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Neuromuscular forms of glycogen branching enzyme deficiency

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Deficiency of glycogen branching enzyme is causative of Glycogen Storage Disease type IV (GSD-IV), a rare autosomal recessive disorder of the glycogen synthesis, characterized by the accumulation of amylopectin-like polysaccharide, also known as polyglucosan, in almost all tissues. Its clinical presentation is variable and involves the liver or the neuromuscular system and different mutations in the *GBE1* gene, located on chromosome 3, have been identified in both phenotypes. This review will addresses the neuromuscular clinical variants, focusing on the molecular genetics aspects of this disorder.

Key words: Glycogen storage disease, branching enzyme, *GBE1* gene, metabolic myopathy

Glycogen branching enzyme

The Glycogen branching enzyme (GBE catalyzes the last step in glycogen biosynthesis by transferring short glucosyl chains (about six α-1,4-linked glucosyl units in length) in α,1-6 glucosidic links to naked peripheral chains of nascent glycogen. Its deficiency causes accumulation of an abnormal glycogen – similar to amylopectin – with less branching points, more 1,4 linked glucose units and longer outer chains than normal glycogen (1).

Polyglucosan

This abnormal glycogen, made of long chains of glucose units infrequently branched, known as polyglucosans, is intensely positive to periodic acid-Schiff stain and partially resistant to diastase digestion. Ultrastructurally, it consists of filamentous and finely granular material. Polyglucosan accumulates in skin, liver, muscle, heart and central nervous system, but to different degrees (1).

Polyglucosan deposition is not peculiar of GSD-IV, but can be found in other disorders, such as phosphofructokinase (PFK) deficiency and Lafora disease. As previously discussed, the polyglucosan deposition in PFK deficiency is caused by the alteration of the normal ratio of glycogen synthase and branching enzyme (2).

Clinical presentation

The typical presentation of GSD-IV, originally described by Andersen in 1956 (3), is characterized by failure to thrive, hepatosplenomegaly, and liver cirrhosis leading to death in early childhood. Non-progressive hepatic form is rarely reported (4). However, the neuromuscular system can be primarily involved, and three clinical variants based on age at onset can be identified: i) congenital, ii) juvenile, and iii) adult. The congenital phenotype can, in turn, be subdivided into two clinical subgroups. The first is characterized by severe perinatal disorder presenting as fetal akinesia deformation sequence (FADS) with by multiple congenital contractures (arthrogryposis multiplex congenita), hydrops fetalis, and perinatal death. The second is characterized by severe congenital myopathy, inconsistently associated with cardiopathy, often simulating Werding-Hoffman disease (5-8).

The juvenile phenotype is dominated by myopathy (7, 9) or by cardiopathy (10).

The adult form can present as isolated myopathy (11) or as a multisystem disorder with central and peripheral nervous system dysfunction (adult polyglucosan body disease, APBD) (12, 13).

However, it may be wiser to consider GSD-IV as a clinical *continuum*, with different degrees of involvement of each organ system, rather than splitting the disease in separate clinical variants (14).

Biochemical analysis

The diagnosis is confirmed by the determination of the branching enzyme activity in affected tissues. However, the commonly use assays are indirect and not sensitive enough for precise assessment of low levels of branching activity (15).

Molecular Genetic

Human GBE is a monomeric protein, which consists of 702 amino acids, and contains two highly conserved

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Table 1. Congenital forms: phenotype-genotype correlation.

