Preliminary Evidence for a Cognitive Phenotype in Barth Syndrome

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Barth syndrome (BTHS) is a rare, X-linked, recessive disorder that affects almost exclusively males. It is characterized by short stature, cardioskeletal myopathy, cyclic neutropenia, increased excretion of 3-methylglutaconic acid in the urine, and moderate hypocholesterolemia. The objective of the present study was to assess whether BTHS presents with a cognitive phenotype. Preliminary data were collected from five kindergarten or first-grade boys with BTHS. An abbreviated psychoeducational test battery was administered to each boy, and parents of each boy completed standardized behavior rating scales. Data from 120 boys of similar age or grade level were used for one comparison group; a subset of this sample comprised a comparison group that was individually matched on age and grade level to one of the five boys with BTHS. Preliminary data reflect a higher incidence of cognitive difficulties in boys with BTHS relative to both comparison groups. Boys with BTHS had significantly lower visual spatial skills, but comparable reading-related skills, when compared with either group. Although based on a small sample size, the preliminary data presented in this work are the first indication of a cognitive phenotype associated with BTHS.

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KEY WORDS: Barth syndrome; cardiomypathy; cognitive phenotype

INTRODUCTION

Barth syndrome (BTHS) was first described in 1981 in a large pedigree with affected males and apparently unaffected obligate carrier females [Barth et al., 1983]. This rare, X-linked recessive metabolic disorder is characterized by short stature, cardioskeletal myopathy, cyclic neutropenia, increased excretion of 3-methylglutaconic acid in the urine, and moderate hypocholesterolemia [as reviewed in Barth et al., 1999]. Precise incidence rates of BTHS are not available because of a lack of widespread empirical incidence studies of this disorder. In this work, the notion of a cognitive phenotype of BTHS is explored, following a summary of current knowledge regarding the BTHS physical phenotype, the BTHS genotype, and the biochemical mechanisms that underlie the disorder.

BTHS is a disorder that affects almost exclusively males. Theoretically, it is possible for a female to inherit two BTHS genes and to present with the disorder, although no cases of females with BTHS have been reported to date [Johnston et al., 1997]. As a group, it is common for males with BTHS to become symptomatic in infancy; in some cases symptoms are first reported later in early childhood. These boys are typically reported as small-for-date newborns despite a full-term gestational period. Cardiac dysfunction, particularly abnormal heart wall function or thickness, is frequently the initial clinical manifestation, accompanied by marked delays in motor development and statural growth. During early childhood, boys with BTHS usually fall at or below their age-referenced fifth centile for height and weight [Kelley et al., 1991], with many case study reports indicating growth well below the third centile [Ino et al., 1988; Kelley et al., 1991; Ades et al., 1993; Christodoulou et al., 1994]. Growth curves parallel those for normal growth, through childhood. Later, there is evidence of an increased positive slope during adolescence, with normal adult height being achieved later in development in some individuals [Kelley et al., 1991]. General clinical features also tend to improve with age among children who survive beyond early childhood, but neutropenia and 3-methylglutaconic aciduria usually persist.
The discovery of characteristic biochemical abnormalities associated with the disorder, 3-methylglutaconic aciduria, was reported in 1991 [Kelley et al., 1991]. Since then, DNA studies have indicated a mutation of the G4.5 gene [Bione et al., 1996; D'Adamo et al., 1997; Johnston et al., 1997; Cantlay et al., 1999] located at Xq28 [Bolhuis et al., 1991; Ades et al., 1993; Christodoulou et al., 1994; Gedeon et al., 1995]. Both familial and spontaneous mutations of G4.5 have been reported [e.g., Cantlay et al., 1999]. At present, DNA testing is available for diagnosis and carrier testing. At the First International Barth Syndrome meeting in June, 2000, Drs. Vreken and Barth reported that the G4.5 gene functions as a fatty acyl transferase in the synthesis of cardiolipin, and that BTHS cells and tissues are very deficient in cardiolipin. This makes BTHS the first known defect in the biosynthesis of cardiolipin, an important structural lipid in the mitochondrial inner membrane, electron transport chain, and other cellular membranes involved in tissue development and function. Different G4.5 mutations may be associated with variation and severity of the clinical phenotype, although some researchers have not found support for a genotype–phenotype association [Johnston et al., 1997; Cantlay et al., 1999]. For instance, Johnston and colleagues did not find any association between mutation variant and severity of cardiomyopathy or its age at onset, or with severity of neutropenia or magnitude of 3-methylglutaconic aciduria/acidaemia. Thus, the genotype and physical phenotype have both been described and reported, although precise associations between the two currently remain under investigation.

