Genetic characterization of the Albanian Gaucher disease patient population

Paskal Cullufi | Mirela Tabaku | Virtut Velmishi | Agim Gjikopulli | Sonila Tomori | Ermira Dervishi | Aferdita Tako | Anika Leubauer | Ana Westenberger | Claudia Cozma | Christian Beetz | Peter Bauer | Stefan Wirth | Arndt Rolfs

1Pediatric Department, University Hospital “Mother Teresa”, Tirana, Albania
2CENTOGENE GmbH, Rostock, Germany
3Institute of Neurogenetics, University of Lübeck, Lübeck, Germany
4Department of Pediatrics, HELIOS University Hospital Wuppertal, Centre for Clinical and Translational Research, Wuppertal, Germany
5Medical Faculty, University of Rostock, Rostock, Germany

Correspondence
Christian Beetz, CENTOGENE GmbH, Am Strande 7, 18055 Rostock, Germany.
Email: christian.beetz@centogene.com

Communicating Editor: Roberto Giugliani

Abstract
Gaucher disease (GD) is a recessive metabolic disorder caused by a deficiency of the GBA gene-encoded enzyme β-glucocerebrosidase. We characterized a cohort of 36 Albanian GD patients, 31 with GD type 1 and 5 affected by GD types 2, 3, and an intermediate GD phenotype between type 2 and type 3. Of the 12 different GBA alleles that we detected, the most frequently observed was p.Asn409Ser, followed by p.[Asp448His;His294Gln]. The prevalence of the p.Leu483Pro allele was approximately 10-fold lower than reported in other populations. We identified a novel pathogenic missense variant (c.1129G>A; p.Ala377Thr). All five of our non-type 1 patients had genotypes consisting of the p.[Asp448His;His294Gln] allele in combination with another severe GBA allele. The median Lyso-Gb1 level of treated patients carrying the p.[Asp448His;His294Gln] and no p.Asn409Ser allele was significantly higher than that of treated individuals homozygous or compound heterozygous for the p.Asn409Ser allele. In conclusion, the most important distinguishing features of the Albanian GD patient population are the underrepresentation of the p.Leu483Pro allele and an unusually high number of p.[Asp448His;His294Gln] alleles originating from a common Balkan founder event. The presence of at least one p.Asn409Ser allele is associated with mild disease and low Lyso-Gb1 biomarker levels, while compound heterozygosity involving p.[Asp448His;His294Gln] and no p.Asn409Ser entails severe phenotypes and high Lyso-Gb1 levels.

KEYWORDS
Albanian population, Balkan region, Gaucher disease, GBA, genotype-phenotype correlation, Lyso-Gb1

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. JIMD Reports published by John Wiley & Sons Ltd on behalf of SSIEM.
1 | BACKGROUND

Gaucher disease (GD) is an autosomal recessive metabo-
lolic disorder caused by a deficiency of the lysosomal
enzyme, β-glucocerebrosidase (also called acid β-glucosi-
dase), responsible for the hydrolysis of the sphingolipid
glucosylceramide. A decrease in β-glucocerebrosidase
activity is particularly perilous for macrophages as it
leads to a progressive accumulation of glucosylceramide
in their lysosomes. The swollen macrophages, termed
Gaucher cells, subsequently infiltrate the liver, spleen,
and bone marrow and elicit a variety of GD pheno-
types.1,2 Another consequence is the accumulation of the
sphingolipid glucosylsphingosine (also known as Lyso-
Gb1), which represents both a diagnostic and monitoring
biomarker.3,4

Three main clinical types (1-3) of GD are distin-
guished based on age at onset and involvement of the
nervous system. While absence of neurological symptoms
defines GD type 1, nervous system involvement defines
types 2 and 3. Type 2 is distinguished from type 3 by
onset in infancy and, if untreated, by a severe course and
often fatal outcome.5

β-Glucocerebrosidase is encoded by the GBA gene
and to date, ~500 pathogenic or likely pathogenic GBA
alleles have been reported.6 Of note, a difference in the
distribution of pathogenic GBA alleles has been observed
in different ethnic populations7-10 and attributed to foun-
der effect.10,11

We investigated a large, ethnically homogeneous
group of consecutively ascertained Albanian GD patients
and report the types and frequencies of GBA alleles spe-
cific for this patient population. We also suggest a
genotype-phenotype correlation for this cohort.

