

# High <sup>64</sup>Cu uptake and retention values in two clinically atypical Menkes patients

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**Abstract**

We have investigated two previously published atypical Menkes patients with <sup>64</sup>Cu uptake and retention studies. Both of these analyses gave significantly increased results in the range seen for classical Menkes patients. <sup>64</sup>Cu uptake analyses on female relatives gave the same uptake pattern as seen for other families with classical Menkes disease.

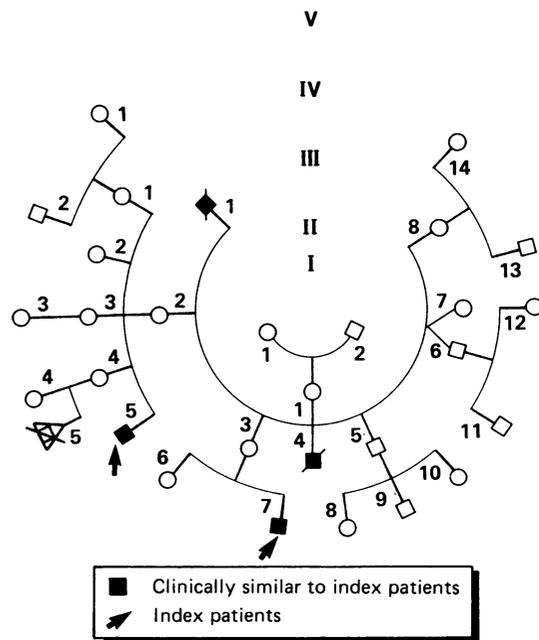
Menkes disease (McKusick 30940) is an inherited disorder of copper metabolism transmitted as an X linked recessive trait. Patients show specific symptoms of copper deficiency, such as lack of keratinisation and pigmentation of hair, degenerative changes of the elastic tissue in the aorta and blood vessels, and scorbutic changes. In addition, progressive psychomotor retardation, seizures, and temperature instability are seen, and affected males rarely survive for more than three years.<sup>1</sup> The disturbance of copper metabolism is characterised by increased copper accumulation in multiple cell types in the body and in culture,<sup>2-5</sup> and this provides a useful diagnostic marker for both postnatal and prenatal diagnosis.<sup>6</sup>

The clinical symptoms may vary in Menkes disease and several reports describing milder forms have been published.<sup>7-16</sup> Haas *et al*<sup>17</sup> described a family with four affected boys with some symptoms of Menkes disease. Two of the boys were extensively studied. The disease showed X linked recessive

inheritance. The boys presented with marked psychomotor retardation with seizures, low serum copper and ceruloplasmin levels, and a block in gut copper absorption. The two boys differed from the classical Menkes patients by having no hypothermia and longer survival than usual. The facies and skin were normal, and there was no radiological evidence of any bone changes. We have performed <sup>64</sup>Cu uptake studies on fibroblast cultures from these two patients. Both uptake and retention values were indistinguishable from those of classical Menkes patients.

**Case reports**

Four affected males were diagnosed in this family (figure). The two most thoroughly investigated of the patients were IV-5 and IV-7. V-5 was a male fetus of which therapeutic abortion was performed



Pedigree of the reported family. The previously published family pedigree<sup>17</sup> has been followed up.

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after knowledge of the fetal sex.  $^{64}\text{Cu}$  uptake results from fetal skin fibroblasts were completely normal.

#### IV-5

For the early clinical details, see Haas *et al.*<sup>17</sup> Pregnancy, delivery, and birth weight were normal. At the age of 3½ months developmental delay was diagnosed, including hypotonia and athetoid movements. After the age of 3 years seizures started, apparently associated with fever. At the age of 14 years there was spasticity of the extremities with increased reflexes, and the patient has never been able to walk. He had pectus excavatum, but no radiological bone abnormalities were found. No hair, skin, or head abnormalities were observed, except for mild micrognathia. The testes had descended normally. He was severely mentally retarded with dysarthria and a vocabulary of about 50 words.

#### IV-7

For the early clinical details, see Haas *et al.*<sup>17</sup> Pregnancy was complicated by a threatened abortion at 14 weeks' gestation, but normal spontaneous delivery at term resulted in a boy with a birth weight of 2920 g. At 6 months of age developmental delay was observed with hypotonia, lack of visual attention, and later nystagmus. From the age of 1 year 2 months to 3 years seizures occurred, but they ceased spontaneously without treatment. At the age of 17½ years there was mild spasticity of the extremities with brisk reflexes, and the patient has never been able to sit unsupported. He was severely mentally retarded with no speech. The hair was coarse and slightly stiff, but no skin abnormalities were present. There was microcephaly and EEG was abnormal. The testes are undescended.

### Results

$^{64}\text{Cu}$  uptake studies performed on the fibroblasts from the two patients gave the following results (table 1). For both patients (IV-5 and IV-7), the  $^{64}\text{Cu}$  uptake was significantly increased compared to the controls. The results are clearly in the range characteristic of Menkes disease. For both the fathers, the  $^{64}\text{Cu}$  uptake was increased but to a lesser extent than in the two patients. After 24 hours additional growth in unlabelled medium both patients retained a considerable amount of  $^{64}\text{Cu}$ , whereas the fibroblasts from both fathers were capable of removing most of the  $^{64}\text{Cu}$  uptake. Both patients thus showed significantly increased  $^{64}\text{Cu}$  uptake and retention.

