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Original paper

The Latest Insights into the Genetic Landscape and Clinical Profile of Gaucher Disease in Albania

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Abstract

Background and aim: This study analyzed the clinical and genetic characteristics of Gaucher disease (GD) in an Albanian population.

Materials and methods: A total of 52 GD patients to non-consanguineous marriages and of the Albanian ethnicity were included in the study and categorized into different types of GD, with Type 1GD being the most common. Clinical symptoms such as splenomegaly, hepatomegaly, and thrombocytopenia were commonly observed.

Results: The study identified 18 different genotypes and 15 different mutations, including two novel mutations. The N370S mutation was the most frequent, followed by D409H and L444P. The presence of the N370S mutation was associated with milder disease, while other mutations reflected the variability in disease severity. A family with four distinct genotypes exhibited variability in GD manifestations. The double mutant allele D409H;H255Q, was found in a significant prevalence of patients and associated with an intermediate Type 2-3 GD phenotype. The L444P mutation was less frequent in the Albanian population.

Conclusion: These findings contribute to understanding the clinical and genetic aspects of GD in this population.

Keywords: *Gauche Disease, GD type1,2,3 and 2-3, GBA1 gene mutations, Genotypes, N370S, D409H:H255Q*

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Introduction

Gaucher Disease (GD, OMIM #230800, ORPHA355), initially characterized by Philippe Gaucher in 1882, is a rare autosomal recessive genetic metabolic disorder.

The estimated incidence of GD in the general population ranges from 1 in 40,000 to 1 in 60,000 births, with certain cohorts showing a range of 1 in 50,000 to 100,000 live births [1], [2], [3]. However, in the Ashkenazi Jewish population, the incidence is notably higher, affecting approximately 1 in 800 individuals [1], [2], [3]. In the Albanian population, the estimated incidence is around 1 in 50,000 births [4].

GD is the one of the most common lysosomal storage disease and it is caused by a deficiency of a specific enzyme called glucocerebrosidase or β-glucosidase that is located within lysosomes, resulting in the accumulation of glucosylceramide, primarily in macrophages [3]. These anomalous cells, recognized as Gaucher cells, infiltrate various organs, especially the bone marrow, spleen, and liver, thereby giving rise to the characteristic clinical manifestations observed in the GD patients. Moreover, the deficiency of glucocerebrosidase also results in the accumulation of another sphingolipid, namely glucosylsphingosine or Lyso-Gb1, which has proven to be a valuable biomarker for the diagnosis of GD, prediction of disease progression, and monitoring of treatment efficacy in GD patients [3].

The β-glucocerebrosidase is encoded by *GBA1* gene, situated on chromosome 1 (1q21) and more than 500 pathogenic or likely pathogenic GBA1 variants have been reported to date. The distribution of these GBA mutations varies among different ethnic populations, often due to founder effects [5], [6], [7]. Some mutations, such as c.1226A>G (N370S), c.1448T>C (L444P), c.84dup, c.115+1G>A (IVS2+1G>A), and RecNciI, have greater prevalence [5], [6].

GD is recognized as a highly heterogeneous disorder, encompassing a broad spectrum of genetic and clinical and variability [1], [2], [3], [4], [5], [6].

Clinical presentations of Gaucher disease (GD) manifest diversely, including hepatosplenomegaly, cytopenia, bone pain or bone crisis, and, in particular forms, neurological impairment. Three major phenotypic presentations are typically acknowledged: Type-1 GD, termed non-neuronopathic GD, and Type-2 and Type-3, referred to as neuronopathic GD. In addition, Type 2-3 phenotype represents a rare form of GD, that is neither typical for type 2, nor for type 3 GD [2], [3], [8].

