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PRENATAL DIAGNOSIS OF PIBIDS

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SUMMARY

In a well-documented PIBIDS family, two investigations of DNA excision repair showed a severe defect in lymphocytes from the index case (residual repair activities were 10·6–12·1 per cent). The values for the mother, father, and sister were within the normal range when compared with a healthy control. In the pregnant mother, a prenatal diagnosis of PIBIDS was made by measuring UV-induced unscheduled DNA synthesis in cultivated amniotic fluid cells. Results ranged between 12·5 and 26·1 per cent depending on the UV doses applied and were consistent with an affected fetus. The parents opted for a termination of pregnancy. Following a therapeutic abortion, fetal skin fibroblasts were tested and showed a severe DNA excision-repair defect of 9·2–13·5 per cent of residual activity.

KEY WORDS PIBIDS Prenatal diagnosis Excision repair

INTRODUCTION

Allen (1971) and Jackson *et al.* (1974) described a recessive autosomal syndrome (McKusick No. 23405, 1983) associating brittle hair (B), intellectual impairment (I), decreased fertility (D), and short stature (S). The acronym BIDS was suggested (Baden *et al.*, 1976). Price *et al.* (1980) referred to brittle hair as trichothiodystrophy (TTD) including trichoschisis as described by Pollitt *et al.* (1968): fragility, low sulphur content, abnormal hair pattern under polarization microscopy as alternative light and dark bands, and ichthyosis. Jorizzo *et al.* (1980) added one 'I' for ichthyosis and proposed the acronym IBIDS. As suggested from Price *et al.*'s case reports (1980), an association with photosensitivity was pointed out by Van Neste *et al.* (1979, 1982), especially with ultraviolet B (UVB) light photosensitivity (Lucky *et al.*, 1984). From patients described by Tay (1971), a unifying concept was proposed by Happle *et al.* (1984) as 'Tay's syndrome' and by Crovato *et al.* (1983) as PIBIDS (Photosensitivity, Ichthyosis, Brittle hair, Intellectual impairment, Decreased fertility, Short stature). A severe DNA excision-repair defect was described in lymphocytes (Vasseur *et al.*, 1985) taken from a young infant previously assumed to be a xeroderma pigmentosum (XP) and further diagnosed as PIBIDS (Van Neste *et al.*, 1985; Vasseur *et al.*, 1990); this was confirmed in keratinocytes (Van Neste *et al.*, 1985) and skin fibroblasts (Rebora *et al.*, 1986). A relationship between PIBIDS and XP was suggested (Van Neste *et al.*, 1985) and confirmed when excision-repair complementation group D (XP-D) was described in PIBIDS (Stefanini *et al.*, 1986; Vasseur *et al.*, 1989; Van Neste *et al.*, 1989).

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In a family with a medical history of PIBIDS, we investigated DNA excision repair by the use of UV-induced unscheduled DNA synthesis (UDS) and in addition performed a prenatal diagnosis of PIBIDS using UDS on amniotic fluid cells.

CASE REPORT

The index case was born in 1977 after a 38-week gestation. Maternal high blood pressure, albuminuria, and intrauterine growth retardation were noticed during pregnancy. Apgar scores were 3 at 1 min and 6 at 5 min. The baby weighed 1730 g and measured 43 cm in length. Hypotrichosis and brittle hair were obvious at birth. Intense photosensitivity, ichthyosis, and mental retardation were noted from the first months. The PIBIDS syndrome was suggested and confirmed by observing a DNA excision-repair defect in lymphocytes (Vasseur *et al.*, 1985). In 1990, prenatal diagnosis was proposed to the pregnant mother. Amniocentesis was performed at 17 weeks' gestation.

METHODS

DNA excision repair was investigated by UV-induced UDS on lymphocytes of the index case and the parents, amniotic fluid cells from the pregnant mother, fetal skin fibroblasts after therapeutic abortion, and on normal control cells.

