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SYSTEMATIC REVIEW

Mucolipidosis type II and type III: a systematic review of 843 published cases

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PURPOSE: Mucolipidosis (ML) II, MLIII alpha/beta, and MLIII gamma are rare autosomal recessive lysosomal storage disorders. Data on the natural course of the diseases are scarce. These data are important for counseling, therapies development, and improvement of outcome. The aim of this study is to gain knowledge on the natural history of ML by obtaining data on survival, symptom onset, presenting symptoms, diagnosis, and pathogenic variants associated with the MLII or MLIII phenotype.
METHODS: A systematic review on all published MLII and MLIII cases between 1968 and August 2019 was performed.
RESULTS: Three hundred one articles provided data on 843 patients. Median age at diagnosis: 0.7 for MLII and 9.0 years for MLIII. Median survival: 5.0 for MLII and 62.0 years for MLIIIII. Median age of death: 1.8 for MLII and 33.0 years for MLIII. Most frequent causes of death in all ML were pulmonary and/or cardiac complications. Pathogenic variants were described in 388 patients (*GNPTAB:* 571, *GNPTG* 179).
CONCLUSION: This review provides unique insights into the natural history of MLII and MLIII, with a clear genotype–phenotype correlation with the most frequent pathogenic variant c.3503_3504del in MLII and in MLIII alpha/beta c.22A>G for *GNPTAB*. All pathogenic *GNPTG* variants resulted in MLIII gamma.

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INTRODUCTION

Mucolipidosis (ML) type II (OMIM 252500), type III alpha/beta (OMIM 252600), and type III gamma (OMIM 252605) are rare autosomal recessive lysosomal storage disorders, with an estimated combined incidence of 0.22 to 2.70 per 100,000 live births[1–6]. As these diseases are very rare, data on the age of death and the natural course of the diseases are solely based on single case studies, small case series, or expert opinions.

Mucolipidosis, which is associated with significant morbidity and mortality, presents as a broad clinical spectrum with MLII at the severe end of the phenotypic spectrum and MLIII at the milder end.

Patients with the most severe form of MLII typically present prenatally or within the first months of life with a diversity of severe symptoms such as marked dysmorphic features, cardiac involvement, respiratory symptoms, dysostosis multiplex, severe growth abnormalities, and mental and motor developmental abnormalities. These patients generally die in early childhood[7–9]. MLIII has a much broader phenotypic spectrum than MLII, ranging from severely affected patients, MLIII alpha/beta patients who die in childhood (these patients may be regarded to have an intermediate form of ML, MLII/III alpha/beta[10,11]), to milder patients who survive into late adulthood[11–14]. MLIII gamma patients are generally the least severely affected of all ML patients[15]. MLIII patients often present at an older age than patients with MLII, with characteristic but less pronounced and less rapidly progressive dysmorphic features and skeletal changes, restricted joint mobility, short stature, and carpal and/or tarsal tunnel syndrome. Most patients with MLIII have normal intellectual capacity. Even though symptoms in MLIII may not result in mortality, they may cause severe morbidity and have a great impact on quality of life[16].

In MLII or MLIII the activity of the enzyme N-acetylglucosamine-1-phosphotransferase (GlcNAc-PTase) is absent or decreased, respectively. GlcNAc-PTase is a hexameric ($\alpha_2\beta_2\gamma_2$) enzyme that is encoded by two genes: *GNPTAB* (encoding for membrane-bound α- and β-subunits) and *GNPTG* (encoding for soluble γ-subunits)[14,17]. Pathogenic variants in *GNPTAB* that result in absent or significantly reduced GlcNAc-PTase activity, such as frameshift and nonsense variants, are related to the severe MLII phenotype[11,14]. If at least one of the two *GNPTAB* variants leads to residual enzymatic activity, the associated clinical phenotype is the more attenuated MLIII alpha/beta subtype[11,14]. Variants in *GNPTG* are associated with the milder MLIII gamma subtype.

In the *cis*-Golgi network GlcNAc-PTase catalyzes the first step of the formation of mannose 6-phosphate (M6P) on specific lysosomal soluble hydrolases by the transfer of a GlcNAc-1-phosphate residue from UDP-GlcNAc. A second enzyme (N-acetylglucosamine-1-phosphodiester α-N-acetylglucosamidase) removes the terminal GlcNAc, uncovering the M6P[18]. M6P is the essential targeting signal for the transport of more than 70 soluble lysosomal enzymes from the *trans*-Golgi network via endosomes to the lysosomes via two M6P receptors (MPR46 and MPR300)[19,20]. A small amount of newly synthesized lysosomal enzymes escape the M6P receptor binding and are secreted. M6P receptors (MPR300), exposed at the cell surface, recapture these lysosomal

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enzymes allowing their transport to lysosomes[21]. Without M6P, newly synthesized lysosomal enzymes are missorted into the extracellular space, and are therefore not able to breakdown their specific substrates in lysosomes[14,20,22]. These intralysosomal substrates accumulate in connective tissues, cartilage, bones, ligaments, and other tissues and are likely responsible for the clinical symptoms in MLII and MLIII patients. There are currently no curative or disease-modifying treatments available for both MLII and MLIII, but given the rapid development of novel therapies for monogenetic inborn errors of metabolism it is likely that therapies for ML, like gene therapy, will be available in the near future. To set up treatment studies and develop evidence-based clinical guidelines and follow-up protocols, thorough knowledge of the natural history of ML is essential. This would ideally be done with an international prospective or well-defined retrospective cohort study. However, given the extreme rareness of the disease this is very difficult to achieve. All published retrospective cohorts are small, with less than 70 patients, despite being multicenter[11,16,23–26]. A systematic review of all published cases can provide insights in the natural history, but has not yet been performed.