Type-1 Gaucher Disease (GD1) represents the most common form, accounting for 90% to 95% of cases in Europe and North America that is characterized by the absence of neurological impairment. The clinical presentation of GD1 exhibits significant variation, ranging from asymptomatic individuals throughout their lifespan to early-onset forms detected in childhood as well as affecting the quality of life and causes substantial morbidity. Studies report median ages of diagnosis ranging from 10 to 20 years old. The most frequently observed clinical features of GD1 include splenomegaly, hepatomegaly, fatigue, growth retardation, delayed puberty, bleeding problems, anemia, and an increased prevalence of gallstones compared to the general population. Painful bone crises, with varying degrees of severity, are common symptoms likely due to the ischemic vaso-occlusive phenomena. Pulmonary and renal involvement rarely present in GD1 [2], [3], [8].

Type-2 Gaucher Disease represents less than 5% of cases in most countries but can account for up to 20% in certain cohorts. It is characterized by severe neurological impairment that start in infants aged 3 to 6 months, along with systemic involvement and hepatosplenomegaly [2], [3], [5], [8], [9].

Type-3 Gaucher Disease, also known as juvenile or subacute neurological GD, constitutes about 5% of cases but can be as high as 33% in some specific cohorts. It presents with both visceral

manifestations similar to GD1 and oculomotor neurological involvement, typically occurring before the age of 20 [2], [3], [5], [8], [9].
This study focuses on Albanian GD patients, aiming to investigate specific *GBA*1 alleles and their correlation with the disease phenotype.

Material and Methods

In this study, we present for the first time the molecular findings of 52 Gaucher Disease (GD) patients diagnosed in Albania. All 52 GD patients, including the 36 patients previously reported were recruited from the Gaucher unit at University Hospital Center "Mother Teresa" in Tirana, Albania, which is the only specialized unit for GD in the country [4], [10], [11], [12]. Genomic DNA was extracted from peripheral blood in dried blood spot and the molecular genetic analysis were developed and the performance was validated by CENTOGENE Gmbh. The identified variants were labeled according to the Human Genome Variation Society recommendations [13] and Lyso-Gb1 levels were quantified as described previously [14]. The genetic profiles of our patients are shown in tab.1.

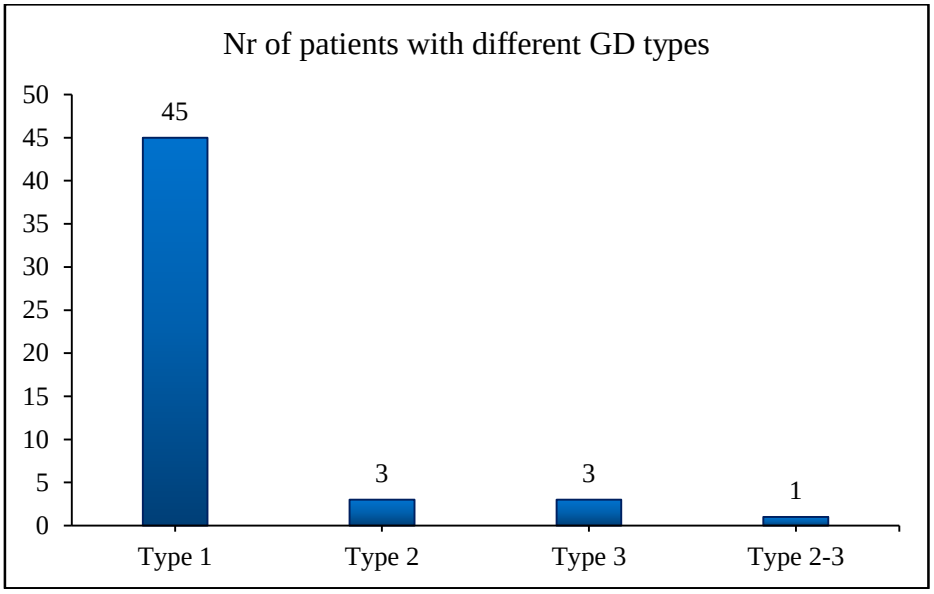
Tab 1. GD type, genotypes and nr of our cohort patients.

