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Neurodevelopment and brain growth in classic Menkes disease is influenced by age and symptomatology at initiation of copper treatment

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Abstract

Menkes disease is an X-linked recessive disorder of brain copper metabolism caused by mutations in an essential mammalian copper transport gene, *ATP7A*. Untreated affected individuals suffer failure to thrive and neurodevelopmental delays that usually commence at 6 to 8 weeks of age. Death by age three years is typical. While provision of working copies of *ATP7A* to the brain by viral vectors is a promising strategy under development, the only treatment currently available is subcutaneous copper injections. These can normalize circulating blood levels and may replete brain copper depending on the molecular context, e.g., the severity of ATP7A mutation, and presence of mosaicism. In this paper, we summarize somatic growth and neurodevelopmental outcomes for 60 subjects enrolled in a recently concluded phase I/II clinical trial of copper histidine for Menkes disease (ClinicalTrials.gov Identifier: NCT00001262). Primary outcomes indicate highly statistically significant improvements in gross motor, fine motor/adaptive, personal-social, and language neurodevelopment in the cohort of subjects who received early treatment prior to onset of symptoms (n=35). Correlating with these findings, quantitative parameters of somatic growth indicated statistically significant greater growth in head circumference for the initially asymptomatic group, whereas weight and height/length at age three years (or at time of death) did not differ significantly. Mortality at age 3 was higher (50%) in subjects older and symptomatic when treatment commenced compared to the asymptomatic group (28.6%). We conclude that early copper histidine for Menkes disease is safe and efficacious, with treatment outcomes influenced by the timing of intervention, and ATP7A mutation.

Keywords

Menkes disease; ATP7A; copper; neurodevelopment; brain growth

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Introduction

Successful management of orphan pediatric neurometabolic diseases is often complicated by difficulty in the early diagnosis and institution of effective therapy before irrevocable brain damage. Without reliable newborn screening to detect asymptomatic affected infants, early diagnosis relies upon a high index of suspicion based on positive family history, or astute clinical judgment by neonatal care providers.

Menkes disease, an X-linked recessive disorder of brain copper metabolism, is one such condition for which early diagnosis is crucial for any prospect of meaningful long-term outcome. First described in 1962 [1], the illness is caused by mutations in a highly evolutionarily conserved copper-transporting P-type ATPase, ATP7A [2–5]. Treatment for Menkes disease with copper replacement was first suggested by Danks et al. [6] and has been applied by others [7–12]. Clinical outcomes in response to various copper regimens have been mixed, however, and the need for alternative or supplemental remedies has been cited [13–15].

In a Phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT00001262), we evaluated the effects of a specific copper treatment regimen on neurodevelopment and somatic growth in 60 patients with a proven diagnosis of Menkes disease.

Materials and Methods

Patients

Fifty-seven individuals identified as having classic Menkes disease based on evidence of disturbed copper transport, including biochemical findings of reduced dopamine-beta-hydroxylase activity [16,17] and clinical stigmata of reduced lysyl oxidase activity [18–22] were enrolled in a National Institutes Health protocol (ClinicalTrials.gov Identifier: NCT00001262) with the informed consent of their parents or legal guardians. The subjects were followed at 4 to 6 month intervals by a single investigator, and for up to three years. Subjects were grouped into two main categories: early treatment, beginning at less than 1 month of age (N=35), and patients whose treatment commenced later, and after appearance of symptoms (N=22). We also studied three older subjects with milder Menkes disease phenotypes.

Copper Histidine Treatment

Patients received daily subcutaneous injections of copper histidine (Food and Drug Administration Investigational New Drug 34,166; holder, S.G. Kaler; prepared by the National Institutes of Health Pharmaceutical Development Service) for up to three years, as previously described [12,14].

Denver Developmental Screening Test

We assessed neurodevelopment by administration of the Denver Developmental Screening Test to quantify gross motor, fine motor-adaptive, personal-social, and language development during serial visits to the National Institute of Health Clinical Center for a duration of up to three years (depending on survival).

Growth Measurements

We measured weight, length, and head circumference during serial visits to the National Institute of Health Clinical Center for durations of up to three years (depending on survival).

