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Cockayne's syndrome: correlation of clinical features with cellular sensitivity of RNA synthesis to UV irradiation

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Abstract

Cockayne's syndrome (CS) is a rare autosomal recessive disorder with dwarfism, mental retardation, and otherwise clinically heterogeneous features. In cultured CS fibroblasts, the failure of RNA synthesis to recover to normal rates after UV-C irradiation provides a useful and relatively simple diagnostic test. We have measured post-UV-C RNA synthesis in 52 patients for whom a clinical diagnosis of CS was considered a possibility. Twenty-nine patients showed the defect characteristic of CS cells, and 23 had a normal response. We have attempted to correlate the cellular diagnosis with the different clinical features of the disorder. Clinical details of the patients were obtained from referring clinicians in the form of a questionnaire. Our results show that, apart from the cardinal features of dwarfism and mental retardation, sun sensitivity correlated best with a positive cellular diagnosis. Pigmentary retinopathy, gait defects, and dental caries were also good positive indicators, although several patients with a positive cellular diagnosis did not have these features.

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Cockayne's syndrome is a rare, clinically heterogeneous, autosomal recessive disorder. Severe growth failure and mental retardation are characteristic, while other features commonly include pigmentary retinopathy, cataracts, hearing loss, gait defects, dental caries, a characteristic wizened appearance, and photosensitivity. Nance and Berry[1] recently reviewed 140 published cases and suggested that, for classical CS, the clinical diagnosis requires growth failure and neurodevelopmental delay together with three of the following: retinopathy/cataracts, hearing loss, dental caries, photosensitivity, and characteristic facial appearance. There is an uncommon and more severe form of the disease which has earlier onset with low birth weight and little postnatal physical or mental development.

Cellular studies on fibroblasts cultured from CS patients (reviewed by Lehmann[2]) have shown hypersensitivity to the lethal effects of UV-C irradiation.[3] In this respect they resemble cells from the highly cancer prone genodermatosis xeroderma pigmentosum (XP), but whereas XP patients have pronounced defects in either excision repair or post-replication daughter strand repair of ultraviolet DNA damage, CS cells show no such defect. After UV irradiation, RNA synthesis in CS cells is depressed in a similar manner to that in normal cells. RNA synthesis recovers rapidly in normal cells but in CS cells remains depressed.[4] Recent results have shown that the rapid recovery of RNA synthesis in normal cells can be attributed to preferential rapid repair of DNA damage in transcribed regions of DNA, in contrast to much slower repair in the bulk of the DNA.[5] In CS cells this preferential repair of transcribed DNA does not take place, damage in these regions being repaired at the same (slow) rate as in the bulk of the DNA.[6] This failure to effect the rapid repair of crucial regions of the DNA is presumably the cause of the hypersensitivity of the cells to the lethal effects of UV light. In a few instances patients with the clinical features of both CS and XP have been reported. These patients have been assigned to XP complementation groups B,[7] D,[8 9] and G (Vermeulen and Hoeijmakers, personal communication) and show the close mechanistic relationship between the two disorders.

The failure of RNA synthesis to recover in CS cells after UV-C irradiation has provided a relatively simple and rapid test for diagnosing CS both pre- and postnatally.[10] The RNA synthesis test has also provided an assay for genetic heterogeneity. Cell fusion studies have shown that 10 patients with CS (without the additional features of XP) could be assigned to two complementation groups, A and B.[11 12] There are no obvious clinical differences between patients in the two groups.

In this study we have received cultures from 52 patients in several different countries in whom a clinical diagnosis of Cockayne's syndrome was considered a possibility. After UV irradiation, defective recovery of RNA synthesis was found in 29 of these cultures, confirming the diagnosis of CS, while in the other 23 the response was normal. Clinicians, on whose patients we carried out the tests, were asked to fill in a questionnaire concerning their clinical features, and in this paper we attempt to correlate the clinical features of the patients with the results of the cellular test.

Materials and methods

FIBROBLAST CULTURES

Patients who had some or all of the clinical features of CS and were resident in Europe, the Middle East, or the USA were studied.