GD type and Genotype	Nr of pat
type 1 N370S/D409H:H255Q	21
type 1 N370S/L444P	4
type 1 N370S/D409H	4
type 1 N370S/ N370S	3
type 1 D409H:Leu422Profs/Arg87Trp	3
type 1 N370S/Arg87Trp	2
type 1 A377T/ D409H:H255Q	1
type 1 N370S/R463H	1
type 1 N370S/pArg*86	1
type 1 N370S/L444P, A495P	1
type 1 N370S/L444P, R368H	1
type 1 N370S/S146L	1
type 1 N370S/D409H:Leu422Profs	1
type 1 N370S/R484H	1
type 2 D409H:H255Q/D409H:H255Q	2
type 2 IVS+IG>A/ D409H:H255Q	1
type 3 F213I/ D409H:H255Q	2
type 3 L444P/ D409H:H255Q	1
type 2-3 D409H:H255Q/D409H:H255Q	1

Results and Discussion

A wide variety of GD-related characteristics was clinically present in our patients. Based on clinical classification, the patients were categorized as follows: 45 patients (86.5%) had Type 1 GD, 3 patients (5.8%) had Type 2 GD, 3 patients (5.8%) had Type 3 GD, and 1 patient (1.9%) had Type 2-3 GD (graph 1). The clinical findings of our patients are consistent with those of survey

conducted from different international centers, which identify that splenomegaly, hepatomegaly and thrombocytopenia, are the most frequent clinical symptom [15]. It is important to note that all 52 patients were born to non-consanguineous marriages and belonged to the Albanian ethnicity.



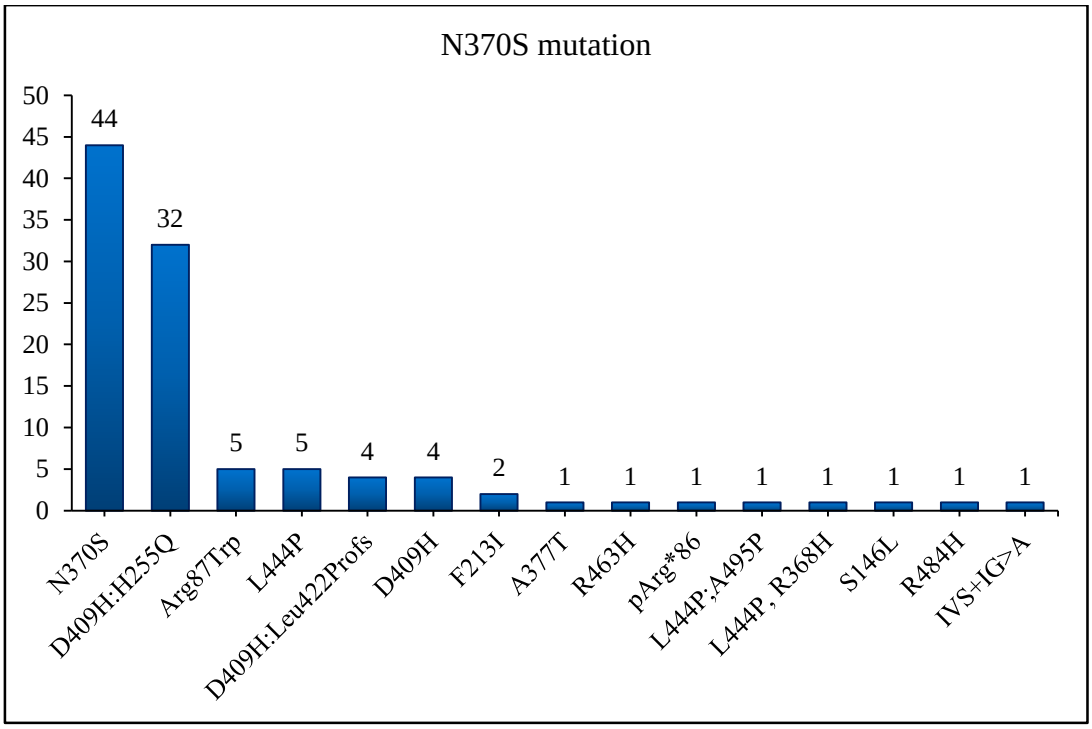
Graph 1. Patients and GD types.