The aim of this study was therefore to gain knowledge on the natural history of ML by obtaining more robust data on survival, symptom onset, presenting symptoms, diagnosis, and genetic pathogenic variants associated with the MLII or MLIII phenotype, by performing a systematic review on all published MLII and MLIII cases and case series.

MATERIALS AND METHODS

A systematic search of the following electronic databases was conducted on the 17 September 2019: Medline, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and Google Scholar. The search strategy was structured using the keywords "Mucolipidosis type 2," "Mucolipidosis type 3," "Pseudo-hurler," I-cell," "Leroy." Details on the search strategy are shown in S1. Two reviewers (E.J.D. and E.O.) independently screened titles and abstracts of all articles to determine if articles were suitable for inclusion. All disagreements were discussed between the two reviewers, and all persisting disagreements were resolved by discussion with a third reviewer (M.W.). Publications were included if they contained clinical information on patients with the MLII or MLIII phenotype. There were no restrictions on the method used for diagnosis, which has only been genetically only possible since 2000/2005 when the *GNPTAB* and *GNPTG* genes were identified[17,27]. Many other papers published before 2000 describing ML patients, even the first historical reports, could be included as the clinical symptoms are very clear and enzyme missorting was measured in patients' blood, urine, and/or fibroblasts. No specific search duration was defined and all eligible papers published before the search date were included. Publications lacking clinical information or information on the phenotype of the patient were excluded, as were articles that included patients with an unclear phenotype or focused only on the MLI and IV phenotype; in vitro studies or animal, prenatal, molecular, and genetic models; or biomarkers. Only publications written in the English language were included. We tried to avoid double registration of patients, which may happen if data of one single patient are included in multiple articles, as much as possible by checking information on author, country, hospital, and patient characteristics.

Data extraction

The following data were extracted from included articles and entered into a database: demographic characteristics including phenotype, gender, symptom onset defined as the age at first presentation in the hospital, age at diagnosis, latest reported age, age at death, parental consanguinity, and genetic variants. In addition, information on presenting symptoms defined as symptoms reported at first hospital evaluation, potential life-extending procedures, and cause of death were collected. Potential life-extending procedures were defined as procedures that might have had an effect on the life expectancy of the patient.

In the analysis of the data we focused on the MLII and MLIII subgroups and did not incorporate data of the ML intermediate phenotype, as this subtype was introduced in 2010 and 2014 and the number of patients ($n = 14$) in our study were very small[10,11].

Annotation of variants

All published *GNPTAB* and *GNPTG* variant annotations were checked for accuracy and compared with a reference sequence. NM_024312.4 was used as a reference sequence for *GNPTAB* and NM_032520.4 was used as a reference sequence for *GNPTG*. Position c.1 represents the first nucleotide of the translocation start codon ATG located in exon 1. Variant annotations were described according to the current recommendations of the Human Genome Variation Society nomenclature guidelines[28].

Statistical analyses

The data were analyzed using the statistical software packages SPSS statistics 25 and R version 3.6.1. Descriptive statistics and frequencies were used calculating median, interquartile range (IQR), range, and percentages. For comparison, continuous data were compared with a Mann–Whitney *U*-test. Categorical data were compared with a chi-squared test. Analyses of more than two groups were performed with a Kruskal–Wallis test. Survival analyses were performed using the Kaplan–Meier and log-rank test. When the age of death was unknown, the record was censored in the analysis as the latest reported age. Furthermore, we analyzed survival hazard ratios (HR) by periods of 20 years (before 1980, between 1981 and 2000, and after 2001) with a Cox proportional hazards model. Two-sided P values ≤ 0.05 were considered significant.

RESULTS

A total of 1,666 abstracts were retrieved from the systematic search after deduplication. Subsequently, 1,301 records did not meet the inclusion criteria based on title and abstract screening. Of the 365 records that underwent full text screening, 64 did not meet the inclusion criteria and were also excluded, resulting in 301 publications that were included for data extraction (Fig. 1, and S2 for the list of the references of the included publications). The reasons for exclusion were (1) no clinical patient characteristics or only description of averages ($n = 42$), (2) unclear phenotypes or MLI or MLIV phenotype ($n = 12$), (3) focus on prenatal studies ($n = 9$), and (4) focus on animal studies ($n = 1$). All included articles were published between 1968 and August 2019.

Distribution of the MLII and MLIII phenotype and general characteristics

The 301 selected articles provided data on 843 patients with ML. Sixty-one percent ($n = 516$) of these patients had the MLII phenotype and 37.1% ($n = 313$) the MLIII phenotype (Table 1). Fourteen percent of the cases were published before 1980, 20.9% between 1981 and 2000, and 65.1% after 2001 (Table 1). Over time, the relative percentage of patients diagnosed with MLIII increased (Table 1).

The articles originated from 34 different countries, mostly from the United States, Japan, Italy, and China, followed by Israel, Brazil, and Turkey (S3).

The gender of the patients was reported in 626 of the cases as approximately 50% male and 50% female and there was no significant difference in distribution among MLII and MLIII phenotypes ($P = 0.677$, Table 1).

