Cockayne Syndrome: Clinicopathologic and Tissue Culture Studies of Affected Siblings

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Abstract. Two siblings with Cockayne syndrome (CS) had extremely severe and early onset cachectic dwarfism, developmental delay, cataracts, microcephaly, peripheral neuropathy, and spastic quadriplegia. In order to study the inherited DNA-repair defect known to be present in cultured CS cells, a lymphoblastoid line was established from the younger sibling. Tissue culture studies revealed the line to have a hypersensitivity to the lethal effects of 254-nm ultraviolet radiation (UV) equivalent to that of lymphoblastoid lines from CS patients who had either the usual severity or a very mild form of CS. Autopsy of the older sibling at six years of age showed the brain to be severely atrophic, with particularly severe cerebellar atrophy. There was a marked reduction in the number of granule cells in the cerebellum and irregular patchy myelination throughout the brain. Many astrocytes contained either a large, bizarre-shaped nucleus or multiple nuclei. Some Purkinje cells of the cerebellum and pyramidal neurons of the hippocampus were binucleated. It is suggested that the DNA-repair defect of CS causes abnormalities in nuclear DNA replication and cell division which result in cell death and in the observed nuclear abnormalities.

Key Words: Cockayne syndrome; DNA repair; Dwarfism; Microcephaly; Nuclear atypia; Ultraviolet radiation.

INTRODUCTION

Cockayne syndrome (CS) is a rare autosomal recessive syndrome characterized by cachectic dwarfism, cutaneous photosensitivity, and progressive neurologic abnormality (1–9). Microcephaly, psychomotor retardation, extrapyramidal movement disturbances, sensorineural deafness, optic atrophy, pigmentary retinopathy, and peripheral neuropathy are among the prominent neurological signs. Cultured skin fibroblast and lymphoblastoid lines from all CS patients who have been studied have a hypersensitivity to the lethal effects of ultraviolet radiation (UV) and to UV-mimetic chemicals (9–13). Neuropathological studies of CS have revealed extensive demyelination, cerebellar atrophy, and intracranial calcification (4, 7, 8, 14–28). We now report the clinical features of two siblings with an unusually severe and early onset form of CS. We also report the autopsy of one sibling and the tissue culture study demonstrating the UV hypersensitivity of a lymphoblastoid line established from the other sibling.

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RESULTS

Case Histories

Case 1: This girl was the first-born child and weighed 3,350 g at birth. Her head circumference at birth was 32.5 cm (10th percentile). Congenital cataracts had been surgically removed. At six months of age she was evaluated for her microcephaly. Plain skull X-ray studies did not show premature closure of the cranial sutures. An electroencephalogram revealed no specific abnormality. Her developmental milestones were slow, and she was never able to sit alone.

At age two years, four months, her weight was 7.2 kg, height 69 cm, and head circumference 40.0 cm. Examination revealed sparse, white, dry hair, microphthalmia, micrognathia, pectus excavatum, and thin pale skin with diminished pigmentation. She babbled but had no words, and her Denver Developmental Screening Test Score was eight months. She had roving nystagmoid eye movements but turned to light and had full extraocular movements. She responded to a loud bell. She had slight to moderate generalized spastic tone in all extremities and flexion contractures. Deep tendon reflexes were hypoactive and plantar stimulation produced no toe movement. Motor nerve conduction velocities were slowed with values of 29 m/second (s) in the right median nerve, 30 m/s in the right peroneal nerve, and 28 m/s in the right tibial nerve.

At three years of age her weight was 6.0 kg and height 75 cm. At three years, three months, repeat motor nerve conduction studies showed conduction velocities of 28 m/s in the right ulnar, right median, and right tibial nerves and 24 m/s in the right peroneal nerve. At four years her weight was 6.5 kg and height was 77 cm. Lumbar puncture cerebrospinal fluid protein was 96 mg/dl. At four years, five months, a pure tone audiogram revealed 45-50 decibel threshold in the right ear and a 35 decibel threshold in the left ear at 500, 1,000, and 2,000 Hz. Although the parents stated that the child had been taken outdoors in the sun during the summer in North Dakota, she apparently had never experienced acute sunlight sensitivity. After an afebrile upper respiratory infection at age six years, she developed acute respiratory distress, pallor, and hypothermia and was hospitalized. Lumbar puncture cerebrospinal fluid contained three lymphocytes/mm³, the protein was 24 mg/dl, and the glucose was 59 mg/dl. She had a progressive downhill clinical course culminating in renal and pulmonary failure, and she died within 24 hours (h) of admission to the hospital.