Among the patients, 17 patients from seven families were identified as having a first-degree relationship (parents- children; brother-sister; brother-brother and sister-sister).

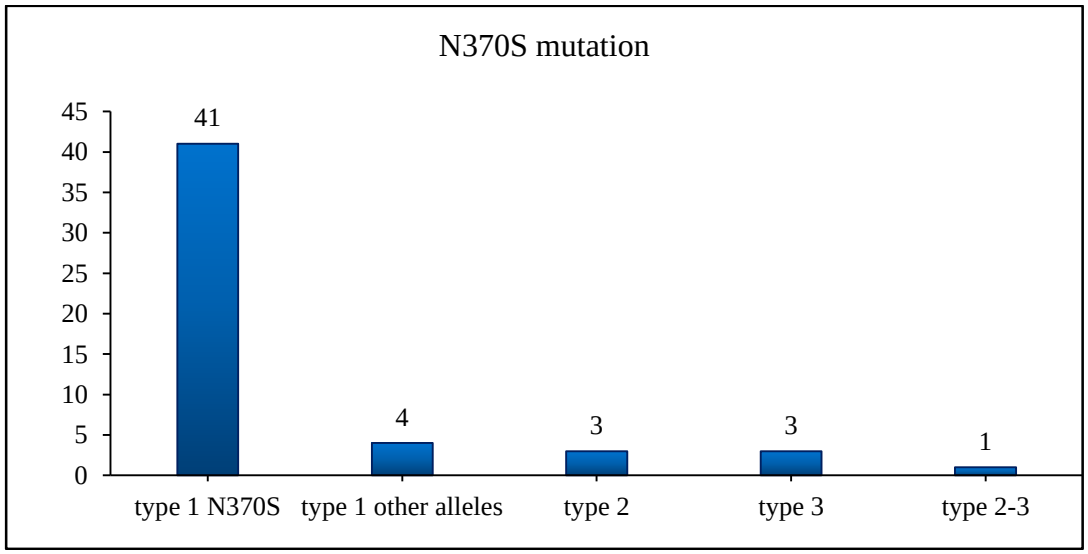
The mean age at the time of diagnosis varied depending on the GD type: for Type 1 GD, the age ranged from 4 to 78 years; for Type 2 GD, the age ranged from 1 to 6 months; and for Type 3 GD, the age ranged from 4 years to 21 years old. The age at onset and disease severity displayed high variability and partially depended on the genotype of the patients [1], [2], [3].

The relationship between genotype and phenotype in Gaucher disease is not always evident, however, there are several correlations that have been observed as well as the heterogeneity in disease manifestations and severity can also be observed even in the patients with the same mutation.

Mutation analysis revealed 18 different genotypes and 15 different mutations among the patients (graph 1). Two novel mutations (ALA377Thr; ALA495Pro) were also identified. The most frequent mutation observed was the N370S mutation, which was present in 44 out of 104 alleles (42.30%) and found in 41 out of 52 patients (78.8% of patients) (graph 2, 3). The prevalence of the N370S mutation in the Albanian population was comparable to that reported in studies from neighboring and other European countries, such as Greece, Romania, and Hungary [4], [16], [17], [18]. In non-Jewish GD patients, the prevalence of the N370S mutation ranges from 40% to 45%, while in Ashkenazi Jews GD patients, it accounts for approximately 70% of cases [3], [4]. Although the N370S mutation is characteristic of Type 1 GD patients, not all Type 1 GD patients carried this mutation.