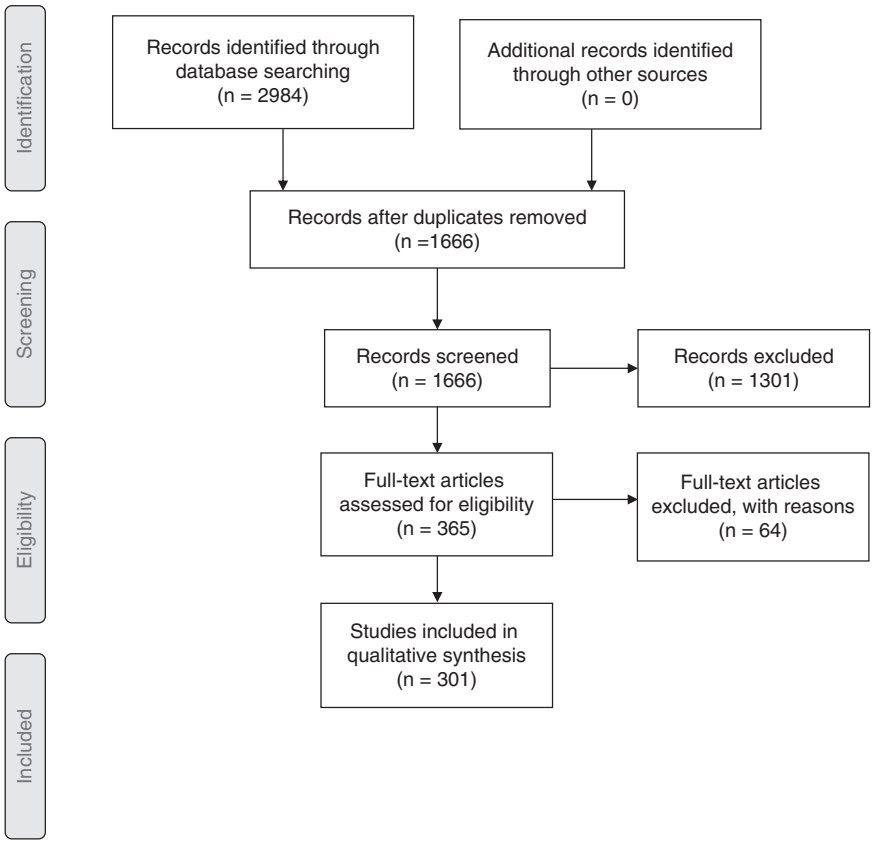

Fig. 1 PRISMA flow diagram. Following deduplication, 1,666 unique articles were screened against eligibility criteria, resulting in 301 articles for inclusion.

The median age at time of description in the publications was 1.5 years for the MLII phenotype (IQR: 0.6–4.0 years, age range: 0.0–20.0 years) and 14.0 years for the MLIII phenotype (IQR: 8.7–21.0 years, age range: 0.1–86.0 years) ($P < 0.001$) (Table 1). Most MLII patients presented with symptoms immediately after birth. The median age of symptom onset was 0.0 years (IQR: 0.0–0.3 years, range: 0.0–4.0 years). The median age of symptom onset for MLIII patients was 3.0 years (IQR: 2.0–6.0 years, range: 0.0–47.0 years) ($P < 0.001$) (Fig. 2). The median age at diagnosis for MLII patients was 0.7 years (IQR: 0.2–1.4 years, range: 0.0–7.0 years) and 9.0 years for MLIII patients (IQR: 6.0–15.0 years, range: 0.1–64.0 years) ($P < 0.001$) (Fig. 2). Age at diagnosis for MLII and MLIII patients did not change over time (MLII: $P = 0.423$, MLIII: $P = 0.811$).

Presenting symptoms

Presenting symptoms were provided for 406 of 829 patients (215 MLII, 181 MLIII; S4). The most frequently reported presenting symptoms in MLII patients (reported in >10% of patients) were dysmorphic facial features (47.4%), (motor and/or cognitive) developmental delay (24.2%), bone abnormalities (20.0%), growth retardation (12.6%), abnormal shape of the skull (10.7%), and restricted joint range of motion (10.7%). The most frequently reported presenting symptoms in MLIII patients were hand deformities (such as claw hands, stiffness of hands, carpal tunnel syndrome, inability to make fists) (47.6%), restricted joint range of motion (30.9%), and growth retardation (13.3%) (S4).

Survival

Age of death was reported for 184 MLII and MLIII cases, of whom 166 were patients with MLII. In total 32% of the MLII patients had died at time of description (166/516 cases) versus 4% of patients with the MLIII phenotype (12/313 cases). Median survival calculated with a Kaplan–Meier analysis was 5.0 years (95% CI 3.8–6.2 years) for the MLII phenotype and 62.0 years (95% CI 52.8–71.2 years) for the MLIII phenotype (Table 1). The median age of death was 1.8 years (IQR: 0.2–4.1 years, age range 0.0–14.0 years) for the MLII phenotype and 33.0 years (IQR: 20.0–54.0 years, age range 0.1–62.0 years) for the MLIII phenotype ($P < 0.001$) (Table 1). The Kaplan–Meier curve of the survival of both phenotypes is shown in Fig. 3a.

When the survival data was subdivided in three subgroups based on the year the article was published (before 1980, between 1981 and 2000 and after 2001), a significant better overall survival was found over time using a Cox regression analysis correcting for the phenotype ($P < 0.001$) (Fig. 3b). At any time point patients with MLII or III had a lower risk of dying if the publication date was after the year 1980. In particular, in the period 1981 to 2000 patients had 23% (HR: 0.77; 95% CI: 0.52–1.14) less risk of dying, and patients in the period after 2000 had 49% (HR: 0.51; 95% CI: 0.35–0.73) less risk of dying compared to the period before 1980.

