Compound heterozygous p.L483P and p.S310G mutations in GBA1 cause type 1 adult Gaucher disease: case report with a novel mutation

Xiaoling Wen (517349427@qq.com)
Second Hospital of Shanxi Medical University https://orcid.org/0000-0001-7300-4027
Yaozi Wang
Second Hospital of Shanxi Medical University
Xialin Zhang
Shanxi Academy of Medical Sciences: Shanxi Bethune Hospital
Zhijuan Zhang
Second Hospital of Shanxi Medical University
Xiaxia Liu
Second Hospital of Shanxi Medical University
Haiyan Lu
Children's Hospital of Shanxi
Guoping Hao
Children's Hospital of Shanxi
Xiaohuan Wang
Children's Hospital of Shanxi
Linhua Yang
Second Hospital of Shanxi Medical University
Ruijuan Zhang
Shanxi Academy of Medical Sciences: Shanxi Bethune Hospital https://orcid.org/0000-0003-1147-9240

Research Article

Keywords: Gaucher disease, Parkinson's disease, lipid metabolism, molecular mechanism

Posted Date: April 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1559790/v1

Abstract

Background

Gaucher disease (GD) is a rare autosomal recessive hereditary lysosomal storage disease. It is caused by glucocerebrosidase (*GBA1*) gene mutation that leads to decreased activity of acid β-glucosidase (glucocerebrosidase [GCase]). GD genotype-phenotype correlation is not fully understood. The aim of the current study was to identify and characterize compound heterozygous mutations of *GBA1* in a type 1 adult GD patient.

Results

Using enzymological studies and whole exome sequencing, the patient with type 1 GD was found to becompound heterozygous for *GBA1* missense mutations in exon 10 c.1448T > C (p.L483P) and exon 7 c.928A > G (p.S310G). The patient's mother who had Parkinson disease history was heterozygous for p.S310G mutation. The asymptomatic father was heterozygous for p.L483P mutation. The sister did not carry either mutation. Structural modeling showed both p.S310G and p.L483P are far away from the GCase active site. The p.S310G mutation in domain 1 could cause decreased stability between the α2 and α3 helices of *GBA1*. The p.L483P mutation in domain 2 could reduce the van der Waals force of the side chain and disrupt the C-terminal -sheet.

Conclusions

The p.L483P/p.S310G novel compound heterozygous mutation underlies type 1 GD and likely impacts GCase protein function. This is the first description of p.S310G associated with mild type 1 GD in the context of coinherited p.L483P mutation.

Background

Gaucher disease (GD) is a rare autosomal recessive hereditary lysosomal storage disease with a global incidence of about 1/40,000–1/60,000[1]. It arises from glucocerebrosidase (*GBA1*) gene mutations resulting in the accumulation of glucosylceramide in the reticuloendothelial system, leading to anemia, low platelet counts, and damage to the liver and spleen. *GBA1* (RefSeq: NG_009783.1) located at chromosome 1q21, spans 7.6 kb with 11 exons. There are 459 reported *GBA1* mutations, including point mutations, splicing, insertions and deletions, frameshift mutations, and recombination alleles, however not all are pathogenic[1]. GD is heterogeneous and classified into three types based on neurological severity[2, 3]. In type 1, neuronal features are not observed, and patients can be asymptomatic and present at any age[4]. Both type 2 and 3 GD have neurological involvement and present in infancy, but only type 2 results in early death.

Two significant pathogenic mutation are *GBA1* c.1226A > G (p.N370S) and c.1448T > C (p.L483P) which lead to type 1 and type 2 GD, respectively, in Ashkenazi Jewish populations[5]. Surprisingly, *in vitro* expression experiments demonstrated these *GBA1* mutation are relatively stable and active[6, 7]. *In vivo*, GCase is synthesized on the rough endoplasmic reticulum (ER) and then transported to the lysosome. *GBA1* mutations cause GCase cannot exit the ER and are destroyed by the ubiquitin-proteasome system[8].Thus, these GD patients have reduced amounts of acid β-glucosidase (glucocerebrosidase [GCase]) in the lysosome.

The current study examined the etiology of type 1 GD in a female adult patient and found double heterozygous for p.L483P and c.928A > G (p.S310G) mutations in *GBA1*. The p.L483P mutation is common in the Asian population, and homozygous p.L483P mutations result in a severe GD phenotype with neurological manifestation and early death[9]. The patient, with heterozygous mutation p.L483P/p.S310G had milder phenotype and did not have clinical GD symptoms until adulthood. The p.S310G mutation has only been reported in Parkinson's disease (PD)[10]. Its molecular pathogenesis remains unclear. In the current study, the effects of p.L483P/p.S310G on clinical manifestations of GCase protein and GD were preliminarily explored through gene diagnosis and protein structure analysis.

Materials And Methods

Patient

A 46-year-old female patient, presented with asymptomatic splenomegaly (ultrasound: spleen length 154mm, width about 41mm) in December 2016. She was hepatitis B surface antigen (HbsAg) positive, and her blood routine was normal. In December 2018, she complained of persistent right hip joint pain without mobility problems. Radiographic studies did not reveal significant hip joint or femur abnormality. Review ultrasound in March 2019 showed an enlarged spleen, (length 153mm, width about 57mm), and thrombocytopenia (52×10^9/L). In November 2019, another ultrasound showed continuing enlargement of the spleen (length 249mm, width about 66mm), and further decreased platelet count (40×10^9/L). With the exception of her mother's PD diagnosis, her family history was insignificant.

