Syndrome of the month

Menkes disease

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The first report of an X linked disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration by Menkes¹ in 1962 was a thorough description of the phenotype and neuropathology, recognising the inheritance pattern and a likely metabolic aetiology.

It was a decade later that Danks *et al*^{2,3} established that this was a disturbance of copper metabolism and deficiency of copper dependent enzymes. The homology to mottled mouse mutant brindled was recognised and the copper deficiency was confirmed in the mouse.⁴ The 1970s and 1980s saw intense investigation of the mottled mouse in an effort to delineate the nature of the primary defect causing the copper disturbance.

Many clinical cases were reported. The frequency of Menkes syndrome was established as 1:200 000⁵ and it was recognised that the occipital horn syndrome (X linked cutis laxa or EDS IX) had similar biochemical changes.⁶ The gene was isolated in 1992 by three research

groups.⁷⁻⁹ The clue came from an expressing heterozygous female found to have an X;2 translocation.¹⁰ The deduced gene product, a transmembrane copper transport protein, clarified the nature of the copper disturbance.⁸

Phenotype of Menkes disease

These babies are often born prematurely. Although many are normal at birth with fine, normal looking hair (fig 1A, B), they may have neonatal problems of temperature instability, jaundice, and feeding difficulties. Many acquire head control and responsive smile, but by 3 months of age they develop seizures and loss of the achieved milestones and head control. They have truncal hypotonia and progressive spasticity of the limbs with often marked failure to thrive. The hair is hypopigmented, lusterless, and breaks off, particularly over scalp areas of maximum friction (fig 2). The hair feels like "steel wool" to touch owing to pili torti (fig 3). Eyebrows and eyelashes are affected. The skin

Figure 1A Hypotonic face with broad cheeks and sparse eyebrows and eyelashes. The scalp is rough to touch because of broken scalp hair.

Figure 1B Dry, twisted, hypopigmented, broken hair.

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Figure 2 Dry hair with patchy hypopigmentation and pili torti, broken in areas exposed to abrasion.

is hypopigmented, hyperextensible, and the joints are hypermobile.⁶

The bones are osteoporotic with flared metaphyses of long bones, rib fractures, and there may be Wormian bones on skull x ray. The blood vessels are tortuous and elongated with irregular lumen owing to defective development of the elastic lamina and thickened intima (fig 4). Diverticulae of the urinary tract

Figure 3 Microscopic view of a hair showing pili torti.

Figure 4 Irregular lumen of arteries.

are common. In the brain and cerebellum there is patchy neuronal loss with glial proliferation and atrophy. Subdural and cerebral bleeds may occur.

There is progressive deterioration and death occurs usually by 3 years of age. With excellent care, some children have survived longer.¹⁶

A milder phenotype has been reported.¹¹ This patient presented at 2 years of age with marked cerebellar ataxia and mild developmental delay and was noted to have the typical hair changes. He had a lesser copper deficiency but abnormal copper uptake and mild vascular changes of Menkes disease. He was treated and has not had a neurodegenerative course but ataxia and borderline abilities persist.

Heterozygotes are usually neurologically normal but may have patchy pili torti and patchy failure of pigmentation. Copper accumulation in fibroblasts is also patchy and multiple skin biopsies are needed to identify carriers. A single study will only detect half the carriers.⁶

Biochemical findings

Serum copper and ceruloplasmin levels are low and copper content in liver and brain is also reduced. Copper accumulates in intestinal and renal tubular cells with raised levels of copper bound to metallothionein in the cytoplasm. Copper is thus trapped in the gut mucosa and renders the baby copper deficient.³ It has been proposed that the lack of copper transport protein blocks the egress of copper from cells and probably the flux into mitochondria and organelles where copper is incorporated into copper dependent enzymes.⁸ Results from mouse research suggest that copper also accumulates in cerebral blood vessels and glial cells¹² rendering the brain copper deficient. Copper transport is affected in all tissues except the liver.¹¹ When copper is available, there is normal uptake in fibroblasts, but reduced efflux, hence the increased accumulation in fibroblasts and in the placenta in pregnancy. Increased copper accumulation has been the diagnostic test in antenatal diagnosis of Menkes disease.

Copper dependent enzyme levels are low and are believed to explain the pathogenesis of the disorder.¹³ Lysyl oxidase, important in cross linking of collagen and elastin, explains the connective tissue findings. Low levels of cytochrome c oxidase may be responsible for the temperature instability. Tyrosinase deficiency explains the hypopigmentation of skin and hair. Copper is needed for cross linking of keratin, hence the pili torti. Deficiency of cytochrome c oxidase, superoxide dismutase, and dopamine betahydroxylase may cause the neurodegenerative changes.

Copper treatment

Because of poor gut absorption, copper is administered parenterally as copper histidine (by intramuscular injection daily). This readily corrects the serum and liver copper levels but does not restore the levels of all copper dependent enzymes in other organs.³

When the treatment is started before onset of

Figure 5 Facial hypotonia less marked in a mildly affected child on copper treatment.

seizures and neurodegeneration, it has been shown to prevent neurodegeneration and prolong survival⁶ (fig 5). Studies in mice suggested that very early treatment may be of even greater benefit and several cases have been treated from birth after elective/early delivery (one in utero). Patients treated very early have had only mild ataxia, no seizures or neurodegeneration, and have developed the skeletal changes of the occipital horn syndrome. Postural hypotension was present and successfully treated with L-DOPS, a compound which can be converted to noradrenaline without dopamine beta-hydroxylase (D M Danks, personal communication).

The Menkes gene

The Menkes gene has been localised to Xq13.3 and the gene has been recently identified.⁷⁻⁹ It is expressed in most tissues except the liver. The product is a 1500 amino acid copper binding protein believed to have six copper binding sites and six to eight transmembrane domains (four loops). The 8.5 kb mRNA is reduced or absent in most Menkes patients. It corresponds to a genomic region of 150 kb including about 20 exons. The first exon contains most of the 5' non-coding sequence, separated by a large intron (about 40 kb) from the second exon which is the start of the coding sequence. The large (4 kb) 3' untranslated region is encoded in one exon.

Seventy percent of Menkes patients express little or no mRNA. It is believed that splice site or promoter mutations are involved in most

cases; deletions in the gene have also been identified. A candidate deletion of two amino acids has been defined in the Brindled mouse mutant (JFB Mercer, unpublished data). Splice mutations have been documented in the Blotchy mouse, homologous to occipital horn syndrome.^{14,15}

Prenatal diagnosis

Before the gene was identified, the diagnosis of Menkes disease in pregnancy relied on showing increased accumulation of copper in chorionic villi or abnormal accumulation of copper in cultured amniotic or CVS cells.

There can be variation in the results dependent on culture conditions and it has been recommended that efflux of copper in the next 24 hours is a more reliable measure. Copper contamination is a problem with direct assay of CVS.⁶ The tissue needs careful handling with copper free reagents and containers. The concurrent analysis of normal control CVS is helpful. Copper uptake is measured per mg protein per 24 hours. While Menkes patients show a greater than 10 fold increase in copper levels in amniotic cells, up to 20% of carriers have copper levels in the normal range. The most reliable way of establishing if an at risk female fetus is a carrier is to measure placental copper content at the time of her birth.

Molecular methods can now be used once the mutation in the family has been established. Since each family would need to be investigated for their mutation, copper studies, performed with care, may be more readily applicable.

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