ATP7A Mutation Analysis

We performed *ATP7A* mutation analysis using reverse transcription-polymerase chain reaction (RT-PCR) and manual sequencing as described [23], or polymerase chain reaction of genomic DNA and automated DNA sequencing (NINDS Sequencing Facility, James Nagle, Ph.D., Director), as described [24].

Statistical Analysis

Two-tailed Student t-tests were performed to compare outcome parameters between Group I (N=22) and Group II (N=35). P values less than 0.05 were considered statistically significant.

Results and Discussion

The neurodevelopmental levels achieved by age three years (or by time of death), and the weight, length, and head circumference percentiles for each subject at completion of the trial, relative to age- and gender-matched healthy controls, are tabulated in Table 1. We found statistically significant increases in all four major spheres of neurodevelopment (gross motor, fine motor-adaptive, personal-social, and language) in the earlier treated subjects (Group II). In terms of somatic growth, only occipitofrontal circumference (OFC) was significantly greater in Group II (P=0.0009). Death by age three years occurred more frequently (50%) among patients who had already developed signs and symptoms by the time of enrollment in the copper treatment trial (Group I), versus 28.6% among patients asymptomatic at entry (Group II).

Menkes disease is caused by mutations in a highly conserved copper-transporting ATPase, *ATP7A* [5]. Responses to early copper replacement treatment for this illness have been reported previously, with the observation that patients with *ATP7A* mutations retaining some capacity for copper transport generally have the most favorable prospect for successful neurological outcomes [13,14, 25–28]. Our tabulation of these 60 subjects, some of whom were previously reported in various other contexts [12,14,17–23,25–30], confirms this impression.

The cumulative data presented here also reinforce the concept that early copper treatment, prior to appearance of signs and symptoms, is often associated with partial clinical benefit regardless of the underlying *ATP7A* mutation. We previously documented that early diagnosis and treatment improved brain electrical activity and decreased seizure occurrence in classical Menkes disease, irrespective of the precise molecular defect [30]. These and other more subtle desirable outcomes, such as normal head growth (reflecting normal brain growth) (Table 1), highlight the importance of effective Menkes disease newborn screening, which will likely require molecular approaches.

The relatively high rate of under-three mortality for this orphan disease regardless of when copper injection treatment is initiated also indicates the need for supplemental therapeutic approaches. These include viral gene therapy, which aims to provide working copies of ATP7A to the brains of affected subjects, which appears quite promising in preclinical animal model studies [15,31].

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Table 1

Neurodevelopmental Outcomes and Growth at 36 mos of age (or at Death) after Copper Histidine Treatment in Menkes disease.

Subject	Gross motor (mos)	Fine motor (mos)	Personal social (mos)	Language (mos)	Weight (centile)	Length (centile)	OFC (centile)	*ATP7A* mutation	Death ≤3 yrs
Group I									
I-01[26]	3	4	6	6	0	0	0	IVS8 AS dup5	
I-02[26]	2	2	2	2	0	0	0	IVS8 AS dup5	
I-03[18,28]	1	1	1	1	10	0	25	R201X	D
I-04[12]	2	2	2	3	0	0	0	Q724H	
I-05	1	1	1	2	0	0	10	4195del4	D
I-06	1	1	1	1	10	5	10	2926/7GG>TT	D
I-07	1	1	1	1	15	10	0	2926/7GG>TT	D
I-08	1	2	1	1	0	40	0	IVS6 DS,+1g>a	D
I-09	2	2	1	1	15	15	25	ND	D
I-10	2	2	1	2	0	0	0	Del exon 1	
I-11	10	1	1	2	5	50	15	2233 delT	
I-12	6	8	15	12	50	0	0	A629P	
I-13	2	3	10	10	0	0	5	G728D	
I-14	5	2	5	4	0	0	0	ND	D
I-15	1	4	5	5	3	60	10	Q724X	
I-16	2	2	1	1	0	10	25	IVS21 AS -1, G>A	D
I-17	2	3	4	5	50	50	40	IVS12 DS +1, g>a	D
I-18[27]	2	2	4	2	50	75	50	G727R	D
I-19	1	4	5	3	5	5	5	R201X	
I-20	4	1	2	1	10	5	0	S487X	
I-21	2	1	1	2	25	15	25	Q843X	D
I-22	1	4	4	4	0	0	0	ND	
Mean	2.455	2.409	3.364	3.227	11.273	15.455	11.136		11 of 22
SD	2.154	1.652	3.499	2.943	17.097	23.192	14.551		50%

