested after an incubation time of 4 hours following UVB- or sham-irradiation and total RNA was extracted by employing the RNA Minikit (Qiagen, Hilden, Germany). Generation of cDNA was carried out employing a gene specific primer mix for Atlas® Human 1.2 arrays (Clontech, Heidelberg, Germany) containing 1,185 known human genes according to the manufacturers protocol. Amplified cDNA was $^{32}$P-labelled and hybridised to nylon array membranes for 24 hours followed by Phosphorimager® (Molecular Dynamics, Heidelberg, Germany) analysis of signal intensities. Quantification was carried out with AtlasImage® Software normalizing values of cells from XP-patients and from patients with normal DNA repair but increased skin cancer risk to values from normal control cells, thus comparing normalised ratios of expression levels.

**Real time RT-PCR**

Real time RT-PCR was carried out by using the commercially available TaqMan Gene Expression Assay by Applied Biosystems containing 250 nM final concentration FAM™ dye-labeled TaqMan® MGB probe, 900 nM final concentration unlabeled PCR primer oligonucleotides as well as PCR reagents to facilitate real time RT-PCR for interleukin 2 receptor alpha, according to the manufacturers protocol. PCR reactions were set up in triplicates and performed three times separately for XP-patients as well as DNA repair proficient patients. Data are presented as means ± SD.

**Statistical analysis**

For description of the repair capacity (UDS) by non-linear least squares we fitted the following model to the data:

$$y = a \left[ 1 - \exp(-bx) \right],$$

where $y$ is the UDS in %, $x$ is the UVC-dose in J/cm², $a$ is the asymptotic UDS for large doses and $b$ is the rate of approach to the asymptotic value. The inverse of $b$ multiplied by natural log of 2 ($=0.693$) equals the dose $D_{50}$ at which 50% of the asymptote is reached. For the 7 normals/patients matched pairs we tested whether the means of the difference of the parameters $a$ and $b$ are equal to zero employing one-sample t-test.

Three separate experiments were carried out for each cell line, expression levels of investigated cells were normalized to values from aged matched normal controls and means were generated from this data. Each described set of data and genes determined by array analysis were included in further statistical analysis for which at least two data points were available. In XP cells, for both cells from each complementation group this minimum of two data points had to be available. Gene expression of XP cells was compared with that of patients by Student’s t-test for all genes included in the subset of 144 genes by using the statistical software package JMP (www.jmp.com). 20 of these genes showed a p-value $<0.05$ as shown in Table 2. In order to achieve normally distributed variates logarithmic transformations of the original data were used. Statistical significance was determined by calculating the q-values for each gene on the basis of the corresponding
Table 2. Genes with differential gene expression following exposure to UVB.

