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Title: *SORD*-related peripheral neuropathy in a French and Swiss cohort: clinical features, genetic analysis and sorbitol dosage.

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Abstract

Background

Biallelic variants in *SORD* have been reported as one of the main recessive causes for hereditary peripheral neuropathies such as Charcot-Marie-Tooth disease type 2 (CMT2) and distal hereditary motor neuropathy (dHMN) depicting lower limb (LL) weakness and muscular atrophy. In this study, phenotype and genotype landscapes of *SORD*-related peripheral neuropathies were described in a French and Swiss cohort. Serum sorbitol dosages were used to classify *SORD* variants.

Methods

Patients followed in neuromuscular reference centres in France and Switzerland were ascertained. Sanger sequencing and NGS were performed to sequence *SORD* and mass-spectrometry was used to measure patients' serum sorbitol.

Results

Thirty patients had *SORD* peripheral neuropathy associating LL weakness with muscular atrophy, foot deformities (87%), sometimes with proximal LL weakness (20%) or distal upper limb weakness (50%). Eighteen had dHMN, nine had CMT2 and three had intermediate CMT. Most of them had a mild or moderate disease severity. Sixteen carried a homozygous c.757delG (p.Ala253Glnfs*27) variant, and 11 carried compound heterozygous variants among which four variants were not reported yet: c.403C>G, c.379G>A, c.68_100+1dup and c.850dup. Two unrelated patients from different origins carried a homozygous c.458C>A variant and one patient carried a new homozygous c.786+5G>A variant. Mean serum sorbitol levels were 17.01 mg/L ± 8.9 SD for patients carrying *SORD* variants.

Conclusions

This *SORD*-inherited peripheral neuropathy cohort of 30 patients shows homogeneous clinical presentation and systematically elevated sorbitol levels (22-fold) compared to controls with both diagnostic and potential therapeutic implications.

INTRODUCTION

Charcot-Marie-Tooth disease (CMT) and distal Hereditary Motor Neuropathy (dHMN) are the most common types of hereditary peripheral neuropathies(1). CMT is responsible for motor and sensory disabilities, usually affecting children and young adults. It can be secondary to either myelin (CMT1) or axonal (CMT2) damages and has a X-linked, recessive or dominant inheritance(2). Clinically, patients present with lower limb weakness and amyotrophy, foot deformities and in some cases, sensory symptoms. In CMT2, there is predominant axonal injury responsible for a decrease in the compound muscle action potential leading to muscle weakening and atrophy as well as decreased sensory action potentials, which may or may not be clinically significant. The detection rate for causal variants is estimated at 36% for axonal CMT(3).

dHMN differs from CMT2 in that only the peripheral motor axons are damaged leading to distal limb muscle weakness, sparing the sensory nerves. Inheritance is either autosomal recessive, dominant or X-linked(4). Common causative genes responsible for both dHMN and CMT2 such as *HSPB1*, *IHGMBP2* and *GARS1* have been described and therefore a phenotype overlap can be observed between these two forms of peripheral neuropathies(1),(5).

Both phenotypes have been associated with variants in the *SORD* gene as described by Cortese et al., in 2020(6). They identified 37 patients with the homozygous pathogenic variant c.757delG (p.Ala253Glnfs*27) in exon 7 and eight patients with a compound heterozygous variant in *SORD*. The carrier frequency in healthy controls for the c.757delG variant is estimated at 0.0004 in GnomAD (v2.1.1) (115 alleles over 277146) with one homozygous carrier reported (GnomAD v2.1.1). A total of 18 variants have been inventoried, summarized by Liu et al.(7) and one case described by Dong et al.(8) carried a compound heterozygous variant c.[404A>G;c.908+1G>C] without the c.757delG variant.

Recent studies(9),(5),(10),(8),(11) have further described the phenotype associated with *SORD* variants. The onset is generally around the second decade of life and is characterized with rather mild or moderate motor deficiency of the lower limbs.

In this observational descriptive study, 30 French and Swiss patients were clinically reported with peripheral neuropathies associated with homozygous and compound heterozygous pathogenic variants in the *SORD* gene and comparison of our findings with those reported in the literature was made. Furthermore, five novel pathogenic variants were identified and non-fasting serum sorbitol dosages were performed and helped in their classification as serum sorbitol is elevated when the variant is responsible for a *SORD* impairment as reported by Cortese et al(6).

PATIENTS AND METHODS

Design of the study cohort and biomolecular analysis of the *SORD* gene.

The study cohort gathered patients from France and Switzerland. Clinical features were collected by French and Swiss neurologists, molecular diagnosis was established in Switzerland and in Lyon and Marseille, France. Data were collected from January 2021 to June 2022 and for some CMT patients undiagnosed at the molecular level, past medical records were consulted, some of them dated back to 1991.

Thus, the molecular analysis was achieved for 768 patients with CMT clinical presentation. DNA was obtained from peripheral blood (Biobank CRB Assistance Publique des Hôpitaux de Marseille (CRB TAC AP-HM, Marseille, France [BIORESOURCES]). Patients underwent either Sanger or NGS panel sequencing. Sanger sequencing was performed in a molecularly undiagnosed cohort analysed retrospectively presenting a CMT2 or a dHMN with an early onset of disease (before 50 years old) and a compatible recessive mode of inheritance or sporadic cases. For that, a nested PCR using the same primers as Cortese et al.(6) was designed.

NGS panel sequencing with genes related to CMT diseases was done both in a molecularly undiagnosed cohort of patients with *SORD* phenotype analysed retrospectively and in a cohort of patients with broader CMT2 symptoms analysed

prospectively. To note, variants identified by NGS panels were confirmed with Sanger sequencing.

Patients with a *SORD* biallelic variant were referred as *SORD*+/+ and control patients were referred as *SORD*wt/wt.