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Subject	Gross motor (mos)	Fine motor (mos)	Personal social (mos)	Language (mos)	Weight (centile)	Length (centile)	OFC (centile)	*ATP7A* mutation	Death ≤3 yrs
Group II									
II-01[12]	12	15	15	10	5	0	10	Q724H	
II-02[17,30]	5	4	6	7	0	0	5	W1187X	D
II-03[12]	2	3	2	2	0	0	40	Q724H	D
II-04[26]	36	36	36	36	0	0	60	IVS8 AS dup5	
II-05[30]	5	4	4	4	0	0	0	IVS7 AS -1G>C	
II-06[28]	36	36	36	36	25	15	50	R201X	
II-07[30]	13	15	24	20	50	10	75	Q197X	
II-08[19,30]	4	4	4	5	0	0	0	K1037N	D
II-09[25]	3	3	5	5	0	0	50	Del exon 1	D
II-10[14]	4	6	6	8	5	10	75	2757/8 delAG	D
II-11[14]	12	16	24	16	0	0	0	G666R	
II-12[14,30]	7	11	13	8	50	5	50	Del exon 7-19	
II-13[14]	4	21	15	12	0	20	25	Del exon 1	
II-14	3	3	5	4	5	5	50	IVS7 AS -1, G>C	
II-15[14]	36	36	36	33	40	25	40	IVS9 DS +6 t>g	
II-16[14]	24	30	28	27	80	15	50	3936/7 delT	
II-17[14]	2	2	2	2	0	5	0	3061 del T	
II-18[14]	34	36	36	32	20	10	40	G666R	
II-19[21]	3	2	4	3	0	5	0	IVS11 SA -1, G>A	
II-20[20]	30	30	32	24	0	75	25	ND	
II-21[14]	12	16	15	14	0	0	0	del 4246-4260	
II-22[14]	1	1	1	1	10	25	0	Q1383X	D
II-23[14]	2	4	3	6	0	10	0	3061 del T	
II-24[21,27]	30	30	38	30	0	0	60	G727R	
II-25[14,30]	2	4	3	4	25	10	0	Del ex 20-23	
II-26[29,30]	1	1	2	1	5	5	50	Del ex 13-14	D

Subject	Gross motor (mos)	Fine motor (mos)	Personal social (mos)	Language (mos)	Weight (centile)	Length (centile)	OFC (centile)	*ATP7A* mutation	Death ≤3 yrs
II-27[30]	11	15	17	24	5	5	50	IVS15 DS -1, G>A	D
II-28	24	28	34	30	50	5	90	Del exon 1	
II-29[22]	10	14	19	17	0	0	0	L625X	D
II-30[21]	15	18	24	20	3	5	25	Del ex 2-14	
II-31	24	28	24	32	5	5	50	1020 dup5	
II-32[27]	32	34	38	34	10	10	50	G727R	
II-33	2	3	3	2	5	5	25	ND	D
II-34	24	28	32	20	25	5	60	Del 2-23	
II-35	16	30	32	24	0	0	60	Del exon 1	
Mean	13.743	16.200	17.657	15.800	12.086	8.286	33.286		10 of 35
SD	12.200	12.762	13.482	12.034	19.589	13.501	27.060		28.6%
P values: Group I v II	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.8735	0.1453	0.0009		
Group III									
III-01[23]	27	32	35	31	15	75	50	S833G	
III-02	10	15	12	20	0	10	0	ND	
III-03[23]	10	6	6	12	0	0	5	IVS21 DS +3, a>t	
Mean	15.667	17.667	17.667	21.000	5.000	28.333	18.333		0 of 3
SD	9.815	13.204	15.308	9.539	8.660	40.723	27.538		0%

Group I: Copper histidine treatment beginning after 1 month of age and onset of symptoms. **Group II**: Classic Menkes disease: Copper histidine treatment beginning within 1 month of age. **Group III**: Milder variants of Menkes disease: Copper histidine treatment beginning late after onset of (milder) symptoms. D=deceased; ND=not determined. Superscripts refer to previous reports (see References) in which the respective subjects were included.