Clinical Report

Non-Lethal Congenital Hypotonia Due to Glycogen Storage Disease Type IV

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Glycogen storage disease type IV (GSD-IV) is an autosomal recessive genetic disorder due to a deficiency in the activity of the glycogen branching enzyme (GBE). A deficiency in GBE activity results in the accumulation of glycogen with fewer branching points and long, unbranched outer chains. The disorder results in a variable phenotype, including musculoskeletal, cardiac, neurological, and hepatic involvement, alone or in continuum, which can be identified at any stage of life. The classic form of GSD-IV is a hepatic presentation, which presents in the first 18 months of life with failure to thrive, hepatomegaly, and cirrhosis that progresses to liver failure, resulting in death by age 5 years. A severe congenital musculoskeletal phenotype with death in the neonatal period has also been described. We report an unusual case of congenital musculoskeletal presentation of GSD-IV with stable congenital hypotonia, gross motor delay, and severe fibro-fatty replacement of the musculature, but no hepatic or cardiac involvement. Molecular analysis revealed two novel missense mutations with amino acid changes in the GBE gene (Q236H and R262C), which may account for the mild phenotype.

Key words: glycogen storage disease (GSD); congenital myopathy; glycogen branching enzyme (GBE); Andersen disease

INTRODUCTION

Glycogen storage disease type IV (GSD-IV; OMIM 232500), also known as Andersen disease, is an autosomal recessive genetic disorder that results from a deficiency in the activity of the glycogen branching enzyme, α-1,4-glucan:α-1,4-glucan 6 glucosyl-transferase [Chen, 2001]. The glycogen branching enzyme catalyzes the last step in glycogen biosynthesis by attaching short glucosyl chains in α-1,6 glucosidic links to naked peripheral chains of nascent glycogen [Bruno et al., 1999; Tay et al., 2004], resulting in a branched polymer with increased water solubility. The effect of abnormal branching enzyme function is an abnormal glycogen structure with fewer branching points and long, unbranched outer chains.

The human glycogen branching enzyme gene (GBE1) located at chromosome 3p12, codes for the glycogen branching enzyme and has a coding sequence of 2106 base pairs. Mutations in the GBE1 gene result in glycogen storage disease type IV [Bao et al., 1996; Bruno et al., 1999; Bruno et al., 2004]. The phenotype is variable with hepatic, cardiac, neurologic, and musculoskeletal involvement, alone or in continuum [McConkie-Rosell et al., 1996; Bruno et al., 1999; Maruyama et al., 2004; Tay et al., 2004]. Congenital to adulthood presentations have been described. Affected persons demonstrate a broad spectrum of severity and organ involvement. Thus, many variants of GSD-IV have been described, resulting in a rather confusing classification system.

We report on a child with a congenital neuromuscular phenotype of GSD-IV characterized by congenital hypotonia, and severe replacement of muscles with fat and fibrous tissue. To date, there

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has been no evidence of cardiomyopathy or hepatic disease. Her features are similar to that of children with other neuromuscular disorders. Unlike patients with the severe lethal congenital neuromuscular form of GSD-IV, our patient has a stable hypotonia and mild phenotype. Our findings suggest that deficient GBE activity not only has varying effects on specific organs, but also upon specific tissues. Additionally, further evidence of a genotype-phenotype correlation in GSD-IV is revealed.

CLINICAL REPORT

Clinical Presentation

Our patient (Fig. 1) was the product of an uneventful 39-week pregnancy in a 30-year-old gravida 2, para 2 female. Family history was unremarkable and parents were not related. Delivery was via cesarean section for breech presentation. Birth weight was 3.9 kg (90th centile), length 53 cm (90th centile), and OFC 37 cm (90th centile). Physical examination shortly after birth revealed mild hypotonia, mild frontal bossing, deeply set eyes, overfolded helices, mild micrognathia, bilateral simian creases, and broad great toes. Cardiovascular and abdominal examinations were normal.

Significant hypotonia persisted and muscle atrophy was associated with gross motor delays, requiring physical therapy. At 1 year of age, she was able to roll from front to back, sit unsupported without difficulty, and scoot on her bottom, but was unable to crawl, pull to a stand, or walk. She was unable to lift her arms above her head.

At 2½ years of age, her gross motor skills have plateaued and she primarily moves about with the aid of a motorized wheelchair. Her cardiovascular and abdominal exams continue to be unremarkable.