SORD-mutated patients' enrolment and data collection.

Patients were identified during national meetings of the FILNEMUS network (French rare neuromuscular diseases Healthcare Network) gathering French molecular biology laboratories specialized in neuromuscular diseases with clinical neurology departments.

Disease severity was assessed using the validated CMT Neuropathy Score (CMTNS) or CMT Examination Score (CMTES)(12). Cases were classified as mild (CMTNS 0 to 10 or CMTES 0 to 7), moderate (CMTNS 11 to 20 or CMTES 8 to 16) or severe (CMTNS 21 to 36 or CMTES 17 to 28) according to their neurological examination.

All patients had electrodiagnostic studies by nerve conduction study and electromyography.

To note, Patient 18 in this cohort has already been described by Bernard et al.(13) (showing an unsusual phenotype which could be classified either juvenile Amyotrophic Lateral Sclerosis or dHMN with pyramidal signs).

A pooled analysis comparing our findings with those of the literature (PubMed keywords used: SORD, neuropathy, peripheral neuropathy) was performed with the clinical features collected.

A written and signed approval has been collected from the patients following French recommendations and in agreement with the local ethics committee rules. The study was ethically approved by the university ethic committee of Marseille under the registered number PADS22-191.

Sorbitol measurement in patients' serum with peripheral neuropathies

Non-fasting blood sorbitol was measured using HPLC-MS/MS in two French laboratories (Paris and Marseille). Sorbitol dosages were available for 55 patients: 22 patients with biallelic variants in *SORD*, 32 patients without *SORD* variants, identified as control patients (*SORD*wt/wt) and one unaffected heterozygote relative of patient 27.

Paris's laboratory used Li et al.'s(14) method and Marseille's laboratory used a method derived from Li et al.'s based on a HPLC-tandem mass spectrometry as well. The two methods were compared using 12 sera tested by the two laboratories: 6 from *SORD*+/+ patients and 6 from *SORD*wt/wt patients.

HPLC conditions of Marseille's laboratory: Acquity column BEH amide 1.7µm (2.1 × 100mm) eluent A acetonitrile heated at 35°C, eluent B 10mM ammonium acetate, isocratic elution 85% A and 15% B, flow rate, 0.6 ml/min. The retention time for sorbitol was 1.2 min. MS/MS conditions: H-ESI interface in negative ion mode, 4200V, ion transfer tube and vaporizer heated at 350°C. MRM transitions were 180.85/88.97 for sorbitol and 187.138/91.899 for internal standard.

Sample preparation: serum and plasma samples were collected from patients in Marseille to make a comparison of the two matrices (performed on 11 *SORD*wt/wt patients and 5 *SORD*+/+ patients). Serum and plasma samples underwent protein precipitation with acetonitrile as follows: 50µl of plasma/serum were mixed with 150µl acetonitrile and 25µl of internal standard (D-SORBITOL U-13C6, 98%+; Cambridge Isotop Laboratories) diluted to tenth. Samples were centrifuged at 13000rev/min for 10 minutes. 200µL of supernatant were injected in the liquid chromatography. Neither laboratory used Oasis HLB cartridges as recommended by Cortese et al. Calibration curve (0.05 to 50mg/L) was done using standards prepared in water supplemented with internal standard in Marseille and in *SORD*wt/wt patients' serum supplemented with sorbitol in Paris. Internal quality controls were also prepared in water for each experiment.

Statistical Analysis

Clinical variables were reported as mean ± standard deviation (SD) and range for continuous variables and percentages for categorial ones. Continuous variables from

sorbitol measurements were compared with a two-tailed Student's t test as specified in figures' legends. A p-value <0.05 was retained as significant.

RESULTS

Study cohort and number of CMT patients involved.

Thirty patients with biallelic *SORD* variants were eventually retained for the clinical and sorbitol dosage evaluation, see flow chart in figure 1.

Identification of biallelic *SORD* variants in undiagnosed inherited neuropathies.

Regarding patients with undiagnosed neuropathy analysed retrospectively, 9.4% of them (N=16/170) eventually had biallelic *SORD* variants. For the patients analysed prospectively, 2.3% (N=14/598) had biallelic variants in *SORD*. In total, we diagnosed *SORD* variants in 3.9% (N=30/768) patients of our cohort, 25 were diagnosed with NGS panels and five with Sanger sequencing, see figure 1. We have not experienced a false negative result in SORD analysis by panel that has secondarily been found positive by Sanger sequencing.

As a result, thirty patients were identified (P1 to P30) from 29 different families with biallelic variants in the *SORD* gene (NM_003104.6) including 27 French and 3 Swiss patients, 22 were sporadic cases and eight had family history of peripheral neuropathy, see data in table 3 supplementary appendix. Of all the patients, 27 carried the deletion c.757delG; p.Ala253Glnfs*27; chr15:45361217 (hg19, rs55901542), 16 in homozygous state and 11 were compound heterozygous. Two patients (P3 and P25) carried a homozygous c.458C>A (p.Ala153Asp) and one patient (P30) carried a new homozygous variant c.786+5G>A (p.?) altering the splicing. All those variants are shown in figure 2 and were classified as pathogenic by Varsome(15), see table 2.

The familial inquiry was available for six index patients (P3, P6, P12, P19, P24 and P27) and the pedigree was available for 21 families (see figure 3), consistent with the recessive mode of inheritance of *SORD*-associated peripheral neuropathy.

Pooled analysis and phenotype of patients carrying biallelic variants in *SORD.*

All clinical features of the 30 patients are summarized in table 1 and in table 4 supplementary appendix comparing our results with those published earlier by Cortese et al.(6), Dong et al.(8), Laššuthová et al.(10), Liu et al.(7), Frasquet et al.(9) and in table 3 supplementary appendix.