UDS on blood cells

Leucocytes from heparinized blood samples were suspended in 0·5 ml aliquots of TC 199 medium supplemented with 20 per cent fetal calf serum (FCS), and irradiated with a 6 W, 254 nm UV lamp (Bioblock). UV doses ranged from 0 to 20 J/m^2 as measured by a VLX 254 radiometer (Bioblock). At 3 h DNA repair incubation was performed in 20 per cent FCS TC 199 containing 5 μCi/ml [^{3}H]thymidine (specific activity 83 Ci/mmol, Amersham). An excess of unlabelled thymidine was added before harvesting. The medium was discarded and the cells were washed three times, fixed in methanol–acetic acid (3:1), and spread onto slides.

UDS on amniotic fluid cells and skin fibroblasts

Primary cultures of amniotic fluid cells and skin fibroblasts were initiated in tissue culture flasks (Falcon) containing 8 ml of Chang medium (Hana Biologic Inc.). Subcultures were performed on slides set in 3 cm Petri dishes and maintained for 2 days in RPMI medium supplemented with 20 per cent FCS. In order to label the S-phase, cells were incubated for 1 h with TC 199 medium containing 1 per cent FCS and 5 μCi/ml [^{3}H]thymidine (labelled TC 199) and then UV-irradiated from 0 to 50 J/m^2. After a 3 h DNA repair incubation in 'labelled TC 199' with 10 mM hydroxyurea (Sigma), an excess of unlabelled thymidine was added before harvesting and followed by three washes. The slides were then immersed for 15 min in methanol–acetic acid (3:1) and air-dried.

Autoradiographic studies

Autoradiographic studies were standardized for lymphocytes, amniotic fluid cells and skin fibroblasts. Preparations were first washed three times in 2 × SSC (1 × SSC

Table 1. Quantitation of DNA excision repair by UDS on lymphocytes from the PIBIDS index case. I_1 is the average background. Standard errors are given in parentheses

	Average number of grains per lymphocyte			
	Before irradiation (background) (I_1)	After 6 J/m^2 UV irradiation (I_2)	Specific excision-repair label (I_2-I_1)	% Excision repair (%)
Experiment 1				
Control	5·90 (1·24)	19 (1·17)	13·10 (1·70)	100·0
Index case		7·3 (0·56)	1·40 (1·36)	10·6 (11·17)
Experiment 2				
Control	3·23 (0·29)	35 (1·51)	31·77 (1·53)	100·0
Index case		7·1 (0·42)	3·87 (0·51)	12·16 (1·9)

is 0·15 M NaCl and 0·015 M sodium citrate) containing 10 mM sodium pyrophosphate, pH 7, and then given a second wash with distilled water. Slides were air-dried after dehydration in an ethanol series (70–90 per cent) and then dipped in Kodak NTB 2 liquid nuclear emulsion (Eastman Kodak) and stored at 4°C for a 3–30 day exposure. The autoradiographs were processed according to the supplier's protocol. The quantitative analysis of UDS was as previously described (Vasseur *et al.*, 1990). For UDS on blood cells, only mononuclear cells were considered.

RESULTS

Index case and familial reports

DNA excision repair has been investigated twice in lymphocytes from the index case, her parents, and her elder sister. For each patient the autoradiographic grain number was averaged after UDS, from 50 unirradiated cells and from 50 UV-irradiated cells (6 J/m^2), each at random. The results were compared with those from a healthy control. In the first investigation, an average background (I_1) of 5·9 grains per cell was determined, and after 6 J/m^2 UV irradiation, 7·3 grains per cell (I_2) were scored for the index case versus 19 for the control. Specific excision-repair labels (I_2-I_1) were 1·4 grains per cell for the index case and 13·1 for the control (Table 1). In the second investigation, I_1 was 3·23 grains per cell; I_2-I_1 was 31·77 grains per cell for the control and 3·87 for the index case (Table 1). In both experiments, the control excision-repair values were taken as the 100 per cent reference level. The index case presented a severe DNA excision-repair defect with only 10·6 (experiment 1) or 12·1 per cent (experiment 2) activity. The results for the parents and sister remained in the control range (data not shown).

