Specific Cognitive Deficits in Young Children with Cystinosis: Evidence for an Early Effect of the Cystinosin Gene on Neural Function

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Objectives   Infantile nephropathic cystinosis is associated with a specific cognitive deficit in visual spatial processing in older children and adults. The cause of this deficit is unknown. This study was designed to determine whether the cognitive deficit is present in young children with cystinosis, suggesting an early effect of the genetic disorder on brain development.

Study design   Young children (n = 25; age, 3-8 years) with cystinosis and 25 matched control subjects underwent cognitive testing, including tests of intelligence, visual perceptual, visual spatial, and visual motor functions.

Results   Children with cystinosis performed significantly more poorly on tests of visual spatial and visual motor function than did control subjects. Visual perceptual abilities were equivalent in the 2 groups.

Conclusion   The same pattern of visual spatial deficit is present in young children with cystinosis as has previously been demonstrated in older children and adults, which suggests that there may be an influence of the cystinosis gene on brain development, rather than an adverse effect of prolonged cystine accumulation in the brain during childhood. *(J Pediatr 2007;151:192-6)*

Nephropathic cystinosis is an autosomal recessive disorder of cystine transport. The gene, CTNS, mapped to chromosome 17p13,[1-3] codes for a lysosomal membrane transporter protein, cystinosin. When there is an inactivating mutation of the cystinosin gene, cystine cannot be transported across the lysosomal membrane.[4] Sequestration and accumulation of cystine within lysosomes occurs as a result.

Infantile nephropathic cystinosis presents within the first year of life, with failure to thrive and signs of a generalized defect of renal tubular reabsorption (renal Fanconi syndrome). The natural history of untreated cystinosis is that of progressive renal failure in the first decade of life, with renal transplant required by 10 to 12 years of age. However, treatment with cysteamine, a cystine-depleting agent, can delay the progression of the renal disease for many years.[4]

In untreated individuals with cystinosis, cystine deposition is present in virtually all organs, including the brain.[5,6] Generalized cerebral atrophy and central volume loss have been documented.[7-9] To date, the youngest child with reported cerebral atrophy was 7 years old and in renal failure.[7] Of most functional significance is the documentation of a specific cognitive impairment in visual spatial processing on a foundation of normal intellectual function.[10-12] This deficit is associated with intact visual perception, but impaired spatial processing and memory[10,12] and is associated with deficits in mathematical skills.[13] Two studies have found cognitive impairment and more severe cortical atrophy on neuroimaging studies of school-age children and adults with cystinosis. Both studies were cross-sectional studies, and it is not known whether the anatomic changes preceded or followed the cognitive impairments.[14,15]

The cause of the observed cognitive deficit is unknown. Neuropathological reports are scarce, but because of widespread cystine accumulation[5-7,9,16] in the brains of patients with cystinosis, it is possible that deposition may lead to progressive cellular damage and subsequent impairment in function.

Alternatively, the CTNS gene might influence some aspect of brain development. This could occur as a result of in utero cystine deposition during a critical time in brain development. If cumulative cystine deposition during childhood is responsible for the cognitive differences observed in subjects with cystinosis, it might be expected that young children, especially those treated early with cysteamine, would not demonstrate such

MFVPT	Motor-Free Visual Perception Test	VMI	Visual Motor Integration
PIQ	Performance IQ	WISC	Wechsler Intelligence Scale
VIQ	Verbal IQ		

cognitive profiles. If, however, the gene itself exerts some influence over brain development, the cognitive differences would most likely be present (and potentially nonprogressive) early in life.

Although overall intellectual function is within the normal range in young children with cystinosis, we have recently demonstrated a discrepancy such that non-verbal IQ indices (Performance IQ and Processing Speed Index) were significantly lower than verbal IQ indices.[17] This pattern closely resembles the IQ profile found earlier in older individuals with cystinosis[10] and suggests that the cognitive profile may emerge early in development.

To examine this issue more specifically, this study tested visual perceptual and visual spatial abilities in young children with nephropathic cystinosis.

