



Biochemical and genetic data for the largest global Fabry cohort reported to present

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Introduction: Fabry disease is an X-linked inherited lysosomal storage disease characterized by a deficient alpha-galactosidase caused by mutations in GLA gene.

Materials and methods: Fabry diagnosis is performed in a high-throughput stepwise manner: (a.) males- enzymatic activity, lyso-Gb3 quantification, followed by GLA gene sequencing and, (b.) females- GLA gene sequencing followed by lyso-Gb3. In the development and validation of the enzymatic assay we observed that alpha-galactosidase determination in DBS alone is insufficient for a precise diagnosis of Fabry disease, both in females (ppv 78%) and males (ppv 94.2%) due to multiple variables: leukocyte count for different individuals; hematocrit level; sample handling, lyonisation effect in females. To eliminate the differences between samples several optimizations were introduced: chemical blank for each sample, a standard curve measured in the presence of blood extract and the ratio alpha-galactosidase to another lysosomal enzyme (beta-glucuronidase). Lyso-Gb3 was measured using mass spectrometry (LC/MRM-MS) from DBS extract.

Summary: Mild mutations or late onset patients present levels of lyso-Gb3 in normal range (21.59 % of all Fabry male cases). However, by combining the data from three different biochemical parameters (Lyso-Gb3, alpha-galactosidase and ratio alpha-galactosidase / beta-glucuronidase) we can distinguish between the cohorts of normal controls, mild (or late onset) Fabry cases and affected Fabry cases. The biochemical diagnosis was confirmed in all cases by genetic analysis. We report here the screening of over 170.000 individuals that led to the identification of over 3,600 affected individuals and over 1,100 carriers. We sequenced over 85,000 alleles, resulting in the identification of 529 unique pathogenic variants (40% of which never published before).