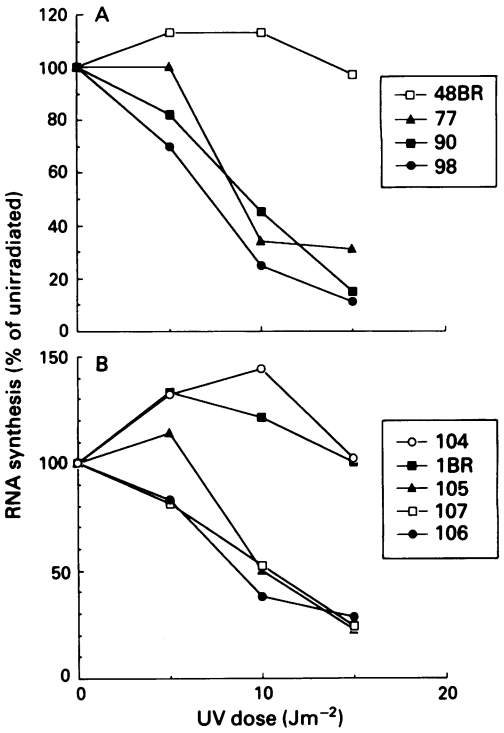
Fibroblast cultures set up either in the donating laboratories or in our own laboratory were routinely cultured in Eagle's MEM supplemented with 15% fetal calf serum.

RNA SYNTHESIS AFTER UV IRRADIATION

The procedure used was similar to that described in Lehmann *et al.*[10] In brief, cells were seeded at 3×10^4 per 3 cm petri dish on day 1 and the complete medium was replaced with fresh medium containing 0·5% serum on day 2 to bring the cells to a state of non-proliferation. On day 5 the plates were UV irradiated with doses of 0, 5, 10, and 15 Jm^{-2}, and the next day RNA was labelled with a four hour pulse of ^{3}H-uridine. The cells were harvested by scraping them off the dishes and the radioactivity incorporated into RNA was measured as described previously. All experiments contained, as control cultures, one normal cell strain and one previously identified CS strain.

QUESTIONNAIRE

A retrospective questionnaire was sent to all donating laboratories requesting detailed information on clinical features relating to the skin, neurological system, eyes, teeth, and growth/development. All questions were of the yes/no/unknown form. Out of 64 questionnaires sent out, 52 were returned.



RNA synthesis after UV irradiation. Non-proliferating cells were UV irradiated with different doses and RNA synthesis was measured 24 hours later as incorporation of ^{3}H-uridine into acid insoluble material. Results are expressed as % of the incorporation in unirradiated cells. Two typical experiments are shown. 1BR and 48BR are cell strains from normal donors. The patient numbers of the other cell strains are indicated.

Results

RNA SYNTHESIS

The response of RNA synthesis to UV was tested in fibroblasts cultured from each patient. Results of two characteristic experiments are shown in the figure. RNA synthesis in cell strains from patients 77, 90, and 98 (figure A) and 105, 106, and 107 (figure B) was very sensitive to UV irradiation, consistent with a diagnosis of CS, whereas in patient 104 (figure B), the diagnosis was not confirmed, the response being similar to that of normal donors. The rates of RNA synthesis after a UV dose of 15 Jm^{-2} are summarised for all patients in tables 1 and 2, columns 5. In almost all cases a clearly defined result was obtained, either normal (>60% of unirradiated level after 15 Jm^{-2}) or defective (<40% of unirradiated level after 15 Jm^{-2}). In only one or two cases (patients 86 and 89) was there any indication of an intermediate response. These were closer to the normal than to the defective response and have been regarded as normal for the purpose of this study. Further investigations would be needed to clarify the significance of these observations.