Auxiliary examination: blood routine: WBC 3.59×10^9/L, HGB 102.00g/L, PLT 34.00×10^9/L; bone mineral density testing on thoracolumbar and bilateral hip joints detected decreased bone mineral density; CT showed bilateral hip joint changes; MRI showed abnormal signal in the medullary cavity of the lower segment of the right femur, bone infarction, and abnormal signal in the medullary cavity of the left lower femur. Bone marrow cytomorphology examination: Gaucher-like cells accounted for 4.4%.

GD was suspected based on the patient's anemia, thrombocytopenia, splenomegaly and bone marrow cytomorphology examination **(Fig.1)**. To confirm the diagnosis, further enzymology and genotype tests were performed on the patient and patient's family.

Fig.1 Bone marrow cytomorphology (HE, 100×).

Enzymology test

Samples for leukocyte GCase activity was assayed by mass spectrometry on samples extracted from dried blood discs (or dried blood spots, DBS). The diagnosis of GD can be made when the GCase activity in peripheral blood leukocytes reduces to less than 30% of the normal value.

Genotype diagnosis

Genomic DNA was extracted from EDTA anticoagulated peripheral blood samples of the proband, her parents, and her younger sister were collected in EDTA anticoagulation tubes, using the QIAamp column method (QIAamp DNA Blood Mini Kit, 51104). After quality inspection on the extracted DNA, high-throughput whole-exome sequencing was conducted, and the Sanger sequencing was adopted for validation.

Protein structure analysis

The GCase protein sequence (NP_000148.2) and structure information (https://www.rcsb.org/structure/6T13) were obtained from the PDB database. The homology modeling software, SWISS-MODEL, was used to predict the structure of mutant GBA. RING 2.0 was used for intramolecular force analysis and visual image analysis using PyMOL program.

Treatment In January 2020, Imiglucerase was administered intravenously. The bone pain was relieved, and the blood routine was rechecked for many times, and the platelet was continuously lower than 30 × 10^9 / L. There was no significant improvement in spleen size..

Results

Enzymology and genetic analysis results of the proband and the family members

The patient's GCase activity of peripheral blood leukocytes was 3.8 nmol/(mg·h) (reference range: 10-25 nmol/[mg·h]). *GBA1* gene test showed heterozygous mutations of c.1448T>C (p.L483P)/c.928A>G (p.S310G) (**Fig. 2&3**). The diagnosis was GD (type 1). The father was a p.L483P heterozygote carrier, and the mother a p.S310G heterozygote carrier. The younger sister did not carry either mutation. Predictions made by PolyPhen-2, SIFT and Mutation Taster suggested that the p.L483P and p.S310G mutations might be pathogenic. According to ACMG guideline[11], the p.L483P was evaluated as pathogenic variant, and the p.S310G was probably pathogenic variant.

Fig.2 Family pedigree of the proband. The first-generation members have no information. The second-generation is the father of proband is heterozygous for exon 10 c.1448T>C (p.L483P) mutation, and the mother is heterozygous for exon 7 c.928A>G (p.S310G) mutation. The third generation is the proband who was compound heterozygous for p.L483P/p.S310G . Her sister was normal and did not carry either mutation.

Fig.3 DNA sequencing analysis of *GBA1* gene. **a** exon 7 c.928A>G (p.S310G) novel heterozygous missense mutation compared to **b** the corresponding wild-type sequence. **c** exon 10 c.1448T>C (p.L483P) heterozygous mutation, compared to **d** the corresponding wild type sequence.

Protein structure analysis

The GCase protein belongs to the GH30 family of glycoside hydrolases. Different from the traditional three-domain classification method, domain I and domain II were reclassified as domain 2, and the previous domain III was classified as domain 1[12,13].The GCase protein contains two domains, in which the active sites E274 and E379 are located in the β -folded plates β4 and β7 of Domain 1. The mutation point p.S310G was located at α-helix α4. Although it appeared to be closer to E274 in the secondary structure. In the three-dimensional structure, p.S310G was actually located at the bottom of α4 and E274 at the top of β4. The distance between the two was relatively far. The mutation point p.L483P was located at the β pleated-sheet βs6 in Domain 2, which was far away from the two active centers. Therefore, being far away from the GCase active centers, the mutation points p.S310G and p.L483P were predicted not to have a significant impact on the catalytic properties of the protein.

The p.S310G mutation was located at α-helix α4. In wild type, the C-β atom of the side chain of Ser residue formed the van der Waals force with the C-γ2 atom of the Val253 side chain and the C atom of the Leu307 main chain. In addition, the N atom of the Ser residue main chain formed a hydrogen bond with a length of 3.5 Å and 3.2 Å with the O atom of the Thr306 and Leu307 main chains, respectively; the O atom of the main chain formed a hydrogen bond with a length of 3.3 Å with the N atom of the His313 main chain. After Ser310 mutated into Gly310, the hydrogen bond of the main chain remained, while the van der Waals force formed by the side chain and surrounding residues disappeared.
The p.S310G mutation weakened the force between this point and Val253, which was located above the α helix α3, therefore might affect the stability between α2 and α3(**Fig. 4**).