Twenty-one patients were from Europe (70%) eight from North Africa (26%) and one patient was from Reunion Island, Indian Ocean (4%). Of note, 73% (N=22/30) of cases were sporadic without evidence of family history. The male/female sex ratio was equal to 2. Mean age at disease onset was 12.36 ± SD 5.99 y.o and the mean age at examination was 31.3 ± SD 13.38 y.o.

The clinical diagnosis was dHMN in 18 patients (60%), axonal CMT (CMT2) in 9 patients (30%) and intermediate CMT in 3 patients (10%). All patients had distal lower limb weakness and six (20%) also had associated proximal lower limb weakness. For 43% (n=13/30) patients, the posterior compartment of the leg was affected: four patients (P1, P5, P6, P28) had the triceps surae more affected than the tibialis anterior, nine patients had both the triceps surae and the tibialis anterior affected similarly (P13, P14, P17, P18, P19, P22, P26, P27, P30) and seven patients had the tibialis anterior mostly affected (P4, P7, P8, P10, P12, P23, P24). Twenty-eight patients (93%) had concomitant distal lower limb amyotrophy. Twenty-six patients (87%) presented pes cavus or other foot deformities like hammer toes.

Disease-related symptom severity was classified as mild according to the CMTNS and CMTES scores in 25/30 patients (83%) and moderate in 5/30 (17%).

Patients with the homozygous c.757delG variant were the only ones to have scoliosis (four patients among the 16 homozygous c.757delG: P7, P21, P24, P26) and reduced pinprick superficial sensation of the lower limbs (three patients among the 16 homozygous c.757delG: P17, P19, P22), and they mainly presented dHMN (12 patients among the 16 homozygous c.757delG) rather than CMT2 or intermediate CMT.

Patients P3 and P25 with the homozygous c.458C>A (p.Ala153Asp) and patient 30 with the homozygous c.786+5G>A didn't show a different phenotype than those carrying either the homozygous c.757delG or compound heterozygous with c.757delG.

On nerve conduction studies, 67% (n=20/30) of the patients had a motor phenotype and 33% (n=10/30) a both motor and sensory one. Only three patients had reduced motor nerve conduction velocity of the median nerve.

Patients' sorbitol blood levels

Regarding the correlation between plasma and serum sorbitol levels, there were no statistically significant differences between the two matrices regarding control (p=0.62) and *SORD*+/+ patients (p=0.82), suggesting the analysis can be performed on both, see figure 4C and 4D.

Regarding the correlation between the two dosing laboratories, there were no statistically significant differences both for control (p=0.16) and *SORD*+/+ patients (p=0.80) as shown in figure 5 supplementary appendix. The control patients didn't show increased sorbitol level in any laboratories and *SORD*+/+ patients had increased sorbitol levels in Paris and Marseille in the same range of values.

Twenty-two *SORD*+/+ patients had a mean increased serum sorbitol level of 17.01 mg/L ± 8.9 SD. Among them, 11 patients carrying the homozygous c.757delG variant had mean serum sorbitol levels of 17.2 mg/L ± 4.24 SD and 10 patients carrying compound heterozygous variants in *SORD* had mean serum sorbitol levels of 17.3 mg/L ± 12.7 SD; the slight difference between both groups was not significant (p=0.74), see figure 4. One patient carrying the homozygous c.458C>A had a sorbitol level of 11.35 mg/L. To note, patient 16 with c.[458C>A;757delG] had the highest sorbitol level of 51.9 mg/L and had an early onset of disease at eight years old. He was the only patient of the cohort to have reduced pinprick superficial sensation in the upper limbs without any muscular atrophy and all deep tendon reflexes were present (as they also were for P2, P7, P9, P18). He presented a mild CMT2.

For the 32 control patients, the mean sorbitol level was 0.79 ± 0.52 (range: 0.27-2.17) mg/L. The unaffected heterozygote member of family 27 carrying only the c.553G>A

Het (p.Gly185Arg) variant had also normal sorbitol levels (1.13 mg/L) in accordance with the autosomal recessive inheritance of *SORD.*

Serum sorbitol levels of patients with biallelic *SORD* variants were 22-fold higher than controls ($p<0.0001$). No *SORD*+/+ patients had normal serum sorbitol level.

Regarding the time from onset of the disease to sampling, three groups of patients were analysed (5 to 10 years, 10 to 15 years and > 15 years). There was no statistically significant difference of sorbitol levels between the different groups, see figure 5C supplementary appendix.

DISCUSSION

In this study, 30 patients from France and Switzerland with peripheral hereditary neuropathies related to the *SORD* gene were reported.

Regarding the molecular diagnosis, five new variants were discovered, c.403C>G, c.379G>A, c.68_100+1dup, c.850dup together with c.757delG and a homozygous c.786+5G>A variant which has not been reported in the literature and has never been identified in GnomAD(16) (v2.1.1). The patients' phenotype with those new variants was not different from the rest of the cohort carrying other variants.

The age of onset of the disease was early in our cohort and was around 12 years old while it can be much higher in the CMT field(17). Our results are consistent with those already described in the literature, although dHMN phenotypes were more frequent in our cohort than in previously reported ones: 60% had dHMN compared to 40% and 11% respectively in Cortese et al. and Laššuthová et al.'s studies, 30% had a CMT2, compared to 51% and 77% respectively in Cortese et al. and Laššuthová et al.'s studies, and 10% had an intermediate CMT compared to 9% in Cortese et al. and 11% in Laššuthová et al.'s studies. It is important to mention that the posterior compartment of the lower legs seemed to be frequently involved and in four patients it was even more important than the anterior one. We also confirmed that *SORD*-associated peripheral neuropathies are generally mild or moderate in severity as 83% of patients had a mild neuropathy and 17% had a moderate one, consistent with Cortese et al.'s study showing mainly mild neuropathies in 67% of patients, whereas 31% had moderate forms.