Causes of death

The cause of death was reported in 98 of the 217 cases (MLII: 93, MLIII: 5). The most frequent reported causes of death in MLII patients were pulmonary and or cardiac complications (pneumonia [32/93], respiratory failure [24/93], cardiac failure [15/93], and a combination of both [7/93]). Other reported causes of death were multiorgan failure (6/93) and complications after stem cell transplantation (3/93). Reported causes of death in MLIII patients were cardiac failure (2/5), pneumonia (1/5), complications of heart transplantation (1/5), and renal insufficiency (1/5).

Table 1. Demographics of the mucolipidosis types II and III (MLII and MLIII) phenotype.

	Whole group ($n = 843$)				MLII ($n = 516$)				MLIII ($n = 313$)				*P* value
	Median	IQR	Range	*N*	Median	IQR	Range	*N*	Median	IQR	Range	*N*	
Age at description (years)	4.1	1.0–12.0	0.0–86.0	702	1.5	0.6-4.0	0.0–20.0	411	14.0	8.7–21.0	0.1-86.0	280	<0.001[a]
Age at symptom onset (years)	0.3	0.0–3.0	0.0–47.0	283	0.0	0.0–0.3	0.0–4.0	167	3.0	2.0–6.0	0.0–47.0	107	<0.001[a]
Age at diagnosis (years)	1.8	0.4–7.0	0.0–64.0	418	0.7	0.2–1.4	0.0–7.0	239	9.0	6.0–15.0	0.1–64.0	167	<0.001[a]
Age at death (years)[d]	2.0	0.5–5.0	0.0–62.0	184	1.8	0.2–4.1	0.0–14.0	166	33.0	20–54.0	0.1–62.0	11	<0.001[b]
	Percentage median			***N***	**Percentage median**			***N***	**Percentage median**			***N***	**CI**
Consanguinity (%)	38.3%			444	36.5%			260	42.6%			176	0.202[b]
Gender (% female)	49.8%			626	48.9%			358	50.6%			255	0.677[b]
Survival in years (95% CI)[e]	54.0 (29.5–78.6)			702	5.0 (3.8–6.2)			411	62.0 (52.8–71.2)			280	<0.001[c]
	Whole group				**MLII**				**MLIII**				**Ratio MLII: III**
Publication before 1980 (% cases)	116 (14.0%)				86 (74.1%)				30 (25.9%)				2.9:1
Publication 1981–2000 (% cases)	173 (20.9%)				116 (67.1%)				57 (32.9%)				2.0:1
Publication 2001–2019 (% cases)	540 (65.1%)				314 (58.1%)				226 (41.9%)				1.4:1

CI confidence interval, *IQR* interquartile range.
[a]Calculated with Mann–Whitney *U*-test.
[b]Calculated with chi-squared test.
[c]Calculated with log-rank test.
[d]Censored data not included.
[e]Censored data included.

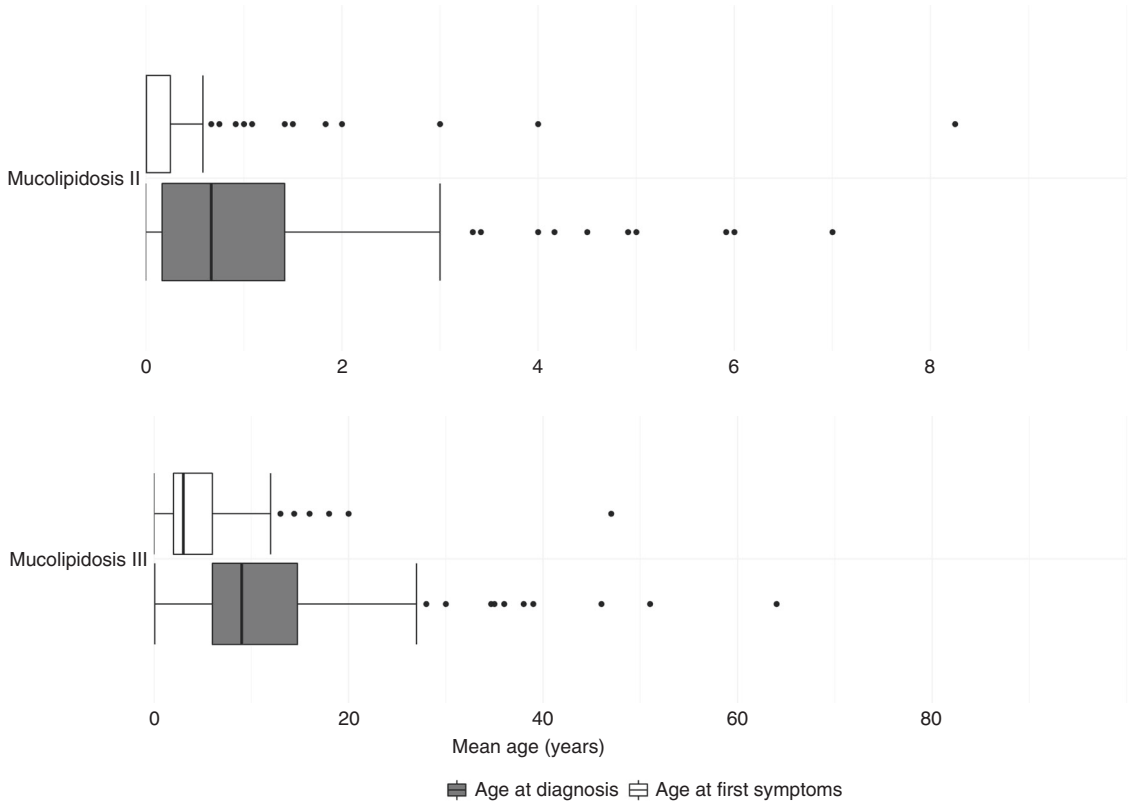

Fig. 2 Age at symptom onset and age at time of diagnosis of mucolipidosis (ML) type II and III. Age at symptom onset and age at time of diagnosis are presented as boxplots. The vertical line represents the median, the box the 25th to 75th percentile, the whiskers 1.5 times the interquartile range (IQR), and dots outliers outside this range. The *y*-axis represents the age in years. Of note, the median of age at first symptoms in MLII is 0 and therefore only half a box is presented.