Fig.4 Molecular contacts of residue 310. **a** wild type GCase protein. **b** Mutant Type.

p.L483P was located in the loop region connecting βs5 and βs6 (near βs6). In wild type, the side chain of Leu483 formed abundant van der Waals forces with the surrounding residues. The C-δ1 atom of its side chain could form van der Waals forces with C-δ2 atom of Leu104, C-γ1 atom of Val499, C-γ1 atom of Val507, and C-δ2 atom of Leu509, and the C-δ2 atom could form van der Waals forces with C-γ atom of Asn501 and C-β atom of Ser523. Those van der Waals forces were formed due to the interactions between the side chains. When Leu483 mutated into Pro483, the N-Cα rotation of Pro was bound by the pyrrolidine ring in its structure, thereby having less conformational freedom. This structure limited the diversity of its spatial conformation, especially in the loop region. The existence of Proline could help to stabilize the loop region which was originally more flexible. Therefore, theoretically, the p.L483P mutation located in the loop region, should have enhanced the stability of this region. But due to side chain changes, the original 6 van der Waals forces were reduced to 3, and the retained van der Waals forces were formed between C-γ of Pro483 and C-δ2 of Leu104 and C-γ1 of Val507, and one between C-β and C-γ1 of Val499. Thus it can be seen that the introduction of Pro stabilized the conformation of the loop

region, but the reduced van der Waals forces of the side chain might also affect the function of the protein structure (**Fig. 5**).

Fig.5 Molecular contacts of residue 483.**a** wild type GCase protein. **b** Mutant Type.

Discussion

GD is a rare inborn error of metabolism, secondary to *GBA1* gene mutations that lead to reduced GCase activity. The substrate glucocerebroside (also known as glucose ceramid) therefore accumulates in macrophage lysosomes to form Gaucher cells. Gaucher cells accumulates widely in the liver, spleen, bones, lungs, brain as well as other tissues and organs resulting in the progressively worsening dysfunction. Based on different clinical manifestations, GD can be divided into three types, of which type 1 (non- neuropathic) is the most common, accounting for about 95%[14]. GD can occur at all ages, with widely varied clinical manifestations. No primary central nervous system involvement is found in type 1 GD and enzyme replacement therapy has been the main treatment.

Previous study suggested that *GBA1* mutations were closely related to PD[5]. Carriers of *GBA1* gene mutations, such as p.N370S and p.L483P mutations, were found to increase the risk of PD disease by 2.16 times, with the characteristics of early onset age and declined cognitive ability. The possible pathogenic mechanism was that the mutations increased α-synaptic nucleoprotein aggregation[5, 15–17]. Coexistence of p.L483P with recombinant alleles, splice variants, or known severe missense alleles such as p.D488H and p.R159W has been reported to commonly lead to neuronopathic GD. When coexisting with mild mutations such as p.N409S and p.P305A, it manifests as non-neuronopathic GD[18, 19]. P.L483P has long been considered a serious pathogenic mutation, however, p.L483P has been reported to be associated with delayed onset of neurological symptoms in type 3 patients in Japanese patients[20], which may be related to possible genetic heterogeneity among different ethnic groups. Enzyme replacement therapy can significantly improve hematologic and other systemic symptoms in patients with type I GD, but its efficacy in patients with type 3 GD remains controversial. It has been reported in the literature that some type 3 patients developed symptoms of nervous system involvement after a median treatment period of 7.6 years[21]. In this study, the patient was treated with imiglucerase for 2 years, and the platelets continued to fail to recover, and the splenomegaly was not improved significantly. The proband with type 3 GD is the most common pathogenic mutation p.L483P, and her mother carries the p.S310G mutation has been confirmed to be a PD patient. Therefore, this proband is a high-risk patient for PD, and should be alert to the combination of neurological symptoms. The p.S310G mutation has only been reported in PD and to the best of our knowledge is first identified in GD. But its molecular mechanism and significance in GD still remain unclear. In our patient with type 1 GD the p.L483P mutation alone should not be sufficient to result in GD phenotype, suggesting that p.S310G mutation was contributory to her type 1 disease manifestation.