Regarding the serum sorbitol levels, *SORDwt/wt* patients had mean 0.79 ± 0.52 (range: 0.27-2.17) mg/L. We found different sorbitol normal values in the literature. Grosz et al.(18) measured the plasma sorbitol level of one control patient which was 0.2 mg/L, consistent with the 0.164 ± 0.044 mg/L published by Preston et al.(19) (13 healthy control patients) and the 0,2 ± 0,068 mg/L reported by Shetty et al.(20) (12 patients measured).

Li et al.(14) published that the basal sorbitol level should be 0.72-1.46 µM which is approximately equivalent to 0.13-0.27 mg/L knowing that sorbitol's molar mass is 182.17g/mol. Cortese et al.(6) derived their dosing method from Li et al.'s but found basal sorbitol level of 0.046 ± 0.004 mg/L (10 control patients) which is 3 to 6 times lower.

The Human Metabolome Database reported various normal blood values : <2,198 µM equivalent to < 0.4 mg/L in a series of 33 patients published by Vanholder et al.(21) and another normal value of 13 (range 4 - 24) µM equivalent to 2,4 (0.7 – 4,4) mg/L. Finally, Hwang et al.(22) reported plasma sorbitol level of 0.02mmol/L in 25 pregnant women equivalent to 3.6 mg/L. As a result, our findings spread between the lowest and the highest sorbitol normal values found in the literature. The differences may be due to different extraction methods: for instance, the Oasis cartridges recommended by Cortese et al. were not used in this study.

SORD+/+ patients had sorbitol values of 17.01 mg/L ± 8.9 SD (range: 10-51,9) mg/L compared to the 14.82 mg/L ± 0.780 SD of Cortese et al.(6)The lowest value was 10 mg/L which is still almost 5 times higher than the highest normal sorbitol level of 2,17 mg/L. The personal history of diabetes was available for 23 patients, 18 of whom had a sorbitol dosage performed and none was diabetic suggesting the increased sorbitol levels were not due to diabetes.

Furthermore, the result of this potential biomarker must be correlated to the identification of biallelic *SORD* variants before making the diagnosis. Biomarkers play an interesting role in medicine as their follow up can be achieved when a clinical trial is launched to monitor the efficacy of a treatment. For instance, deoxysphingolipids have shown their usefulness in HSAN(23).

Patient 16 had the highest sorbitol level ever reported in the literature. His diagnosis was established at 8 years old which is earlier than the diagnostic age of the cohort

(mean SD 32±13.7 y.o.) and he was 56 years old when the sorbitol sampling was done. We hypothesized that the sorbitol level was increasing along the course of the disease but the comparison over time of sorbitol levels between the different groups showed no significant differences between various individuals suggesting sorbitol level remains stable, but this should be assessed by a prospective study evaluating sorbitol levels at different times within a same patient.

Laššuthová et al.**(10)** recommended the use of more specific primers they designed to prevent the sequencing of the pseudogene *SORD2P* and therefore having a false positive diagnosis. The primers used in our study were similar to those designed by Cortese et al. Nevertheless, in all 22 patients identified with biallelic *SORD* variants, sorbitol levels were pathologically increased. If our primers were to amplify and find a variant in the pseudogene *SORDP2* instead of *SORD*, it should not have enzymatic consequences and sorbitol levels should not be altered by a *SORDP2* impairment. They also suggest the use of the primers designed by Cortese et al. may result in an allele drop out as it lacks specificity to *SORD* and might hybridize with *SORDP2*. However, in our study, we were able to identify 11 compound heterozygous patients with increased sorbitol levels suggesting the two variants identified belonged to the two *SORD* alleles. Furthermore, patient 27 carrying c.[553G>A;757delG] transmitted to his son only the c.553G>A variant and patient 6 with c.[458C>A;757delG] inherited the c.757delG variant from his father and the c.458C>A variant from his mother proving variants were in trans in patients 27 and 6.

The polyol pathway transforms glucose into fructose in two steps: first glucose is metabolised into sorbitol by the aldose reductase and second, sorbitol is transformed into fructose by the sorbitol dehydrogenase. The aldose reductase inhibitors (ARI) lead to a decrease in sorbitol. Oyama et al.**(24)** explained that sorbitol accumulation in human umbilical vein endothelial cells results in increased osmotic pressure and oxidative stress leading to cell injury such as microvascular endothelial damages observed in diabetic neuropathy. Ranirestat, an ARI, was reported by Sekiguchi et al.**(25)** to decrease the erythrocyte sorbitol concentration (closely related to the nerve sorbitol content**(26)**) by 75.82% versus placebo group of diabetic patients. They also

demonstrated that ranirestat was responsible for an increase of 0.52 m/s in nerve conduction velocity after one year of treatment, counterbalancing the annual decrease of 0.5 m/s mentioned by Hotta et al.**(27)** According to the studies conducted by Hotta et al**(27)**,**(28)**. and Bril et al.**(29)**, the use of fidarestat, epalrestat and ranirestat, improved diabetic patient-reported neuropathy symptomatology and nerve conduction velocity. However, ARI may have limited impact as when the neuropathy is settled, the damages might be irreversible because peripheral nerves affected are not readily regenerated**(30)**. Thus, the treatment should be delivered as soon as possible after disease onset and neurologists should be aware of the clinical phenotype and perform a sorbitol and/or a molecular analysis.