2 | PATIENTS AND METHODS

All 36 participants (including the 21 previously reported
patients12-14) were recruited at the University Hospital
Center “Mother Teresa” in Tirana, Albania. Genomic
DNA was prepared from dried blood spot samples
(CentoCard®). Genotyping was a three-step procedure:
(a) an initial long-range PCR was used to specifically
amplify the whole GBA gene (but not the GBAP
pseudogene), (b) in a second PCR, the product from step 1
was used as a template to amplify all exons of GBA
individually, and (c) the products from step 2 were
paired-end sequenced on an Illumina MySeq with >20x
coverage for all exonic nucleotides plus the 10 neighbor-
ing intronic/UTR nucleotides. The two novel GBA vari-
ants were annotated and scored using PolyPhen2,15
SIFT,16 and combined annotation dependent depletion.17

Variant frequencies in the general population were esti-
mated using the Genome Aggregation Database.18 The
identified variants are labeled according to the Human
Genome Variation Society recommendations.19 Upon the
presence of two heterozygous variants, phasing was
determined by checking individual reads (when both var-
iants were in the same exon) and/or by considering the
genotypes of parents (in the familial samples); in the
remaining such cases, the indicated in trans constellation
was concluded from the presence of a GD-compatible
phenotype and an increased value for the biomarker
Lyso-Gb1. Lyso-Gb1 levels were quantified as described
previously.3 Lyso-Gb1 values above the cutoff of
6.8 ng/mL were considered pathological. At the time of
measuring the Lyso-Gb1 values, 24 patients were treated
with taliglucerase alfa. A nonparametric Mann-Whitney
U test was used for statistical analysis.

3 | RESULTS

Through the only national GD center in Albania, we
identified 36 patients that had a median age at examina-
tion of 19 years (interquartile range [IQR]: 12.0-35.5 years; range: 3 months-68 years) (Table S1). Among
these 36 individuals, 28 were unrelated index
patients (4 familial and 24 sporadic; Table S2) and 8 were
affected family members. Notably, two unrelated index
patients formed one of the families through marriage.14
The three remaining families consisted of sibling pairs.

The molecular genetic testing identified 12 different
GBA alleles in 27 of our index patients, while one variant
in one index patient could not be phased. The most com-
mon GBA allele (A7; 42.6%) contained a p.Asn409Ser
change and was found in 21 index patients (twice homo-
zygously) and in 8 different genotypes (eg, in patients
1, 2, 5, 6, 17, and 32-34, Table S2). The second most fre-
quent allele (A9; 38.9%) encompassing p.Asp448His and
p.His294Gln variants was identified in 20 index patients
and in six genotypes (eg, in patients 6, 19, 25-27, and 36,
Table S2). All other GBA alleles were found in a heterozygous state in single index patients. In addition to the p.[Asp448His;His294Gln] allele, two more alleles consisted of two alterations: A8 (likely “RecΔ55 conversions”\textsuperscript{20}), with p.Leu422Profs*4 and p.Asp448His, and A11, a recombinant allele (historically known as RecNci\textit{I}) with p.Leu483Pro and p.Ala495Pro variants derived from the GBA pseudogene. As mentioned above, the genotype of one index patient (patient 14, Table S2) contained three changes, however, the p.Arg368His alteration could not be phased, and thus it remains unclear whether it comprises an allele with p.Asn409Ser or p.Leu483Pro. Of note, the marriage of two of our index patients (patients 31 and 32, Table S2) resulted in three affected offspring (patients 33-35) with novel genotypes emerging from the combination of their parental alleles, as previously reported.\textsuperscript{14} Thus, overall, our patients harbored 15 different genotypes (Table S2).

Two of the missense variants we identified (p.Ala377Thr and p.Arg368His) were not previously reported in GD patients. The p.Ala377Thr change has been found in\textit{trans} with another pathogenic allele and was thus classified as pathogenic (Appendix S1). The aforementioned p.Arg368His change was found in\textit{cis} with another pathogenic variant and therefore its pathogenicity could not be assessed (Appendix S1).