In this study, in order to further understand the significance of p.L483P/p.S310G compound heterozygous mutations in GD, the GCase protein structure analysis was conducted to probe into the pathogenic

characteristics of p.L483P/p.S310G mutations from the level of protein. The GCase protein belongs to the glycoside hydrolases GH30 family. The definition of the GH30 family protein structure was first reported in 2010[13]. Based on the overall characteristics of the GH30 family protein, this study divided the GCase protein into two domains. *GBA1* mutations can exert significant influences on the structure and function of the protein, including reducing the stability of domain III, premature termination of translation, and interference with catalytic activity and others[22–24]. Protein structure simulation was found that p.L483P and p.S310G mutation points were far away from the GCase active center, suggesting they should have little effect on the catalytic properties of the GCase protein. This also conformed to the patient's characteristics of late age of onset with mild clinical phenotype. Located in domain 2, the p.L483P mutation was believed to affect the structure and function of the protein by reducing the van der Waals forces of the side chain. Earlier studies believed that p.L483P mutation could lead to decreased enzymatic activity[25], affect the catalytic activity of GCase[26], disrupt the hydrophobic core and domain folding[17] or alter protein stability by reducing intramolecular hydrogen bonding[22]. Our study is different from the previous study, which further enriches the understanding of the impact of L483P mutation on protein structure. In this paper we provided the first observed influence of the p.S310G mutation on the GCase protein. The p.S310G mutation in domain 1 could cause decreased stability between α2 and α3 of α helix of GCase protein, thus affecting the function of the GCase protein.

Conclusions

The current study verified that the p.L483P/p.S310G novel compound heterozygous mutation was the cause of GD, This mutation caused the disease probably by interfering the biological function of the GCase protein. A follow up study will be performed to assess the risk of PD in the p.L483P variant patient.

Declarations

Acknowledgments.

We thank the patient and her family members for participating in this study. This work was supported by funds from the Shanxi Key Research and Development Project(201903D321133) and Shanxi Bethune Hospital's talent introduction scientific research start-up fund project(2021RC038).

Authors contributions

RJZ, XLW and XLZ designed the study. XLZ and XLW performed the experiments. XLW and YZW wrote the manuscript draft. All authors contributed to revision the manuscript.

Availability of data and materials

Data are available by request.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all study participants. This study was approved by the Ethics Committee of The Third Hospital of Shanxi Medical University (Approval No. SBQKL-2021-052) and in accordance with the Principles of the Declaration of Helsinki.

Consent for publication

The consent for publication has been obtained from the patient and her family members.

Competing interests

The authors declared that they have no competing interests.

Author details

[1]Department of Hematology, The Second Hospital of Shanxi Medical University, 382 Wuyi road, Taiyuan 030001, Shanxi, China. [2]Department of Hematology, The Third Hospital of Shanxi Medical University, The Shanxi Bethune Hospital, The Shanxi Academy of Medical Sciences, The Tongji Shanxi Hospital, 99 Longcheng street, Taiyuan 030032, Shanxi, China. [3]Department of Hematology, The Children's Hospital of Shanxi, 13 Xinmin north street, Taiyuan 030006, Shanxi, China.

References

1. Stenson PD, Ball EV, Mort M, et al. Human Gene Mutation Database (HGMD): 2003 update. Hum Mutat. 2003;21:577–81. https://doi.org/10.1002/humu.10212.
2. Guggenbuhl P, Grosbois B, Chalès G. Gaucher disease. Joint Bone Spine. 2008;75:116–24. https://doi.org/10.1016/j.jbspin.2007.06.006.
3. Horowitz M, Zimran A. Mutations causing Gaucher disease. Hum Mutat. 1994;3:1–11. https://doi.org/10.1002/humu.1380030102.
4. Bultron G, Kacena K, Pearson D, et al. The risk of Parkinson's disease in type 1 Gaucher disease. J Inherit Metab Dis. 2010;33:167–73. https://doi.org/10.1007/s10545-010-9055-0.
5. Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. Lancet Neurol. 2012;11:986–98. https://doi.org/10.1016/s1474-4422(12)70190-4.
6. Alfonso P, Rodríguez-Rey JC, Gañán A, et al. Expression and functional characterization of mutated glucocerebrosidase alleles causing Gaucher disease in Spanish patients. Blood Cells Mol Dis. 2004;32:218–25. https://doi.org/10.1016/j.bcmd.2003.10.010.
7. Liou B, Kazimierczuk A, Zhang M, et al. Analyses of variant acid beta-glucosidases: effects of Gaucher disease mutations. J Biol Chem. 2006;281:4242–53. https://doi.org/10.1074/jbc.M511110200.

