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Homozygosity for the common GAA gene splice site mutation c.-32-13T>G in Pompe disease is associated with the classical adult phenotypical spectrum

Olimpia Musumeci [a], Andrea Thieme [b], Kristl G. Claeys [c,d], Stephan Wenninger [e], Rudolf A. Kley [f], Marius Kuhn [g], Zoltan Lukacs [h,i], Marcus Deschauer [j], Michele Gaeta [k], Antonio Toscano [a], Dieter Gläser [g], Benedikt Schoser [e,*]

[a] *Department of Neurosciences, University of Messina, Messina, Italy*
[b] *Department of Neurology, Erfurt, Germany*
[c] *Department of Neurology, RWTH Aachen University, Aachen, Germany*
[d] *Institute of Neuropathology, RWTH Aachen University, Aachen, Germany*
[e] *Department of Neurology, Klinikum Munich, Friedrich-Baur-Institute, Germany*
[f] *Department of Neurology, University Hospital Bergmannsheil, Ruhr-University Bochum, Germany*
[g] *Genetikum, Center for Human Genetics, Neu-Ulm, Germany*
[h] *Institute of Clinical Chemistry, Germany*
[i] *Department of Pediatrics Hamburg, University Medical Center Hamburg, Germany*
[j] *Department of Neurology, Halle-University, Halle, Germany*
[k] *Department of Radiology, School of Medicine, University of Messina, Italy*

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Abstract

Homozygosity for the common Caucasian splice site mutation c.-32-13T>G in intron 1 of the GAA gene is rather rare in Pompe patients. We report on the clinical, biochemical, morphological, muscle imaging, and genetic findings of six adult Pompe patients from five unrelated families with the c.-32-13T>G *GAA* gene mutation in homozygous state. All patients had decreased GAA activity and elevated creatine kinase levels. Five patients, aged between 43 and 61 years (median 53 years), initially presented with myalgia, hyperCKaemia, and/or exercise induced fatigue at an age of onset (12–55 years). All but one had proximal lower limb weakness combined with axial weakness and moderate respiratory insufficiency; the sixth patient presented with hyperCKaemia only. Muscle biopsies showed PAS-positive vacuolar myopathy with lysosomal changes and reduced GAA activity. Muscle MRI of lower limb muscles revealed a moderate adipose substitution of the gluteal muscles, biceps femoris and slight fatty infiltration of all thigh muscles. One MRI of the respiratory muscles revealed a diaphragmatic atrophy with unilateral diaphragm elevation. So, the common Caucasian, so called mild, splice site mutation c.-32-13T>G in intron 1 of the GAA gene in a homozygote status reflects the full adult Pompe disease phenotype severity spectrum.

Keywords: GAA-MLPA; GAA mutation; Glycogen storage disease type 2; Homozygosity; Pompe disease

1. Introduction

The human structural gene encoding glucosidase alpha (GAA) is located at chromosome 17q25.2–q25.3 and contains 19 coding exons, and a first non-coding exon [1–4]. GAA cDNA encompasses about 3.6 kb with 2856 nucleotides of coding sequence, predicting a protein of 952 amino acids with a molecular mass of 105 kDa for the non-glycosylated protein [5–8]. This enzyme is synthesized as a 110-kDa glycoprotein precursor that matures into a multi-subunit complex through multiple proteolytic and carbohydrate moiety modifications [9–11]. At present, more than 500 different sequence variants are listed in the Rotterdam Pompe disease mutation database. About 2/3 of these variants have been demonstrated to be pathogenic [[12], www.pompecenter.nl]. The most frequent mutation of juvenile and adult Caucasian Pompe patients is the leaky splice mutation c.-32-13T>G in intron 1 of *GAA* (formerly termed IVS1-13T>G).

* Corresponding author. Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University Munich, Ziemssenstr. 1a, D-80336 Munich, Germany. Tel.: +49 89 4400 57400; fax: +49 89 4400 57402.
E-mail address: bschoser@med.uni-muenchen.de (B. Schoser).

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This common splice site mutation gives rise to alternatively spliced transcripts, including a deletion of the first coding exon 2, but still allows for the production of a low amount of normally processed mRNA and therefore enzyme activity [6,12–14]. Homozygosity for this most common Caucasian GAA gene splice site mutation is an unexpected rare event compared to the appraised frequency in the population with regard to the predicted incidence of the disease ranging between 1:40,000 and 1:250,000 [[12], www.pompecenter.nl, [15]]. In the fast growing cluster of publications related to Pompe disease over the past 20 years, four singles and three siblings of Pompe patients with a homozygous c.-32-13T>G mutation are reported so far [16–19].