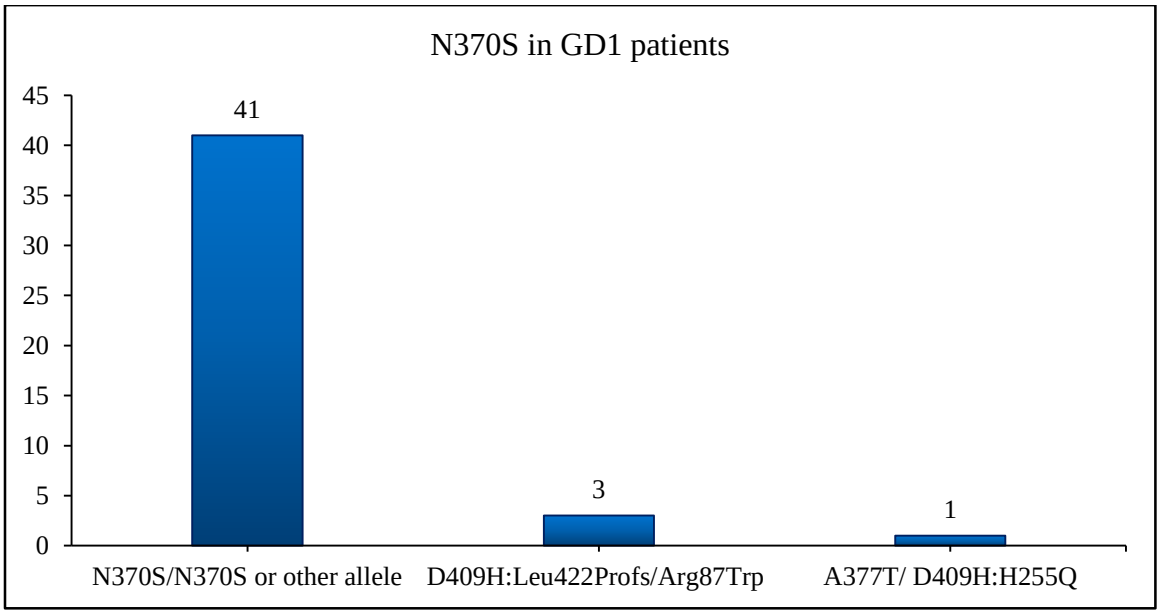


Graph 2. Nr of alleles with N370S mutation among 104 alleles of 52 patients



Graph 3. 41 GD1patients who harbor N370S mutation out of a total of 52 patients

It was found in 44 out of 90 alleles (48.9%) among GD1 alleles and in 41 out of 45 patients (91.1%) (graph 4). In our cohort of patients, this mutation was not identified in four patients classified as type 1. The N370S mutation was found to be as homozygous in three patients and as heterozygous in 38 patients (tab.2). The clinical phenotype of homozygous patients was mostly mild, while the phenotype of heterozygotes depended on the pathogenicity of the other allele.