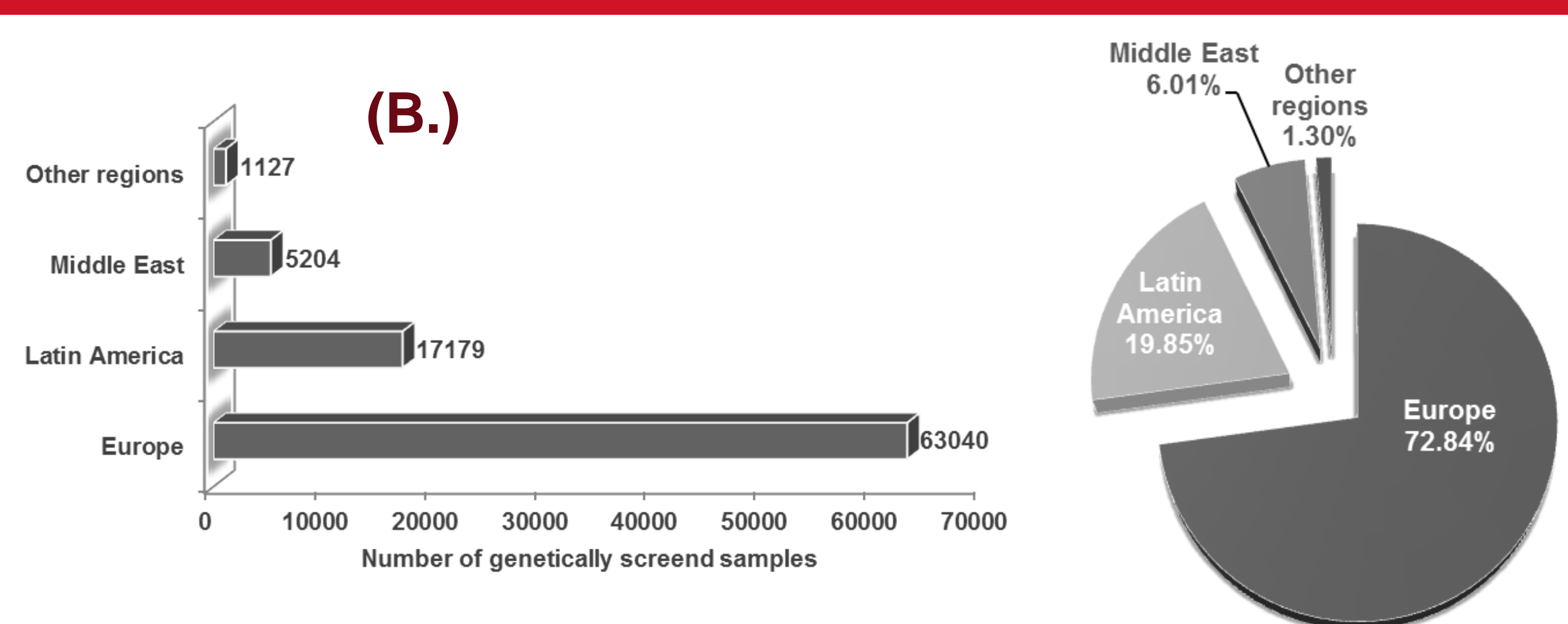