With respect to a cognitive phenotype, there have been no empirical studies addressing whether BTHS leads to specific intellectual deficits or delays. Summaries of BTHS, and several case reports, commonly include reference to “normal cognitive development” [Gibson et al., 1993; Christodoulou et al., 1994]. Kelley et al. [1991] described patients with BTHS as having “normal or only minimally delayed cognitive development.” In cases reported by Ades et al. [1993] or Christodoulou et al. [1994], the few references to intellectual function described these functions as “normal.” The only reference to “mental deficiency” that has appeared in a review of BTHS literature is associated with cardiac transplantation, with an implication that the deficiency was secondary to the intervention vs. primary to BTHS [Barth et al., 1999].

It is not surprising that the cognitive features of BTHS have not yet been explored, because of the importance of first addressing potentially fatal physical characteristics of the disorder. In view of the initial success in medical treatment of children who receive a BTHS diagnosis, it is now important to address aspects of these children’s future and their quality of life, including cognitive development and function. The frequent references to “normal cognitive function” in the BTHS literature suggest that if there is a BTHS cognitive phenotype, it does not lead to mental retardation in early childhood, unlike the phenotype for males with fragile X syndrome [Bailey et al., 1998]. There are disorders with a relatively specific cognitive phenotype that occurs in the absence of mental retardation, including early-treated phenylketonuria (PKU) [Welsh et al., 1990], Turner syndrome [Rovet, 1993], and approximately half of females with fragile X syndrome [Rousseau et al., 1994]. For instance, girls with Turner syndrome demonstrate a slight lowering of intellectual performance relative to parents and siblings; more importantly, this “lowering” is, in most cases, specific to and more markedly apparent in spatial reasoning and mathematical skills [Mazzocco, in press; Rovet et al., 1994]. It is possible that a mild yet specific cognitive phenotype is associated with BTHS, which would be consistent with reports of “normal or minimally delayed cognitive development.” In view of models from other subtle phenotypes, this possibility is worthy of investigation, and was examined in the present study. Moreover, an investigation of this X-chromosome-related disorder may contribute to the identification of possible X-chromosomal genes, metabolic, or nutritional influences on cognitive development and function.

**METHODS**

**Participants**

These data were collected in conjunction with the First International Barth Syndrome conference, which was held June 17, 2000, at the Kennedy Krieger Institute, Baltimore, Maryland. There were 30 families in attendance, and each included one child or more with BTHS. Only those children who had just completed kindergarten or first or second grade were recruited, because comparison data for this age group were available from a larger, longitudinal study of primary school academic achievement [described in detail in Mazzocco and Myers, in press]. In the BTHS group, a total of seven families in attendance had children in this grade-level range. Of these, six agreed to have their sons participate; one child refused to participate (due to a preference to remain in the playroom). Thus we were able to include five boys with BTHS in this group. Data on each boy’s age and grade level appear in Table I.

The children in the available comparison group included 120 boys and 129 girls who were enrolled as volunteer participants in the larger, longitudinal study as kindergartners from one large suburban school district [Mazzocco and Myers, in press]. Each child in the longitudinal study was recruited in somewhat random fashion, in that participation was offered to parents of all kindergartners at seven elementary schools within a neighboring suburban school district. Thus, the longitudinal sample is a normative sample, and each participant was evaluated annually. From this longitudinal sample, we selected two comparison groups for the present study. The matched comparison group was comprised of boys who could be well matched on age (within 4 months) and grade level at testing, to one or more of the five boys in the BTHS group. Only 12 boys met these criteria because many of the boys with BTHS were old relative to their grade level. The second sample was comprised of all 120 boys in the longitudinal study, with data from first grade used for a
subset of the boys and data from kindergarten for the remaining boys. This mixed grade level was employed because both grade levels were represented in the BTHS group.

**Procedures**

Each boy with BTHS was individually administered an abbreviated version of the psychoeducational assessment battery used in the ongoing study, in one to two sessions that required no more than 2 hours. Two of the three examiners who participated in the overall ongoing study worked with the five boys in the BTHS group. Each child in the larger study was also individually evaluated during two to three sessions, for approximately 3 hours.