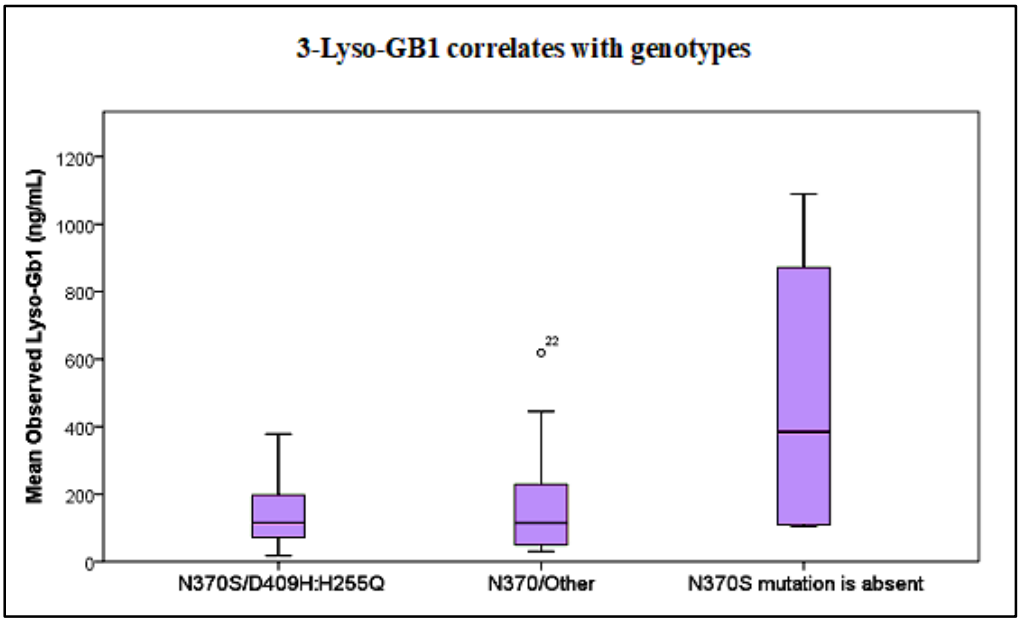


Graph 4. N370S mutation among other mutations in GD1 patients

Tab 2. N370S mutation as homozygous and as heterozygous in GD1 patients

Genotype	Nr of patients
type 1 N370S/D409H:H255Q	21
type 1 N370S/R463H	1
type 1 N370S/pArg*86	1
type 1 N370S/L444P, A495P	1
type 1 N370S/ N370S	3
type 1 N370S/L444P, R368H	1
type 1 N370S/S146L	1
type 1 N370S/Arg87Trp	2
type 1 N370S/D409H:Leu422Profs	1
type 1 N370S/L444P	4
type 1 N370S/R484H	1
type 1 N370S/D409H	4

Overall, the patient cohort displayed a high degree of phenotypic heterogeneity in relation to the N370S mutation. The presence of at least one N370S allele is associated with mild disease and low levels of the Lyso-Gb1 biomarker. On the other hand, when the N370S allele is absent, severe phenotypes and high Lyso-Gb1 levels are commonly observed (graph 5).