CONCLUSION:

This study identifies a new European cohort of 30 patients carrying variants in the *SORD* gene. Five new variants were described: c.403C>G (p.His135Asp), c.379G>A(p.Gly127Arg), c.68_100+1dup, c.850dup and c.786+5G>A and the first three cases with homozygous c.458C>A (p.Ala153Asp) and homozygous c.786+5G>A were reported.

The serum sorbitol dosage is a functional test demonstrating the impairment of the sorbitol dehydrogenase. This dosage has three interests. Firstly, it allows geneticists to classify variants as pathogenic when sorbitol levels are increased. Secondly, it could be a first screening test achieved faster than the molecular one. Thirdly, it could be envisaged as a biomarker to monitor a therapeutic trial.

This study could be the starting point for a new clinical trial using aldose reductase inhibitors for those 30 patients.

Conflict of Interest: the authors have stated explicitly that there are no conflicts of interest in connection with this article.

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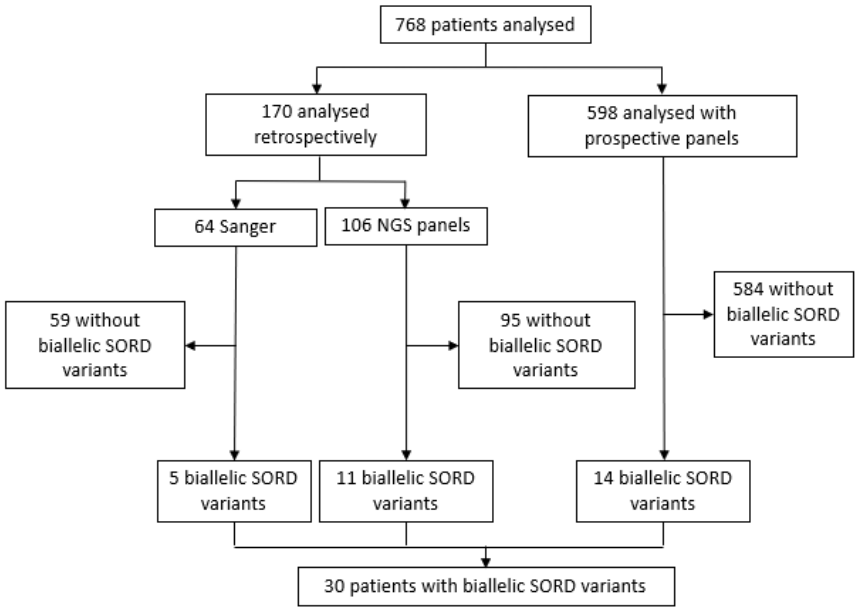


Figure_1: flow chart diagram representing our cohort of interest. In total, 30 patients had biallelic SORD variants and were included in our study.

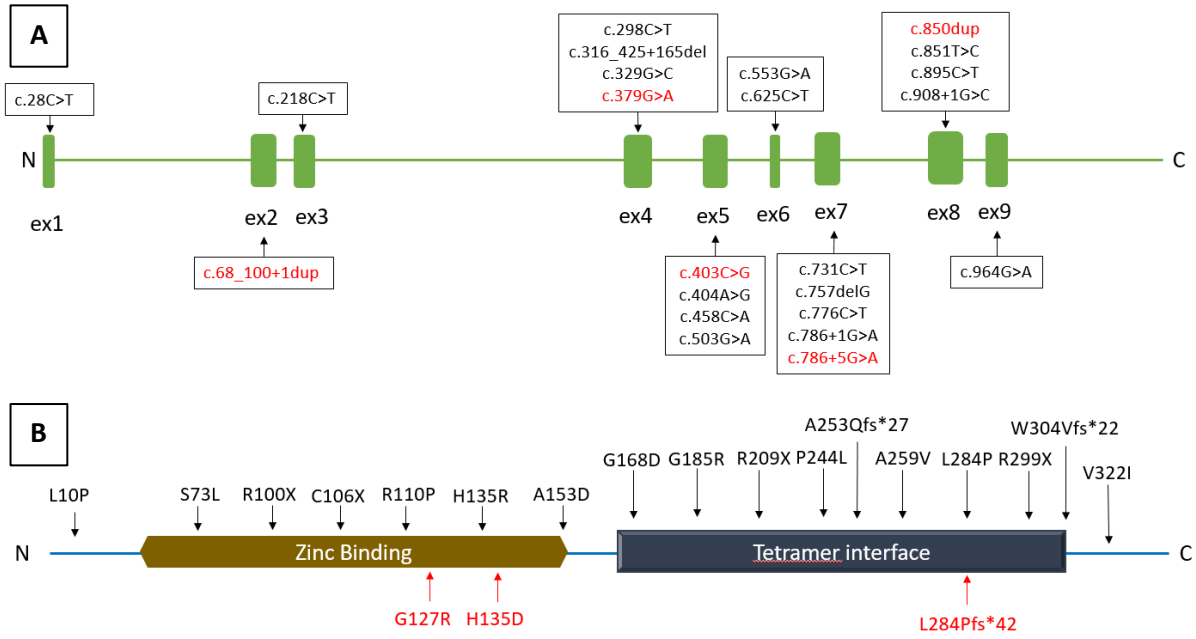


Figure_2: Representation of the *SORD* gene and the Sorbitol Dehydrogenase enzyme. A/ shows the positions of all the SORD variants identified in the literature. Five individuals (probands of families 1, 14, 23,28, 29) carried the c.757delG(p.A253Qfs*27) variant with a second unreported variant including c.379G>A (p.G127R) for family 14 and 23, c.403C>G(p.H135D) for family 1, c.68_100+1dup (p.?) for family 28, c.850dup for family 29 (p.L284Pfs*42) and proband of family 30 carried a homozygous c.786+5G>A(p.?). All new variants are marked in red. B/ represents the variant localisations in the protein.