METHODS

Participants

Children with nephropathic cystinosis (n = 25; age range, 3 years 1 month-8 years 0 months), and 25 matched control children (age range, 3 years 3 months-7 years 9 months) participated in the study. Children with cystinosis were identified primarily through the National Cystinosis Foundation, the Cystinosis Research Network, and the Cystinosis Research Foundation, all of which maintain close contact with many families in which cystinosis occurs. Children from many parts of the country were brought to San Diego with a parent to participate in the study. All children had a diagnosis of infantile nephropathic cystinosis confirmed by clinical presentation and by assays documenting elevated leukocyte cystine concentrations. Children were excluded from the study when they were in renal failure, were acutely ill, or had any other condition that might affect cognitive function. The mean age of the children when cystinosis was diagnosed was 17.3 ± 9.6 months; the mean age of the children at onset of treatment with cysteamine was 20.7 ± 11.7 months. Renal function data were not available for all patients with cystinosis. However, none were on dialysis at the time of testing, and all parents reported that their children were not in renal failure to their knowledge.

Because of the relatively small sample size and the use of some non-normed tests (or use of data from participants out of the age range of the normative data for some tests), a case-control study design was selected. Control participants were recruited from the community through advertisements in parent magazines, through local health fairs, and through fliers placed in pediatricians' offices. The cystinosis and control participants were matched for sex, within 8 months of chronological age, and within 1 point for socioeconomic status on the basis of the Hollingshead Four Factor Index of Social Status.[18] All control subjects had normal developmental histories and no evidence of medical or neurological conditions that might affect cognitive function, such as seizures, head trauma leading to prolonged loss of consciousness, attention deficit-hyperactivity disorder, or learning disabilities.

Informed consent was obtained according to University of California San Diego Human Research Protection Program procedures, and the study was approved by the Human Research Protection Program. This study was part of a larger longitudinal study of brain structure and cognition in cystinosis.

Cognitive Measures

INTELLIGENCE. All children received an age-appropriate Wechsler Intelligence Scale (WPPSI-III, WISC-III, or WISC-IV).[19-21] Only 4 children received the WISC-IV (all from the cystinosis group). Because the WISC-IV does not yield verbal IQ (VIQ) or performance IQ (PIQ) scores, the WISC-IV Verbal Comprehension Index was substituted for VIQ and the WISC-IV Perceptual Reasoning Index was substituted for PIQ.

VISUAL PERCEPTION TASKS. Gollin Incomplete Figures[22]: This is an object recognition task in which participants are shown incomplete line drawings of familiar objects presented in 5 gradations of completeness. The participant must mentally "fill in" missing visual information. For each test item, the most incomplete picture was shown first, for 3 seconds, and the participant was asked to guess what it might be. More pictures in subsequent gradations of completeness were also shown for 3 seconds until the participant was able to correctly identify the object. The test consisted of 1 practice item followed by 21 test items. The total number of pictures needed to identify all the items was calculated. The possible range of scores was 21 to 105, with a higher score representing poorer performance.

Motor-Free Visual Perception Test (MFVPT)[23]: The MFVPT is a test of visual perception that does not require motor skills. Test items are categorized into 5 groups, including Spatial Relationships, Figure-Ground, Visual Discrimination, Visual Memory, and Visual Closure. Raw scores were used because many of our subjects were younger than the normative data cutoff (4 years 0 months). The possible range of raw scores was 0 to 36, with higher scores representing better performance.

The Beery-Buktenica Developmental Test of Visual-Motor Integration, 5th Edition (VMI) Visual Perception Supplement[24]: The VMI Visual Perception supplement is a test of visual perception without motor requirements that uses smaller versions of the VMI figures as test stimuli. In this task, the participant was required to find the test stimulus among the choices. Raw scores were converted to standard scores, with a mean of 100 and a SD of 15.

VISUAL SPATIAL TASKS. Kaufman Assessment Battery for Children Spatial Memory[25]: This test is used to assess short-term spatial recall of simultaneously visually presented material. The participant was shown a page with familiar pictures for 5 seconds and then was shown an empty grid and asked to point to those boxes on the grid that correspond to the exact positions of the previously shown pictures. Raw scores were

Table I. Summary of demographic variables for the cystinosis and control groups

	Cystinosis (n = 25)	Control (n = 25)	P value
Mean age at testing	5 years 5 months ± 1 year 5 months	5 years 4 months ± 1 year 3 months	NS
Mean socioeconomic status*	2.32 ± .90	1.84 ± .94	NS
Sex	13 males, 12 females	13 males, 12 females	—
VIQ	93.40 ± 12.73	114.00 ± 12.65	<.0001

*Based on the Hollingshead Four Factor Index of Social Status (Hollingshead, 1975), with 1 being the highest and 5 being the lowest socioeconomic status.