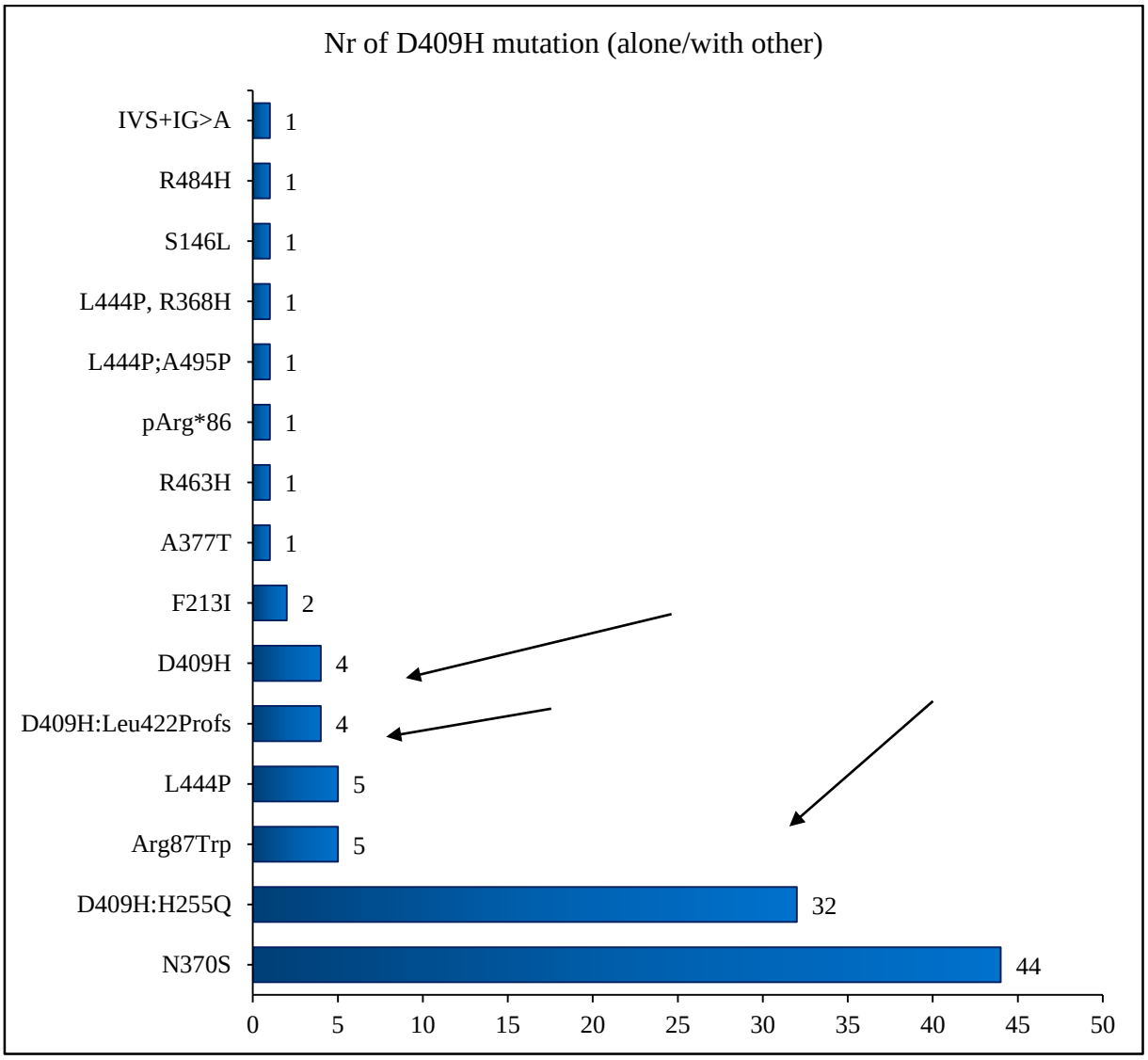


Graph 5. Correlation of Lyso-GB1 with genotypes

The D409H mutation was detected in a significant prevalence, accounting for 40/104 alleles (38.5%) (graph 6). It was the second most frequent mutation observed. The D409H mutation alone was detected in 4/104 alleles as well as the D409H:Leu422Profs mutation that was found in 4/104 alleles. Additionally, the double mutant allele (D409H;H255Q/D409H;H255Q) was detected in 32/104 alleles (30.7%), in total of 29 patients (29/52 patients =55.8%), in heterozygosity in 26 patients and in homozygosity in 3 patients (tab.3). Interestingly the double mutant allele (H255Q;D409H/ H255Q;D409H) was identified in heterozygosity in patients of all types of GD but in homozygosity only in patients with Type 2 and Type 2-3 GD (tab.3). We found in our cohort one of the three reported cases worldwide of patients of Albanian origin who are homozygous for D409H;H255Q who present with an intermediate Type 2-3 GD phenotype. These cases along with other reported ones from other authors provide strong evidence supporting the idea that GD is a continuum of phenotypes rather than a disease with significant distinctions between clinical manifestations [19], [20], [21]. Further research is needed to understand the specific effect of this homozygosity on this particular phenotype. The available data, along with other published findings, support the hypothesis of a Balkan origin for this double mutation, suggesting a single Albanian origin [4], [21], [22].

Tab 3. GD types and double mutation (D409H;H255Q) in 29 patients out of a total of 52 patients.