| Gene name                                      | P       | Code | Genbank Locus | Swissprot | Classification                                                                 |
|------------------------------------------------|---------|------|---------------|-----------|--------------------------------------------------------------------------------|
| Excision repair cross-complementation 1 (ERCC1)| 0.0289096 | C01n | M13194 2067   | P07992    | DNA damage signaling/repair proteins and DNA ligases                           |
| mutL (E. coli) homolog 1                       | 0.0051168 | C06n | U07418 4292   | P40692    | DNA damage signaling/repair proteins and DNA ligases, stress response proteins  |
| CDC-like kinase 1                              | 0.0161107 | A14i | L29222 1195   | P49759    | cell cycle-regulating kinases, nuclear proteins                               |
| prothymosin alpha                              | 0.0167602 | A03i | M26708 5757   | Q15249    | other cell cycle proteins, oncogenes and tumour suppressors                    |
| Ribosomal protein S19                         | 0.0039686 | A07I | M81757 6223   | P39019    | other cell cycle proteins, oncogenes and tumour suppressors                    |
| Mitogen-activated protein kinase kinase 11     | 0.0025372 | B04k | L32976 4296   | Q16584    | death kinases, intracellular kinase network members (non-receptor protein kinases) |
| Ubiquitin C*                                   | 0.0008739 | G11  | M26880 7316   |           | stress response proteins, cytoplasmic proteins, nuclear proteins              |
| v-jun avian sarcoma virus 17 homolog           | 0.0482950 | A10c | J04111 3725   | P05412    | oncogenes and tumour suppressors, apoptosis associated proteins                |
| Glucocorticoid receptor DNA binding factor 1   | 0.0364432 | D07I | M73077 2909   | Q14452    | transcription activators and repressors, nuclear proteins                     |
| EGF-response factor 1                          | 0.0491504 | D06j | X79067 677    | Q07352    | transcription activators and repressors, nuclear proteins                     |
| Colony stimulating factor 1*                   | 0.0010858 | F03e | M37435 1435   | P09603    | growth factors, cytokines, and chemokines, extracellular secreted proteins    |
| Hepatoma-derived growth factor                 | 0.0184383 | F05e | D16431 3068   | P51858    | growth factors, cytokines, and chemokines, extracellular secreted proteins    |
| Interleukin 2 receptor alpha                   | 0.0153361 | E01I | X01057 3559   | P01589    | interleukin and interferon receptors; plasma membrane proteins;              |
| Interleukin 1 beta                             | 0.0068164 | F10i | K02770 3553   | P01584    | interleukins and interferons; extracellular secreted proteins;               |
| Ribosomal protein L13a                        | 0.0371257 | G45  | X56932 2352   | P40429    | other immune system proteins                                                  |
| Major histocompatibility complex I C           | 0.0305518 | G31  | M11886 3107   | P10321    | major histocompatibility complex; plasma membrane proteins;                  |
| Tubulin alpha                                  | 0.0246099 | G29  | K00558 10376  | P04687    | cytoskeleton/motility proteins, cytoplasmic proteins, cytoskeletal proteins   |
| Calpain 2 (m/l) large subunit                  | 0.0390684 | C14h | M23254 824    | P17655    | calpains, cysteine proteases, cytoplasmic proteins                           |
| Insulin-like growth factor binding protein 3*  | 0.0002142 | F12h | M31159 3486   | P17936    | hormones, extracellular secreted proteins                                     |
| Heat shock 27kD protein 1                      | 0.0381607 | F05b | X54079 3315   | P04792    | heat shock proteins; cytoplasmic proteins                                     |

Genes are clustered according to their respective classification. Genes with correlation to clinical severity of XP complementation groups as well as patients with normal DNA repair and increased skin cancer risk are shown in bold. P-values of respective genes are given as generated by Student’s t test and only genes with a p-value < 0.05 are listed.
p values based on the false detection rate (FDR) as developed for array analysis.\textsuperscript{21} We followed exactly the method proposed by Storey and Tibshirani except that we replaced the cubic spline by an exponential function in determining the proportion of genes with no effect. In our data set, this method revealed p-values smaller than 0.0025 to be statistically significant (as denoted by an asterisk in Table 2). Of the genes with $p < 0.05$ six showed a direct association of gene expression with the clinical severity of XP complementation groups. These associations were illustrated by the 95% confidence intervals of the complementation group specific means as calculated by a one-way analysis of variance (Fig. 3).

**Results**

**Confirmation of UDS levels in employed cells**
To ensure deficient DNA repair in XP cells as well as normal repair in cells derived from patients with increased skin cancer risk UDS was carried out in the cells employed (Fig. 1a). For XP cells UDS was abnormal as published previously. For fibroblasts from normals and patients with increased skin cancer risk, the asymptotic value of UDS for large doses (10 J/cm²) did not differ and was within the normal range (means $\pm$ SE: 87.1 $\pm$ 8.6% and 90.5 $\pm$ 8.3%, respectively). The D50 for fibroblasts from normals and patients were 1.04 and 2.24 respectively (Fig. 1b). The ratio of the two D50 values was 2.06 (95% confidence interval 1.24 to 3.41; $p = 0.0175$). For pairs of patients the power for this observed difference was 77%. In order to detect a difference of 20% in the asymptotic UDS value one would need 26 matched patients pairs with a power of 80% and a significance level of 5%.