Pt	Pregnancy				Birth	Clinical features			Outcome	Form	Genetics	Ref.
	HF	IGR	↓FM	PH		hypotonia	mech vent.	cardiopathy	arthrogr.				
1	+	-	-	-	34 w	+	+	-	+	death 4 d	FADS	p.R48fsX/p.R48fsX	7
2	-	+	-	-	36 w	-	-	-	-	death in utero	FADS	p.H545R/p.H545R	7
3	-	+	-	+	ND	-	-	-	+	death at birth	FADS	p.H545R/p.H545R	7
4	+	+	+	-	-	-	-	-	-	death in utero	FADS	p.G299X/p.A491T	18
5	ND	ND	+	+	34 w	+	+	-	-	death 4 w	Congenital	p.R262_S331del/?	17
6	-	-	+	-	35 w	+	+	+	-	death 4 mo	Congenital	pE288fsX/?	19
7	-	-	-	+	33 w	+	+	-	-	death 41 d	Congenital	p.D413fsX/p.L490fsX	8
8	-	-	+	+	35 w	+	+	-	-	death 5 w	Congenital	p.W332_M539del	8
9	-	-	+	+	ND	-	-	-	-	death at birth	Congenital	p.W332_M539del	8
10	-	-	-	+	36 w	+	+	-	-	death 4 mo	Congenital	p.V144_S261del/p.E592X	7
11	-	-	+	+	35 w	+	+	-	-	death w	Congenital	p.V144_S261del/p.E592X	7
12	-	-	+	-	32 w	+	+	-	-	death 4 w	Congenital	p.H243R/p.R637X	7
13	-	-	+	+	36 w	+	+	+	-	death 2 mo	Congenital	p.L490fsX/p.R637X	20
14	-	-	-	-	39 w	+	+	+	-	alive 2.5 years	Cong. Mild	p.Q236H/p.R262C	14

HF = hydrops fetalis; IGR = intrauterine growth retardation; ↓FM = decrease fetal movements; PH = polyhydramnios; mech. vent. = mechanical ventilation; arthrogr. = arthrogryposis; d = days; mo = months; w = weeks; ND = not described.

domains that have sequence similarities to the isoamylase N-terminus and to -amylase (15). It is encoded by a single gene, *GBE1*, which has been cloned, sequenced, and localized on chromosome 3p12 (16).

The GBE amino acid sequence shows a high degree of conservation throughout species (15).

Since the first description of a molecular defect in 1996 by Bao and colleagues (17), different mutations have been identified throughout the *GBE1* gene in all different phenotypes.

In patients with the neuromuscular variant of GSD-IV, up to December 2006, 21 mutations in the *GBE1* gene have been identified by sequencing genomic DNA and/or mRNA: 4 nonsense mutations, 9 missense mutations, 3 small deletion, 2 large deletions, 1 insertion, and 2 splicing mutations, spanning the entire coding region (Table 1). It is interesting to note that 24% of all mutations are in exon 12, which appears to be a "hot-spot" for *GBE1* gene mutations.

The congenital form: clinical and genetic correlation

To date, 14 infants with the congenital form have been characterized genetically (Table 2).

We have described the first case of FADS with genetically confirmed GBE deficiency in a baby girl carrying a homozygous missense mutation at the donor site of intron 1 of the *GBE1* gene (7). In the parents of two deceased infants with FADS and GSD-IV, we identified an apparently mild missense homozygous mutation: using a human branching enzyme model based on the known three-dimensional *E. coli* structure, we have shown that this mutation seriously alters the enzyme protein (7).

In one family, three consecutive *foeti* were affected by GSD-IV and in all of them the 12-week-US examination revealed cervical cystic hygromas. In the first pregnancy, hydrops fetalis was present since the second trimester, while fetal akinesia developed in the second pregnancy (18).

Bao and Nambu independently reported two infants with neonatal hypotonia and dilated cardiomyopathy, who died in the first months of life (17, 19). They harboured a splice-site mutation leading to exon skipping in the first case, and a homozygous single-nucleotide deletion in the second (17, 19). Two other cases presented with severe neonatal hypotonia and died within 40 days of life: the first had two single-base pair deletions and the second had a large homozygous deletion encompassing exons 8 to 12 (6).

We reported three additional patients (two of them siblings) with decreased fetal movements and polyhydramnios, severe hypotonia at birth requiring mechanical ventilation, who died at ages ranging from 4 weeks to 4 months. The two siblings showed a nonsense mutation and a large deletion, while the third patient was a compound

Table 2. The *GBE1* mutations in GSD-IV patients with neuromuscular presentation.