Report of the prenatal diagnosis

DNA excision repair was analysed in amniotic fluid cells (Figure 1). Two experiments were performed in amniocytes from the pregnant mother: the first one

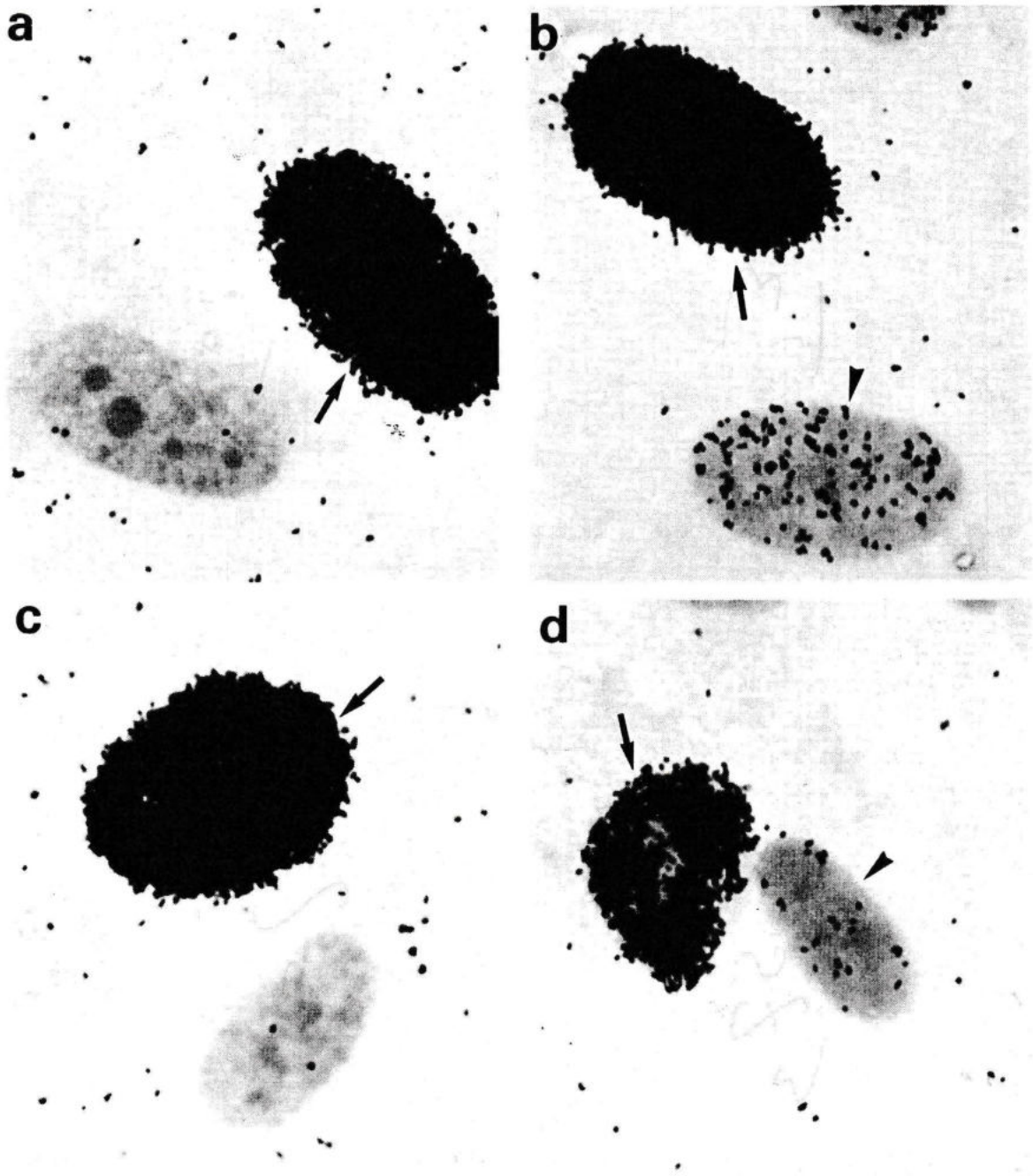

Figure 1. UDS on amniocytes after 30 J/m^2 UV irradiation and thymidine incorporation by the repair process. (a) Unirradiated controls; (b) irradiated controls; (c) unirradiated PIBIDS cells; (d) irradiated PIBIDS cells. Arrows: Labelled cells with scheduled DNA synthesis (S-phase); arrow-heads: labelled cells with unscheduled DNA synthesis

