Acta Myologica • 2007; XXVI; p. 72-74

Clinical, biochemical and genetic features of glycogen debranching enzyme deficiency

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Deficiency of debrancher enzyme causes Glycogen Storage Disease (GSD) type III, an autosomal recessive disorder, characterized by tissue accumulation of abnormally structured glycogen. This report reviews current clinical and molecular knowledge about this disorder and describes the variability at phenotype and genotype levels of a large group of Italian GSDIII patients.

Key words: Glycogen storage disease, AGL, metabolic myopathy

Introduction

Glycogen Storage Disease type III (GSDIII; Cori-Forbes Disease; OMIM 232400) is an autosomal recessive disorder due to the deficiency of amylo-1,6-glucosydase, 4-α-glucantransferase enzyme (AGL, or Glycogen Debrancher Enzyme, GDE) which degrades glycogen branches releasing glucose in a two step reaction catalysed by its two distinct activities. GSDIII was first observed in the '30s by van Creveld; in 1952 Illingworth and Cori described the abnormal structure of GSDIII glycogen (1). In 1953 Forbes correlated the abnormal glycogen structure with the typical symptoms of GSDIII (2). The *AGL* gene was cloned in 1992 (3).

The main clinical phenotypes of this disease are due to involvement of liver and/or muscle. Phenotypic expression is highly variable. GSDIII features can be distinguished in two presentations, according to patient's age. Infancy and childhood are characterised by recurrent fasting hypoglycemia, seizures, hepatomegaly, decreased muscle tone and growth retardation. During childhood and early adulthood the symptoms seem to regress and most patients have only minimal signs of liver disease (4). The predominant symptoms in the adult form are distal weakness, affecting calves and peroneal muscles mostly, and proximal weakness at a variable degree with a slow disease progression. Back pain and fatigue may be present. A number of patients show serum creatine kinase (CK) increase of 5-45 folds. Neuropathy may occur due to glycogen storage in Schwann cells and axons. Hepatic dysfunction persists in few patients and cardiomyopathy, if present, is rarely severe.

Debranching enzyme is a single 1532 aminoacid chain weighing about 165 kDa and consisting of two independent catalytic activities: oligo-1,4-1,4-glucantransferase [EC 2.4.1.25] and amylo-1,6-glucosidase [EC 3.2.1.33], localised in two distinct protein regions (5, 6). Patients with debrancher deficiency are classified into four types: IIIa: lack of both glucosidase and transferase activity in liver and muscle; IIIb: lack of both activities in liver only; IIIc: selective loss of glucosidase activity; IIId: selective loss of transferase activity. Debranching enzyme is encoded by 85-kb *AGL* gene on chromosome 1p21. Six transcript isoforms have been isolated, alternatively spliced in 5' of the gene: the main of them, isoform 1, is ubiquitous and is about 7.0 kb long. A large genetic screening led to the observation that GSDIIIb patients had mutations in exon 3 (7), whereas GSDIIIa arose with downstream mutations. At present, a formal demonstration of this is still lacking.

Clinical data

We reviewed clinical, biochemical and genetic data of 51 patients (26 males and 25 females) with GSD III from Centers throughout Italy. All had absent or severely reduced debranchig enzyme activity, either on red blood cells or muscle tissue. Median age of patients was 25.8 years (range: 2-75).

Liver damage and structure were monitored over the entire lifetime of these patients: we observed an inverse correlation between aspartate and alanine amino transferase (AST, ALT) levels and age, with high transaminase levels in the first decade and in particular in the first three years of age and a progressive reduction in adulthood. Liver echography was useful to differentiate patients with mild or severe liver involvement. We considered four degrees of liver involvement as evaluated by echocardiography: a) normal; b) patients with mild hepatomegaly and diffuse homogeneous hyperechogenicity, classified as having mild liver disease; c) patients with hepatome-

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galy and inhomogeneous hyperechogenicity classified in the moderate liver disease group; d) hepatic involution, cirrhosis or liver transplant were considered indicators of severe liver disease. Among 44 patients we found 2% with normal liver echography, 78% with mild, 16% with moderate and 4% with severe liver disease. In the first decade most patients (86%) showed mild liver disease, while only 5% of patients showed normal liver echography; 9% of patients had moderate hepatic involvement. In the second decade 100% of patients had signs of mild liver disease. In the third decade 66.6%, 16% and 16% of patients presented respectively mild, moderate and severe hepatic involvement, while none had normal liver imaging. In patients aged over 30 years, none had normal liver findings, 55%, 36% and 9% of this age group had respectively mild, moderate and severe liver disease. Only two patients developed liver failure and needed liver transplantation at 23 and 32 years of age respectively.