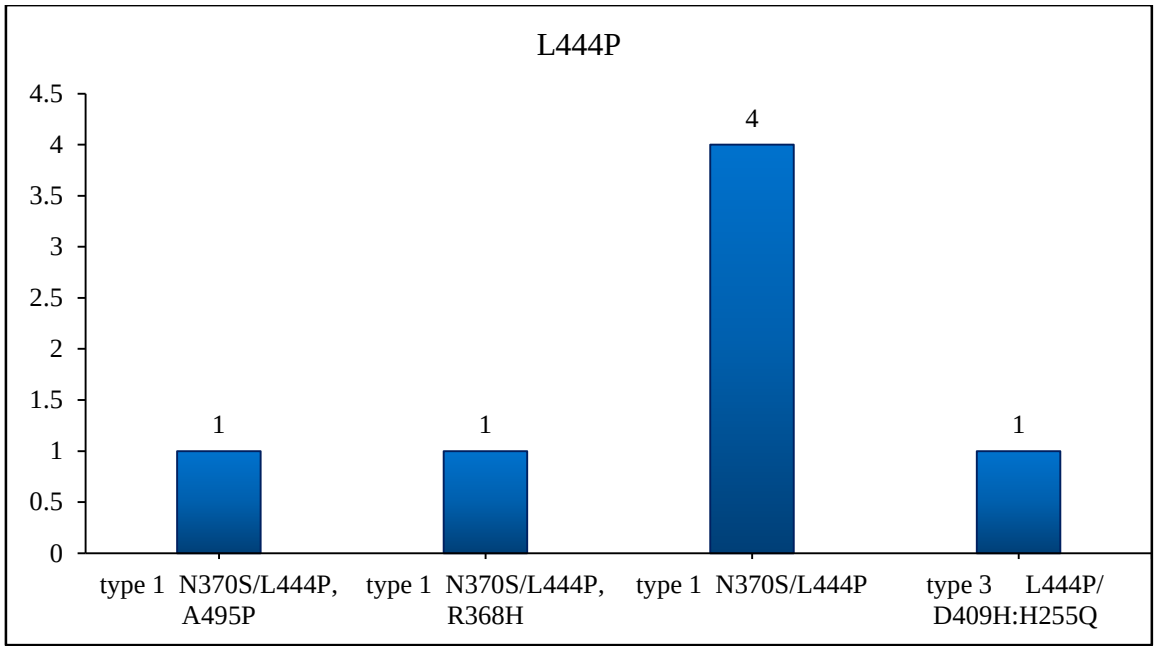
GD type and D409H:H255Q mutation		**Nr of patients**
type 1	N370S/**D409H:H255Q**	21
type 1	A377T/ **D409H:H255Q**	1
type 2	***D409H:H255Q/D409H:H255Q***	2
type 2	IVS+IG>A/ **D409H:H255Q**	1
type 3	F213I/ **D409H:H255Q**	2
type 3	L444P/ **D409H:H255Q**	1
type 2-3	***D409H:H255Q/D409H:H255Q***	1



Graph 6. D409H mutation (alone/with other mutation) in a total of 104 alleles.

The most prevalent genotype in the Albanian population is N370S/D409H:H255Q, accounting for 40.38% of cases (tab.1). This genotype is associated with a mild form of the disease.

The third most frequent mutation observed was L444P, which was found in 7/104 identified alleles, corresponding to seven patients (graph 7). The frequency of this allele was almost much lower than that observed in other neighbor populations. This mutation was identified alone in five patients and as a double mutation in two patients. Among these, six patients had Type 1 GD, and one patient had Type 3 GD [18], [22].



Graph 7. L444P mutation in GD patients

Conclusion

In conclusion, this study expands the understanding of the clinical and genetic characteristics of GD in the Albanian population. Novel mutations were found, and the prevalence of known mutations was determined. The variability in disease manifestations and severity highlights the complex relationship between genotype and phenotype in GD. Further research is needed to elucidate the specific effects of certain mutations on phenotype expression.

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Conflict of interests

The authors declare no conflict of interest.

Ethical approval

The study was performed in accordance with the ethical standards of the national ethics committee and received their approval. Patient`s parent consent for publication: Obtained

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