used 10 and 30 J/m^2 UV doses and a 3 h repair incubation (experiment 3) and the second experiment used 10, 30, and 50 J/m^2 UV doses and a 5 h repair incubation (experiment 4). Control amniocytes from two 17-week pregnancies were processed in parallel.

For experiment 3, Table 2 shows minor specific excision-repair labels in amniocytes from the pregnant mother, as compared with the control: 29 grains per cell versus 160·8 after 10 J/m^2 UV irradiation and 33·8 versus 268·8 after 30 J/m^2 UV irradiation. In experiment 4, weak excision-repair labels were also observed in the pregnant mother's amniocytes whatever the UV dose, as compared with the values from the control (Table 2). Since control excision-repair values were taken as the 100

Table 2. Quantitation of DNA excision repair on amniocytes from the pregnant mother following a 3 h (experiment 3) and a 5 h (experiment 4) repair incubation. I_1 is the average background. Standard errors are given in parentheses

	Average number of grains per amniocyte			
	Before irradiation (background) (I_1)	After 6 J/m^2 UV irradiation (I_2)	Specific excision-repair label (I_2-I_1)	% Excision repair (%)
Experiment 3				
10 J/m^2				
Control	20·2 (2·39)	181 (8·23)	160·8 (8·57)	100·0
Pregnant mother		49·2 (3·00)	29 (3·83)	18·0 (3·34)
30 J/m^2				
Control	20·2 (2·39)	289 (15·9)	268·8 (16·07)	100·0
Pregnant mother		54 (2·32)	33·8 (3·33)	12·5 (1·99)
Experiment 4				
10 J/m^2				
Control	44·5 (3·63)	211 (9·32)	166·5 (10·00)	100·0
Pregnant mother		88 (5·96)	43·5 (6·97)	26·1 (5·75)
30 J/m^2				
Control	44·5 (3·63)	266 (14·68)	221·5 (15·12)	100·0
Pregnant mother		85 (5·86)	40·5 (6·89)	18·2 (4·35)
50 J/m^2				
Control	44·5 (3·63)	344 (36·99)	299·5 (37·162)	100·0
Pregnant mother		90 (5·55)	45·5 (6·63)	15·3 (4·09)

per cent reference levels, the amniocytes from the pregnant mother displayed a severe DNA excision-repair defect. Indeed, residual excision-repair activities were 18 and 12·5 per cent in experiment 3 and 26·1, 18·2, and 15·3 per cent in experiment 4. The excision-repair defect was confirmed after the therapeutic abortion (experiment 5): skin fibroblasts from the aborted PIBIDS fetus and a healthy fetus were irradiated with 5, 10, and 30 J/m^2 UV doses. As shown in Table 3, the specific excision-repair labels for the PIBIDS fetus were greatly depressed as compared with the healthy control (I_2-I_1 were 2·9, 6·1, and 12·6 versus 29·2, 66·3, and 93·1 grains per cell). Residual excision-repair activities were 9·9, 9·2, and 13·5 per cent in agreement with the previous prenatal diagnosis.

