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# Novel *GAA* sequence variant c.1211 A > G reduces enzyme activity but not protein expression in infantile and adult onset Pompe disease

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ABSTRACT

Pompe disease is a clinically and genetically heterogeneous autosomal recessive disorder caused by lysosomal acid α-glucosidase (GAA) deficiency. We report on two affected members of a non-consanguineous Caucasian family, including a classical infantile-onset patient with severe cardiomyopathy (IO) and his paternal grandmother with the adult-onset (AO) form. Two compound heterozygous sequence variants of the *GAA* gene were identified in each patient by mutation analyses (IO = c.1211A > G and c.1798C > T; AO = c.1211A > G and c.692 + 5G > T). For this study, the biochemical phenotype resulting from the missense mutation c.1211A > G in exon 8, which converts a highly conserved aspartate to glycine (p.Asp404Gly), was of specific interest because it had not been reported previously. Western blotting revealed a robust expression of all GAA isoforms in quadriceps muscle of both patients (fully CRIM positive), while enzymatic activity was 3.6% (IO) and 6.6% (AO) of normal controls. To further validate these findings, the c.1211A > G sequence variant was introduced in wild type *GAA* cDNA and over-expressed in HEK293T cells. Site-directed mutagenesis analyses confirmed that the mutation does not affect processing or expression of GAA protein, but rather impairs enzyme function. Similar results were reported for c.1798C > T (p.Arg600Cys), which further supports the biochemical phenotype observed in IO. The third mutation (c.692 + 5G > T, in intron 3) was predicted to affect normal splicing of the GAA mRNA, and qPCR indeed verified a 4-fold lower mRNA expression in AO. It is concluded that the novel sequence variant c.1211A > G results in full CRIM but significantly lower GAA activity, which in combination with c.1798C > T leads to infantile-onset Pompe disease. We surmise that the difference in disease severity between the two family members in this study is due to a milder effect of the intronic mutation c.692 + 5G > T (vs. c.1798C > T) on phenotype, partially preserving GAA activity and delaying onset in the proband (paternal grandmother).



## 1. Introduction

Pompe disease (OMIM # 232300), also known as glycogen storage disorder type II (GSD II), is an autosomal recessive disorder caused by a deficiency of the glycogen hydrolysis enzyme acid α-glucosidase (GAA, EC.3.2.1.3). The onset and clinical course of GSD II are correlated with the level of residual GAA activity, with the most severely affected infants exhibiting a near complete loss of enzyme function, causing a rapid progression and premature death due to cardiomyopathy and cardiorespiratory insufficiency (classical infantile form Reuser et al., 1995; van den Hout et al., 2003). At the opposite end of the GSD II disease spectrum (Güngör and Reuser, 2013), patients retain some enzyme function, resulting in symptoms manifesting in adulthood and a slower progression of the disease (adult onset). In the adult form, death may result from pulmonary failure secondary to diaphragmatic weakness as late as the seventh decade, which is preceded by progressive muscle dysfunction leading to wheelchair- and ventilator-dependency. Interestingly, cardiac involvement is now being identified in patients other than those with the classic infantile form, making it increasingly difficult to set boundaries between clinical subtypes in GSD II (Beckemeyer et al., 2013).

To date, over 460 sequence variants of the *GAA* gene have been reported and approximately 30–55% of these are associated with severe pathology (www.pompecenter.nl), but genotype–phenotype correlations are complicated by the fact that most patients are compound heterozygotes. Inter-individual variations in genetic background, epi-

*Abbreviations:* 4-MU, 4-methylumbelliferone; A, adenosine; AO, adult onset; Asp, aspartic acid; Arg, arginine; C, cytidine; cDNA, DNA complementary to RNA; CON, control(s); CK, creatine kinase; CRIM, cross-reactive immunologic material; Cys, cysteine; ERT, enzyme replacement therapy; FEV, forced expiratory volume; FVC, forced vital capacity; G, guanosine; *GAA*, acid α-glucosidase (gene); GAA, acid α-glucosidase (protein); Gly, glycine; GSD II, glycogen storage disorder type II; HET, heterozygous; HRP, horseradish peroxidase; IO, infantile onset; IVS, intervening sequence; kDa, kilodalton(s); NaAc, sodium acetate; PCR, polymerase chain reaction; RT, room temperature; T, thymidine; TRIS, tris-buffered saline; 3'UTR, three prime untranslated region; WB, western blotting.

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genetic factors, and health-related behaviors undoubtedly also add to the clinical heterogeneity observed in this population (Kroos et al., 2012). In the absence of mutations seen in homozygosity, bioinformatic software tools and site-directed mutagenesis can be utilized to predict pathogenicity and to reconstruct the phenotype in mammalian cell lines. In this paper, we present a previously unreported missense mutation in exon 8 of the *GAA* gene (c.1211A > G [p.Asp404Gly]), which is associated with distinctly different Pompe phenotypes in the first (adult onset) and third (classic infantile onset) generation of a non-consanguineous Canadian Caucasian family. To elucidate the biochemical basis underlying the clinical heterogeneity of affected family members, GAA mRNA/protein abundance and enzyme function were assessed and the phenotype was reconstructed by site-directed mutagenesis and transfection of HEK293T cells.

