



Commentary

Silent but significant - A synonymous SNV alters prognosis in Pompe disease


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Pompe disease (PD) is one of about 50 rare genetic diseases subsumed as lysosomal storage disorders (LSDs). Deficiency of lysosomal acid α -glucosidase (GAA), which hydrolyzes lysosomal glycogen, leads to aberrant glycogen storage in lysosomes and cytoplasm throughout multiple tissues. Enzyme replacement therapy (ERT) by alglucosidase alfa is approved by FDA since 2006 for infants and 2010 for late-onset PD with significant effects on survival and symptom progression [1]. PD is an autosomal recessive disorder with considerable allelic heterogeneity, including missense and nonsense variants, rearrangements and splicing variants with no clear genotype-phenotype correlations yet in case of non-classic infantile patients.

Leucocyte GAA activity is reduced and urine glucose tetrasaccharide (Glc₄) increased in PD patients. The latter is additionally useful to monitor ERT treatment response. The level of GAA reduction and/or Glc₄ increase may distinguish between early and late onset disease and thus be of value for determining the disease phenotype and prognosis in newly diagnosed patients [2].

Although an early start with ERT suggests the best prognosis, treatment is currently not recommended prior to symptom onset. Currently, there is no biomarker indicating disease conversion, so there is still an urgent need for prospective clinical exams to identify the clinical conversion for the start of ERT. This is especially difficult as late-onset disease often manifests with a range of phenotypic presentation and severity [3].

In PD, the c.-32-13T>G (IVS1) variant occurs most frequently in the Caucasian region and affects mRNA splicing of GAA [2]. High variant prevalence in founder populations is a common phenomenon in rare monogenic diseases. Such founder populations may serve as subjects of investigation for mechanistic studies, since sufficiently powered sample sizes are achieved for epidemiologically relevant examinations. For the LSDs Gaucher and Fabry disease, frequent alleles and haplotypes were reported to similarly manifest with phenotypic variation [4–6]. In the example of Fabry disease, the haplotypes seem to also affect

splicing and gene expression. These cases, however, require further work-up beyond conventional sequencing techniques, and, hence, rarely benefit everyday clinical practice.

In the current issue of *EBioMedicine*, Bergsma and colleagues assessed phenotype variability of the frequent IVS1 allele, which occurs in 90% of Caucasian late onset patients in PD by searching for intragenic modifiers in a cohort of 143 compound heterozygous and 10 homozygous IVS1 patients [7]. An area of 4.4 kilobase pairs of genomic DNA around IVS1 were screened for cis-site single nucleotide variants (SNVs) by allele-specific long range PCR. Only one of the 21 identified SNVs was located on the IVS1 allele. So far, the known synonymous SNV c.510C > T (rs564758226) has received little attention. In the compound heterozygous patients, the IVS1/c.510C > T haplotype was uniquely present in 9/33 (27%) patients with childhood onset, but was absent from 110 patients with onset in adulthood. Median age at symptom onset was lower in patients with IVS1/c.510C > T haplotype compared to IVS1. Importantly, the second allele did not play a significant role. In homozygous IVS1 patients, the c.510C > T variant was absent in 4/4 (100%) asymptomatic individuals and present in 3/6 (50%) symptomatic patients. Consistently with these results, an overall reduced GAA enzyme activity was found in fibroblasts from patients carrying the IVS1/c.510C > T haplotype compared to patients without c.510C > T. Mechanistically, in patients with the IVS1 allele, wild type mRNA splicing is reduced to 10–15% [8], whereas in patients harboring the IVS1/c.510C > T haplotype, leaky wild-type splicing was further reduced. Since mRNA splicing can be cell type-dependent, it is important to note that defective splicing has also been detected in myoblasts, the cells of primary damage in PD.

In summary, Bergsma and colleagues propose an extended interpretation of a synonymous SNV that significantly influences age of onset and severity of PD. In IVS1 compound heterozygous patients the presence of c.510C > T could turn the late onset variant into an infantile or childhood onset. Additionally, homozygous IVS1 patients had an increased risk of developing symptoms if the c.510C > T modifier was present. Even though GAA activity differed depending on the presence of c.510C > T, the observed difference will not help to distinguish these individuals in a diagnostic setup. Knowledge of the genetic modifier, on the other hand, can aid to improve prognosis, patient counseling and the decision making on when to start ERT treatment.

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One additional advantage of the study is that the findings are easily implementable in standard DNA diagnostics using the dideoxy method or pyrosequencing, which are established in every genetic diagnostic laboratory, as the c.510C > T is an exonic variant. Nevertheless, one drawback of allele-specific long-range PCR is the limitation of gene coverage. To extend the specifically targeted regions (the *GAA* gene locus spans approximately 20 kilobase pairs) a nanopore technology has recently been exploited to study haplotypes of the highly complex *GBA1* gene in Gaucher disease [9].

The article highlights how merging clinical and molecular investigations allows us to accelerate and refine diagnostic processes. Ultimately, this results in a much more accurate prediction of disease onset, severity and progression, as well as improvement of rational therapy decisions benefitting patients.

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