Table II. Visual perception and visual spatial multivariate analysis of covariance results, individual test means and SDs, estimated marginal means and SDs, and significance values

	Mean ± SD		
Multivariate model and individual tests	Cystinosis (n = 25)	Control (n = 25)	Significance
Visual Perception MANCOVA			NS
Gollin Incomplete Figures Test raw score	62.2 ± 10.1	55.9 ± 9.9	—
(Estimated marginal mean)	(61.4 ± 2.3)	(56.7 ± 2.3)	
Motor Free Visual Perception Test raw score	18.4 ± 7.5	23.9 ± 7.7	—
(Estimated marginal mean)	(19.4 ± 1.8)	(22.9 ± 1.8)	
VMI: Visual Perception Supplement standard score*	86.2 ± 17.1	109.8 ± 15.7	—
(Estimated marginal mean)	(94.3 ± 3.1)	(101.7 ± 3.1)	
Visual Spatial MANCOVA			P = .02
K-ABC Spatial Memory raw score	5.0 ± 4.1	8.3 ± 4.2	NS
(Estimated marginal mean)	(5.4 ± 1.0)	(7.9 ± 1.0)	
Woodcock Johnson Spatial Relations Standard Score*	97.6 ± 18.1	115.1 ± 9.1	P = .031
(Estimated marginal mean)	(100.6 ± 3.2)	(112.1 ± 3.2)	
VMI standard score*	84.0 ± 12.2	103.5 ± 13.7	P = .009
(Estimated marginal mean)	(87.6 ± 2.9)	(100.0 ± 2.9)	

Blank spaces represent subtests for which follow-up analyses were not applicable because the multivariate test was not significant.
MANCOVA = Multivariate analysis of covariance; K-ABC = Kaufman Assessment Battery for Children.
*Tests for which VIQ was a significant covariate.

used because many of our subjects were younger than the normative data cutoff (4 years 6 months). The possible range of raw scores was 0 to 21, with higher scores representing better performance.

Woodcock-Johnson: Spatial Relations[26]: This is a test of visual spatial ability that does not require motor involvement. The participant was required to select, from a series of shapes, component parts required to make a whole. Raw scores were converted to standard scores with a mean of 100 and a SD of 15.

The Beery-Buktenica Developmental Test of VMI, 5th Edition[24]: The VMI is a test of visual motor skills with a visual spatial component.[24] The participant was required to copy a series of increasingly complex geometric figures. Raw scores were converted to standard scores with a mean of 100 and a SD of 15.

Statistical Analyses

Potential group differences (cystinosis versus control) on the demographic variables of age at testing, socioeconomic status, and VIQ were analyzed with t tests. A multivariate analysis of covariance framework was used to analyze the cognitive data. VIQ was chosen as a potential covariate (instead of PIQ or FSIQ), because PIQ (and therefore FSIQ)

may be abnormally low because of visual perceptual or visual spatial deficits in the cystinosis group. In the overall between-group analyses, the dependent variables were cognitive test scores, and the independent variable was group membership (cystinosis versus control).

RESULTS

The cystinosis and control participants were not significantly different in the demographic variables of age at testing, socioeconomic status, or sex. There was a significant difference in the groups on VIQ, with the cystinosis group scoring in the average range, although lower than control subjects. VIQ was therefore included in the model as a covariate. See Table I for group means, SDs, and significance values for the demographic and intelligence variables.