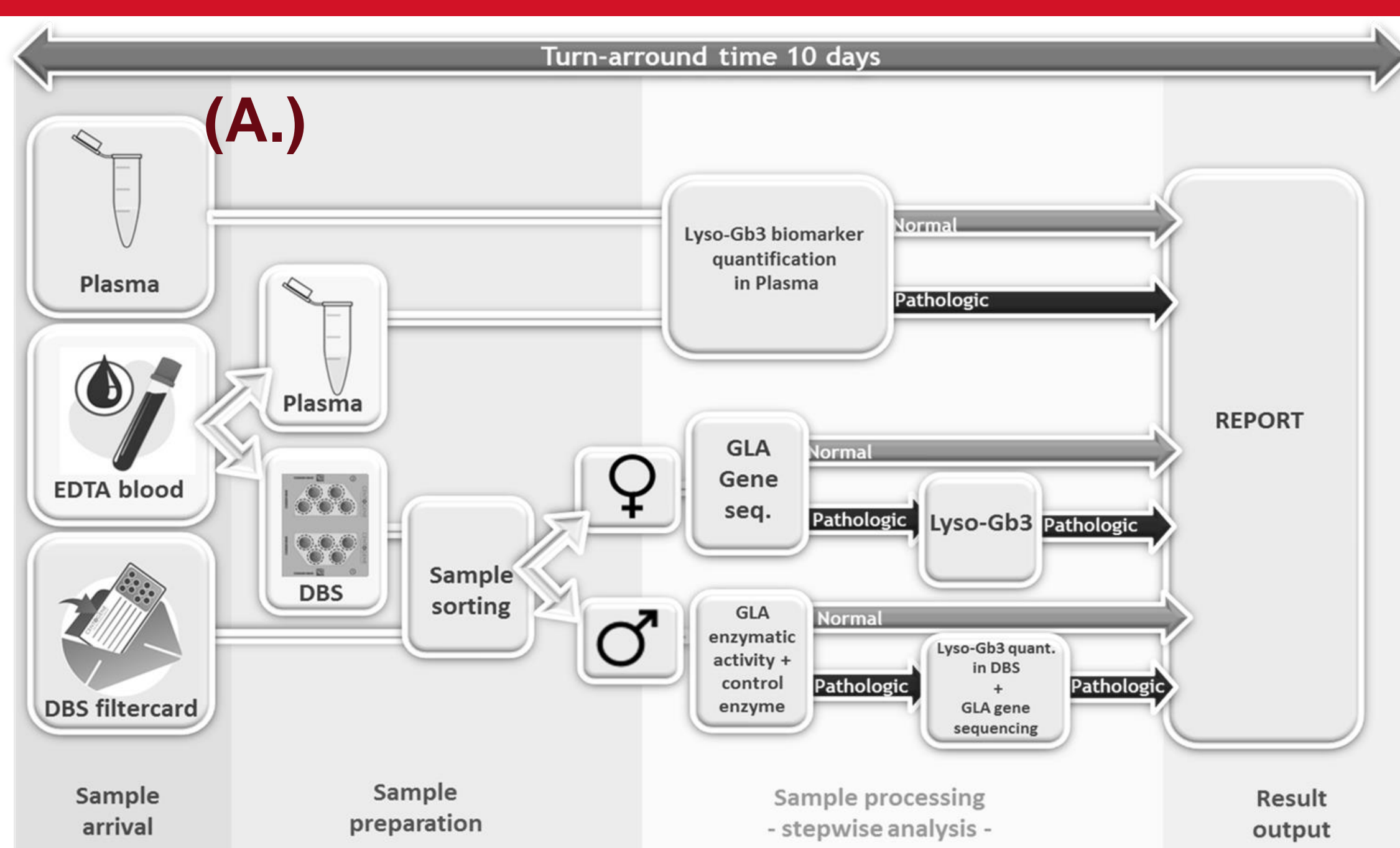