The abbreviated battery was comprised of a measure of expressive vocabulary, a comprehensive math assessment, five visual spatial tasks, and three reading-related tasks. For each child, an age-appropriate measure of expressive vocabulary was administered; the vocabulary subtest from the fourth-edition Stanford Binet (SB-IV) was used with children under 6 years [Thorndike et al., 1986], and the Wechsler vocabulary subtest was used with children ages 6 and 7 years [Wechsler, 1999]. The second-edition Test of Early Math Ability (TEMA-2) [Ginsburg and Baroody, 1990] was administered as a measure of formal and informal early math concepts, facts, and skills. The visual perceptual measures included the four motor-reduced subtests of the second-edition Developmental Test of Visual Perception (DTVP-2) [Hammill et al., 1993], and the Beery Visual Motor Integration (VMI) task [Beery, 1997]. The DTVP-2 subtests administered each required a single selection from three to five choices. The four tasks involved matching figures on the basis of direction (Position in Space), identifying shapes in embedded designs (Figure Ground), Visual Closure of abstract designs, and matching shapes (Form Constancy). Finally, the VMI involved copying simple and complex geometric figures. To measure reading-related skills, the Woodcock-Johnson revised letter word identification (LWID) and word attack (WA) subtests [Woodcock and Johnson, 1989] were administered. The LWID task involves use of symbols, reading letters, and recognizing single words. Word attack performance provides a measure of phonological decoding skills used when sounding out unfamiliar nonwords. The third reading-related measure was a rapid automatized naming (RAN) task [Denckla and Rudel, 1976]. Three RAN subtests were administered, each of which involved rapidly naming colors, numbers, or letters. For each subtest, response times were recorded. From each of these described measures, except the RAN, a standardized, age-referenced score was derived. The order of test administration was fixed for the boys with BTHS. Although additional measures were also administered to boys in the comparison groups, the sequence was preserved across the comparison and BTHS groups.

In addition to administering cognitive tasks to the children, we asked parents of all participants to complete two widely used standardized behavior checklists. The Child Behavior Checklist (CBCL) [Achenbach and Edelbrock, 1983] is designed to screen for childhood psychopathology or significant behavioral difficulties. Conners’ Parent Form [Conners, 1997] is used to measure indications of attention difficulties, hyperactivity, academic difficulties, and risk for meeting attention deficit hyperactivity disorder (ADHD) criteria.

**RESULTS**

In view of the small sample of boys with BTHS, statistical analyses should be interpreted with caution. Nonparametric procedures were used because of the inability to determine normality of data with a sample

![Table I. Cognitive Performance Scores in Boys With BTHS and Controls*](image-url)
size of five boys. The Mann-Whitney $U$ test was used as a measure not influenced by outliers. In cases of tied ranks, tied $P$ levels are reported. Two sets of analyses were carried out to examine cognitive performance and behavior rating data in boys with BTHS vs. the two comparison samples described above (Participants section), including 1) the total comparison group of 120 boys, and 2) the subset of 12 matched boys.

**Cognitive Performance**

Average scores on the vocabulary measure suggest that the boys with BTHS have average intelligence, or at least an average verbal I.Q. score (Table I). Relative to the 120 boys in the overall comparison sample, boys with BTHS did not differ on measures of reading and pre-reading skills, nor were there any trends toward group differences, $P$ values $= .25–.87$. On these measures, scores from the BTHS boys were comparable to the mean reported for the overall group of boys. Although no significant group differences emerged on the TEMA-2, four of the five BTHS boys had TEMA-2 scores below 93, and these scores were $.58–.97$ standard deviations (SDs) below the overall comparison group mean of 101.2, $P = .19$. Four of the boys with BTHS had below average VMI scores that fell $.8–1.9$ SDs below the overall comparison boys’ mean score, $U = 109.5, P < .02$. On a motor-reduced task of visual closure, four boys with BTHS scored $.66–1.76$ SDs below the overall comparison sample boys’ mean score, $U = 65.5, P < .01$. Group differences were also seen for the DTVP-2 figure-ground subtest, $U = -116.0, P < .02$. A similar pattern of results emerged when the boys with BTHS were examined relative to the 12 age- and grade-level-matched boys derived from the overall comparison sample (results in Table I). Each significant finding from the first set of analyses persisted, with the exception of a group difference on figure-ground performance.