Potential life-extending procedures

Eighty-six patients underwent a potential life-extending procedure. Commonly reported potential life-extending procedures in MLII patients were stem cell transplantations (i.e., either hematopoietic stem cell transplantation [HSCT], umbilical cord blood transplantation [UCT], or bone marrow transplantation [BMT]) (32/70), tracheostomy (9/70), gastric tube placement (7/70), surgery for craniosynostosis (7/70), surgical procedures to correct cervical spine abnormalities (atlantoaxial dysfunction, cervical subluxation or dislocation, cervical decompression) (5/70), and cardiac interventions (valve replacement, coronary artery bypass, drainage of pericardial effusion) (4/70). From the 32 patients who received a stem cell transplantation (SCT), 14 were reported to have died. Their median age of death was 2.9 years (range: 0.6–10.1 years), which was comparable with the MLII patients who did not receive a SCT (median: 2.7, range: 0–14 years, $P = 0.148$). Median survival of MLII patients who received a SCT was 9.0 years (95% CI 4.42–13.58) and 5.0 years (95% CI 3.91–6.09) for the MLII patients who did not receive SCT. However, this difference is not statistically significant ($P = 0.239$). Potentially life-extending procedures in MLIII patients were less frequently reported and included cardiac surgeries (valve replacement, heart transplantation, implantable cardioverter defibrillator surgery) (7/16), surgical procedures to correct cervical spine abnormalities (cervical decompression and preventing atlantoaxial luxation) (5/16), and stabilization of severe kyphosis (2/16).

Genetic variants

Data on consanguinity was available for 436 of the cases; 170 patients (39.0%) were reported to have consanguineous parents (95 MLII and 75 MLIII patients) (S5 and S6). Pathogenic variants were described for 388 (46.8%) of all patients (208 MLII, 180 MLIII). These included 571 variants in *GNPTAB* and 179 variants in *GNPTG*. Biallelic variants were known for 362 patients (93.3%); for the other 26 patients a second pathogenic variant was not identified. A homozygous pathogenic variant was present in 189 of the patients (53.9% MLII, 49.7% MLIII). There was no significant difference in the occurrence of homozygosity between the two phenotypes ($P = 0.426$). An overview of combinations of pathogenic variants of *GNPTAB* and *GNPTG* with a frequency of 2 or more is given in Fig. 4. The type of pathogenic variant and the effect on protein level are shown in S5 and S6.

Several single *GNTPAB* variants were found in both the MLII and the MLIII phenotype. It appears that specific combinations of these variants correlate with the MLII or with the MLIII alpha/beta phenotype (Fig. 4a). For example, a combination of two nonsense and/or frameshift variants in *GNPTAB* resulted in the MLII phenotype and the combination of one of these variants with a missense or splice variant was found in the MLIII alpha/beta phenotype (S5 Table 2).[13,29–32] The homozygous frameshift variant c.3503_3504del ($n = 47$) was most frequently found in MLII patients and was reported earlier by Velho et al.[14]. Other homozygous variants (in total $n = 28$) that resulted in the MLII phenotype were nonsense c.136C>T, c.1090C>T, c.3487_3490del, c.3565C>T, frameshift c.1314_1315del, c.3503_3504del; missense c.242G>T; and splice c.3135+1G>T, c.3434+1G>A.

The frameshift variants c.3503_3504del in combination with c.3443_3446del ($n = 4$) resulted in a MLII phenotype indicating that the latter variant is also a severe variant, while the c.3503_3504del variant combined with the missense variants