Figure 1: Fabry screening (2006-2017): A. Fabry screening protocol ; B. Geographical origin of the samples of the genetically samples for which GLA sequencing was performed

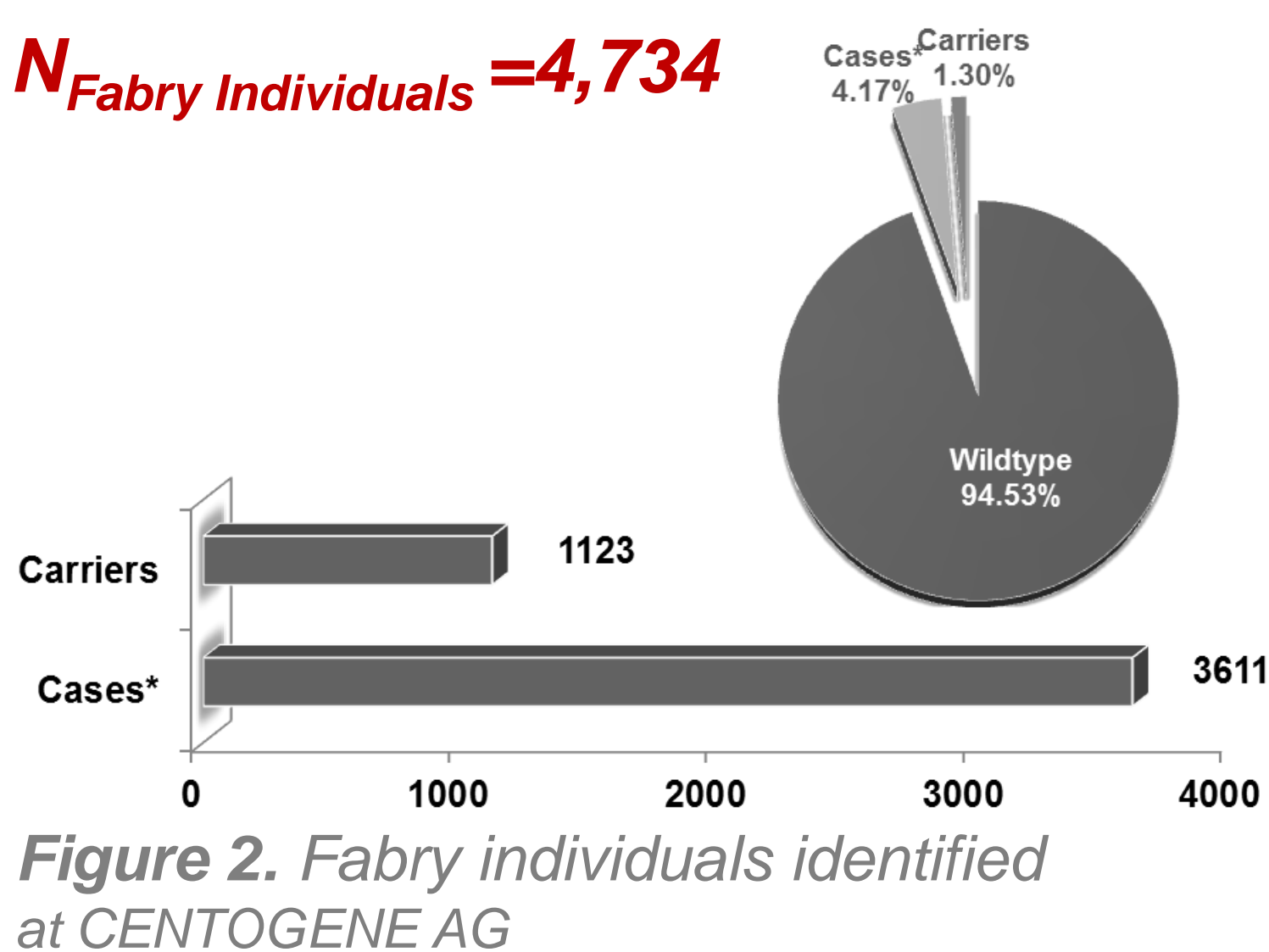


Figure 2: Fabry individuals identified at CENTOGENE AG

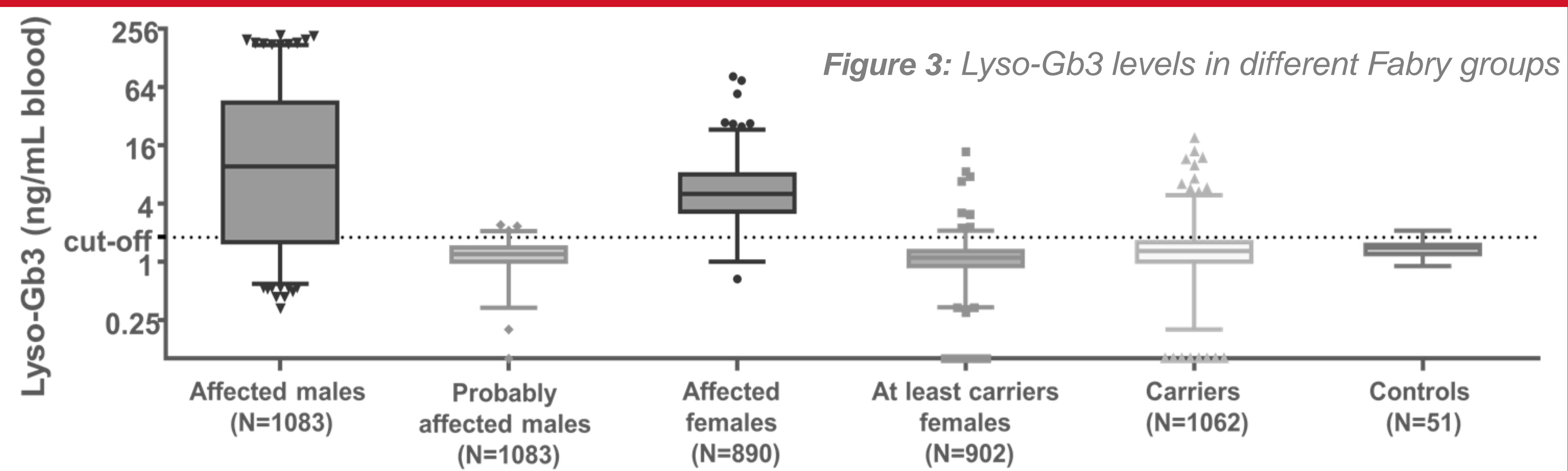