The majority of our 36 patients (n = 31; 86.1%, belonging to 23 different families) were affected by GD type 1, including all of the individuals (n = 27) that carried at least one p.Asn409Ser allele (Tables S1 and S2). In all five (13.9%) of the more severely affected patients, the p.[Asp448His;His294Gln] allele was compound heterozygous with another severe GBA allele (GD type 3: p.Phe252Ile or p.Leu483Pro, GD type 2: c.115+1G>A, and an intermediate GD phenotype between type 2 and type 3: p.[Asp448His;His294Gln]) (Tables S1 and S2). The median Lyso-Gb1 level in 18 treated patients with available values of this biomarker (Table S2, Figure 1) and at least one p.Asn409Ser allele (64.8 ng/mL; IQR: 32.1-88.8 ng/mL) was significantly lower (P = .0188516) than the median Lyso-Gb1 level measured in five treated individuals with at least one p.[Asp448His;His294Gln] allele and no p.Asn409Ser allele (104 ng/mL; IQR: 95.5-249.5 ng/mL). Given that only a single individual with at least one p.[Asp448His;His294Gln] allele and no p.Asn409Ser allele (776.0 ng/mL) was untreated, we could not perform the statistical analysis between the untreated groups. However, the median Lyso-Gb1 level in eight untreated patients with at least one p.Asn409Ser allele was 139.5 ng/mL (IQR: 67.2-238.0 ng/mL).

Short case reports of the patients with GD types other than one are given in Appendix S1.

4 | DISCUSSION

Our study represents a comprehensive description of the allelic spectrum of GBA in Albanian GD patients. Considering that there is only a single GD center in Albania, our cohort likely includes most, if not all patients diagnosed with GD in this land. Although this indicates a country-specific prevalence of ~1 in 80 000, this figure may be an underestimate. Namely, many Albanian patients are likely misdiagnosed given that they might not be able to reach our center and therefore may not be correctly examined and treated. Studies of the frequency of GD in newborns indicate that it may be between ~1 in 15 000\textsuperscript{21} and ~1 in 60 000.\textsuperscript{22}

The two most frequent alleles were p.Asn409Ser and p.[Asp448His;His294Gln] (Table 1).

When considering only GD type 1 patients (n = 24), p.Asn409Ser was present in 22 (91.7%) and p.[Asp448His;His294Gln] in 16 (66.7%) individuals. Interestingly, in other studies the two alleles most frequently reported

\[\text{FIGURE 1} \quad \text{Lyso-Gb1 values according to genotype. Measurements of Lyso-Gb1 biomarker from dried blood spot samples for each patient are plotted against their genotype. Green diamond signs indicate that the patient was undergoing treatment at the time the sample was taken. Red triangles depict Lyso-Gb1 values in untreated patients.}\]
are p.Asn409Ser (75%-100%) and p.Leu483Pro (22%-35%) while alleles containing the p.Asp448His change were found in less than 1% to 2% of patients.7,10,23,24 Thus, in the Albanian population, the frequency of patients with the p.Asn409Ser allele is comparable to that reported worldwide. In contrast, the p.Leu483Pro allele is considerably underrepresented and the p.[Asp448His;His294Gln] allele is overrepresented, with no allele harboring only the p.Asp448His variant identified among our patients. Importantly, such a high prevalence of the p.[Asp448His;His294Gln] allele in individuals of the same ethnicity indicates a shared allele/haplotype (and kinship) among all the carriers and thus corroborates a likely founder allele originating in the Balkans11,25,26 or plausibly even Albania. As a matter of fact, the low frequencies of alleles other than p.[Asp448His;His294Gln] in comparison to other populations are likely due to the commonness of this founder allele.

Two more alleles consisting of two variants each (recombinant RecNciI: p.[Leu483Pro;Ala495Pro] and likely recombinant: p.[Leu422Profs*4;Asp448His]14) were identified one time each.