8. Schapira AH, Gegg ME. Glucocerebrosidase in the pathogenesis and treatment of Parkinson disease. Proc Natl Acad Sci U S A. 2013;110:3214–5. https://doi.org/10.1073/pnas.1300822110.
9. Tammachote R, Tongkobpetch S, Srichomthong C, et al. A common and two novel GBA mutations in Thai patients with Gaucher disease. J Hum Genet. 2013;58:594–9. https://doi.org/10.1038/jhg.2013.60.
10. Nuytemans K, Rajabli F, Bussies PL, et al. Novel Variants in LRRK2 and GBA Identified in Latino Parkinson Disease Cohort Enriched for Caribbean Origin. Front Neurol. 2020;11:573733. https://doi.org/10.3389/fneur.2020.573733.
11. Rehder C, Bean LJH, Bick D, et al. Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23:1399–415. https://doi.org/10.1038/s41436-021-01139-4.
12. Dvir H, Harel M, McCarthy AA, et al. X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. EMBO Rep. 2003;4:704–9. https://doi.org/10.1038/sj.embor.embor873.
13. St John FJ, Gonzalez JM, Pozharski E. Consolidation of glycosyl hydrolase family 30: a dual domain 4/7 hydrolase family consisting of two structurally distinct groups. FEBS Lett. 2010;584:4435–41. https://doi.org/10.1016/j.febslet.2010.09.051.
14. Biegstraaten M, Cox TM, Belmatoug N, et al. Management goals for type 1 Gaucher disease: An expert consensus document from the European working group on Gaucher disease. Blood Cells Mol Dis. 2018;68:203–08. https://doi.org/10.1016/j.bcmd.2016.10.008.
15. Behl T, Kaur G, Fratila O, et al. Cross-talks among GBA mutations, glucocerebrosidase, and alpha-synuclein in GBA-associated Parkinson's disease and their targeted therapeutic approaches: a comprehensive review. Transl Neurodegener. 2021;10:4. https://doi.org/10.1186/s40035-020-00226-x.
16. Creese B, Bell E, Johar I, et al. Glucocerebrosidase mutations and neuropsychiatric phenotypes in Parkinson's disease and Lewy body dementias: Review and meta-analyses. Am J Med Genet B Neuropsychiatr Genet. 2018;177:232–41. https://doi.org/10.1002/ajmg.b.32549.
17. Liu LY, Liu F, Du SC, et al. A Novel Functional Missense Mutation p.T219A in Type 1 Gaucher's Disease. Chin Med J (Engl). 2016;129:1072–7. https://doi.org/10.4103/0366-6999.180523.
18. Phetthong T, Tim-Aroon T, Khongkraparn A, et al. Gaucher disease: clinical phenotypes and refining GBA mutational spectrum in Thai patients. Orphanet J Rare Dis. 2021;16:519. https://doi.org/10.1186/s13023-021-02151-2.
19. Chiong MAD, Racoma MJC, Abacan MAR. Genetic and clinical characteristics of Filipino patients with Gaucher disease. Mol Genet Metab Rep. 2018;15:110–15. https://doi.org/10.1016/j.ymgmr.2018.03.010.
20. Tajima A, Yokoi T, Ariga M, et al. Clinical and genetic study of Japanese patients with type 3 Gaucher disease. Mol Genet Metab. 2009;97:272–7. https://doi.org/10.1016/j.ymgme.2009.05.001.

21. Lee NC, Chien YH, Wong SL, et al. Outcome of early-treated type III Gaucher disease patients. Blood Cells Mol Dis. 2014;53:105–9. https://doi.org/10.1016/j.bcmd.2014.05.007.
22. Kim YM, Choi JH, Kim GH, et al. The GBA p.G85E mutation in Korean patients with non-neuronopathic Gaucher disease: founder and neuroprotective effects. Orphanet J Rare Dis. 2020;15:318. https://doi.org/10.1186/s13023-020-01597-0.
23. Riboldi GM, Di Fonzo AB. GBA. Gaucher Disease, and Parkinson's Disease: From Genetic to Clinic to New Therapeutic Approaches. Cells. 2019;8:364. https://doi.org/10.3390/cells8040364.
24. Thirumal Kumar D, Eldous HG, Mahgoub ZA, et al. Computational modelling approaches as a potential platform to understand the molecular genetics association between Parkinson's and Gaucher diseases. Metab Brain Dis. 2018;33:1835–47. https://doi.org/10.1007/s11011-018-0286-3.
25. Malini E, Grossi S, Deganuto M, et al. Functional analysis of 11 novel GBA alleles. Eur J Hum Genet. 2014;22:511–6. https://doi.org/10.1038/ejhg.2013.182.
26. Fishbein I, Kuo YM, Giasson BI, et al. Augmentation of phenotype in a transgenic Parkinson mouse heterozygous for a Gaucher mutation. Brain. 2014;137:3235–47. https://doi.org/10.1093/brain/awu291.