Muscular weakness and disability were evaluated using a modified Walton Functional Rating Scale (8), to take into consideration signs of distal lower limb weakness. We observed a direct correlation between age and disease progression, with higher functional rating scores in older patients. Functional impairment is very mild in patients younger than 35 years, who mainly have scores lower than 2. Older patients present a higher variability of the functional score, ranging from 3 to 10. CK values of the entire series were instead higher among children and young adults. They ranged from 29 to 3097 I.U. with a mean value of 899 in the first decade, from 57 to 6574 (mean: 2763 I.U.) in the second decade, from 87 to 3422 in the third decade (mean: 1047 I.U.). Older patients showed a decrease of CK values probably due to progressive muscle loss.

Signs and symptoms of heart function were also evaluated: 45% of patients had normal echographic findings, 54% had mild hypertrophy and 1% had moderate hypertrophy. None of the patient in our group had severe cardiac hypertrophy.

Molecular data

AGL gene was analyzed by direct sequence of the coding region and splicing sites. 35 patients could be completely characterized (69%), whereas only one allele was identified in 7 patients (14%), while 9 patients (17%) resulted negative. The majority of changes are represented by mutations giving rise to null alleles. The IVS21 + 1G/A intronic change is the most frequent mutation in our series (23.4%). Missense mutations amount to 25% of total. Identified mutations are widespread along the whole gene and no particular hot spot could be found.

Grouping mutation type by severity (null *vs*. missense) and gathering clinical and genetic data, it came out that null patients have higher probability to develop more severe myopathic and hepatic involvements. Anyway, exceptions in both directions exist. Furthermore, as with other genetic diseases, the difficulty in establishing genotype-phenotype correlations is something well known with GSDIII. IVS21 + 1G/A is a good example in this sense (9). Among our patients, we observed the case of three genotypically identical adult patients, which were homozygous for the exon 21 skipping. All of them developed a severe myopathy, and hepatopathy, though at quite different ages and degree, but only the older of them suffers from cardiopathy. A further complication comes from the presence of intrafamilial clinical variability.

As far as different types of GSDIII are concerned, all our informative patients (those older than 30: n = 19) are affected by GSD type IIIa.

Genetic screening on ethnically different populations has shown that only very few mutations are common in a considered geographic area, the great majority being private mutations. The only mutation shared by Caucasians coming from different countries is R864X, identified in Mediterranean and North American population. The mutation IVS6 + 3 A/G accounts for 11.7% of mutated alleles in Mediterranean families (10).

Therapy and perspectives

Therapy is not available for debranching enzyme deficit. To avoid fasting hypoglycaemia in infancy, dietary measures have been prospected. Frequent daytime high-protein feedings (45% carbohydrate, 25% protein, 30% fat) and supplementation of uncooked corn starch before sleep showed to be effective in young patients with regard to metabolic control and growth retardation. Effects of dietary measures on myopathies in adults are less well established even if there are reports of improvement in patients following high-protein diet (11).

Beside the described marked clinical and genetic variability, environmental effects on the GSDIII pathogenesis are not elucidated at present. The creation of animal models for this disorder might reveal to be useful in defining pathogenesis and care.

Acknowledgements

We would like to thank the patients and their families, the support of the "Associazione Italiana Glicogenosi" and the following Colleagues: Mirella Filocamo, Maja Di Rocco, Rosanna Gatti Institute G. Gaslini, Genoa, Italy; Carmelo Rodolico, Olimpia Musumeci, Antonio Toscano, Department of Neurosciences, University of Messina, Messina Italy; Daniela Melis, Michelina Sibilio, Pediatric Institute, University Federico II, Naples, Italy; Rossella Parini, Clinica De Marchi, Milan, Italy; Marta Torcoletti, Sabrina Paci, Division of Pediatrics, San Paolo Hospital, Milan, Italy; Maria Alice Donati, Meyer Children's Hospital, Florence, Italy.

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