Case 2: This second-born child weighed 3,780 g at birth. Head circumference at birth was 32 cm. He had congenital cataracts which had been removed at three months of age. At age 28 months he had a bilateral orchiopexy for cryptorchidism. At age 20 months motor nerve conduction studies showed a right ulnar nerve velocity of 38 m/s, right median nerve 34 m/s, right peroneal nerve 34 m/s, and right tibial nerve 31 m/s. He was scooting around the floor at four months of age, but later developmental milestones were markedly delayed. By age three years he was beginning to vocalize the sounds “wa,” “da,” and “ba,” but language progressed no further.

At age four years and eight months, two months after the death of his sister, he was hospitalized for an evaluation. His weight was 6.5 kg, height 76 cm, and head circumference 41 cm. He had scanty gray hair and most of this teeth were misaligned and carious. His ears appeared prominent. He had significant hypogonadism. The extremities were very thin with little subcutaneous fat and marked muscle atrophy. On the Bayley Scales of Infant Development he received a raw score of 48, equivalent to the level of function of a four-month-old infant. He had continuous wandering
nystagmoid eye movements and blinked to light and threat. He had spastic tone in all four extremities, slightly more in the legs than the arms, and flexion contractures were apparent in all four extremities. Deep tendon reflexes were symmetrically brisk and equal in upper and lower extremities. There was minimal great toe extension with plantar stimulation (Babinski reflex). Brain stem auditory-evoked response testing gave no reproducible responses on the right side, while on the left side the absolute latencies of wave I (2.8 Ms) and wave V (6.8 Ms) were normal (as was the I-V interwave latency of 4 Ms). The following laboratory values were obtained at age four years, eight months: creatinine clearance 20 ml/min; plasma renin 2.8 ng/ml/h; serum luteinizing hormone (LH) 5 µg/dl; serum follicle stimulating hormone (FSH) 4 µg/dl; serum prolactin 17 ng/ml; serum cortisol 13.9 µg/ml; serum alpha-1-antitrypsin 270 mg/dl; serum IgG 899 mg/dl, IgA 465 mg/dl, and IgM 129 mg/dl. Thyroid stimulating hormone (TSH) was 5.8 IU/ml at age five years, four months, and 1.7 IU/ml at age six years, five months.

The presence or absence of cutaneous sunlight sensitivity could not be established since the family took the patient outside only rarely and then completely clothed.

Plain skull X-ray films showed a small cranium relative to the size of the face and a markedly thickened cranial vault. Spine X-ray films showed diffuse osteoporosis, moderate kyphosis, and slight low thoracic scoliosis (convex to the left). A CT scan of the head (Fig. 1) showed marked cerebral gyral atrophy with prominence of the sulci, moderate ventricular enlargement, marked brain stem atrophy, large cystic atrophy of the left cerebellar hemisphere, moderate right cerebellar atrophy, calcification bilaterally in the basal ganglia and in the dentate nuclei of the cerebellum, and a small calcification in the right frontal lobe.

He was rehospitalized at age six years, five months after an apparent seizure. An electroencephalogram showed a slow background rhythm, persistent low-amplitude delta activity over the right hemisphere, frequent paroxysmal runs of bilateral high-amplitude theta and delta activity, and occasional bisynchronous sharp transients. After release from the hospital on phenobarbital, he died at home. No autopsy was performed.