Figures

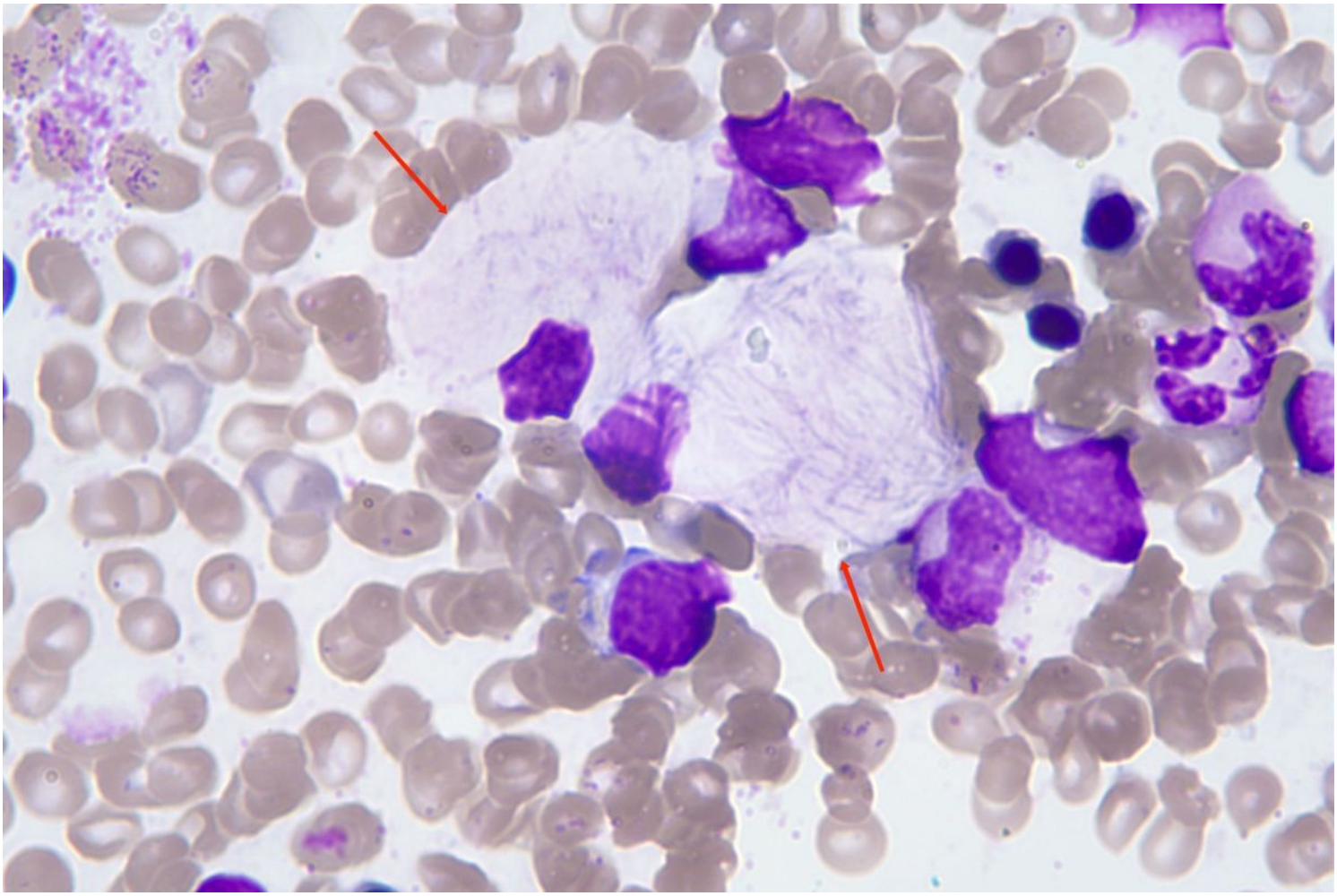

Figure 1

Bone marrow cytomorphology (HE, 100×).

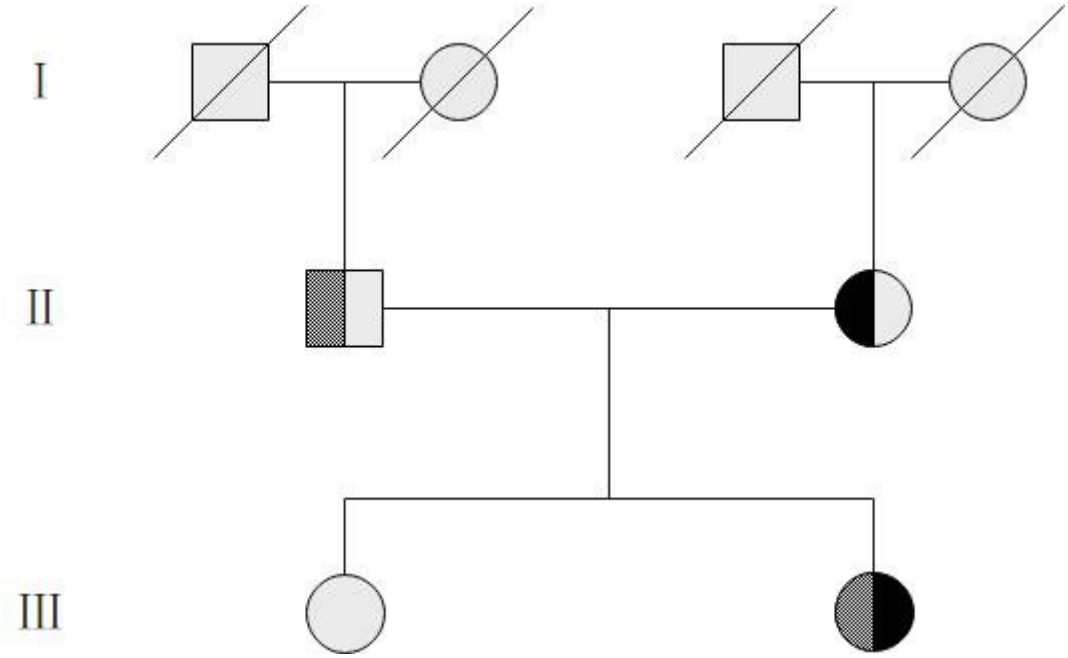

Figure 2

Family pedigree of the proband. The first-generation members have no information. The second-generation is the father of proband is heterozygous for exon 10 c.1448T>C (p.L483P) mutation, and the mother is heterozygous for exon 7 c.928A>G (p.S310G) mutation. The third generation is the proband who was compound heterozygous for p.L483P/p.S310G . Her sister was normal and did not carry either mutation.