**Identification of a defined subset comprising genes with differential expression after UVB with p-values $< 0.05$**
Differential gene expression in cells from patients with XP complementation groups of different clinical severity compared to normal cells was measured by Atlas Human 1.2 Arrays after sham- or UVB-irradiation with 100 mJ/cm² containing 1,185 known genes. The signal intensity for control housekeeping genes showed no variation between experiments, indicating comparable hybridization levels for all experiments (Fig. 2a, Array picture of normal cells; 2b, Array picture of XP cells). Detected levels of gene expression in sham irradiated cells did not show any patterns attributable to clinical severity or other parameters. However, when measuring expression levels in UVB-exposed cells, differential gene expression could be detected in 144 genes comprising adequate data sets. Of these 144 genes, a total of 20 genes showed a p-value $< 0.05$ (Table 2). Most of the genes detected by array analysis could be classified in functionally relevant pathways such as DNA damage signalling and repair, cell cycle control, apoptosis, protooncogenes and tumour suppressor genes, transcription activators and basic transcription factors as well as cytoskeleton/motility proteins (Table 2). Of these 20 genes, determination of positive false detection rates (pFDR) controlling for multiple measurements as described by Storey et al.\textsuperscript{21} revealed a number of three genes (Insulin like growth factor, ubiquitin C, colony stimulating factor 1), denoted by an asterisk in Table 2, to be statistically significant in our data set.

**Association of gene expression levels with clinical severity of XP complementation groups**
When comparing clinical severity of XP symptoms with levels of gene expression after UVB-irradiation, a monotone association could be detected in six genes (Fig. 3). Of these six genes five (Excision repair cross complementing repair (ERCC)-1, interleukin 2 receptor alpha, prothymosin alpha, hepatoma derived growth factor, tubulin alpha) showed an increasing tendency of gene expression with a low expression level in complementation group XP-A, intermediate expression level in XP-D and a high expression level in XP-F (Fig. 3 a). The sixth gene (ubiquitin C) showed a decreasing tendency of gene expression with high levels in XP-A, intermediate in XP-D and low levels of expression in XP-F, respectively (Fig. 3 b). The six genes showing association with the clinical severity of the XP-complementation groups involve genes in functionally relevant pathways including DNA repair (ERCC-1), immunosurveillance (interleukin 2 receptor alpha), cell cycle control (prothymosin...
Figure 1. Measurement of UDS in cells from patients with at least 2 skin tumours before the age of 40. A) Assessment of UDS by BrdU-incorporation for fibroblasts from normal individuals, patients with increased skin cancer risk and XP-patients. Cells from patients with increased skin cancer and XP were age matched to normal fibroblasts e.g. F1 is matched to Patient 1 and F8 to XP19BR. All seven patients show normal levels of UDS with values of 50% of normal control cells or above at 10 J/cm². Cells from patients of XP complementation groups XP-A, XP-D and XP-F served as negative controls with values staying below 30% of normal control cells. Values for UDS-associated BrdU-incorporation are shown in % relative to values of control cells ±SD. B) Mean values of UDS. Data are given as means of normal and patient cells. The maximum value of UDS repair capacity (asymptotic value) at dose 10 J/cm² is the same in normals and patients but normal cells reach this value already at lower doses.
Association of XP clinical phenotype and gene expression

Confirmation of results in XP cells by array analysis in cells from patient with no known defect in DNA repair but increased skin cancer risk

Genes with expression levels in association with the clinical severity of different XP complementation groups could be confirmed in cells from patients with normal UDS but increased skin cancer risk (Fig. 3). As with XP cells, array analysis showed comparable expression levels for housekeeping controls and no discernible patterns in sham-irradiated cells (Fig. 2c). In UVB-irradiated cells the previously detected 20 genes were also regulated with no association of clinical phenotype to either number or type of tumours or age of the patients (Table 3, bold; Fig. 3). Particularly, the tendency identified in cells from different XP complementation groups was continued in these cells in an extrapolating manner. In all six genes expression levels were next to the expression levels of the mildest XP complementation group, XP-F (Fig. 3).

Confirmation of array data by real time RT-PCR

The expression levels detected by array analysis could be confirmed by real time RT-PCR. Figure 4 shows gene expression levels for all genes identified by array analysis, thus confirming the data generated by array analysis (Fig. 4).

