A quantitative-PCR protocol rapidly detects aGAL deletions/duplications in patients with Anderson-Fabry disease

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The Anderson-Fabry disease (AFD, MIM #301500) is a rare form of X-linked (Xq22.1) glycosphingolipidosis due to a reduced/absent activity of the lysosomal enzyme-galactosidase A (GLA) with systemic involvement.

In about 3-5% of AFD cases is reported only the involvement of the myocardium leading to left ventricular hypertrophy [LVH]. After a multidisciplinary workup, the diagnosis of AFD can be readily made in male patients by measuring the GLA activity with enzymatic assays on peripheral blood mononuclear cells (PBMC) and sera samples. However, this analysis can fail in case of mutations leading to a residual GLA activity. In addition, heterozygous female patients might show lower but normal ranges of GLA activity. Mutation analysis by sequencing the GLA gene is the golden standard to confirm the clinical diagnosis.

The GLA gene is made up of 7 exons for a total size of 12kb. A comprehensive list of reported mutations associated with AFD can be seen at: The Human Gene Mutation Database, National Center for Biotechnology Information Database and Cincinnati Children’s Research Foundation Database). Moreover, alternative splicing due to mid-intronic mutations or intra-gene rearrangements leading to a deletions or duplications of single up to several exons have been described. Direct conventional sequencing might fail in identifying such mutations in heterozygous females because of the presence of the wild type GLA allele. By means of the MLPA technique (Multiple Ligation-dependent Probe Amplification) the detection rate of the GLA mutations has been improved.

In this report we evaluated the efficacy of the Quantitative Real Time PCR (qRT-PCR) on a cohort of 100 female patients presenting with hypertrophic cardiomyopathy (HCM) with no classical sarcomeric mutations and additionally tested for the GLA gene with Sanger sequencing with negative results. By applying the qRT-PCR method, we eventually found two patients with a deletion respectively of the exon 6 and 7 of the GLA gene.