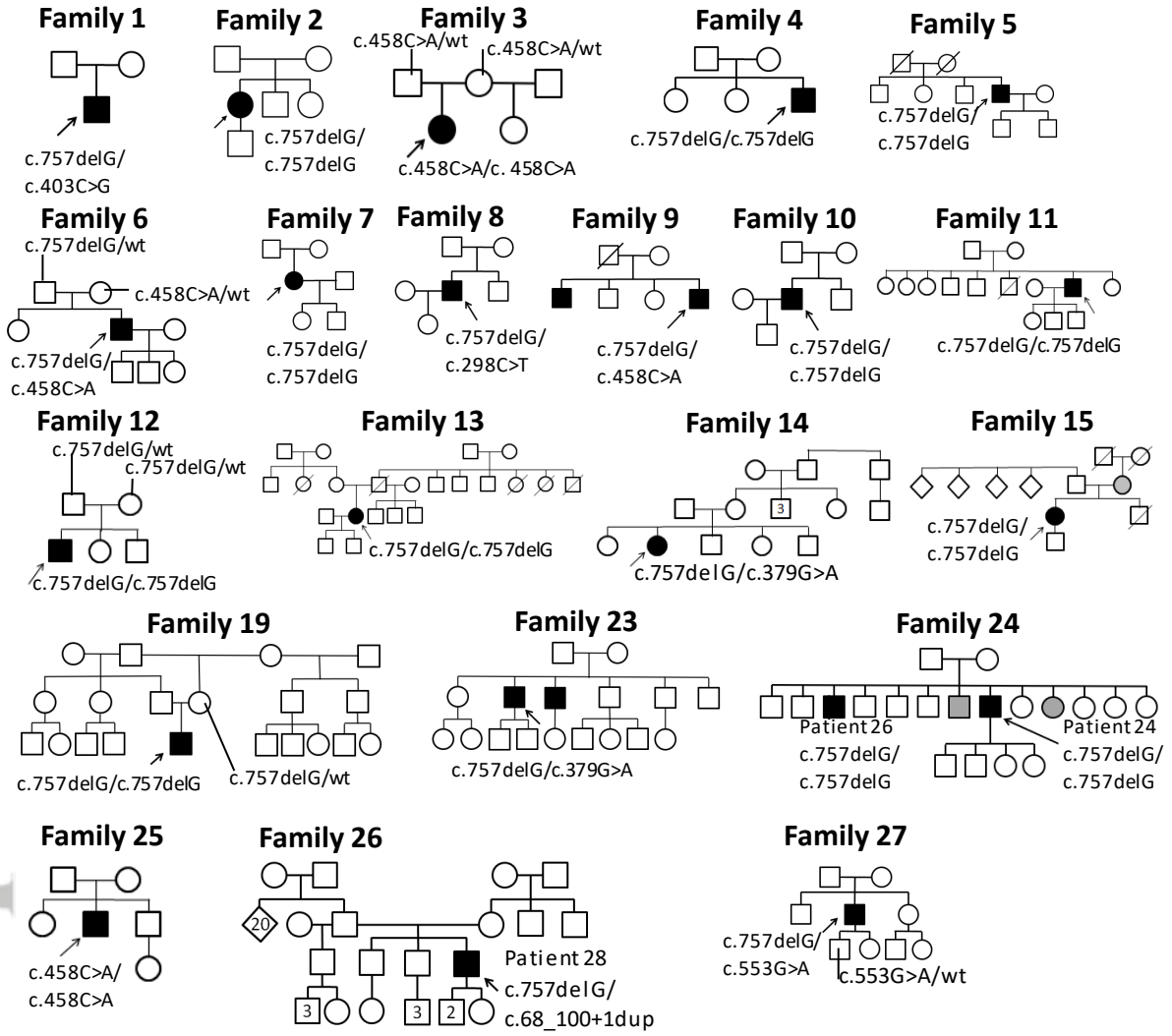


Figure_3: Pedigrees and genotypes of 21 families in our study with *SORD* variants. Squares stand for males, circles for females and diamonds for unspecified gender. Arrows represent index patients. Squares or circles crossed by a diagonal line are used for deceased individuals. Patients are represented with filled black shapes. Individuals with grey shapes show uncomplete phenotype. The number inside the shapes represents the number of individuals. wt: wild type.