Discussion

The risk of a certain individual to develop skin cancer is determined by genetic and environmental factors. The exact relative influence of each factor as well as the susceptibility of one individual to a given amount of environmental factors is currently unclear. These results indicate that it is possible to identify a defined subset of genes whose expression levels are associated with the clinical severity of different XP-complementation groups. Furthermore, the identified genes can be applied to a background with no known defects in DNA repair but increased skin cancer risk.
Excision repair cross-complementing repair 1 (ERCC1).

Interleukin 2 receptor alpha.

Prothymosin alpha.

Hepatoma derived growth factor.
Genes identified by our analysis have been found by different approaches from other groups. Serewko et al. investigated the differential gene expression of epidermal cells during squamous cell carcinoma development and identified cell cycle genes, EGF receptors, calpains, growth factors, MAP kinases and insulin like growth factor receptors, also applicable to seven of the 20 genes identified in our study. Furthermore, a study by Dong et al. investigated metastatic murine squamous carcinoma cells by differential display where 3 of the six genes identified by us to be associated with the clinical phenotypes of patients were the same in that study. Genes found to be differentially expressed after UVB and statistically significant were also identified by further studies.15,23

We have previously shown that ICAM-1 expression is associated with the skin cancer risk in cells from patients with XP. It was unclear however, whether this holds true for cells from patients with normal DNA repair but increased skin cancer risk. In those studies UVB-mediated suppression of interferon-γ induced ICAM-1 expression was investigated. In the present study constitutive ICAM-1 expression was measured by array technology. This did not show any association of expression levels after UVB-irradiation with the clinical phenotypes of XP patients or patients with normal DNA repair. This is most likely due to differences of the experimental design, since it has been shown that in a number of cells such as keratinocytes and fibroblasts, constitutive ICAM-1 expression can not or only at higher doses be suppressed by UV-irradiation. It has to be noted that identification of differential gene expression by array analysis always implies the risk of false positive detection or spurious association even though we controlled for this by performing three
independent experiments for two XP-cell lines and seven patients with normal DNA repair and statistical evaluation of positive false detection rate.\textsuperscript{21} Bearing all this in mind, however, these results may nevertheless indicate, that the investigation of a single gene e.g. ICAM-1, may be sufficient to distinguish between cancer prone XP and non-cancer prone TTD but it may not be sufficient to identify an increased skin cancer risk in individuals with no known defects in DNA repair capacity. In our present study, simultaneous measurement of 1,185 genes by array technology did reveal six genes that are indeed associated with the skin cancer risk of investigated individuals. This may indicate a higher sensitivity of array assessment as compared to measurement of any single gene, this being the case even in a background with no known defects in DNA repair.

In the present study the NER gene ERCC-1 was found to be associated with the clinical phenotypes of DNA repair deficient and proficient patients. Since it has been shown previously that expression of NER genes such as XPC and XPE can be transactivated by p53 after UV-radiation\textsuperscript{10} the present data confirm the notion that NER genes can be differentially expressed and that they may be associated with the clinical phenotype not only in XP patients but also in individuals with no known defect in DNA repair but increased skin cancer risk. Genes identified by a study investigating differential gene expression in cells with the XPB/CS or XPB/TTD allele\textsuperscript{17} also confirmed genes identified by our work (tubulin alpha, MHC-I, Insulin like growth factor) albeit this study employed UVC-irradiation.

Investigation of differential gene expression analysis has been carried out extensively previously with the intention to identify genes regulated by ultraviolet radiation or during skin carcinogenesis.\textsuperscript{14–16,22,24–27} In the present study this intention was only secondary. One of the strengths of array analysis is their application as a diagnostic technology.\textsuperscript{28,29} The present work identified a restricted and defined subset of genes generated from well established syndromes. These genes were then transferred to a background with no known defect in DNA repair. Further studies are necessary to evaluate the prognostic value of this gene subset in the normal population.