CLINICAL SYMPTOMS

The data from the questionnaires are summarised in table 1 for patients with defective RNA synthesis after UV irradiation, and in table 2 for patients with a normal response. All of the patients had growth delay, mental retardation, and microcephaly, and these features are not recorded in the tables. For space considerations the tables do not include all the information obtained from the questionnaire. A few of the patients with defective RNA synthesis had unusual pigmentation. Thus the Turkish patients were described as having brown pigmented areas (patient 77), hypo- and hyperpigmented areas on the face (patient 88), hyperaemia in malar areas and exfoliative dermatitis (patient 98), and hypo- and hyperpigmented areas on the face and extremities (patients 119 and 164). Patient 9 had generalised hyperpigmentation. Patient 11 was described as showing marked tanning, and patients 106 and 136 had dark skin. The negro girl, patient 138, had linear streaks of hypo- and hyperpigmentation on the neck and trunk. Three patients from table 1, the sib pair 35A and 35B, and patient 105 were from Asian families and were rarely allowed out of doors, so that sun sensitivity was not known with certainty. In keeping with earlier published reports in none of the patients referred was skin cancer or internal malignancy recorded.

Discussion

Previous studies have shown that cell strains from bona fide CS patients are sensitive to the lethal effects of UV irradiation and that RNA synthesis fails to recover after UV irradiation,[4] even though there is no gross defect in excision repair of UV damage.[15] The RNA synthesis defect has been attributed to a failure of CS cells to effect the rapid removal of damage from actively transcribed regions of DNA.[6] In the present investigation we have looked for

Table 1 Clinical features of patients with defective post-UV RNA synthesis.

No	Age	Clinician	Country	RNA synth	Pig ret	Cataracts	Deafness	Caries	Tremor	Gait	Sun sen	Facies	Pigment
4	10	E Brett	UK	19 (2)	N		N		Y	Y	Y		Asian
6*	4	P Norris	UK	15 (3)	Y	N			N	Y	Y	N	
7*	12	P Norris	UK	37 (2)	Y	N	Y		N	Y	Y	N	
9*	13	P Norris	UK	26 (5)	Y	Y	Y		N	Y	Y	Y	Y
11	7†	R MacFaul	UK	22 (6)	N	Y	N	Y	N	Y	Y		Y
34	4†	D Donnai	UK	20 (5)		Y		Y	N	Y	Y	Y	
35A	4†	M Patton	UK	28	Y	N	Y	Y	N	Y	a	Y	Asian
35B	4†	M Patton	UK	23			Y	Y	N	Y	a	Y	Asian
38	11	J Wilson	UK	30 (11)		N	Y		Y	Y		Y	
39	20	D Siggers	UK	21 (5)	Y	Y	Y	Y	N	Y	Y	Y	
67	6	C Cohen	Israel	28 (8)	Y		Y	Y		Y	Y	Y	
68	5	C Cohen	Israel	20 (4)	Y		Y			Y	Y	Y	
77	8	M Topcu	Turkey	23 (8)	Y	N	Y	Y	Y	Y	Y		Y
88	9	M Topcu	Turkey	16 (3)	N	N	N	N	Y	Y	Y	Y	Y
90	6	C Cohen	Israel	19 (4)	Y		Y			Y	Y	Y	
97	9	M Kyllerman	Sweden	14	Y	N	Y		Y	Y	Y	Y	
98	2	M Topcu	Turkey	16 (5)	N	N	Y	N		Y	Y	Y	
105	6	J Wilson	UK	32 (8)	N	N	N		Y	Y	a		Asian
106	2†	C Ricci	Italy	36 (8)	N	N	Y		N		N		Y
113A	13	A Lundberg	Sweden	15	Y	N	Y		N	N	Y		
113B	9	A Lundberg	Sweden	22 (6)	Y	N	Y		Y	N	Y	Y	
119	9	M Topcu	Turkey	14 (2)	N	N	N	Y	Y	Y	Y	Y	Y
127	6	C Cohen	Israel	20		Y	N	Y			Y	Y	Y
133	6	K Temple	UK	16 (3)	N	N	N		Y	Y	Y	Y	
136	4	S Robb	UK	19 (2)	N	N	Y		N	Y	Y	Y	Y
138	4	S Holder	UK	16 (2)	N	N	N		Y	Y	Y		Y,Negro
140	8	N Nevin	UK	17 (4)	Y	N	N	N	Y	Y	Y		
161	4	R Greenstein	USA	16	N	Y	N	N	Y	Y	Y	N	
164	5	M Topcu	Turkey	26 (5)		N	N	N	N	Y	Y		Y