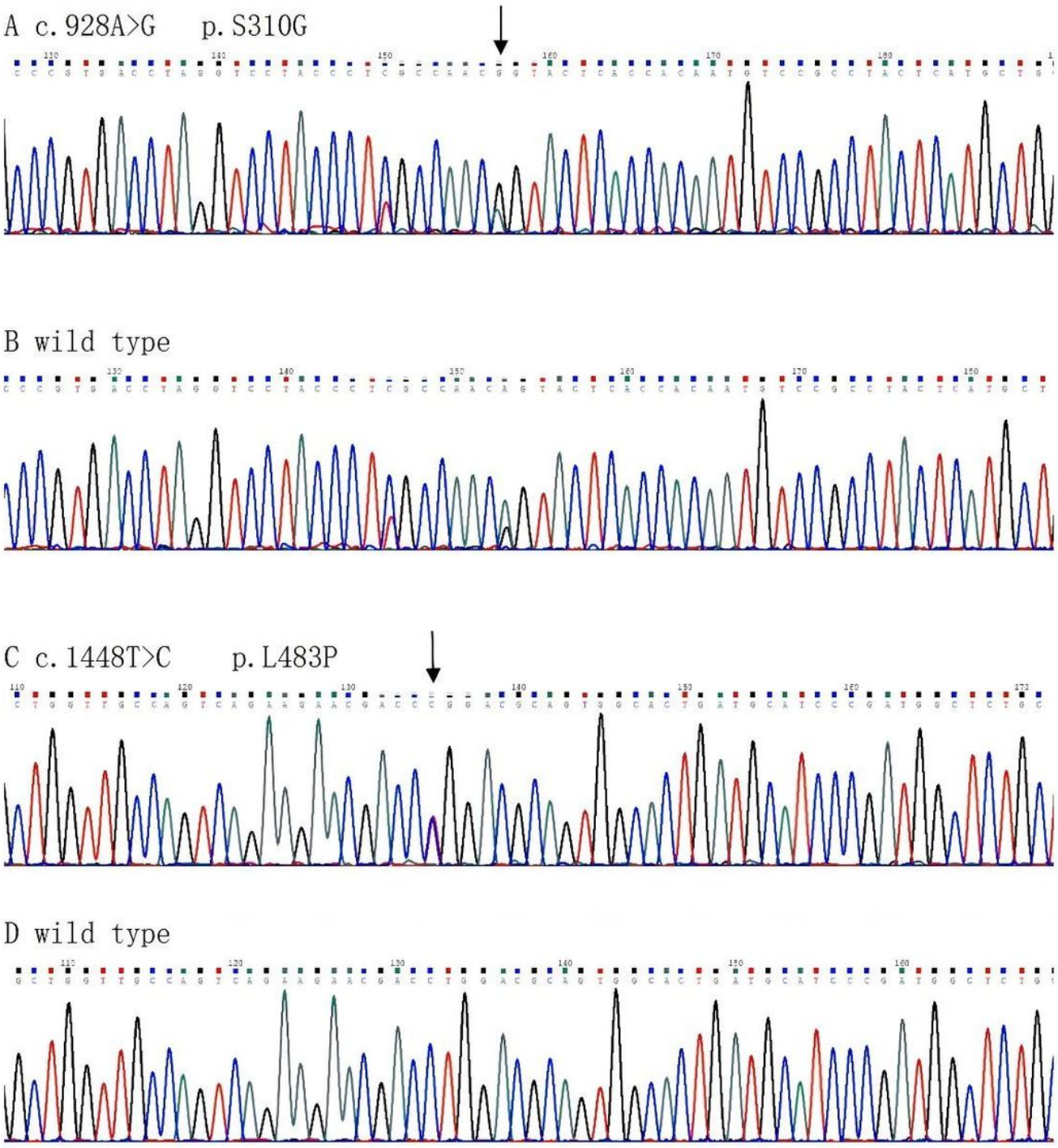

Figure 3

DNA sequencing analysis of *GBA1* gene. **a** exon 7 c.928A>G (p.S310G) novel heterozygous missense mutation compared to **b** the corresponding wild-type sequence. **c** exon 10 c.1448T>C (p.L483P) heterozygous mutation, compared to **d** the corresponding wild type sequence.