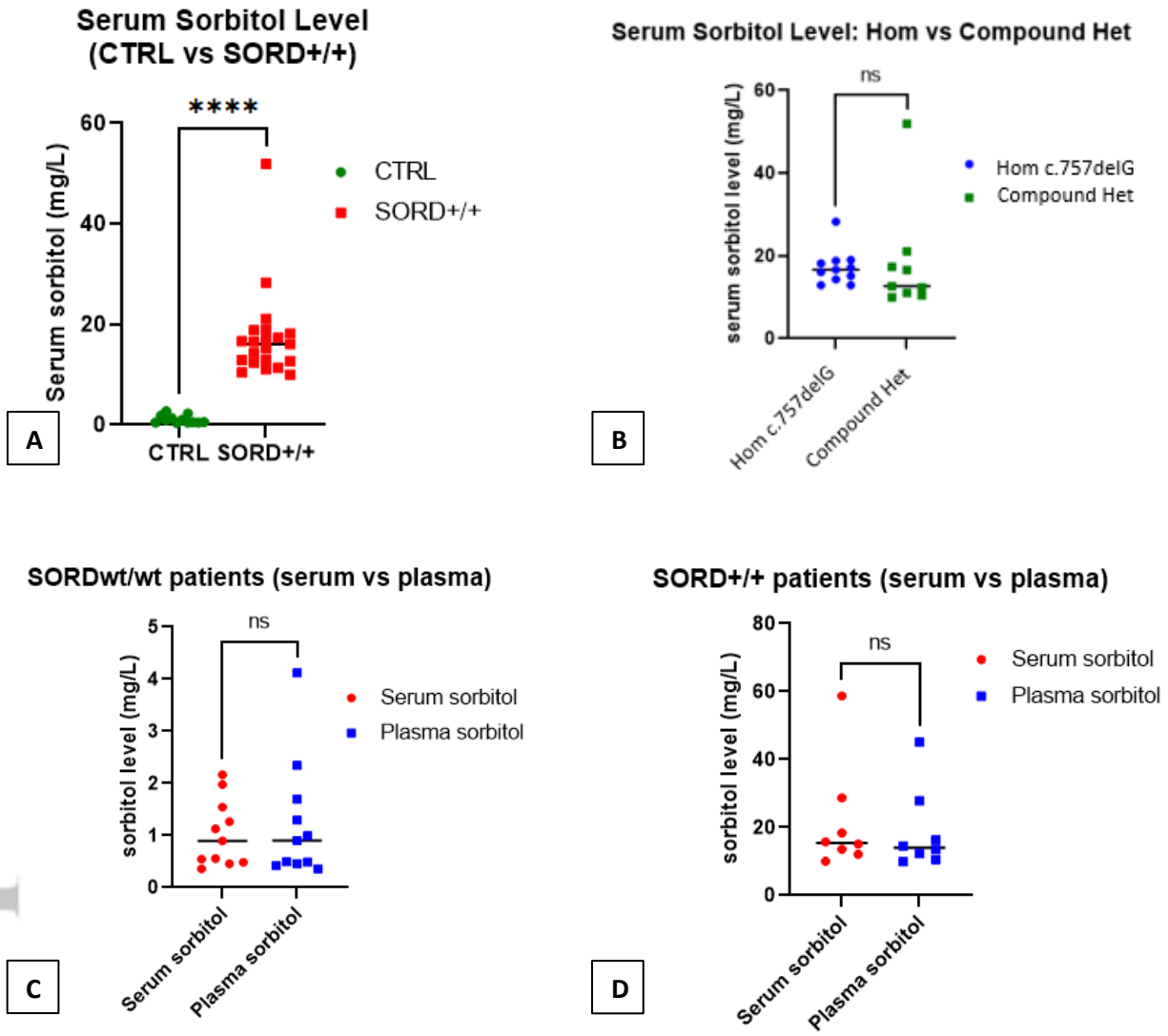


Figure_4: Sorbitol levels (mg/L) measured by high-performance liquid chromatography.
A/ sorbitol levels of patients carrying biallelic variants in *SORD* compared to controls. B/ Comparison of sorbitol levels between patients carrying a homozygous variant c.757delG and patients carrying compound heterozygous variants together with c.757delG. C/ Comparison of serum and plasma sorbitol levels in control patients. D/ Comparison of serum and plasma sorbitol levels in patients with biallelic mutations in *SORD*.
The graphs show the mean ± SD and data distribution (dots) and the p-value of two-tailed t-tests comparing sorbitol levels across groups - **** $p<0.0001$. ns stands for non-significant.

	N=30
Sex (Male)	21/30 (70%)
Family history	7/30 (23%)
Age of onset (years) Mean ± SD(min-max)	12,36 ± 5.99
Normal Psychomotor Development	16/30 (53%)
Age at examination (years) mean ± SD (min-max)	31.3 +/- 13.38 (8-64)
Neuropathy subtype:	
- CMT2	9/30 (30%)
- dHMN	18/30 (60%)
- CMT intermediate	3/30 (10%)
Pes cavus	26/30 (87%)
Abnormal walking	27/30 (90%)
Tiptoe impossible	22/30 (73%)
Walking on heels impossible	27/30 (90%)
Scoliosis	4/30 (13%)
Upper limb weakness	
- Proximal muscle groups	0/30
- Distal muscle groups	15/30 (50%)
Lower limb weakness	
- Proximal muscle groups	6/30 (20%)
- Distal muscle groups	30/30 (100%)
Axial weakness	1/30 (3%)
Facial weakness	0/30

	N=30
Limb tremor	7/30 (23%)
Muscular Atrophy	
- Proximal upper limbs	0/30
- Distal upper limbs	11/30 (37%)
- Proximal Lower limbs	2/30 (7%)
- Distal lower limbs	28/30 (93%)
Absence of Deep tendon reflexes	
- Achilean	24/30 (80%)
- Patellar	8/30 (27%)
- Upper limbs	2/30 (7%)
Reduced vibratory sensation	
- Lower limbs	13/30 (43%)
- Upper limbs	0/30
Reduced pinprick superficial sensation	
- upper limbs	1/30 (3%)
- Lower limbs	3/30 (10%)
Respiratory insufficiency	0/30
Diabetes	0/23
Overweight	5/23 (22%)
Disease severity-CMTNS:	
- Mild	25/30 (83%)
- Moderate	5/30 (17%)
- severe	0/30
Ankle-foot orthoses or other walking aids	16/30 (53%)
Nerve conduction study	
- Abnormal Motor Nerves	20/30 (67%)†
- Abnormal Sensory + Motor Nerves	10/30 (33%)

Table_1: Clinical features of patients with peripheral neuropathies associated with biallelic *SORD* variants of our cohort. n.a: not available, EMG: electromyography, LL: lower limb, RMCV: Reduced Motor Conduction Velocity, RSAP: Reduced Sensory Action Potential, RSNCV: reduced Sensory Nerve Conduction Velocity