Table II shows the visual perception and visual spatial multivariate analysis of covariance framework results, individual test means and SDs, estimated marginal means and SDs, and significance values. Multivariate analyses indicated that the cystinosis and control groups were not significantly different on any visual perception measures. In contrast, the cystinosis group performed significantly more poorly than the control group on the visual spatial measures ($F_{(3,45)}$ = 3.6; P = .020). With tests of between subject effects, it was

revealed that on the visual spatial tasks, the cystinosis group performed significantly more poorly than the control group on Spatial Relations ($F_{(1,47)} = 5.0$; $P = .031$) and the VMI ($F_{(1,47)} = 7.4$; $P = .009$).

DISCUSSION

We demonstrate a significant impairment in visual spatial and visual motor skills in very young children with nephropathic cystinosis, with a relative sparing of visual perceptual skills. VIQ was lower in the cystinosis group than in the control group (although in the average range). Even after co-varying for VIQ, however, the children with cystinosis performed significantly more poorly on visual spatial and visual motor measures than control subjects, whereas visual perceptual skills remained intact. These findings are strikingly consistent with earlier studies demonstrating impaired performance on visual spatial and visual motor tasks in older children and adults with cystinosis.[12,27] Thus, these results and our recent findings of significantly lower PIQ scores on the Wechsler Preschool and Primary Intelligence Scales in younger children[17] argue against progressive cystine accumulation during childhood as the explanation for the observed cognitive differences in cystinosis.

One of the benefits of this study was that we included a very homogeneous group of children with cystinosis, in that all had been treated from an early age with cysteamine. The mean time between initiation of treatment with cysteamine and cognitive testing for this study was almost 33 months. Although there is no means of measuring brain cystine levels in living individuals, almost 3 years of treatment would allow reasonable time for cysteamine to have a beneficial effect on tissue cystine levels. If cystine accumulation in the brain were responsible for the cognitive deficits previously observed in older children and adults, as suggested by Broyer,[16] we would expect that younger children would exhibit few or no cognitive deficits compared with control subjects, in particular because early treatment with a cystine-depleting agent had been initiated.

An alternative explanation of the cognitive dysfunction is a very early (possibly in utero) effect on brain development, either from a direct effect of the abnormal cystinosin gene or as a result of very early deposition of cystine during a critical period of brain development. In this case, the neural changes are not age-dependent in the sense of a cumulative effect of cystine during childhood. The exact mechanism by which cystine damages cells is unknown.[4] We speculate that the early neurologic influence of the metabolic disorder might be particularly injurious to brain myelination. A potential mechanism for white matter damage in utero is damage to pre-oligodendroglial cells, the precursors of myelin-forming oligodendroglia. It is known that such cells are susceptible to oxidative stress[28,29] such as might be caused by a metabolic disturbance caused by the defective cystinosin gene. Defective myelination in turn could adversely affect the development of cortico-cortical projections necessary for complex processing abilities.

A third possibility is that cysteamine, the medication used to clear cystine from the lysosomes, may have some adverse effect on cognitive function. In studies of older children and adults, however, similar results were obtained in patients who had never been given cysteamine,[10,14] suggesting that other factors are more likely responsible for the cognitive deficit.

Finally, it is possible that renal dysfunction could impair cognitive functioning in the children with cystinosis. None of the children in our study were receiving dialysis, and parents of all the children reported that their child had normal or near-normal renal function. However, because we do not have specific data on renal function from all the children at the time of cognitive testing, it is possible that some degree of renal impairment was present that might have contributed to the findings of this study. Future studies will attempt to ascertain the degree of renal dysfunction in children undergoing these studies.

One of the limitations of this study is that it is cross-sectional, so we are unable to compare cognitive performance in the same children with time. Despite this limitation, it is reasonable to at least compare our results to those of earlier studies of older individuals. Longitudinal studies that more directly address this issue are currently in progress. A second limitation is that we do not yet have complete structural data from neuro-imaging on these children to relate functional differences to brain structural alterations. These studies are underway. However, the results of this study are suggestive of a very early adverse effect of the cystinosis gene on brain development, such that cognitive function is impaired even in young children with this disorder. The practical implication of this finding is that children with cystinosis may be at risk for learning difficulties,[13] particularly in subjects with a significant visual spatial component (eg, arithmetic, geography). Early identification of specific cognitive deficits will permit prompt remediation in a strongly language-based curriculum and the use of computer-assisted educational techniques to reduce complications of poor fine motor skills that can make handwriting tedious and illegible.

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