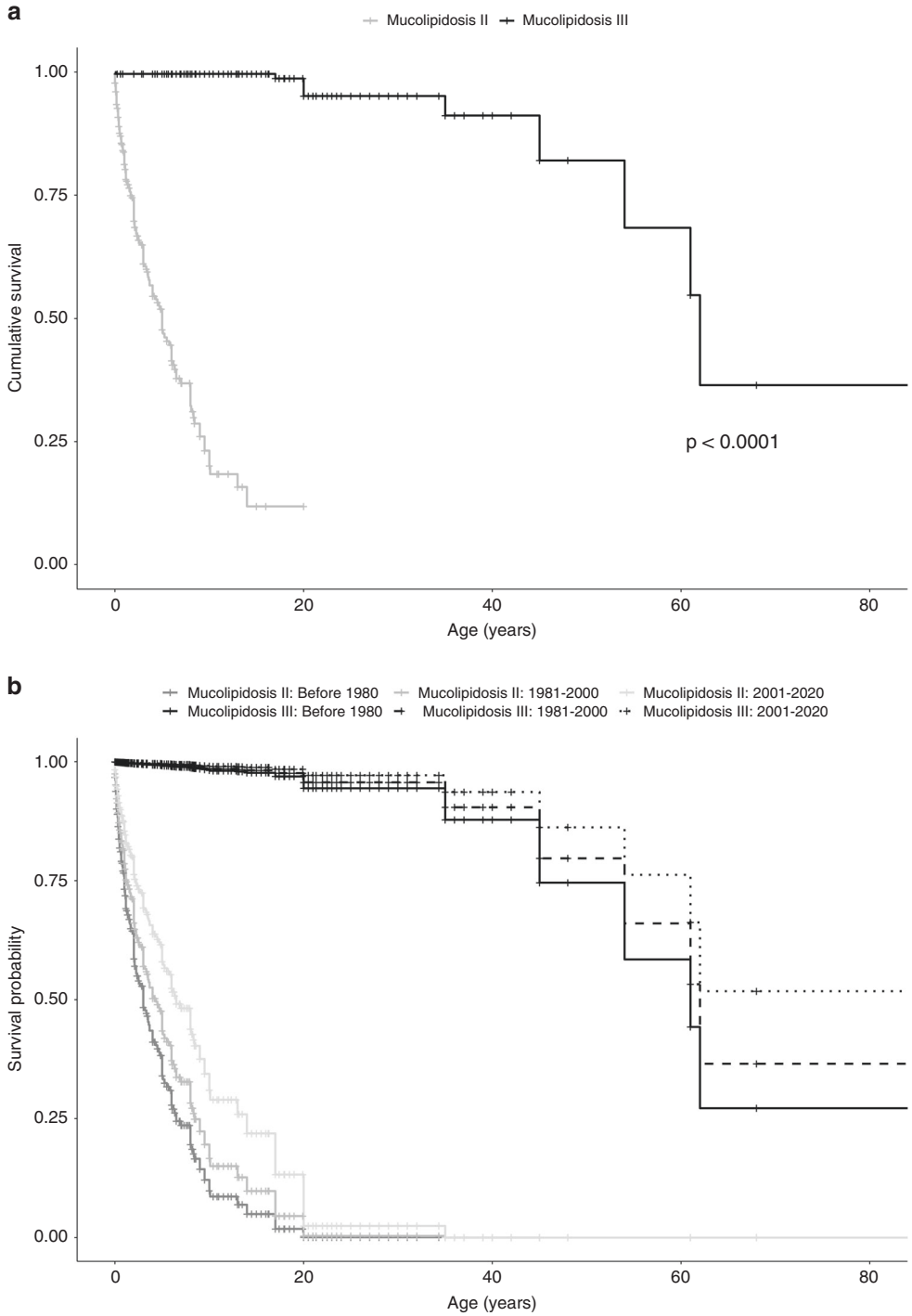

Fig. 3 Kaplan–Meier survival probability curve for the mucolipidosis types II and III (MLII and MLIII) phenotype. (**a**) Kaplan–Meier curve for the comparison of the survival probability of the MLII (gray) and MLIII (black) phenotype (log-rank test: $P < 0.001$). (**b**) Kaplan–Meier survival probability curve for the MLII and MLIII phenotype (log-rank test: $P < 0.001$) over three different time periods: <1980, 1981–2000, and 2001–2020.

c.1196C>T ($n = 4$) or with c.1208T>C ($n = 3$) resulted in the MLIII alpha/beta phenotype (Fig. 4a), indicating that these variants are less severe.

Noteworthy, all pathogenic variants detected in *GNPTG*, even frameshift or nonsense variants, resulted in the MLIII gamma phenotype (Figs. 4b and S6 Table 3). The homozygous nonsense c.323G>A variant ($n = 8$) was most frequently reported in the MLIII gamma patients.

DISCUSSION

As MLII and MLIII are extremely rare diseases, current knowledge about natural history is solely based on case reports, small case series, and expert opinions. This systematic review on ML analyzes the combined data of 843 patients with ML reported in papers published over the last 50 years. Although this study cannot replace a longitudinal natural history study, it provides unique insights in survival, symptom onset, presenting symptoms,