Ex/In	Codon	Coding Sequence	AA change	Phenotype	Reference
1	13	c.38insA	p.D13fsX	Childhood (+ liver)	Bruno 2004
2	96	c.288delA	p.E288fsX	Congenital lethal	Nambu 2003
6	236	c.708G > C	p.Q236H	Congenital mild	Burrow 2006
6	243	c.728A > G	p.H243R	Congenital lethal	Bruno 2004
7	262	c.784C > T	p.R262C	Congenital mild	Burrow 2006
7	299	c.895G > T	p.G299X	FADS	L'hermine-Coulomb 2005
8	329	c.986A > C	p.Y329S	APBD	Lossos 1998
10	413	c.1238delT	p.D413fsX	Congenital lethal	Tay 2004
12	490	c.1468delC	p.L490fsX	Congenital lethal	Tay 2004/Janecke 2004
12	491	c.1471G > C	p.A491T	FADS	L'hermine-Coulomb 2005
12	515	c.1544G > A	p.R515H	APBD	Ziemseen 2000
12	524	c.1570C > T	p.R524X	Childhood	Bruno 2004
12	524	c.1571G > A	p.R524Q	Childhood (+ liver)/ APBD	Bruno 2004/Ziemseen 2000
13	545	c.1634A > G	p.H545R	FADS	Bruno 2004
13	592	c.1774G > T	p.E592X	Congenital lethal	Bruno 2004
14	628	c.1883A > G	p.H628R	Childhood	Bruno 2004
14	637	c.1909C > T	p.R637X	Congenital lethal	Bruno 2004/Janecke 2004
4-6	144-261	c.430_782del	p.V144_S261del	Congenital lethal	Bruno 2004
8-12	332-539	c.993_1617del	p.W332_M539del	Congenital lethal	Tay 2004
Int.1	-	c.143 + 1G > A	p.R48fsX	FADS	Bruno 2004
Int.6	-	c.783-1G > A	p.R262_S331del	Congenital lethal	Bao 1996

Nomenclature of mutations refers to the cDNA sequence with A of the translation initiation codon as + 1; the initiator Met is numbered as + 1.

heterozygote for a nonsense mutation and a missense mutation in a highly conserved amino acid (7). Decreased fetal movements, polyhydramnios, severe hypotonia with respiratory insufficiency requiring ventilatory support, also characterized the clinical presentation of a baby-girl, who died at 2 months of age (20).

Recently, Burrow and collegues reported a child with non-lethal congenital hypotonia without hepatic or cardiac involvement, due to GSD-IV. Genetic analysis revealed the presence of two missense utations in the *GBE1* gene (14).

Adult polyglucosan body disease

Adult polyglucosan body disease (APBD, MIM 263570) is a clinical variant of GBE deficiency. It is a late-onset neurological disease clinically characterized by progressive upper and lower motor neuron involvement, sensory loss, early urinary incontinence, and dementia in about half of the patients (12, 13). Polyglucosan bodies accumulate in the axons and hillocks of neurons in both gray and white matter, at difference from the polyglucosan bodies of Lafora disease, which are never seen in neuronal perikarya.

To date, genetic analysis of the *GBE1* gene identified an homozygous missense mutation – c.986A > C (p.Y329S) – in several Ashkenazi Jewish patients (21). Ubogu and collegues reported a manifesting heterozygous patient with 48% of GBE activity and a single common Ashkenazi-Jewish Y329S mutation (22).

Ziemssen and collegues identified two heterozigous mutations in a non-Jewish patients with reduced GBE activity (c.1544G > A p.R515H.1571G > A p.R524Q) (23).

Prenatal diagnosis

Prenatal diagnosis can be performed by the measurement of the GBE activity in cultured chorionic *villi* cells and in cultured amniotic fluid cells (24), or by DNA analysis in genetically confirmed cases (25).

Animal models

Two naturally occurring animal models with GSD-IV are known, the Norwegian Forest cat and the American Quarter horse. In Norwegian Forest cats, the disease is fatal and affects primarily the striated muscles and the nervous system. A 6.1-kb deletion that causes exon 12

skipping has been identified in the feline *gbe1* gene (26).

A fatal neonatal equine GSD IV, occurring in newborn foals of American Quarter Horses (27), is due to a 102C > A transversion in exon 1 of the equine *gbe1* gene (28).

Conclusion

Although GBE deficiency is usually reported in textbooks as a liver disorder, in the last few years the involvement of the neuromuscular system has become apparent and several cases have been reported in close succession, suggesting that this disease has been underestimated.

GBE deficiency should be included in the differential diagnosis of pregnancies complicated by hydrops fetalis, polyhydramnios, and decreased fetal movements, and in infants with mild to severe hypotonia.

All cases characterized by perinatal death or by fatal infantile hypotonia have been associated with almost complete absence of GBE activity and with severe mutations in the *GBE1* gene.

Reduced enzyme activity and mild or heterozygous *GBE1* mutations result in APBD.

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