Age is the age of the patient in November 1992 or at death. RNA synth = RNA synthesis in cells exposed to 15 Jm^{-2} relative to that in unirradiated cells. The numbers, expressed as percentages, represent means (SD) of one to eight determinations. Pig ret = pigmentary retinopathy, Sun sen = sun sensitivity, Pigment = pigmentary abnormalities or non-Caucasian characteristics, N = no, Y = yes, a = patients never exposed to sun, † (in age column) dead. * Patients 1, 2, 3 in Norris *et al.*[13] Y for yes in the column marked gait indicates that the patient had either limb ataxia, gait abnormality, or was non-ambulatory. Y for yes in the column marked facies indicates that the patient had either 'characteristic facies', prematurely aged appearance, or sunken eyes. Y in the sun sensitivity column indicates an abnormal sun reaction either always or sometimes. An unfilled slot indicates that the information was not provided or not known.

Table 2 Clinical features of patients with normal post-UV RNA synthesis.

No	Age	Clinician	Country	RNA synth	Pig ret	Cataracts	Deafness	Caries	Tremor	Gait	Sun sen	Facies	Pigment
8	6	E Brett	UK	81 (5)	N	N	N		Y	Y			
21	8	E Brett	UK	108 (28)	N	N	N		Y	Y	Y	Y	
43	7	S Holder	UK	69	N	Y	N		N	N	N	Y	
44	9	P Lunt	UK	83 (25)	N	N	N	N	N	N	N	Y	
52*	11	E Boltshauser	Switzerland	83	N	N	N	N	N	N	N	Y	
53*	37	E Boltshauser	Switzerland	63 (5)		Y	Y				N	Y	
56	8	D Viskochil	USA	93	N	N	N		N		N	Y	
63	4	J Hersh	USA	99 (21)	N	N	N	N	N	N	Y	N	
74	4	M Super	UK	62 (2)	N	N	N	N		Y	N	Y	
78	9	G Neri	Italy	69 (4)	N	N	N			Y	N	Y	
83	4	J Tolmie	UK	81 (25)	N	N	N	N	N	N	N	Y	
86	3	P Lunt	UK	44 (10)	N	N	N		N	N	N	Y	
89	7	E Ballesta	Spain	50 (11)	N	N	Y	N	Y	Y	N	Y	
94	3	E Brett	UK	81 (15)	N	N	N		N	N			
96	3	M Kyllerman	Sweden	93	N	N	Y		Y	Y	N	Y	
104	3	M Patton	UK	83 (27)	N	N	Y	N	N	N	Y	Y	
109	1	O Quarrell	UK	70	N	Y		NA		NA	N	Y	
112	6	I Ellis	UK	69 (20)	Blind	Y			N	Y	N		
126	11	D Rita	USA	70 (10)	Y	N	Y		N	Y	Y	Y	Y
135	1	E Thompson	UK	71 (10)	N	Y	N	NA	N	NA	N	Y	
142	12	C Kunzle	Switzerland	60 (6)		N	N			Y	N	Y	
151	6	P Lunt	UK	60 (8)	N	N	N	Y	N	N	N	Y	
160	15	N Nevin	UK	67 (4)	N	Y	Y	N	Y	Y	Y	Y	

Abbreviations as in table 1. NA = not applicable (patient too young). * Patients described in Boltshauser *et al.*[14]

Table 3 Summary of features of patients with defective and normal RNA synthesis.

	Pig ret	Cataracts	Deafness	Caries	Tremor	Gait	Sun sen	Facies
RNA synthesis defective	13/24 (54)	5/23 (22)	16/27 (59)	9/14 (64)	12/24 (50)	25/27 (92)	24/25 (96)	18/19 (95)
RNA synthesis normal	1/20 (5)	6/23 (26)	6/21 (29)	1/9 (5)	5/18 (28)	10/19 (53)	5/21 (24)	19/20 (95)
Q	0·92	−0·12	0·56	0·87	0·44	0·84	0·97	0
χ^2	12·3	0·12	4·5	6·3	2·1	9·8	25	0
Significance level	0·1%	—	5%	1%	—	0·1%	0·1%	—