## 2. Procedures, patients and methods

### 2.1. Procedures

All procedures conformed to the Declaration of Helsinki guidelines and written approval was given by the chairperson of the Hamilton Integrated Research Ethics Board for further confirmation of genetic findings of uncertain significance to MT. Clinical examination (see below) and mutational screening revealed that P1 and P2 share a unique mutation on exon 8 of the *GAA* gene, despite presenting at opposite ends of the GSD II clinical spectrum, which attracted our attention and motivated further study. Genetic testing of asymptomatic family members confirmed that the mutation was transmitted paternally with each parent carrying a different pathological allelic copy (see Fig. 1. for genetic pedigree of the family). In order to establish combined effects of compound heterozygous mutations in P1 and P2, GAA mRNA expression (Fig. 2A), protein levels (Fig. 2B), and enzyme function (Fig. 2C) were assessed in skeletal muscle of both patients. Furthermore, *in silico* analyses and site-directed mutagenesis were completed to predict pathogenicity (Table 1) and to reconstruct the phenotype associated with a specific mutation (Fig. 3 and Supplemental Fig. 1, respectively).

### 2.2. Patient history

The proband (P1) is of north western European decent and was referred to the Neuromuscular Disease Clinic for evaluation of proximal muscle and suspected diaphragmatic weakness at 49 years of age. She presented with slightly elevated plasma creatine kinase (CK) levels (201 U/L) and significant weakness in shoulders, hips, quadriceps, and respiratory muscles/diaphragm. Spriometry showed a forced vital capacity of 1.2 (FVC; 32% of predicted) and a forced expiratory volume at the end of the first second of 0.96 (FEV1; 32% of predicted). She initially started having difficulty climbing stairs and raising from low seats in her late teens, and developed upper limb weakness in her early twenties. Lower limb strength gradually declined and she experienced breathing difficulties in her mid thirties, with wheelchair- and ventilator-dependence occurring in her late forties. A muscle biopsy revealed areas of excessive fibrosis, irregularly shaped myofibers, and extensive vacuolization with an accumulation of glycogen, confirmed to be membrane bound by electron microscopy. Vacuoles were strongly positive for acid phosphatase, indicative of a lysosomal storage disorder. A dry blood spot test indicated low acid α-glucosidase activity and mutational screening of the GAA gene confirmed two pathological sequence variants (c.1211A > G and c.692 + 5G > T). Enzyme replacement therapy with Myozyme®, physical therapy, and respiratory training were initiated without complications. The patient is currently enrolled in a Phase 2a study to investigate the effect of AT2220 (Duvoglustat HCl) on the pharmokinetics of acid α-glucosidase in subjects with Pompe disease (Kishnani et al.).

The grandson (P2) is of First Nations/French (father) and northwestern European (mother) decent and was referred to the Neuromuscular Disease Clinic with a 2-day-history of fever, cough, shortness of breath, and decreased feeding at 5 months of age. Upon examination, the patient exhibited elevated plasma CK levels (1442 U/L), generalized hypotonia, reduced muscle bulk, right upper lobe pneumonia (X-ray), and severely hypertrophic left ventricle with septal thickness (echocardiogram). The pregnancy was unremarkable with a normal neonatal course despite decreased fetal movement in the third trimester, and it was only later in infancy parents noted hypotonia, macroglossia, and feeding difficulties. A muscle biopsy revealed prominent vacuolization with PAS positive material, suggestive of a lysosomal storage disorder and electron microscopy showed extensive Z-band streaming and massive amounts of both membrane and non-membrane bound glycogen. A dry blood spot test confirmed a low level of acid α-glucosidase activity. P2 was diagnosed with classic infantile Pompe disease following mutational screening of the *GAA* gene that yielded two pathological sequence variants, c.1211A > G and c.1798C > T. Myozyme® treatment was successfully initiated and the cardiac variables normalized within six months. Further genetic testing on family members verified that the mutations were in *trans* with the c.1211A > G transmitted paternally and the c.1798C > T inherited from the mother. Of interest, it was only through a Pompe support group discussion that the two patients became aware that they were related and had Pompe disease (i.e., the proband was the paternal grandmother of P2 but contact was lost prior to the diagnoses).

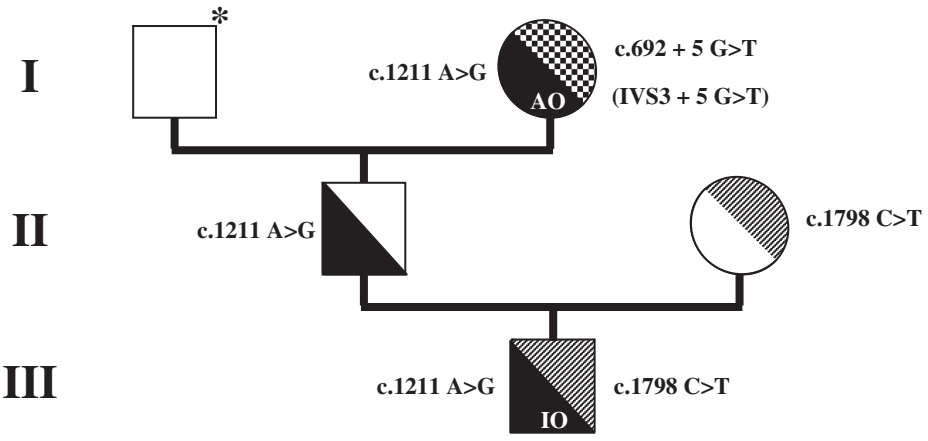


**Fig. 1.** Pedigree of the Canadian Caucasian family with a paternal grandmother affected with adult onset GSD II (AO; generation I), each parent carrying a single mutated GAA allele (carriers; generation II), and one offspring with infantile onset GSD II (IO; generation III). Segregation of the c.692 + 5 G > T (in intron 3), c.1211 A > G, and c.1798C > T alleles of relevant family members are indicated. *Not available for mutational analysis.