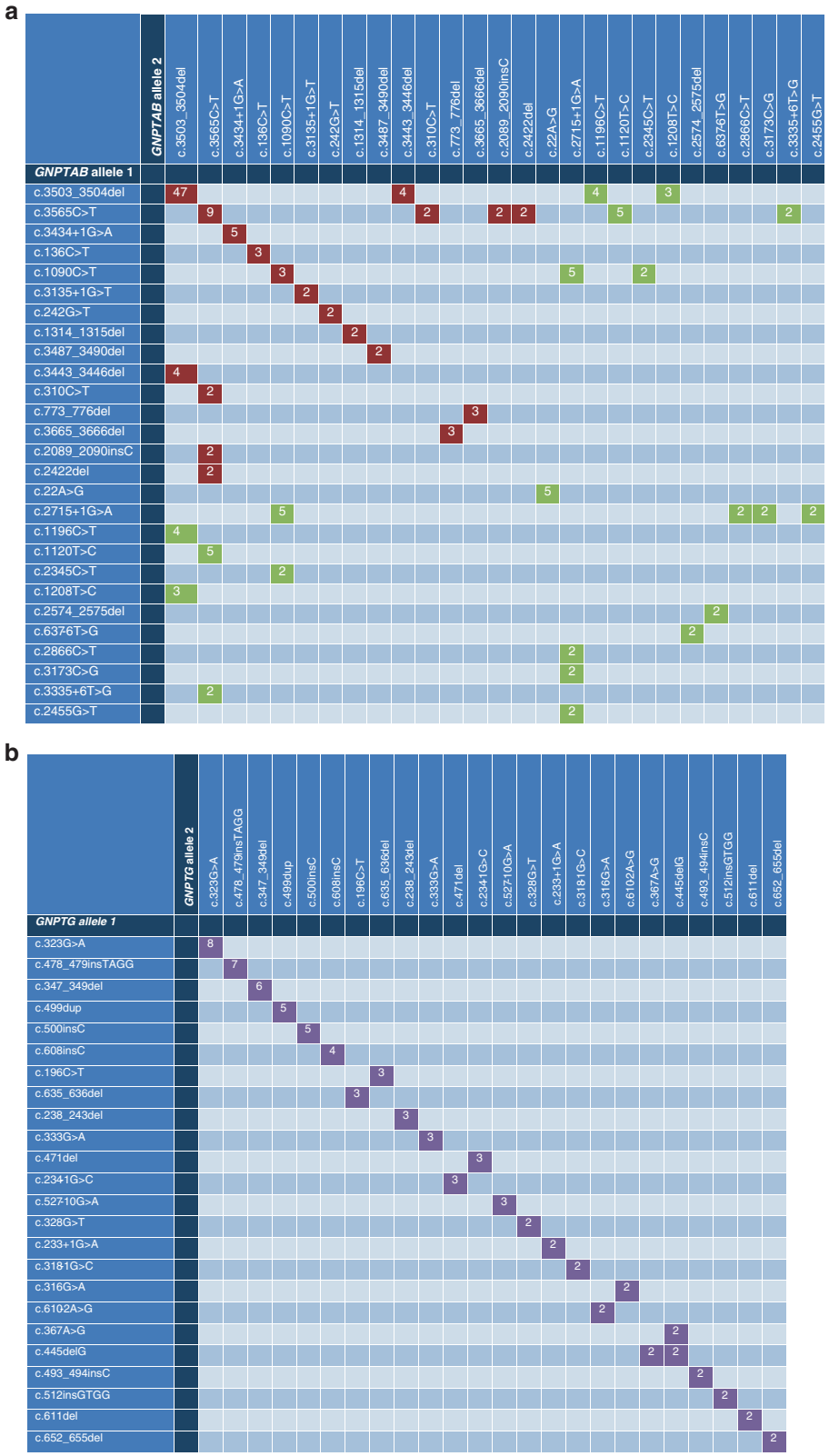

Fig. 4 Common combinations of pathogenic variants in *GNPTAB* and *GNPTG*. (**a**) Overview of common combinations of pathogenic variants in *GNPTAB* in mucolipidosis II (MLII) (red) and mucolipidosis III (MLIII) alpha/beta (green) patients. (**b**) Common combinations of pathogenic variants in *GNPTG* in MLIII gamma (purple) patients.

diagnosis, and distribution of genetic pathogenic variants of both ML phenotypes. This knowledge is not only important for patient counseling, but also for the development of universal follow-up protocols, guidelines, and potential future treatments.

This review presents valuable and novel information about survival and age of death for MLII and MLIII. Because of the large number of patients included in this systematic review, this study could generate a Kaplan–Meier survival curve. Median survival was 5.0 and 62.0 years for MLII and MLIII, respectively, and the median age of death was 1.8 and 33.0 years. There is a significant discrepancy in the survival and age at death in both MLII and MLIII, caused by censoring of the latest reported age when the age of death was unknown. However, it is likely that survival in MLIII may differ from data reported, as information on the age of death of MLIII patients was only available for 22 patients. The oldest MLIII patient alive at time of description was 86 years old[33]. This emphasizes that some patients with a mild MLIII phenotype may reach high ages comparable to the normal population. In contrast, the duration of survival in MLII that we found is probably too long as some papers reported MLII patients who were 20 years of age (range of age at time of description 0–20 years). It is likely that these patients have MLIII, but were misclassified as having MLII.

The main causes of death in MLII and MLIII, such as cardiac failure, respiratory failure, and pneumonia, seem similar. These results, however, should be interpreted with caution because a very limited number of causes of death were reported in MLIII patients ($n = 5$). Interestingly, survival was better after 1980 than before this date. The improved survival rate over time might be explained by advanced supportive care and potentially life-extending procedures, since to date no disease-modifying (apart from SCT) or curative treatments are available. However, the improved survival might also result from underreporting of milder cases in publications in earlier days, or increased reporting of relatively mild cases in more recent years due to improved awareness, recognition, and diagnostics of ML.

Many of the potential life-extending procedures were reported to be performed only in MLII patients. SCT has been reported for 32 MLII patients. SCT is a potentially disease-modifying therapy that aims to provide a constant supply of donor-derived hematopoietic stem cells that produce and secrete lysosomal enzymes with intact M6P. Fourteen of 32 patients who were treated with SCT had died at the time of the report. Median survival of MLII patients who received SCT was higher (9.0 years) than in patients who did not receive a SCT (5.0 years), although this difference was not significant ($p = 0.239$). It is likely that data on SCT are highly biased. The relatively small group of patients who underwent SCT was not randomly selected. The most severely affected patients were most likely not eligible for the transplantation, while SCT may have been considered too high risk for patients with a mild phenotype. Furthermore, only a small number of developed countries have the possibility to perform SCT and these countries might also have better supportive care. Additionally, limited follow-up is available for patients who were transplanted and transplanted patients in whom follow-up was reported still appear to have considerable morbidity caused by ML[26]. To assess if the benefits of SCT in MLII outweigh the risks an international randomized controlled trial would have to be conducted, but it is questionable if this is feasible.

Some of the other potentially life-extending procedures that have been reported in MLII may have improved the quality of life of the patients; however, this has not been evaluated in a standardized manner. It is also possible that the procedures only expanded the life span, while quality of life remained low or got worse. The question therefore remains whether extensive interventions, which may also cause harm and may potentially prolong suffering, are (always) recommended based on the short life expectancy and great morbidity of MLII patients, as confirmed in this study.