Abbreviations as in table 1. The table shows the numbers with the indicated features and the numbers of patients for whom a definite yes or no was entered in the questionnaire for the indicated features. Percentages with the feature are indicated in parentheses. Q = Yule's coefficient of association.

correlations between clinical features of the disease and the defect in post-UV-C RNA synthesis. The clinical features of the 'defective RNA synthesis' and 'normal RNA synthesis' groups are summarised and compared in table 3. All patients had growth and mental retardation, microcephaly, and, in most cases, abnormal facial features, these being in many cases the reasons for the initial suspicion of a diagnosis of CS. It can be seen from table 3 that tremor and cataracts did not differ greatly between the two groups. Pigmentary retinopathy, dental caries, and gait abnormalities were found much more frequently in the 'defective RNA synthesis' than in the 'normal RNA synthesis' group (level of significance: $p \leq 0{\cdot}1\%$). This suggests that these features are good positive indicators for a diagnosis of

CS, although their absence does not exclude it. Patient 4, for example, has a clear positive diagnosis but does not have pigmentary retinopathy, even at the age of 10 years.

The clinical feature which correlated most strongly with defective RNA synthesis was photosensitivity (table 3). This was manifested as a persistent erythema after sun exposure, accompanied in some instances by dermatitis. In only one case (106) with defective RNA synthesis was photosensitivity absent. This patient, from south Italy, was taken to the beach several times and, according to his mother, never developed sunburn or erythema. Only five patients with normal RNA synthesis were photosensitive.

Patient 160 had many features of CS and the referring clinician was confident of the clinical diagnosis of CS. Patients 21 and 104 had an abnormal sun reaction 'sometimes' rather than 'always' according to the questionnaire. Patient 63 had no other characteristic features, the facies were not typical of CS, and the patient has been subsequently diagnosed as having severe Russell-Silver syndrome. Patient 126 had several additional features not normally associated with CS including café au lait spots on half his body and axillary freckling. Finally, patient 78, though not photosensitive, had many typical features of the disorder at the age of 7, and the clinician was very confident that he had CS.

Nance and Berry[1] suggested that, in the absence of laboratory tests, a clinical diagnosis of CS was warranted if dwarfism and mental retardation were accompanied by three out of the following: cataracts or pigmentary retinopathy, photosensitivity, dental caries, characteristic facies, or sensorineural deafness. If it is assumed that the RNA synthesis defect can provide an unambiguous confirmatory diagnosis for CS, we can examine further the correlation between this diagnosis and the clinical phenotype. We can ask if all the RNA synthesis defective persons did indeed have three of these characteristics, and conversely if any of the RNA normal persons had sufficient features for a diagnosis of CS to be made on clinical features alone. Eighteen out of 29 patients with defective UV response in table 1 had three or more of the required characteristics. A further nine had one or two features with a further one or two 'unknown'. Only the Turkish patients 88 and 164 had at most two of these characteristics. Patient 88 did, however, have tremor and gait defects, features which were not listed as criteria by Nance and Berry.[1]

Of the 23 patients listed in table 2 (with normal post-UV RNA synthesis), four patients had three or more of the features required by Nance and Berry,[1] namely patients 53, 104, 126, and 160. Interestingly, except for patient 104, these are all older patients, aged 37, 11, and 15, respectively. The most striking anomaly was patient 160, who had four cardinal features of CS (cataracts, deafness, sun sensitivity, abnormal facies) as well as a tremor and gait defects, but whose cellular RNA synthesis response was normal.

In conclusion, a reduced rate of RNA synthesis has been observed in patients with (in addition to growth and mental retardation, microcephaly, and abnormal facial features), photosensitivity, pigmentary retinopathy, and dental caries. Our investigations indicate that (1) photosensitivity is an almost essential feature, and pigmentary retinopathy and dental caries are good discriminatory indicators for CS (in addition to growth and mental retardation and microcephaly); and (2) in the absence of a specific laboratory test, a patient can be diagnosed with confidence as having CS if these symptoms are present. The status of a small number of subjects who have normal RNA synthesis, but nevertheless have several of the clinical features of CS, remains obscure.

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