Radiographic Evaluation

At 1 year of age, magnetic resonance imaging (MRI) of the upper and lower extremities demonstrated reduced muscle bulk with replacement by diffuse hyper-intense T1-weighted signals, particularly of the large muscle groups, consistent with fatty replacement. Smaller muscle groups, including the gracilis muscles, and intermuscular fascial planes were preserved, bilaterally (Fig. 2A,B). Additional MRI findings included increased signal intensity on fast spin-echo inversion recovery (FSEIR). This suggested edema within the soft tissues, possibly representing ongoing, active disease. Ultrasonography and MRI demonstrated a normal liver. A brain MRI revealed mild prominence of the lateral and 3rd ventricles, but was otherwise normal. Magnetic resonance spectroscopy of the brain was unremarkable. Repeat MRI at 2 years of age revealed a liver of normal size and signal characteristics. However, the spleen was found to be mildly enlarged. The lower extremities continued to exhibit near complete fibrofatty replacement of the large muscle groups in the thigh and buttocks. Previously unaffected muscles appeared to be increased in muscle bulk and normal in signal intensity compared to the prior MRI. The upper extremities were not imaged. Echocardiograms were normal at ages 1 and 2 years.

Biochemical Evaluation

Serum amino acids, acylcarnitine profile, lactate, pyruvate, and urine organic acids were within normal limits. Electron transport chain activity in muscle, thyroid function studies, creatine kinase, and dystrophin studies were also within normal limits. The karyotype was that of a normal 46,XX female. Initial serum screening tests for liver dysfunction were mildly abnormal; however, by 2 years of age, all of her liver function studies were within the normal range. Biochemical studies revealed undetectable levels of GBE in muscle tissue (control 32 ± 10 (mol/min/g), and diminished levels in fibroblasts (75 nmol/min/mg protein; control 1,300 ± 390 nmol/min/mg protein). Muscle phosphorylase and glycogen levels were in normal limits, 8.0 and
0.2% wet weight, respectively. Tissues from the liver and heart were not obtained for evaluation.

**Skeletal Muscle Histology**

An open biopsy of the gastrocnemius muscle was performed. The sample contained fatty and fibrous tissue with very few scattered muscle fibers (predominately type I muscle fibers). Subsequently, an open biopsy of the anterior tibialis muscle was performed, revealing generally normal appearing myofibers, with approximately 5% of the fibers showing partial atrophy and coarse vacuoles containing abnormal glycogen (Fig. 3A). Type I muscle fibers predominated, which is normal for the gastrocnemius muscle. Alcohol PAS and Gram’s iodine stained slides

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**Fig. 2.** T1-weighted MR images of the patient. The chest (A) demonstrates nearly complete bilateral replacement of the normal hypointense signal of the deltoid muscle with the hyper-intense signal of fat (arrow on residual left deltoid muscle strands). The pelvis and thighs (B) demonstrate diffuse displacement of muscle signal intensity with fat signal intensity (arrowheads).

**Fig. 3.** Histology of the anterior tibialis muscle. A. There are occasional myopathic fibers with coarse vacuoles. The storage material is strongly reactive with periodic acid-Schiff stain before and after diastase digestion (B), consistent with amylopectin. Electron microscopy, (C) demonstrates sharply circumscribed non-membrane bound aggregates of filaments surrounded by normal glycogen and mitochondria. Original magnification: 300× (A, B), 3,150× (C).
from the anterior tibialis muscle biopsy demonstrated coarse accumulations of clumped amylopectin only in the abnormal myofibers (Fig. 5B). Oxidative enzyme reactions were normal, but some of the myofibers exhibited coarse clumping of reaction product after staining with succinic dehydrogenase (SDH). The acid phosphatase reaction was negative in the storage foci.

Electron microscopy primarily demonstrated structurally normal muscle fibers with prominent myofibrillar disintegration in occasional myofibers. In rare myofibers that were identified in thick plastic sections as showing focal storage identical to that seen by light microscopy, large sharply circumscribed non-membrane bound aggregates of fibrillar material was found (Fig. 3C). Within the structurally normal myofibers, glycogen and mitochondrial content, appearance, and distribution were unremarkable.

Molecular Study

Sequencing of the \textit{GBE1} gene revealed this patient to be a compound heterozygote for two novel missense mutations: A CDNA 708G > C substitution, resulting in an amino acid change of glutamine to histidine at position 236 (Q236H) and a cDNA 784 C > T substitution resulting in an amino acid change of arginine to cysteine at position 262 (R262C). Both parents were found to be carriers for a single mutation on the \textit{GBE1} gene, neither of which are believed to be polymorphisms.

DISCUSSION

GSD-IV is an autosomal recessive genetic disorder with phenotypes varying in age of onset as well as the organs affected. The ‘classic’ form of GSD-IV presents in the first 18 months of life with failure to thrive, hepatomegaly, and cirrhosis that progresses to liver failure, leading to death by age 5 years. Hepatic and cardiac variants have also been described, varying in severity and age of diagnosis. The cardiac variant is associated with dilated cardiomyopathy and occasionally leads to significant heart disease and death [Guerra et al., 1986; Servidei et al., 1987; Greene et al., 1988; Bruno et al., 1999].