**Behavioral Data**

Among the 120 boys in the overall comparison sample, parents of 118 completed behavioral ratings for their sons. Among the 12 boys in the matched comparison sample, parents of 11 boys completed behavioral ratings. Boys with BTHS did not differ from either comparison group on CBCL measures of withdrawn, anxious, delinquent, or aggressive behaviors, $P > .42$. The only significant group difference of interest pertained to a higher average on “social concerns,” among boys with BTHS vs. boys from the overall comparison sample, $U = 120.5, P < .02$. This difference stemmed from high average to above average ratings for two of the five boys in the BTHS group; the remaining boys had ratings that were well within the average range for boys in their age group. The difference did not remain significant when comparisons were limited to the 12 matched-comparison boys. Consistent with this outcome was the lack of significant differences on oppositional or hyperactive behaviors indicated on Conners’ form, $P > .56$. The rating reflecting academic and cognitive concerns was significantly higher (reflecting more difficulty) in the BTHS group, relative to the overall comparison group, $U = 43.0, P < .01$; and relative to the subset of matched participants, $U = 3.50, P < .01$ (Table II). Indeed, it is noteworthy that only 12 of the 120 boys from our comparison group could be matched to a boy with BTHS, because all five boys with BTHS had not advanced directly from kindergarten to first or second grade. Four repeated an early grade, and one advanced to an intermediate grade (between kindergarten and first grade). Thus, all five were relatively old for their current grade level, and four were reported to be receiving special services in school. The latter frequency rate was significantly higher than that reported for the overall comparison group and for the subset of 12 matched boys (Fisher’s exact test, $P < .01$). Low school attendance due to illness may have influenced

**TABLE II. Selected Behavioral Rating Scores in Boys With BTHS and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Individual Barth syndrome boys</th>
<th>All boys</th>
<th>Matched boys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CBCL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withdrawn</td>
<td>58</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>Anxious/depressed</td>
<td>50</td>
<td>58</td>
<td>50</td>
</tr>
<tr>
<td>Social problems</td>
<td>56</td>
<td>68</td>
<td>50</td>
</tr>
<tr>
<td>Thought problems</td>
<td>50</td>
<td>64</td>
<td>50</td>
</tr>
<tr>
<td>Attention problems</td>
<td>65</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>Delinquent behavior</td>
<td>50</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>Aggressive behavior</td>
<td>53</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Conners’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oppositional</td>
<td>52</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>Cognitive problems</td>
<td>90</td>
<td>53</td>
<td>62</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>46</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>Conners’ ADHD index</td>
<td>72</td>
<td>48</td>
<td>51</td>
</tr>
</tbody>
</table>

*All scores are T scores and are based on a mean of 50 and standard deviation of 10. Scores $> 64$ reflect potentially significant behavior problems.
the need to repeat a school grade. However, school attendance is linked to achievement in both reading and math [Frazier and Morrison, 1998], and reading scores in this small sample were very well within the average range using age-referenced norms.

**DISCUSSION**

**Limitations of the Findings**

There are obvious limitations to the results of the present study, primarily those due to the small sample size of the BTHS group. However, we believe that this small sample is representative of the population of boys with BTHS. It is possible that parents who choose to enroll their children in a study of academic achievement may be more likely to have concerns about their child’s academic performance than are parents who choose not to enroll their child in such a study. However, our preliminary sample of five boys with BTHS represents 71% of the seven boys in our target age group (5–7 years) who were in attendance at the First Barth Conference, and 83% of the boys who were enrolled in the study. Thus, our sample is a good representation of the children at this meeting. Ascertainment bias may have resulted from a nonrandom selection of families in attendance at the meeting. For instance, families at the meeting may have been those with the greatest number or severity of concerns regarding their child. This bias is unlikely to have influenced recruitment for our study, for the following reasons: First, the availability of study participation was not presented to the parents until they arrived at the meeting. Second, travel to the conference may have been affected by the severity of the BTHS, such that children with the most severe cases would be less likely to attend; however, we do not have data to support or counter this notion. Finally, this group of 30 families is believed to represent approximately 40% of BTHS families worldwide, and approximately 60% of the families known in the U.S.

Another limitation of the current study concerns the limited amount of data collected. In particular, it would have been informative to have data reflecting the parents’ cognitive ability. In future research, we hope to collect such data so that child performance can be examined relative to parental scores, as a measure of the degree to which BTHS influences cognitive function and development. Parent–child comparisons of this nature have been successfully used to examine the role of other disorders that affect cognitive development, such as Turner [Mazzocco, 2000] and Fragile X [Reiss et al., 1995] syndromes. In this study, we did not see an association between cognitive scores and each child’s maximum plasma 3MGC level, which may have been due to our inability to detect the degree of cognitive effect in the absence of parent cognitive data.