In our systematic search we found that most patients reported were MLII patients (62.2% of all reported cases). The real life frequency and distribution of both phenotypes needs further investigation. It is likely that patients with the MLII phenotype will less frequently escape from diagnosis, because of the early and severe presentation, while MLIII patients may present at any age[11,16,34]. Most cases of MLIII were published in the period after 2001 (72.2%), indicating improved diagnostic tools, such as untargeted exome sequencing and increased awareness of rare diseases.

Our data also show a significant difference between MLII and MLIII regarding the age of onset of symptoms and age at diagnosis. This difference can be explained by the fact that ML is a progressive disease with a broad clinical spectrum of symptoms, which include common clinical symptoms that may also occur in other diseases. Most patients have symptoms at birth. These symptoms are obvious in severely affected patients, but may be extremely mild in milder phenotypes making the disease difficult to recognize. Moreover, the presenting symptoms differ quite significantly between MLII and MLIII. The typical symptoms of MLII may be very mild or absent in young MLIII patients, who mainly present with progressive hand deformities and restricted joint range of motion. For these symptoms, patients will often consult specialists like rheumatologists and orthopedic or plastic surgeons. As the symptoms caused by MLIII may be very similar to symptoms caused by rheumatologic or orthopedic diseases it is challenging for these specialists, who have no specific expertise with ML, to recognize and diagnose ML. Therefore increasing clinicians' awareness of ML is important to decrease the diagnostic delay and optimized follow-up and treatment in specialized centers.

Finally, we reported on the most frequent genetic pathogenic variants in *GNPTAB* and *GNPTG*. Notably, our study showed that a very high number of patients were the offspring of consanguineous marriages (39.0% of the cases in whom the background of parents was described, 20.5% of the total number of patients). This explains the high frequency of homozygous variants in our study (51.9%). This high frequency of homozygous variants gave us the unique opportunity to study the severity of some particular genetic pathogenic variants as shown in S5 and S6.

In total 571 pathogenic variants in *GNTPAB* and 179 in *GNTPG* were identified. Pathogenic variants in *GNTPAB* either caused the MLII or MLIII alpha/beta phenotype, while pathogenic variants in *GNTPG* always led to a MLIII gamma phenotype. A clear genotype–phenotype correlation was found. Nine pathogenic variants (nonsense c.136C>T, c.1090C>T, c.3487_3490del, c.3565C>T, frameshift c.1314_1315del, c.3503_3504del; missense c.242G>T; and splice c.3135+1G>T, c.3434+1G>A) that caused the severe MLII phenotype, when identified in a homozygous manner, were found. The most frequently encountered variant was c.3503_3504del. This variant leads to a frameshift p.Leu1168Glnfs*5, which explains the deleterious effect and loss of function of the enzyme. In addition we identified six pathogenic variants that led to a MLIII alpha/beta phenotype when combined with one of the severe variants mentioned above. These pathogenic variants were nonsense c.3173C>G, missense c.1120T>C, c.1196C>T, c.1208T>C, c.2345C>T, and splice c.2715+1G>A.

Common variants in *GNPTG* are shown in S6. The most frequently reported pathogenic variant is c.323G>A. Pathogenic variants in *GNPTG* always resulted in the MLIII gamma phenotype as the mildest phenotype of the clinical spectrum. This indicates that *GNPTG* has a less prominent part in the enzyme function[14]. Of note, none of the patients with a *GNPTG* variant had a second pathogenic variant in *GNPTAB*.

Strengths and limitations

The major strength of this study is that via a systematic search of the published literature, we could analyze data of 843 ML patients. Given the fact that ML is an extremely rare disease this is a very large number of patients. Published cohort studies maximally proved data from 61 patients[11]. Therefore this systematic review provides valuable insight into the natural history of ML, and we were able to perform statistical analysis. However, findings should be interpreted with caution as data have solely been extracted from case reports and small case series as large cohort studies and clinical trials are lacking due to the rarity of the disease. Data obtained from case reports/series are not systematically collected and follow-up data are often lacking. Furthermore, data might be subject to publication bias. Patients with more attenuated phenotypes and milder symptoms that might be attributed to something else are probably less likely to be included in publications than patients with a more severe phenotype. This may overestimate the prevalence of more severe symptoms. Furthermore, MLII and MLIII both occur worldwide. It is important to realize that, given the rarity and high level of consanguineous parents in these diseases, the distribution of reports is seriously skewed to countries with better diagnostic resources, which are underrepresented in this review.

In conclusion, this systematic review provides unique insights in the natural history of MLII and MLIII, with a clear genotype–phenotype correlation for pathogenic variants in *GNPTAB*. This information is important for patient/parents counseling and essential reference data for the development of innovative therapies and improvement of outcome of patients in the future. However, we underline the importance of studying the natural course of MLII and MLIII in a well-defined international prospective cohort study as the data derived from this systematic review cannot replace a natural history study.

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AUTHOR CONTRIBUTIONS

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ETHICS DECLARATION

No ethics approval was obtained since the work consisted of a systematic literature search, for which no ethical approval is necessary in the institutions/countries participating in the study.

COMPETING INTERESTS

M.A.E.M.W. received an unrestricted research grant from the Nutricia metabolics research fund. A.T.v.d.P. gives advice to several pharmaceutical companies about the implementation and development of innovative therapies, mainly for Pompe disease, but also for other LSDs and neuromuscular disorders. Furthermore, she received funds for research via agreements between Erasmus MC and pharmaceutical companies. She also advices public or private charities, who aim to improve the care for patients with metabolic diseases. The other authors declare no competing interests.

ADDITIONAL INFORMATION

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