The presence of mild GBA alleles such as p.Asn409Ser in trans with more severe alleles (eg, p.Asp448His) is known to protect patients from developing GD types with neurological involvement.27 Accordingly, our 18 patients carrying both p.Asn409Ser and p.[Asp448His;His294Gln] alleles manifested GD type 1. In contrast, all five of our non-type 1 patients had genotypes consisting of the p.[Asp448His;His294Gln] allele in combination with another severe GBA allele (Appendix S1). Of note, the median Lyso-Gb1 level of patients carrying the p.[Asp448His;His294Gln] and no p.Asn409Ser allele was significantly higher than that of individuals homozygous or compound heterozygous for the p.Asn409Ser allele. This finding not only confirms the neuro-protective nature of the p.Asn409Ser allele and the severe effect of p.[Asp448His;His294Gln] at a biochemical level, but also highlights the usefulness of Lyso-Gb1 as a biomarker in GD.3,28 Although, our patients were treated for different lengths of time, the length of this treatment (taliglucerase alfa) does not significantly impact Lyso-Gb1 levels, as we have recently shown.29

Patients homozygous for the p.[Asp448His;His294Gln] allele present with the most severe GD type 211 or

### Table 1

| Allele number | Allele designation | cDNA level | Protein level | Historical nomenclature | Allele count in index patients\(^a\) | Number of carriers (Het/Hom) |
|---------------|--------------------|------------|---------------|------------------------|---------------------------------|-----------------------------|
| A1            | c.115+1G>A         | p.?        | IVS2+1G>A     | 1                      | 1/0                             |
| A2            | c.256C>T          | p.Arg86\*  | p.Arg47X      | 1                      | 1/0                             |
| A3            | c.259C>T          | p.Arg87Trp | p.Arg48Trp    | 1                      | 1/0                             |
| A4            | c.437C>T          | p.Ser146Leu| p.Ser107Leu   | 1                      | 1/0                             |
| A5            | c.754T>A          | p.Phe252Ile| p.Phe213Ile   | 1                      | 1/0                             |
| A6            | c.1129G>A\(^b\)   | p.Ala377Thr| p.Ala338Thr   | 1                      | 1/0                             |
| A7            | c.1226A>G         | p.Asn409Ser| p.Asn370Ser   | 23                     | 19/2                            |
| A8            | c.[1265_1319del;1342G>C] | p.[Leu422Profs*4;Asp448His] | 55 bp deletion in exon 9 and p.Asp409His | 1 | 1/0 |
| A9            | c.[1342G>C;882T>G] | p.[Asp448His;His294Gln] | p.[Asp409His;His255Gln] | 21 | 19/1 |
| A10           | c.1448T>C         | p.Leu483Pro| p.Leu444Pro   | 1                      | 1/0                             |
| A11\(^c\)     | c.[1448T>C;1483G>C] | p.[Leu483Pro;Ala495Pro] | p.[Leu444Pro;Ala456Pro] | 1 | 1/0 |
| A12\(^d\)     | c.1505G>A         | p.Arg502Gln*2| p.Arg463Gln*2| 1 | 1/0 |

\(^a\) c.1103G>A (p.Arg368His) could not be phased and the index patient (patient 14; Table S2) with this variant was thus not considered above. This variant was found in patient 14 in cis with either a c.1226A>G or a c.1448T>C variant.

\(^b\) Novel variant.

\(^c\) A11 is also known as the recombinant allele RecNciI with the c.1448T>C and c.1483G>C variants derived from the GBA pseudogene.

\(^d\) A truncating variant first reported by Ohshima and colleagues.32
an intermediate GD phenotype between type 2 and type 3\textsuperscript{30} with early neurological impairment but slow progression of neurological symptoms.\textsuperscript{31} Accordingly, the boy from our cohort with the homozygous Balkan/Albania-specific allele presented with an intermediate GD phenotype between type 2 and type 3, including gradually progressing neurological signs such as neck rigidity, head retroflexion, and oculomotor apraxia at the age of 6 months. Now, at 3 years old, his clinical condition is stable with no further neurological deterioration.