### Tissue Culture Studies

**Case 2:** Before the patient's death, skin biopsy and venous blood samples were taken for establishment of fibroblast and lymphoblastoid cell lines at the Institute for Medical Research, Camden, New Jersey. The fibroblast and lymphoblastoid lines are designated RB 5324 and RB 5325, respectively. Lymphoblastoid lines are Epstein-Barr virus-transformed B-lymphocytes which grow as immortal lines in suspension culture. The post-UV survival of a lymphoblastoid line was measured as the viability ratio. The ratio was determined by dividing the concentration of viable (i.e. trypan-blue dye-excluding) cells in an irradiated culture on the third post-irradiation day by the concentration of viable cells in an unirradiated culture on the same day. This lymphoblastoid line cell survival assay has been described in detail previously (13), and it accurately detects hypersensitivity to the lethal effects of UV in cultured cells from patients with xeroderma pigmentosum and CS (13). Dose-response experiments with the lymphoblastoid line from case 2 (CS1FABE) showed a marked hypersensitivity to 254-nm UV (Fig. 2). This hypersensitivity was in the range (13) of that obtained with lymphoblastoid lines from CS2BE and CS3BE, who had typical clinical features of CS, and with the line from an atypical CS patient, who had mild and late onset features of CS.
Fig. 1. Representative CT scan sections from case 2 showing cerebral gyral atrophy with prominence of the sulci, moderate ventricular enlargement, marked brainstem atrophy, large cystic atrophy of the left cerebellar hemisphere, moderate right cerebellar atrophy, calcification in the dentate nuclei of the cerebellum and basal ganglia bilaterally, and a small focal calcification in the right frontal lobe.

Neuropathological Findings

Case 1: The body weight was 6.5 kg, crown-heel length 79 cm, toe-heel length 11.5 cm, head circumference 40.0 cm, chest circumference 38 cm, and abdominal circumference 33 cm. Organ weights were heart 33 g, liver 188 g, right lung 146 g, left lung 113 g, adrenal glands 2.1 g, ovaries less than 1 g, thyroid less than 1 g, spleen 24 g, and kidneys 34 g. There was no specific gross pathological abnormality in any visceral organ. Microscopic sections revealed extensive bilateral bronchopneumonia. There was cortical hypoplasia of the adrenals. Only rare follicle cysts were identifiable in the ovaries. The thyroid showed marked interstitial fibrosis, chronic inflammation, and atrophy of many follicles. There was diffuse basement membrane thickening in many renal glomeruli. Binucleated cells were common in the liver and the renal tubules.
The unfixed brain weighed 450 g. The spinal cord was not examined. The leptomeninges were slightly thickened and fibrotic. The major intracranial blood vessels were grossly normal. There was generalized atrophy of the brain which was particularly severe in the cerebellum. Coronal sections of the cerebral hemispheres revealed marked enlargement of the lateral ventricles (Figs. 3 and 4). The corpus callosum was thin. The hippocampus was atrophic bilaterally. The white matter was decreased in volume and patchily myelinated. The U-fibers were irregularly present throughout the cerebrum. The cerebral peduncles were small (Fig. 3b). Sagittal sections of the cerebellum confirmed the diffuse atrophy.

Microscopic examination confirmed the gross impression of highly irregular and patchy myelination in Luxol fast blue–periodic acid–Schiff–hematoxylin (LFB-PAS-H) stains (Fig. 4) affecting all levels of the brain. Where myelin was present there were normal appearing oligodendrocytes; where myelin was absent, oligodendrocytes were also absent. Astrocytes were prominent throughout the brain. They had variable amounts of cytoplasm, and their nuclei were often large and hyperchromatic in hematoxylin and eosin (H&E) stains (Fig. 5). Many astrocytes contained as many as six to eight nuclei per cell. Astrocytes with two to four nuclei per cell were particularly prominent in all layers of the cerebral cortex.

The cerebellar architecture was grossly distorted (Fig. 6a–d). The granule cell layer and molecular layer were thin, and there was an irregular arrangement of the Purkinje...
Fig. 3. (a) Coronal section of the cerebral hemispheres showing thin corpus callosum, reduced volume of white matter and large lateral ventricles. (b) Midbrain with enlarged aqueduct and small cerebral peduncles.

cell layer with decreased numbers of Purkinje cells. Many Purkinje cells had lobulated nuclei, and occasional Purkinje cells were binucleated (Fig. 6c, d). A small number of Purkinje cells had axonal swellings ("torpedoes") extending into the granule cell layer (Fig. 6b). Abnormal arborization of individual Purkinje cells could be detected with a rapid Golgi stain (29) (Fig. 7). The large dendritic tangles found in association with abnormal Purkinje cell arborization in a previously reported case (8) were not seen in our case. The cerebellar white matter was diffusely depleted.