Figure 3: Lyso-Gb3 levels in different Fabry groups

N_{sequenced alleles} = 85063
N_{unique pathogenic variants} = 529

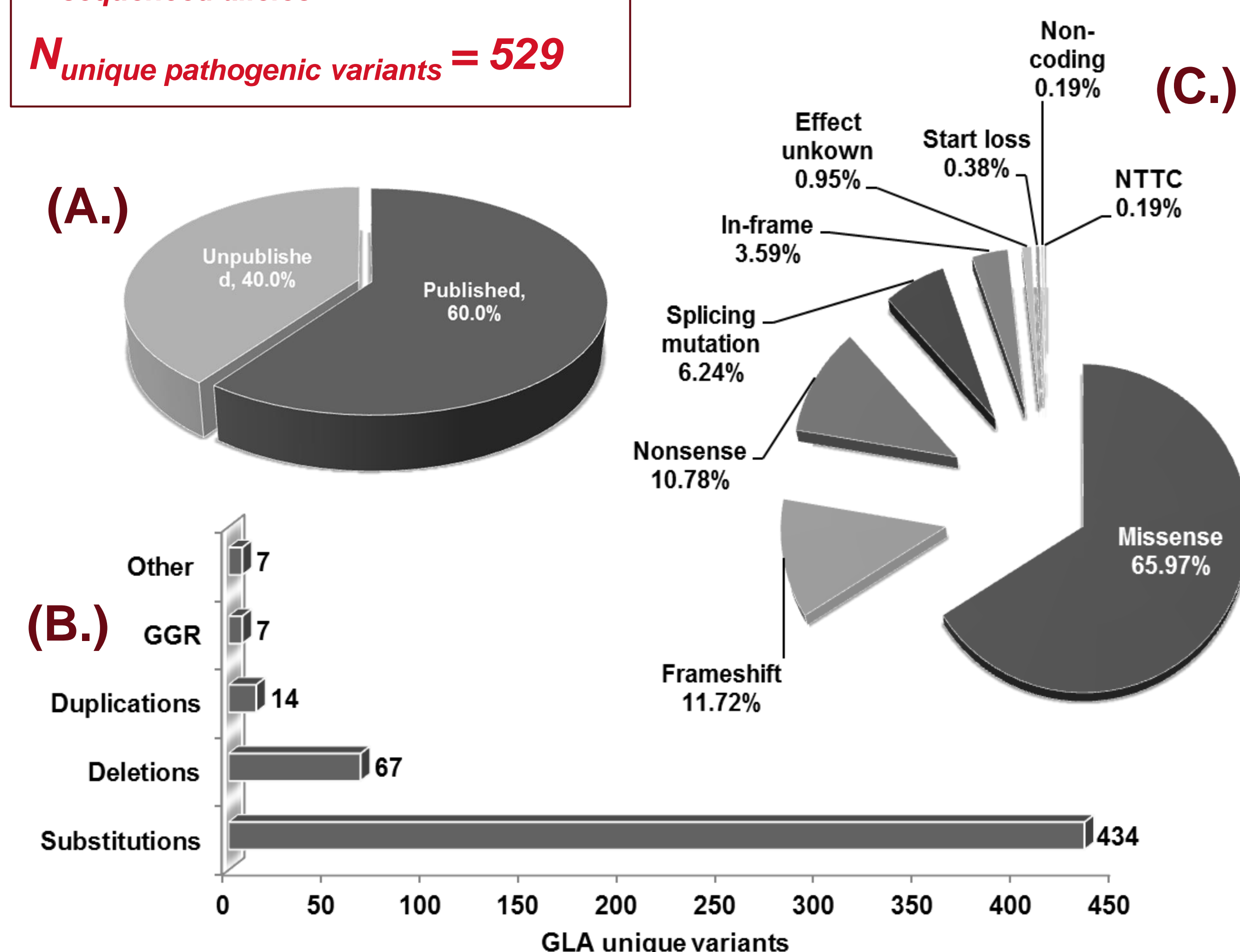
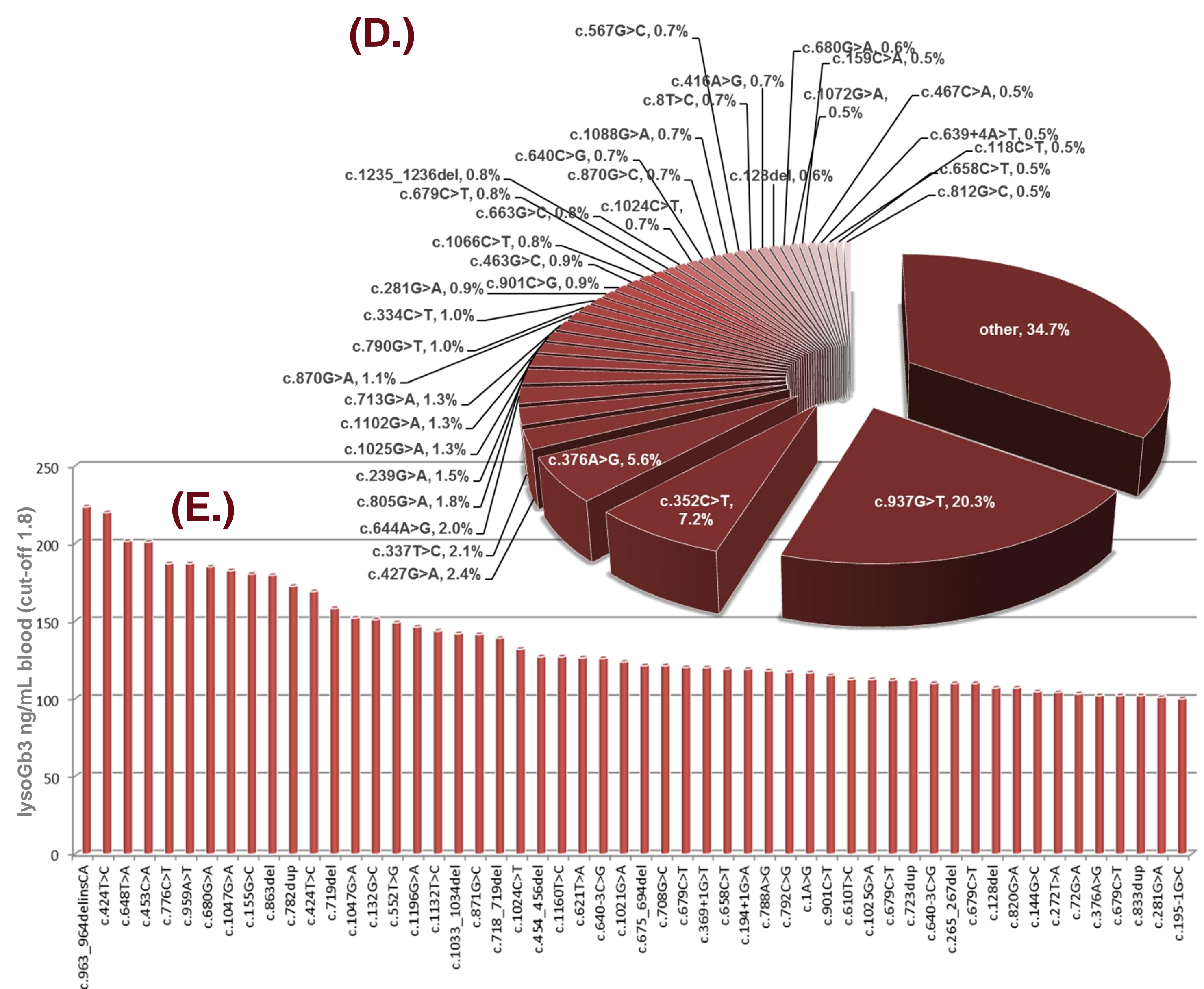


Figure 4: GLA mutations found in the analyzed Fabry cohort: A. Newly detected unique GLA variants; B. Type of GLA mutation; C. Effect of the GLA mutations;



References

- www.centomd.com
- Lukas et al, 2017

Disclosure of conflict of interest:

This study was sustained in part by CENTOGENE AG, Rostock, Author of the presentation, Claudia Cozma, and 3 co-authors are employees of CENTOGENE AG, Rostock, Germany