**Table 3.** Expression levels of differentially expressed genes.

| Gene name                                      | p     | Code    | XP-A  | XP-D  | XP-F  | Patients |
|-----------------------------------------------|-------|---------|-------|-------|-------|----------|
| Excision repair cross-compl. repair 1 (ERCC1) | 0.0289096 | C01n   | -0.143 | -0.090 | -0.045 | -0.001   |
| mutL (E. coli) homolog 1                      | 0.0051168 | C06n   | -0.110 | -0.159 | -0.123 | 0.041    |
| CDC-like kinase 1                             | 0.0161107 | A14i   | -0.165 | -0.313 | -0.140 | 0.054    |
| Prothymosin, alpha (gene sequence 28)        | 0.0167602 | A03i   | -0.411 | -0.335 | -0.203 | -0.105   |
| Ribosomal protein S19                         | 0.0039866 | A07l   | 0.084  | -0.096 | -0.023 | 0.095    |
| Mitogen-activated protein kinase kinase 11   | 0.0025372 | B04k   | -0.093 | -0.233 | -0.147 | 0.059    |
| Ubiquitin C*                                  | 0.0000739 | G11    | 0.080  | 0.079  | 0.030  | -0.064   |
| v-jun avian sarcoma virus 17 homolog          | 0.0482950 | A10c   | -0.016 | -0.173 | -0.037 | 0.081    |
| Glucocorticoid receptor DNA binding factor 1  | 0.0364432 | D07l   | 0.006  | 0.074  | 0.093  | -0.043   |
| EGF-response factor 1                         | 0.0491504 | D06j   | 0.123  | 0.236  | 0.427  | 0.050    |
| Colony stimulating factor 1*                 | 0.0010858 | F03e   | -0.051 | -0.074 | -0.062 | 0.098    |
| Hepatoma-derived growth factor                | 0.0184383 | F05e   | -0.211 | -0.133 | -0.109 | 0.026    |
| Interleukin 2 receptor, alpha                 | 0.0153361 | E01l   | -0.412 | -0.369 | -0.157 | -0.050   |
| Interleukin 1, beta                           | 0.0068164 | F10i   | -0.173 | -0.128 | -0.131 | -0.046   |
| Ribosomal protein L13a                       | 0.0371257 | G45    | -0.163 | -0.248 | -0.156 | -0.015   |
| Major histocompatibility complex, class I, C  | 0.0305518 | G31    | -0.479 | -0.233 | -0.320 | -0.039   |
| Tubulin alpha                                 | 0.0246099 | G29    | -0.418 | -0.338 | -0.182 | -0.139   |
| Calpain 2, (m/I) large subunit                | 0.0390684 | C14h   | -0.135 | -0.059 | -0.062 | 0.023    |
| Insulin-like growth factor binding protein 3* | 0.0002142 | F12h   | 1.045  | 1.253  | 1.220  | 0.389    |
| Heat shock 27K protein 1                      | 0.0381607 | F05b   | -0.115 | 0.053  | -0.036 | 0.096    |

Expression levels of genes are shown for patients with XP-A, XP-D, XP-F and patients with normal DNA repair but increased skin cancer risk. Genes with correlation to clinical severity of XP complementation groups as well as patients with normal DNA repair and increased skin cancer risk are shown in bold. Data is presented as means of three separate experiments from two cell lines of XP patients and seven patients with increased skin cancer risk normalized to age matched normal cells. P-values of respective genes are given as generated by Student’s t test and only genes with a p-value <0.05 are listed.
Figure 4. Real time RT-PCR for confirmation of data generated by array analysis. Expression levels in two cell lines from patients with XP-A, XP-D, XP-F and all cells from individuals with no known defect in DNA repair but increased skin cancer risk (Patients). The logarithms of the data are presented as means ± SD of three separate experiments. Expression levels determined by RT-PCR parallel levels detected by array analysis (compare Fig. 3).
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Abbreviations

Basal cell carcinoma (BCC), Cockayne Syndrome (CS), Cyclobutane-pyrimidine dimer (CPD), Nucleotide excision repair (NER), Squamous cell carcinoma (SCC), Unscheduled DNA-synthesis (UDS), Trichothiodystrophy (TTD), Ultraviolet (UV), Xeroderma pigmentosum (XP)

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