A novel DNA repair disorder with thrombocytopenia, nephrosis and features overlapping Cockayne syndrome


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Cockayne-like syndrome with thrombocytopenia and nephrotic syndrome.

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Abstract
We report four siblings affected by a Cockayne-like syndrome with nephrotic syndrome. The parents were healthy and consanguineous, consistent with autosomal recessive mode of inheritance. UV irradiation of fibroblasts revealed an intermediate sensitivity between normal and standard Cockayne Syndrome (CS) control cells. Two cases of CS in association with nephrotic syndrome have been previously documented, suggesting a Cockayne-like phenotype with nephrotic syndrome represents a novel DNA repair disorder. A genome-wide linkage scan conducted using Affymetrix 10K arrays provided exclusion of the known CS genes in the family, and evidence that the disease gene maps to 1p33-p31.1. Further investigation of additional affected families may facilitate the identification of the gene defect.

Keywords:
Autosomal recessive inheritance, Cockayne Syndrome, DNA repair disorders, Linkage scan,
Nephrotic syndrome, UV irradiation
**Introduction**

Cockayne Syndrome (CS) is a rare autosomal recessive disorder with variable expression. The phenotype classically becomes evident in early childhood and patients present with hyperpigmentation, sunken eyes secondary to subcutaneous lipoatrophy, failure to thrive, short stature and microcephaly. Neurological sequelae include progressive ataxia, global developmental delay, sensorineural deafness and tremors (1-4). While approximately 10% of patients with CS develop renal pathology, this rarely leads to serious complications (5-7).

A diagnosis of CS is made on clinical features and can be confirmed by UV irradiation of fibroblasts from the patient. CS cells fail to restore normal levels of RNA synthesis following UV irradiation when compared to control fibroblasts (8). Mutations in genes CSA and CSB genes, coding for proteins involved in transcription-coupled repair, are thought to be responsible for the Cockayne phenotype in the majority of cases (9). There is poor genotype/phenotype correlation, and identical mutations in CSB may be associated with either CS or Xeroderma Pigmentosum (XP) – another DNA repair disorder (9). Mutations in XPB, XPD and XPG can result in a mixed clinical phenotype with neurological features of CS and skin abnormalities associated with XP (9).

Here we report four siblings affected by a Cockayne-like disorder associated with nephrotic syndrome, and an intermediate fibroblast response to UV irradiation.

**Cases**

The parents of the four affected children are healthy, Pakistani second cousins (figure 1). The two older siblings (III.1 and III.2) were first referred for genetic evaluation aged six and five respectively. They were noted to have a distinctive phenotype with severe global developmental delay, short stature and similar dysmorphic features: progeroid facies
with sunken eyes, frontal bossing, low set ears and microcephaly. There was no history of photosensitivity, poor vision or deafness.

Case 1

The eldest sibling (III.1, figure 1) weighed 2.8kg (9th centile) at birth. At six years, OFC was 47.5cm, height 93cm and weight 13.2kg; all well below the 0.4th centile. In addition to the dysmorphic features he shared with his sister, he also had keratinised papular lesions on his forehead. Previous diagnostic investigations had been inconclusive. He was noted to have a normal karyotype, and computerised tomography (CT) of his brain had revealed “moderately prominent ventricles and sulci”. Urine amino acids were normal and congenital infection screen was negative. He had further investigations at the age of seven, during admission to hospital for steroid-unresponsive nephrotic syndrome. A renal biopsy identified glomerular sclerosis, tubular atrophy, interstitial fibrosis, and hyaline thickening of the arteriolar wall. The patient was thrombocytopenic on admission (platelet count: 50x10^9L) and had a history of easy bruising. A bone marrow biopsy showed hypocellular particulate bone marrow with tri-lineage re-presentation. Normal sensitivity to diepoxybutane excluded Fanconi’s anaemia. Generalised osteoporosis and delayed bone age was apparent on skeletal survey. Recovery of RNA synthesis following UV irradiation of skin fibroblasts gave an intermediate result between normal and known Cockayne controls (Figure 2). At the age of eight he died secondary to complications of nephrotic syndrome.

Case 2

The second child (III.2, Figure 1) was born at 41 weeks by normal vaginal delivery, weighing 3.1kg (50th centile). The neonatal period was complicated by hypotonia, and she required two days admission to the Special Baby Care Unit. At presentation aged five, OFC was 44.5cm and length 87cm. At age seven all growth parameters were below the 0.4th centile. A skeletal survey revealed delayed bone age with non-specific changes. Borderline
growth hormone response was noted, and the patient was started on replacement therapy at the age of nine. She subsequently developed papular lesions on her skin similar to those of her elder brother and persistent thrombocytopenia. Normal sister chromatid exchanges for Bloom syndrome, diepoxybutane sensitivity and chromosomal ionising radiation excluded Bloom Syndrome, Ataxia Telangiectasia and Fanconi’s anaemia respectively. Attempts to establish a fibroblasts culture for UV sensitivity testing were unsuccessful, and parents declined a second attempt. She developed steroid-responsive nephrotic syndrome aged 10, and died secondary to complications at the age of 16.

Case 3

The third child (III.3, Figure 1; Figure 3a) was born at 42 weeks by spontaneous vaginal delivery with no perinatal complications. Her birthweight was 2.57kg (0.4th centile) with a head circumference also at the 0.4th centile. Karyotype and CT brain were normal. By 14 months she had developed the distinctive facial features of her two older siblings. Vision and hearing were normal and there was no evidence of photosensitivity. She exhibited gross motor delay, but otherwise appeared less severely affected than her siblings. UV irradiation of fibroblasts again gave a response intermediate between that of normal and CS cells, similar to her brother. At the age of six she has developed persistent moderate thrombocytopenia and proteinuria.