For juvenile and adult onset Pompe disease, common clinical symptom patterns include: (i) classic limb girdle, axial, and diaphragmatic weakness; (ii) rigid spine syndrome (RSS), scoliosis, and low body mass; and (iii) several cardio-cerebrovascular manifestation patterns. The most common presentation is the limb girdle, axial, and diaphragmatic weakness, which appear in over 80% of juvenile and adult Pompe patients. Less common, possibly underreported and underdiagnosed are the rigid spine, scoliosis, and low body mass phenotype, and the cardio-cerebrovascular manifestation [20]. We here report on six adult patients with Pompe disease and a homozygous c.-32-13T>G mutation.

2. Patients and methods

2.1. Patients

Four patients from four unrelated German non-consanguineous families from Aachen, Bochum, Erfurt, and Munich were included. Furthermore, two Italian brothers, born from healthy consanguineous parents (first cousins) in Sicily, could be included. Both Italian brothers without further details were recently reported [21]. The ethics committee board gave positive ethic board approval for this study. (Ludwig-Maximilians-University Munich, Germany; vote no. 201-14).

2.2. Methods

A summary of clinical assessments and follow-up data of the patients was obtained between 2005 and 2014. A muscle MRI was performed at different time points; however, the most current MRI was performed during the past two years in four patients. Routine diagnostic open muscle biopsy with additional GAA activity and glycogen measurement from muscle tissue was performed in all but one, whereas dried blood spot (DBS) testing of the GAA activity was available from all. As in most patients the parents were no longer available for mutational analysis and proving homozygous GAA gene mutation; multiplex ligation-dependent probe amplification (MLPA) analysis of the GAA gene was performed to rule out large deletions especially of exon 2 [22]. Because there is no commercial MLPA kit available including a probe for exon 2 in *GAA*, we designed self-made probes for each *GAA* coding exon and performed MLPA using the universal SALSA® MLPA® Kit from MRC Holland P200-A1 (for details of the protocol, see www.mlpa.com).

3. Results

3.1. Clinical summary of six patients of five independent European families

All six adult Pompe patients had decreased GAA activity in the DBS test with a reduction of at least more than 30% (range 0.17–2.2 μmol/L/h) compared to normal (>3.3 μmol/L/h). All six patients had elevated creatine kinase levels ranging from 2- to 10-fold of normal. Two women and two men of German origin (patients I.1, II.1, III.1, IV.1), currently aged between 43 and 61 years (median 54 years), initially presented with myalgia, hyperCKaemia, and/or exercise induced fatigue at an age of onset ranging between 12 and 55 years. All but one had mild to moderate proximal weakness predominating in the lower limbs at time of current age. Additionally axial weakness and scapular winging combined with moderate respiratory insufficiency with the need of nightly non-invasive artificial ventilation were found in two patients (I.1, II.1). For further clinical details, see Table 1.

3.2. Patients V.1 and V.2

Patient V.1: A 51 years-old Caucasian man complaining, in the past two years, of exercise-induced muscle cramps and diffuse myalgia. In his past medical history he had a diagnosis of essential tremor, treated with beta-blockers, and reflux oesophagitis treated with proton pump inhibitors. On neurological examination, he had macroglossia, postural hands and head tremor as a “no-no” motion and a mild waddling gait without other signs of muscle weakness. Serum-CK repeatedly was mildly elevated (max. 837 U/L, normal <200 U/L). The electromyography (EMG) was normal. GAA activity measured on DBS was significantly reduced (0.179 μmol/h/L, normal >3.3 μmol/L/h). Muscle biopsy of vastus lateralis muscle showed a moderate vacuolar myopathy with glycogen accumulation. Muscle GAA activity was markedly reduced (13% residual activity). GAA genetic analysis documented the presence of c.-32-13T>G mutation on both alleles, confirming the diagnosis. Furthermore, the patient complained of daytime sleepiness and sleep apnoea and snoring. Respiratory assessment showed a moderate obstructive impairment of the ventilatory pattern without significant variation of %FVC in supine and upright position. Blood gas analysis while breathing room air showed mild hypercapnia and hypoxia. Muscle MRI revealed a moderate adipose substitution of gluteal muscles and slight fatty infiltration of all thigh muscles. Respiratory muscle MRI showed elevation and atrophy of the diaphragm.

Patient V.2: His brother, a 42 year-old man, presented occasional low back pain and myalgia. His neurological examination was normal except for a mild postural tremor on both hands. CK was mildly elevated (630 U/L) and EMG was normal. GAA assay on DBS resulted positive (0.23 μmol/h/L). Genetic analysis confirmed the presence of IVS1 32-13T>G in a homozygous state. Echocardiogram showed slight left ventricular hypertrophy and LVEF of 55%. Muscle MRI was unremarkable. Respiratory assessment did not reveal any respiratory involvement.