We next measured the  $^{64}\text{Cu}$  uptake in fibroblast cultures from most of the female members of this family (table 2). For II-1, who is an obligate carrier

**Table 1**  $^{64}\text{Cu}$  uptake studies in two atypical Menkes patients.  $^{64}\text{Cu}$  uptake and retention analyses were performed as previously described.<sup>6</sup> The results represent the mean of a duplicate analysis.

Subject	$^{64}\text{Cu}$ uptake (ng $^{64}\text{Cu}$ /mg protein/20 h)	$^{64}\text{Cu}$ retained after 24 h in unlabelled medium (%)
Patient 1 (IV-5)	63.8	72.2
Father of patient 1	41.6	17.4
Patient 2 (IV-7)	76.4	77.9
Father of patient 2	48.2	20.9
Control males (n = 21) (mean)	21.1	
(n = 22) (mean)		22.4
95% limits	9.0-33.3	9.0-35.8
Menkes patients (n = 105) (mean)	72.1	
(n = 74) (mean)		71.2
95% limits	34.3-135.1	49.2-93.2

of this X linked disease, we found normal  $^{64}\text{Cu}$  uptake and in her two daughters, III-2 and III-3, who have given birth to one patient each, a normal  $^{64}\text{Cu}$  uptake was found for III-2, whereas a significantly increased  $^{64}\text{Cu}$  uptake was seen in III-3. For IV-2 and IV-3,  $^{64}\text{Cu}$  uptake values above the control limits were seen, suggesting that these two females are carriers.

Although the two patients show some symptoms which are not normally seen in classical Menkes disease, most of their symptoms have been described before either in the occipital horn syndrome or in the previously described atypical Menkes patients (table 3).

### Discussion

The unsuccessful search for pili torti in IV-5 and the very few abnormal hairs (two in 1000) in IV-7

**Table 2**  $^{64}\text{Cu}$  uptake studies in female relatives. The numbers of the subjects tested refer to the family pedigree shown in the figure. The results represent the mean of a duplicate analysis.

Subject	$^{64}\text{Cu}$ uptake (ng $^{64}\text{Cu}$ /mg protein/20 h)
II-1	24.3
III-2	20.1
III-3	70.6
III-7	19.6
III-8	12.7
IV-1	8.1
IV-2	29.2
IV-3	33.0
IV-4	24.7
IV-6	15.3
Female controls (n = 20) (95% limits)	11.5-26.7

Table 3 Comparison of clinical symptoms of the two present patients with occipital horn syndrome and six previously published atypical Menkes patients.

	Menkes syndrome	Occipital horn syndrome <sup>1</sup>	Present two patients	Atypical Menkes patients <sup>5,6</sup>
Low birth weight	+	-	+	2/6
Hypothermia	+	+	-	5/6
Feeding difficulties	+	-	-	4/6
Convulsions	++	-	+	2/6
Abnormal teeth	(+)	-	-	1/5
Abnormal hair	+	-	-	6/6
Dry skin	+	-	-	3/6
Cutis laxa	+	++	-	4/6
Hypopigmentation	+	-	-	5/6
Bladder diverticula	+	++	-	2/5
Cryptorchidism	+	-	+	2/4
Ataxia	ND	-	1+, 1-	3/3
Mental retardation	++	-	++	6/6
Decreased serum copper	+	+	+	6/6
Decreased serum ceruloplasmin	+	+	+	4/5
Increased <sup>64</sup> Cu incorporation	+	+	+	6/6
Abnormal radiographs	+	+	-	2/4
Age at death	< 3 years	Normal	2 > 15 years	6 < 3 years

combined with the low levels of copper and ceruloplasmin in serum was one of the main puzzling original findings in the two patients.<sup>17</sup> Pili torti is closely connected with Menkes disease, but a few Menkes cases have been reported without it.<sup>18,19</sup>

The <sup>64</sup>Cu uptake and retention results obtained in the two patients were indistinguishable from those of classical Menkes patients. Furthermore, when the carrier test used for Menkes disease was applied to female relatives in this family, females with increased <sup>64</sup>Cu uptake values were seen. At the same time, two obligate carriers (II-1 and III-2) showed normal results. This behaviour is often seen in Menkes disease and is probably a reflection of random X inactivation. In the original paper by Haas *et al*,<sup>17</sup> it was found that the copper content in the liver of IV-5 was four times higher than the upper limit of the normal range, whereas the value for IV-7 was within the normal range. This finding is difficult to explain, as Menkes fetuses and patients normally have lower liver copper values than controls.<sup>20</sup> It is known that copper therapy increases the copper content in the Menkes liver, but the effect is not as dramatic as seen here.<sup>20</sup> However, it is not known whether the patient had received any copper therapy at the time of the copper studies.

It is most likely that the two patients suffer from Menkes disease, although many of the symptoms of classical Menkes disease are absent. They also differ from patients with the occipital horn syndrome, which presumably is a mild allelic form of Menkes disease. This might suggest that two different mild forms of Menkes disease exist. The mottled-brindled mouse, which is the animal model of Menkes disease, has five different alleles differing in lethality and severity of symptoms.<sup>21</sup> This suggests a wide variety in the clinical picture. The ultimate proof that the present patients suffer from Menkes

disease has to await the discovery of the basic gene defect and characterisation of the basic gene mutation in this family.

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