**Importance of Studying a BTHS Cognitive Phenotype**

Despite their limitations, these preliminary data comprise the first published indication of a cognitive phenotype associated with BTHS. In this study, the boys with BTHS had significantly weaker visual spatial and visual motor scores relative to boys in the comparison groups. Although not significant, there was also a tendency toward lower math scores among boys with BTHS. It is difficult to assess these and other skills in view of the small sample size, yet it is important to acknowledge that patterns did emerge despite the small sample size. As such, the findings lend support for the need to expand upon studies of BTHS to include investigations of cognitive and academic performance.

Research on the BTHS cognitive phenotype will serve two primary functions. First and foremost, such research efforts will inform and guide the parents, teachers, and clinicians of a child with BTHS. Understanding the BTHS cognitive phenotype permits early identification of, and intervention for, learning difficulties that may compromise the quality of life for children with BTHS and their families. Earlier intervention can have a greater impact on academic success than later intervention, and when subtle features of a phenotype are identified it is possible to recommend specific educational guidelines. This does not imply that children with BTHS, or any other genetic disorder, have academic features unique to those with the disorder; rather, the identified constellation of cognitive and academic strengths and weaknesses can guide intervention recommendations based on what is known about learning disorders with similar learning profiles.

The second function served by research on a BTHS cognitive phenotype concerns our understanding of intellectual development and function in the broader sense. For instance, the metabolic abnormalities observed in BTHS include increased levels of 3-methylglutaconic acid, and decreased levels of cholesterol and taffixin proteins. Associating these features with discrete learning deficits may reveal metabolic or nutritional influences on brain development that are currently not understood. Within that context, such influences on brain development may follow a delay vs. deficit model, which may be linked to the biochemical and clinical improvements reported in BTHS after puberty. Understanding these influences may be informative for sensitive or critical periods during cognitive development. With respect to understanding gene-behavior associations in cognitive development, it is interesting to note the similarity, suggested by the preliminary data, between the Barth and Turner syndrome cognitive phenotypes. Turner syndrome, another X-chromosome-related disorder, is also associated with verbal strengths, and difficulty with aspects of visual spatial skills, executive function [Temple et al., 1996], and mathematics [Mazzocco, in press; Rovet et al., 1994] in the context of normal IQ scores. A similar, but broader and more severe cognitive phenotype is associated with fragile X syndrome, which at Xq 27.3 is proximal to the G4.5 gene linked to BTHS. Thus, as is true for other X chromosome disorders, the study of BTHS may contribute to our understanding of how X chromosome genes influence intellectual development.
Future Directions

Several research directions arise from these preliminary findings. First is the need to replicate the present study with a larger sample, which is challenging in view of the rareness with which BTHS occurs. Replication studies should include assessments of additional neuropsychological characteristics that were not examined with the abbreviated battery employed in the present, preliminary study. Related to this aim is the need for more in-depth assessment of the areas in which deficits are implicated, such as the assessment of different components of visual spatial skills. It will be important to study the development of these features over time, particularly as a function of treatment outcomes, genotype variants, metabolic variables, and the development of other markers of the disorder. For example, an association between cognitive performance and therapeutic compliance has been demonstrated for other disorders with subtle cognitive phenotypes, including early-treated PKU [Welsh et al., 1990; Mazzocco et al., 1992]. Also, the degree of cognitive deficits has been associated with molecular features of the fragile X syndrome [Staley et al., 1993; Abrams et al., 1994]. More distantly related but worthy of exploration is the question of whether subtle effects exist for the heterozygote state of BTHS. We would expect that, as is the case for other X-linked disorders, females with BTHS (if affected) would have milder effects than those seen in males, and that these effects would be associated with X inactivation patterns. In studies of fragile X syndrome in females, the milder phenotype is well substantiated, as is its association with skewing of X inactivation patterns [Abrams et al., 1994]. To date there are no data to suggest female effects in BTHS [Gedeon et al., 1995; Barth et al., 1999], and X inactivation patterns in BTHS heterozygotes have been predominantly skewed towards the normal X [Orstavik et al., 1998]. However, this skewing may be related to age, as most BTHS heterozygotes studied have been adults. These and other important questions remain to be addressed in future studies of BTHS. The preliminary data presented in this report are the first indication of a cognitive phenotype associated with BTHS.

ACKNOWLEDGMENTS

This work was supported by NICHD grant RO1 HD34061-04, awarded to M.M.M.M. We wish to thank all of the families that contributed to this work. We also acknowledge the role of research coordinator Gwen F. Myers in data collection and in other phases of this research; and support from research assistants Laurie A. Thompson and Megan M. Kelly.

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