†3/28 (11%) with RMCV

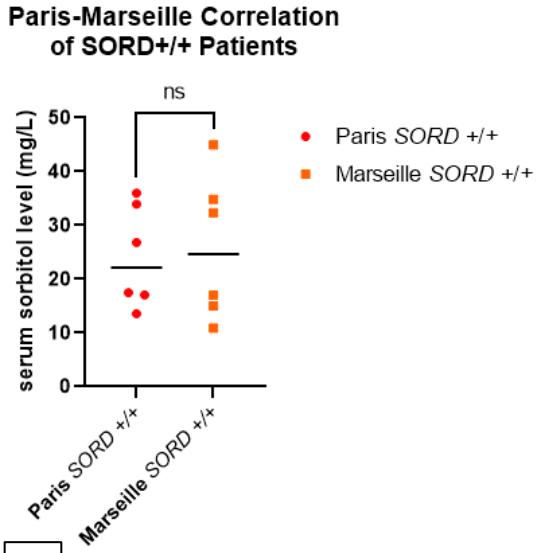
Variants identified	Patients with this variant	GnomAD (v2.1.1) frequencies	Pathoge-nicity score §	Sorbitol dosages
c.68_100+1dup (p.?) †	1 heterozygous	No GnomAD genome entry	Pathogenic	16.6 mg/L when associated with c.757delG
c.298C>T (p.Arg100*) ‡	1 heterozygous	0.007%	Pathogenic	12.4 mg/L when associated with c.757delG
c.329G>C (p.Arg110Pro) ‡	1 heterozygous	No GnomAD genome entry but has been described by Cortese et al.	Pathogenic	10.4 mg/L when associated with c.757delG
c.379G>A (p.Gly127Arg) †	2 heterozygous	No GnomAD genome entry	Pathogenic	10 mg/L when associated with c.757delG (one more patient not available for dosage)
c.403C>G (p.His135Asp) †	1 heterozygous	No GnomAD genome entry	Pathogenic	11 mg/L when associated with c.757delG
c.458C>A (p.Ala153Asp) ‡	3 heterozygous 2 homozygous	0.04%	Pathogenic	When associated with c.757delG: three different dosages of 21.1 mg/L, 12.7mg/L and 51.9 mg/L; When associated with another c.458C>A: 11.35 mg/L (one more patient not available for dosage)
c.553G>A (p.Gly185Arg) ‡	1 heterozygous	0.00003%	Pathogenic	17.34 mg/L when associated with c.757delG
c.757delG (p.Ala253Glnfs*27) ‡	16 homozygous	0.0004%	Pathogenic	Mean ± SD of 17.22 ± 4.24 mg/L (based on the dosage of 11 patients)
c.786+5G>A (p.?) †	1 homozygous	No GnomAD genome entry	Pathogenic	Not available
c.850dup (p.Leu284Profs*42) †	1 heterozygous	No GnomAD genome entry	Pathogenic	9.99 mg/L when associated with c.757delG

Table_2: table summarizing all the variants identified in this study

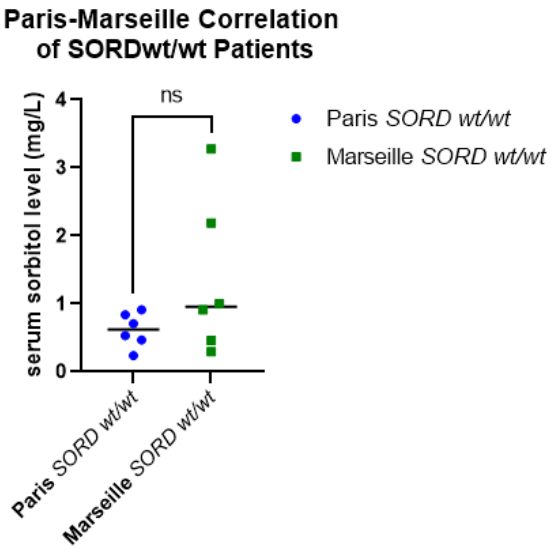
†: variant discovered in this study and never described in the literature

‡: variant already described in the literature

§: Pathogenicity score according to ACMG (Richards et al. 2015)



A



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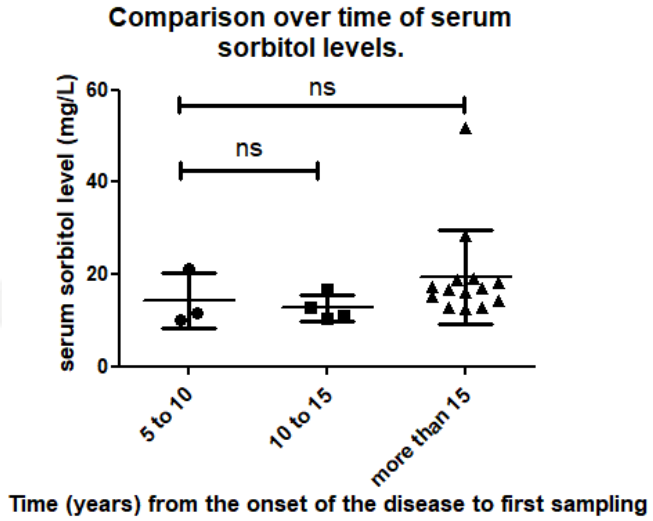

C

Figure_5 supplementary appendix: Sorbitol levels (mg/L) measured by high-performance liquid chromatography between Paris and Marseille comparing the two dosing methods.
A/ Levels of sorbitol levels of patients carrying biallelic variants in *SORD*: 6 positives patients were tested both in Marseille and Paris (p=0.80) B/ Levels of sorbitol levels of patients SORD-mutation free: 6 control patients were tested both in Marseille and Paris (p=0.16). C/ Comparison over time of serum sorbitol levels with one-way ANOVA test and Bonferroni post-hoc test.
The graphs show the mean ± SD and data distribution (dots) and the p-value of two-tailed t-tests comparing sorbitol levels across groups for figure 4A and 4B. ns stands for non-significant.