The cerebral cortex was significantly less disorganized than the cerebellum, and neuronal loss was not apparent except for a reduced number of granule cells in the dentate gyrus of the hippocampus. Cerebral neurons frequently appeared as pairs (Fig. 8a), but occasionally as many as four neurons would be lined up together. In addition, individual neurons containing two nuclei were identified. These binucleated neurons (Fig. 8b–d) were present in all areas of the cerebral cortex examined, including the pyramidal cell layer of the hippocampus.

Calcification was present perivascularly and within the neuropil, mainly in the putamen and cerebellum. Individual calcified Purkinje cells occurred rarely. No significant abnormalities other than irregular myelination were detected in the thalamus, brainstem, or dentate nuclei.

**DISCUSSION**

Cultured fibroblast and lymphoblastoid lines from patients with Cockayne syndrome have a hypersensitivity to the lethal effects of UV-radiation and UV-mimetic chemicals (10–13). This hypersensitivity is the result of a defective system for repairing the UV-type of DNA damage, as evidenced by the CS cell’s defective host-
cell reactivation of UV-damaged, double-stranded DNA viruses (30-33). Of our two siblings with CS, case 2 had cultured cell lines available for testing, and our CS family is the first for which combined clinical, DNA-repair cell culture, and necropsy studies have been reported. The lymphoblastoid cell line from our case 2 had the characteristic hypersensitivity to UV shown by all other CS lines tested (Fig. 2).

This hypersensitivity may reflect the mechanism responsible for CS patients' *in vivo* skin sensitivity to the UV in sunlight (9-11, 13). Most, but not all, CS patients are reported to have a photosensitive dermatitis (7, 8, 26). Some manifest the dermatitis only as acute sun sensitivity (blistering and excessive redness) in infancy or early childhood (9, 13). There was no apparent sunlight sensitivity in our case 1 despite reported exposure to sunlight. Sunlight exposure was apparently minimal for case 2 and may have been insufficient to produce skin changes.

Previous neuropathological studies have included brain biopsy (34), autopsy (4, 8, 14-16, 18, 22-25), and peripheral nerve biopsy (7, 17, 19-21, 26-28, 35). There was slight slowing of motor nerve conduction velocities in case 1 at 28 months of age consistent with a demyelinating neuropathy. The greater slowing found on repeat conduction studies 11 months later was consistent with a progressively worsening disturbance. The normal values for her brother's motor conduction velocities (case 2) found at age 20 months also lend support to the idea that a progressive demyelination takes place in this disorder. Nerve biopsy reports (7, 17, 19-21, 26-28, 35) have shown segmental demyelination, and the nerve conduction studies performed in older patients have all demonstrated slowing of conduction velocity (6, 7, 17, 19, 21, 26, 27, 35-38). The ten cases in which brains have been examined at

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*Fig. 5.* Multiple nuclei in astrocytes of the cerebral cortex (a, b, c) and white matter (d). H&E. Original magnification ×400.
autopsy ranged in age from three years, six months to 31 years. A characteristic of all the previously reported brains and our patient’s brain was the small size. The largest brain weighed 989 g in the case of a 12 year, three-month-old reported by Sugarman et al (23), and the smallest previously reported brain weighed 450 g in the 14 year, nine-month-old reported by Soffer et al (8) and the three year, six-month-old reported by Moyer et al (25). Our six-year-old patient is, therefore, the second youngest recorded autopsy on a CS patient. Our case also had a brain weight of 450 g, which is the normal brain weight for a two and a half-month-old infant, for a body weight of 5 kg, or for a body length of 55 cm; the normal brain weight for age six years would be 1,100 g, and for a crown–heel length of 79 cm the brain should weigh 900 g (39). The small size of the brain is in part the result of the massive loss of cerebral white matter. In our case, as in previous cases, there was irregular, patchy demyelination. The cerebellum had the greatest white matter loss. In the cerebrum there was no distortion of cortical architecture, but in the cerebellum...
Fig. 7. Camera lucida drawing of Purkinje cells showing the abnormal dendritic branching. Denn Rapid Golgi stain.

there was a reduction in the number of Purkinje cells and a marked decrease in number of granule cells. Greater cerebellar than cerebral disorganization has also been found in the previously reported cases.