In conclusion, the most important distinguishing features of the Albanian GD patient population are the underrepresentation of the p.Leu483Pro allele, which, in contrast to p.Asn409Ser and the “Balkan allele,” is known to be a mutational hotspot, and an unusually high number of p.[Asp448His;His294Gln] alleles originating from a common Balkan founder event. The presence of at least one p.Asn409Ser allele is associated with mild disease and low Lyso-Gb1 biomarker levels, while compound heterozygosity involving p.[Asp448His;His294Gln] and no p.Asn409Ser entails severe phenotypes and high Lyso-Gb1 levels. Our study provides the first comprehensive insight into the clinical, genetic, and biochemical spectrum of GD in the Albanian patient population.

**ACKNOWLEDGMENTS**
We thank the patients and their family members for participation.

**CONFLICT OF INTEREST**
Paskal Cullufi, Mirela Tabaku, Virtut Velmishi, Agim Gjikopulli, Sonila Tomori, Ermira Dervishi, Aferdita Tako, Anika Leubauer, and Stefan Wirth,\textsuperscript{4} declare that they have no conflict of interest. Ana Westenberger is a contract worker for CENTOGENE GmbH; Claudia Cozma, Christian Beetz, Peter Bauer, and Arndt Rolfs are employees of CENTOGENE GmbH.

**AUTHOR CONTRIBUTIONS**
Paskal Cullufi, Claudia Cozma, Peter Bauer, Stefan Wirth, and Arndt Rolfs participated in the conception and design of the study, analysis and interpretation of data, and in the critical revision of the manuscript for important intellectual content. Mirela Tabaku, Virtut Velmishi, Agim Gjikopulli, Sonila Tomori, Ermira Dervishi, Aferdita Tako, and Anika Leubauer contributed to the analysis and interpretation of data and to the critical revision of the manuscript for important intellectual content. Ana Westenberger and Christian Beetz participated in the analysis and interpretation of data and drafting the manuscript. All authors approved the submission.

**ETHICS STATEMENT**
The local ethics committee approved the study, and all the patients or their legal guardians gave written informed consent for the molecular analyses.

**ORCID**
Ana Westenberger \(\text{https://orcid.org/0000-0001-8062-6959}\)

Christian Beetz \(\text{https://orcid.org/0000-0001-7061-2895}\)

Peter Bauer \(\text{https://orcid.org/0000-0001-9414-4555}\)

**REFERENCES**

1. Stirnemann J, Belmatoug N, Camou F, et al. A review of Gaucher disease pathophysiology, clinical presentation and treatments. *Int J Mol Sci*. 2017;18(2):441. https://doi.org/10.3390/ijms18020441.

2. Mistry PK, Cappellini MD, Lukina E, et al. A reappraisal of Gaucher disease – diagnosis and disease management algorithms. *Am J Hematol*. 2010;86(1):110-115. https://doi.org/10.1002/ajh.21888.

3. Rolfs A, Giese A-K, Grütner U, et al. Glucosylsphingosine is a highly sensitive and specific biomarker for primary diagnostic and follow-up monitoring in Gaucher disease in a non-Jewish, Caucasian cohort of Gaucher disease patients. *PLoS One*. 2015;8(11):e79732. https://doi.org/10.1371/journal.pone.0079732.

4. Arkadir D, Dinur T, Revel-Vilk S, et al. Glucosylsphingosine is a reliable response biomarker in Gaucher disease. *Am J Hematol*. 2018;93(6):E140-E142. http://www.ncbi.nlm.nih.gov/pubmed/29473199.

5. Pastores GM, Hughes DA. Gaucher Disease: 1993. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20301446.

6. Stenson PD, Ball EV, Mort M, et al. Human gene mutation database (HGMD): 2003 update. *Hum Mutat*. 2003;21(6):577-581. https://doi.org/10.1002/humu.10212.

7. Emre S, Güarak F, Yüce A, Rolfs A, Scott R, Özen H. Molecular analysis of Turkish Gaucher disease patients: identification of novel mutations in glucocerebrosidase (GBA) gene. *Eur J Med Genet*. 2008;51(4):315-321. https://doi.org/10.1016/j.ejmg.2008.02.004.