In addition to the hepatic form, the neuromuscular form is also quite variable in phenotype and has been sub-classified into several different phenotypes. A perinatal form, presenting as fetal akinesia deformation sequence (FADS) has been associated with arthrogryposis, hydrops, polyhydramnios, pulmonary hypoplasia, and death at a very early age, usually due to cardiac or pulmonary compromise [Alegria et al., 1999; Cox et al., 1999; Bruno et al., 2004]. A congenital phenotype has been associated with hypotonia, skeletal muscle tissue atrophy, hepatic and cardiac involvement, and death in infancy, due to cardiac or pulmonary compromise [Bao et al., 1996; Nambu et al., 2003; Bruno et al., 2004; Maruyama et al., 2004; Tay et al., 2004]. The juvenile presentation has been associated with myopathic features that are usually apparent in the first decade of life and is often mild to moderate in severity [Reusche et al., 1992].

Our patient had a neonatal presentation with hypotonia and muscle atrophy; however, her clinical course more closely resembles the juvenile onset phenotype with stable hypotonia and no cardiac and hepatic manifestations. Review of the literature identified several other children with findings similar to our patient’s. Bruno et al. [2004] described a child with congenital hypotonia, developmental delay, waddling gait, absence of hepatic, and cardiovascular manifestations, with molecular and enzymatic testing consistent with GSD-IV. Likewise, Reusche et al. [1992] described three male siblings with microscopic and enzymatic studies consistent with GSD-IV and features of hypotonia, developmental delay, rapid fatigue, and muscle hypotrophy, which were present from an early age in all three children. In that report, two of the siblings exhibited hepatosplenomegaly but no laboratory evidence of hepatic disease and none of the children exhibited cardiovascular disease. Our patient’s neuromuscular phenotype appears to be more severe than that of the previously reported patients.

The relative sparing of type I muscle fibers in these muscle biopsy samples is a finding of unknown significance. Also interesting is the finding of structurally normal glycogen fibers within the same myofibers as abnormal glycogen, suggesting variable enzyme activity within the same muscle fiber.

In our patient, there was generalized hypotonia and replacement of muscle bulk of the upper and lower extremities with connective and adipose tissue. This pattern of involvement is similar to that of other neuromuscular disorders described by Oszaralak et al. [2001] and is most often identified in tissues at the end stages of severe myopathies and muscular dystrophies. Likewise, our patient had relative sparing of smaller muscle groups, including the gracilis muscle, a pattern reported in other neuromuscular disorders by [Schwartz et al., 1991; Oto et al., 2001]. Unlike others with progressive neuromuscular diseases, our patient’s disease is currently stable and the she is thriving.

It has been proposed that the severity of an individual’s phenotype is correlated to the amount of residual glycogen branching enzyme activity, and the particular \textit{GBE1} gene mutation, with null mutations (deletions, insertions, or nonsense mutations) seemingly being associated with more severe forms of GSD-IV [Bao et al., 1996; Bruno et al., 2004; Janecke et al., 2004]. The current branching enzyme activity assay is not sensitive enough to detect low levels of enzyme activity, especially in a tissue of low
branching enzyme activity, such as muscle. The missense mutations of the GBE1 genes in our patient presumably resulted in residual enzyme activity, which may explain why this patient has a milder clinical phenotype. More research, particularly gene sequencing of affected individuals, is necessary to better understand the genotype–phenotype correlations in GSD-IV. Furthermore, additional studies involving children with this newly described phenotype of GSD-IV would be greatly beneficial.

Because the phenotype of GSD-IV is quite variable with regards to age of diagnosis, organ involvement, and severity, a confusing classification system has resulted. It is likely that there are no clear-cut variants to this disease, but rather degrees of involvement of each organ system. For this reason, we propose that it is more appropriate to consider GSD-IV as a continuum of disease rather than discrete variants.

Our patient has a stable myopathy at the time of publication (age 2½ years), and exhibits no cardiac or hepatic pathology, findings that are quite unique for GSD-IV. Considering the characteristically progressive natural history in patients with GSD-IV, this child may eventually experience progression of the myopathy or develop other pathological features associated with the disease. Consequently, her prognosis is unknown and we have recommended periodic echocardiograms and liver ultrasounds as well as bimannual liver function studies to monitor for cardiac and hepatic complications of the disease.

Finally, we recommend evaluations as described above in individuals with similar phenotypes and suggest including GSD-IV within the differential diagnosis of patients with myopathies, regardless of age of onset.

REFERENCES