The striking finding of nuclear abnormalities in astrocytes and neurons in our case has been described previously in only one other case (8). As in the case of Soffer et al (8), we found many astrocytes with a single, large, hyperchromatic, atypical nucleus; other astrocytes were multinucleated. While Soffer et al (8) encountered binucleated neurons in the cerebellum rarely, our case had binucleation frequently not only in Purkinje cells of the cerebellum but also in cerebral neurons. It is probable that the greater degree of these nuclear abnormalities in our patient is due to the fact that our patient, with the exception of the case of Moyer et al (25), had a more severe form of CS than did all other histopathologically evaluated CS patients including that of Soffer et al (8). For example, in contrast to almost all other CS patients who appear normal at birth, our patient was born with clinical features of CS (e.g., head circumference in the 10th percentile, congenital cataracts). Furthermore, our patient’s head circumference never exceeded 41 cm, and she was never able to sit or speak words. Although rare, abnormally large nuclei, presumably of Schwann cells, were found (23) in a mesenteric nerve of a CS patient who had a milder form of CS than our case (sat at 13 months; walked at 36 months; head circumference of 47.5 cm at eight and a half years; cerebrum weighed 899 g at 12 years), no astrocytic nucleomegaly was found. The most severe forms of CS previously documented were those of See et al (37) and that described by Moyer et al (25) in identical twins. The patient of See et al (37) had a head circumference of 27 cm at birth, 38.5 cm at four years, and 39 cm at seven years. The autopsied twin of Moyer et al (25) had a head circumference of 27.5 cm at birth and 36 cm when he died at age three years and six months. His 450 g brain had pathological findings consistent with CS, but, unfortunately, no mention was made of the detailed histological appearance of astrocytes or neurons. It is possible that two siblings reported by Lowry et al (40) may also have had CS of a very severe and early onset form (25, 41-44) although not even the autopsy of one of the siblings unequivocally documented the diagnosis of CS (43).

We suggest that the binucleation of neurons and the nuclear atypia of astrocytes in our patient may result from the accumulation of unrepaired DNA damage due to the defective DNA-repair process which is known to be present in all CS patients and which is reflected by the hypersensitivity of their cultured cells to the lethal effects of UV (Fig. 2). As discussed elsewhere (45), similar nuclear abnormalities
have been described in cells from patients with the ionizing-radiation hypersensitivity diseases ataxia telangiectasia and tuberous sclerosis and also in mammalian fibroblasts that have been supralethally irradiated in vitro with ionizing radiation.

While the DNA in the cells of the nervous system is not exposed to the harmful effects of UV, the DNA is, nevertheless, constantly being subjected to incident damage from active oxygen species, intracellular metabolites, spontaneous hydrolytic reactions, and transcriptional events (46). Repair mechanisms ordinarily correct the DNA damage (46). In CS not only are neurons susceptible to derangement of nuclear material, but, as has been previously suggested (9), myelin-forming oligodendroglia may also accumulate DNA damage and die, resulting in demyelination. Presumably, the involvement of certain cell types but not others may be the result of differences in intracellular or extracellular metabolic microenvironments which will result in different types and spectra of DNA damage in the various cell types.

The significance of the markedly abnormal Purkinje cell dendritic branching (Fig. 7) is not known. Abnormal Purkinje cell dendrites have been previously reported in only one other CS case, that of the rather severely affected patient of Soffer et al (8). Purkinje cell “torpedoes,” also found in our case (Fig. 6), have been reported in two previous CS cases (8, 23). Both abnormal dendritic branching and axonal swellings (“torpedoes”) have been found in the cerebellum of patients with ataxia telangiectasia (47), another disorder with hypersensitivity to DNA-damaging agents (46).

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