8. Gómez G, Arias S, Cárdenas L, et al. GBA mutations in Gaucher type I Venezuelan patients: ethnic origins and frequencies. *J Genet*. 2017;96(4):583-589. http://www.ncbi.nlm.nih.gov/pubmed/28947706.

9. Sheth J, Pancholi D, Mistri M, et al. Biochemical and molecular characterization of adult patients with type I Gaucher disease and carrier frequency analysis of Leu444Pro - a common Gaucher disease mutation in India. *BMC Med Genet*. 2018;19(1):178. http://www.ncbi.nlm.nih.gov/pubmed/30285649.

10. Alonso P, Cenarro A, Pérez-Calvo JI, et al. Mutation prevalence among 51 unrelated Spanish patients with Gaucher disease: identification of 11 novel mutations. *Blood Cells Mol Dis*. 2001;27(5):882-891. https://doi.org/10.1006/bcmd.2001.0461.

11. Santamaria R, Michelakakis H, Moraitou M, et al. Haplotypic analysis suggests a single Balkan origin for the Gaucher disease [D409H;H255Q] double mutant allele. *Hum Mutat*. 2008;29(6):E58-E67. https://doi.org/10.1002/humu.20776.
12. Shehi B, Boçari G, Vyshka G, et al. Gaucher’s disease in Albanian children: Casuistics and treatment. Iran J Pediatr. 2011;21(1):1-7. https://pubmed.ncbi.nlm.nih.gov/23056756/.
13. Velmishi V. Clinical and diagnostic findings of 19 Gaucher patients in Albania. J Liver. 2013;2(2):1-4.
14. Cullufi P, Tabaku M, Beetz C, et al. Comprehensive clinical, biochemical and genetic screening reveals four distinct GBA genotypes as underlying variable manifestation of Gaucher disease in a single family. Mol Genet Metab Rep. 2019;21:100532 http://www.ncbi.nlm.nih.gov/pubmed/31709146.
15. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7(1):Unit7.20. https://doi.org/10.1002/0471142905.hg0720s76.
16. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073-1081. http://www.nature.com/articles/nprot.2009.86.
17. Rentzsch P, Witten D, Cooper GM, et al. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2019;47(D1):D886-D894. http://www.ncbi.nlm.nih.gov/pubmed/30371827.
18. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291. http://www.nature.com/articles/nature19057.
19. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat. 2016;37(6):564-569. https://doi.org/10.1002/humu.22981.
20. iteration of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. Am J Hum Genet. 2006;29(4):591. https://doi.org/10.1007/s10545-006-0316-x.
21. Kumar KR, Ramirez A, Göbel A, et al. Glucocerebrosidase mutations in a Serbian Parkinson’s disease population. Eur J Neurol. 2012;20(2):402-405. https://doi.org/10.1111/j.1468-1331.2012.03817.x.
22. Koprivica V, Stone DL, Park JK, et al. Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. Am J Hum Genet. 2000;66(6):1777-1786. https://doi.org/10.1086/302925.
23. Saville JT, McDermott BK, Chin SJ, et al. Expanding the clinical utility of glucosylsphingosine for Gaucher disease. J Inherit Metab Dis. 2020;43(3):558-563. https://doi.org/10.1002/jimd.12192.
24. Cozma C, Cullufi P, Kramp G, et al. Treatment efficiency in Gaucher patients can reliably be monitored by quantification of Lyso-Gb1 concentrations in dried blood spots. Int J Mol Sci. 2020;21(12):1-9. https://pubmed.ncbi.nlm.nih.gov/32605119/.
25. Michelakakis H, Moraitou M, Dimitriou E, et al. Homozygosity for the double D409H+H255Q allele in type II Gaucher disease. J Inherit Metab Dis. 2006;29(4):591. https://doi.org/10.1007/s10545-006-0316-x.
26. SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.
Appendix S1. Supporting information.

How to cite this article: Cullufi P, Tabaku M, Velmishi V, et al. Genetic characterization of the Albanian Gaucher disease patient population. JIMD Reports. 2021;57:52–57. https://doi.org/10.1002/jimd2.12167.