Case 4

The youngest sibling (III.4, Figure 1) had an uncomplicated delivery. At birth his weight was 3.2kg (25th centile) and head circumference below the 9th centile. By the age of 21 months he had mild developmental delay and similar dysmorphic features to his siblings (Figure 3b, 3c). Like his siblings, he has no evidence of photosensitivity, visual problems or deafness. Although he appears to be affected by the same condition as his three siblings he has not yet developed nephrotic syndrome or thrombocytopenia.
Discussion

Clinical diagnosis

The four siblings described in this report have many of the classic features associated with CS. However, some characteristic findings are missing: they all have normal hearing and vision, and no evidence of sun-sensitivity. Two other children with a diagnosis of CS who have died as a result of nephrotic syndrome have been described in the literature (5,10). In the first case (5), there was a marginal hypersensitivity to sister chromatid exchanges induced by UVC but not to those induced by UVB. In the second case (10) it is stated that “she underwent a skin biopsy that demonstrated ultraviolet-induced damage”, without any data being presented. It is therefore difficult to assess the UV sensitivity status of these patients’ cells.

It is striking that, in addition to having nephrotic syndrome, the child reported by Reiss et al. was phenotypically very similar to the children described in this report: he had many classic CS features but did not have the typical photosensitive rash or deafness. Nephrotic syndrome in this child was unresponsive to steroids (5), whereas the patient described by Funaki et al. was treated with oral prednisolone with good effect (10). Table 1 compares the phenotype of the siblings described in this report and the other reported children with CS and nephrotic syndrome.

The authors are aware of about ten other patients where irradiation of fibroblasts has produced an intermediate response of RNA synthesis recovery following UV irradiation (AR Lehmann, unpublished data). Limited information exists regarding phenotype and results of molecular diagnostic testing in these patients. At least three of the patients appear to have a clear Cockayne –like phenotype. However, none are known to have developed nephrotic syndrome.
Linkage analysis

The parents of these four affected siblings are consanguineous indicating an autosomal recessive mode of inheritance. However, for pure Mendelian inheritance, the probability of all four children being affected is 1 in 256.

To search for a disease locus a genome wide linkage scan of the family was conducted using GeneChip mapping 10K Xba 142 Arrays according to manufacturers protocol (Affymetrix Inc, Santa Clara, CA). Segregation of SNP markers with affection status in the family excluded the possibility that the disease was caused by dominant or recessive mutations in any of the known CS genes, CSA on chromosome 5, CSB on chromosome 10, XPB on chromosome 2, XPD on chromosome 19 and XPG on chromosome 13.

A 27Mb interval of shared homozygosity was identified encompassing 104 informative SNPs (defined by dsSNPs rs2354462 to rs718883) at chromosome 1p33-p31.1 (figure 3). Multipoint linkage analysis assuming a fully penetrant autosomal recessive mode of inheritance was undertaken using the GENEHUNTER programme (11). Allele frequencies for each marker were assumed to be equal and a population disease gene frequency of 0.001 was used to estimate the maximum LOD score. Statistical support for linkage was evaluated over a range of marker allele frequencies. The map order and distances between markers were based on the UCSC Human Genome Browser (http://genome.ucsc.edu/). The multipoint LOD score across the 27Mb region of linkage was 2.4.

Although this LOD score does not attain the classical threshold of significance, it corresponds to a P value of 0.0004; hence it is unlikely to represent a false positive finding. A contiguous gene syndrome cannot be excluded; but any such deletion would have to be <170kb in size, as genotype signatures were obtained from 104 markers mapping to the region of linkage.
One hundred and thirty one transcripts map to the 27Mb region of linkage (UCSC Human Genome Browser, March, 2006). Excluding the predicted or hypothetical genes mapping to the region that have little or no associated information regarding their biological function, there are no obvious candidate genes at present.

**Conclusion**

This report describes a family with four siblings affected by a Cockayne-like disorder associated with nephrotic syndrome. Two previous cases with similar clinical features have been described suggesting that this may be a novel DNA repair disorder. Genome wide linkage analysis indicates the existence of an autosomal recessive candidate locus at 1p33-p31.1. Investigation of additional affected families may further elucidate this emerging phenotype and facilitate identification of the causal gene.
References


Figure 1: Pedigree showing the four affected siblings. No other family members are known to be affected.
Figure 2: Recovery of RNA synthesis following UV irradiation of skin fibroblasts from III.1. Pooled data from five experiments show an intermediate result between normal and Cockayne Syndrome controls.
Summary of data on M.Tayyab (Pooled from E196, E197, E198, E204 and E208)

Recovery of RNA synthesis following UV irradiation

Summary of data on patient III.1

- **Normal**
- **CS control**
- **Patient III.1**
Figure 3(a) Clinical pictures of case 3 at 3 years and 11 months. (b,c) Clinical pictures of case 4 at 1 year and 6 months. The images demonstrate the phenotype: sunken eyes, frontal bossing, low set ears and microcephaly. Informed consent was obtained for publication of these pictures.
Table 1: Phenotype comparison between classic CS and children described in the literature with CS and fatal nephrotic syndrome.
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<tr>
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<th>Case reported by Funaki et al (10)</th>
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<th>III.2</th>
<th>III.3</th>
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<td>+</td>
<td>+</td>
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<td>+</td>
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