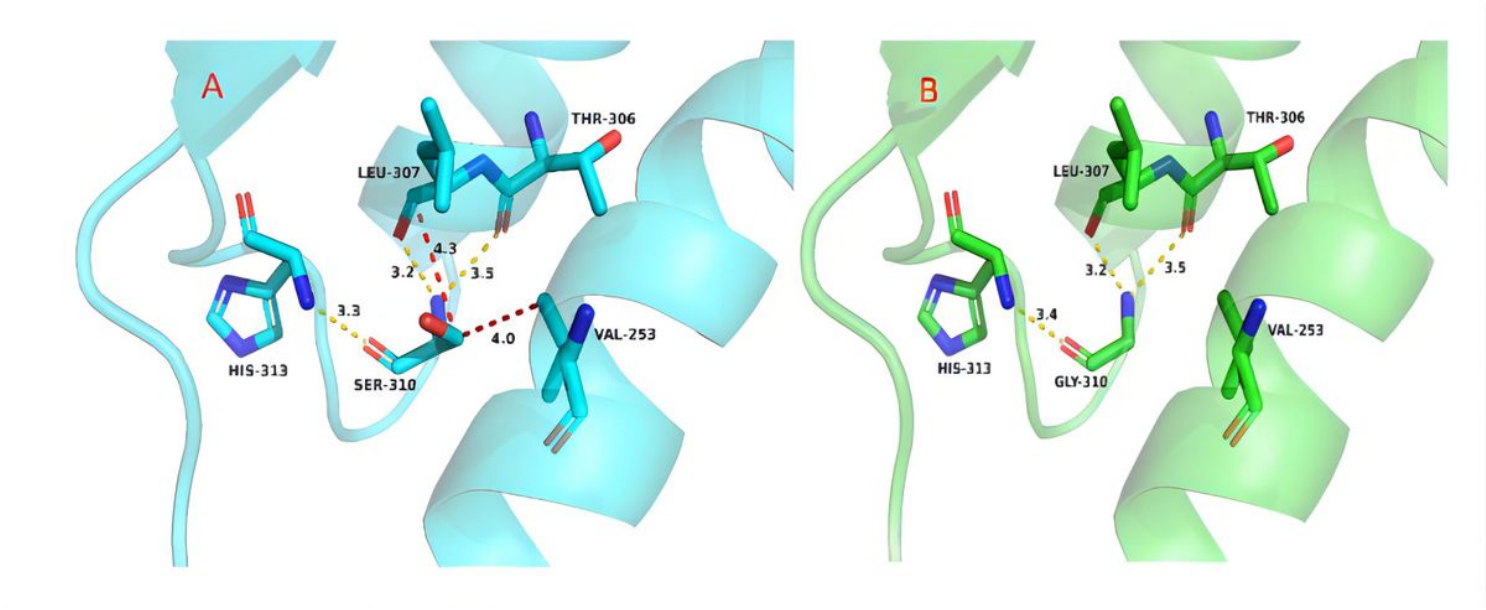

Figure 4

Molecular contacts of residue 310. **a** wild type GCase protein. **b** Mutant Type.

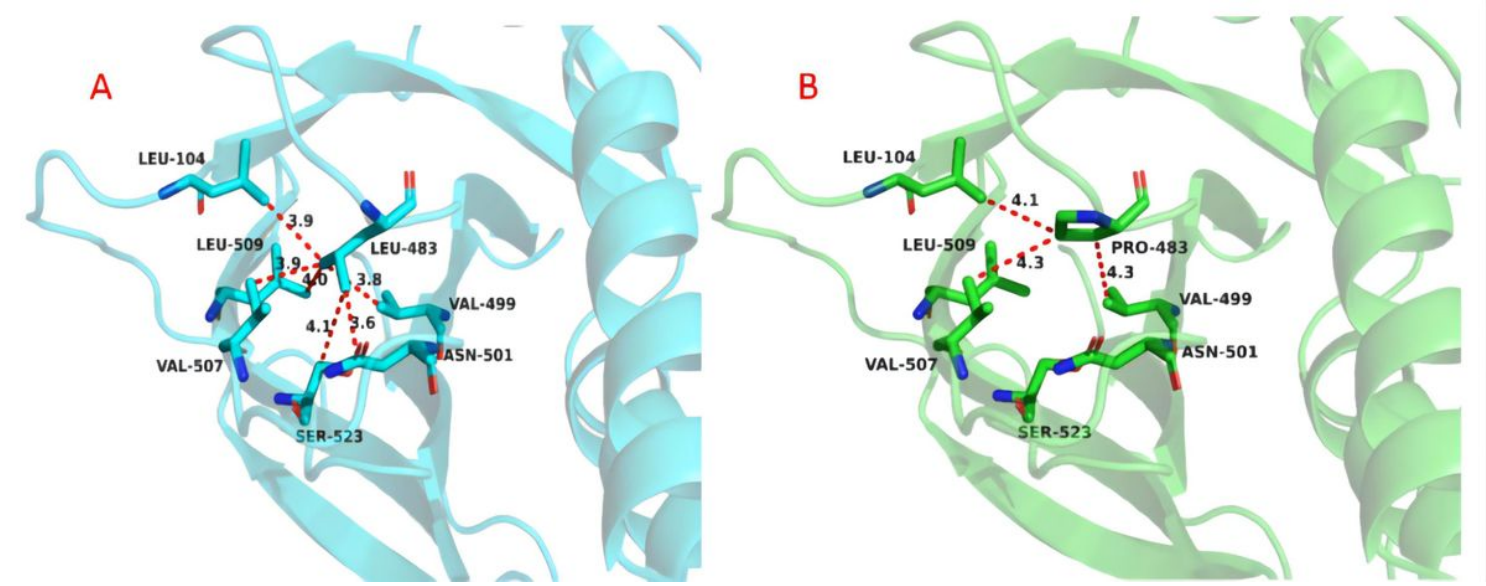

Figure 5

Molecular contacts of residue 483.**a** wild type GCase protein. **b** Mutant Type.