DISCUSSION

UV light is known to induce DNA damage (Helene and Charlier, 1978) and the excision-repair mechanism restores the DNA of normal subjects. Cells with excision-repair defects may leave mutagenic lesions in the DNA. Quantitative

Table 3. Experiment 5: post-abortion corroboration of our previous prenatal diagnosis of PIBIDS. Quantitation of DNA excision repair after a 3 h UDS on fibroblasts from one healthy fetus (control) and the aborted PIBIDS fetus. I_1 is the average background. Standard errors are given in parentheses

	Average number of grains per fibroblast			
	Before irradiation (background) (I_1)	After 6 J/m^2 UV irradiation (I_2)	Specific excision-repair label (I_2-I_1)	% Excision repair (%)
5 J/m^2				
Control	3·5 (0·35)	32·7 (1·47)	29·2 (1·51)	100·0
Aborted fetus		6·4 (0·36)	2·9 (0·50)	9·9 (2·27)
10 J/m^2				
Control	3·5 (0·35)	69·8 (3·26)	66·3 (3·27)	100·0
Aborted fetus		9·6 (0·61)	6·1 (0·70)	9·2 (1·50)
30 J/m^2				
Control	3·5 (0·35)	96·6 (5·36)	93·1 (5·37)	100·0
Aborted fetus		16·1 (0·77)	12·6 (0·84)	13·5 (1·68)

analysis of excision repair is achieved *in vitro* by measuring UV-induced UDS (Cleaver, 1968). This biological diagnosis has been performed on various tissues (Burk *et al.*, 1971; Robbins *et al.*, 1974; Robbins and Moshell, 1979). Excision repair has also been investigated in amniotic cells for prenatal diagnosis of XP (Ramsay *et al.*, 1974). An excision-repair defect has been described in PIBIDS with (Vasseur *et al.*, 1985; Stefanini *et al.*, 1986) and without clinical photosensitivity (Stefanini *et al.*, 1987). In our investigations of excision repair, we used *in vitro* UV-induced damage and measured the incorporation of labelled thymidine during the repair process. [^{3}H]Thymidine incorporation reflects the repair activity of the cells and excision-repair defective cells show less label than normal control cells. In our observation, the excision-repair defect was obvious in the index case (10·6–12·1 per cent repair activity). These results were obtained from white blood cells. It is unknown whether white blood cells may differ in their UDS, and only mononuclear cells were considered. Thus, the interpretation of UDS was predominantly based on the analyis of the lymphocytes.

An excision-repair defect was noticed in the pregnant mother's amniocytes (Table 2). Following a therapeutic abortion, excision-repair values on the resultant skin fibroblasts (Table 3) confirmed the prenatal diagnosis of PIBIDS. The mother and father of the index case are obligatory carriers. Heterozygosity was not obvious from the excision-repair investigations because the values for the parents were in the control range. According to Cleaver (1983), biological carrier detection is not possible with skin fibroblasts. Additional analyses on lymphocytes with increasing UV doses might allow such detection, as previously described (Vasseur *et al.*, 1990).

PIBIDS patients usually exhibit UV photosensitivity with a DNA-related excision-repair defect and diagnosed with UDS. In our observation of PIBIDS, the excision-repair defect was important and the method appeared reliable and reproducible according to the slight standard errors.

UV-induced UDS cannot distinguish between the various diseases with an excision-repair defect (i.e., xeroderma pigmentosum, PIBIDS, trichothiodystrophy). In patients with trichothiodystrophy (TTD), photosensitivity may be present or absent. Photosensitive TTD patients exhibit a molecular excision-repair defect similar to XP group D (Stefanini *et al.*, 1986). In TTD patients without photosensitivity, Stefanini *et al.* (1987) showed after *in vitro* UV-induced DNA damage, an excision repair of cultivated cells ranging from normal to severely altered. Further studies revealed three types of response following UV irradiation (Lehmann *et al.*, 1988; Broughton *et al.*, 1990): patients with a normal response; patients with a response similar to XP group D; and patients with a 50 per cent excision-repair defect, severely reduced RNA synthesis, but normal survival. Such a heterogeneity has not yet been described in PIBIDS; every reported case displays an excision-repair defect. The biological prenatal diagnosis of an excision-repair defect with UV-induced UDS may be performed for PIBIDS detection in families where a PIBIDS index case is available and shows a clear defect of UDS after UV irradiation.

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