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A systematic review and meta-analysis of published cases reveals the natural disease history in multiple sulfatase deficiency

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Abstract

Multiple Sulfatase Deficiency (MSD, MIM#272200) is an ultra-rare lysosomal storage disorder arising from mutations in the *SUMF1* gene, which encodes the formylglycine-generating enzyme (FGE). FGE is necessary for the activation of sulfatases, a family of enzymes that are involved in the degradation of sulfated substrates such as glycosaminoglycans and sulfolipids. *SUMF1* mutations lead to functionally impaired FGE and individuals with MSD demonstrate clinical signs of single sulfatase deficiencies, including metachromatic leukodystrophy (MLD) and several mucopolysaccharidosis (MPS) subtypes. Comprehensive information related to the natural history of MSD is missing. We completed a systematic literature review and a meta-analysis on data from published cases reporting on MSD. As available from these reports, we extracted clinical, genetic, biochemical, and brain imaging information. We identified 75 publications with data on 143 MSD patients with a total of 53 unique *SUMF1* mutations. The mean survival was 13 years (95% CI 9.8-16.2 years). Seventy-five clinical signs and 11 key clusters of signs were identified. The most frequently affected organs systems were the nervous, skeletal, and integumentary systems. The most frequent MRI features were abnormal myelination and cerebral atrophy. Individuals with later onset MSD signs and survived longer than those with signs at birth. Less severe mutations, low disease burden and achievement of independent walking positively correlated with longer survival. Despite the limitations of our approach, we were able to define clinical characteristics and disease outcomes in MSD. This work will provide the foundation of natural disease history data needed for future clinical trial design.

Jutta Gärtner and Tim Friede contributed equally to this work.

KEYWORDS

clinical characteristics, disease outcome, lysosomal storage disorder, meta-analysis, multiple sulfatase deficiency, natural disease history, ultra-rare disease

1 | INTRODUCTION

Multiple sulfatase deficiency (MSD) is an ultra-rare lysosomal storage disorder. It results from mutations in the *SUMF1* gene, which encodes the formylglycine generating enzyme (FGE). FGE post-translationally activates all 17 cellular sulfatases in humans by oxidizing a crucial active site cysteine residue to Cα-formylglycine, a step necessary for catalytic activity. *SUMF1* mutations impair FGE function, thus decreasing all sulfatase activities to variable degrees.[1-3]

Sulfatases are a group of hydrolytic enzymes that degrade sulfated substrates such as glycosaminoglycans (GAG), sulfolipids, and steroid hormones.[4] Insufficient activation of sulfatases in MSD leads to the additive signs from each individual single sulfatase deficiency. Ichthyosis is associated with steroid sulfatase deficiency; Mucopolysaccharidosis (MPS)-like signs such as hepatosplenomegaly and dysostosis multiplex result from deficiency of iduronate sulfatase, arylsulfatase B, and galactose-6-sulfatase; while neurological signs and neurodegeneration are likely to a secondary deficiency in arylsulfatase A, sulfamidase, and *N*-acetylglucosamine-6-sulfatase. As the majority of cellular sulfatases are localized in the lysosome, MSD is classified as a lysosomal storage disorder, even though FGE is located in the endoplasmic reticulum.[4,5]

The first cases of MSD were described in 1965 as a variant of metachromatic leukodystrophy (MLD).[6] Until the discovery of the *SUMF1* gene in 2003, biochemical analysis of reduced sulfatase activities were used to confirm the diagnosis of MSD. Historically MSD has been divided into three subtypes based on age of onset: neonatal, late infantile and juvenile forms.[7] Additionally, residual sulfatase activities may discriminate between severe and milder cases.[8]

Current understanding of MSD disease classifications is derived from small cohorts and single case reports.[5,7,9-12] Prognostic factors of disease severity in MSD, such as age of onset, time of onset of neurodegeneration, mutation severity, and level of residual sulfatase activities are only partially established and sometimes controversially discussed.[5,7,8,10,11] The lack of comprehensive natural disease history studies has limited the full development of potential biomarkers and endpoints for interventional studies.[5,13] It is hypothesized that the same biomarkers that are abnormal in the individual sulfatase deficiency disorders, including measures of sulfatase enzyme activity, glycosaminoglycans, and sulfatides, will be abnormal in MSD. The association of these factors to the clinical phenotype in MSD is unknown.

SYNOPSIS

The article describes results of a meta-analysis from published cases and defines clinical characteristics and disease outcome in multiple sulfatase deficiency.

Where patient numbers are limited or the severity of disease limits participation in centralized prospective natural history studies, systematic reviews and meta-analyses provide a time and cost-efficient method for improving the understanding of the natural disease history of rare diseases as successfully proven in other diseases.[14-18]

In this study, we performed a systematic review and meta-analysis using the data from all previously published reports to characterize the natural history of MSD.

2 | METHODS

2.1 | Literature search and inclusion and exclusion criteria

We searched Pubmed and Embase databases with search terms listed in Table S1 available as of September 1, 2019. We identified 925 publications and 11 additional reports, published between 1965 and 2019. We excluded 466 publications because of the lack of patient data. Full text analysis was carried out in 179 publications. All reports had to have evidence of MSD diagnosis by either molecular testing or reduced activity of at least three sulfatases. Publications that did not allow linking data to individual patients were excluded. We only extracted data from original case reports. Duplicate datasets were omitted when cases were used and cited in different publications or when the first, original published case description was not cited but patient details were identical. Applying these criteria, 104 publications were found not eligible; 75 publications were used for quantitative meta-analysis (Figure S1). Forty-five publications described one case, 14

publications two cases, seven publications three to five cases and nine publications contained data and descriptions of more than five cases (Table S2).

2.2 | Collection and classification of clinical and cranial imaging description

We used published clinical guidelines to identify MSD-specific clinical features.[13] Case descriptions from 137 individuals were searched for key clinical terms (n = 234), then categorized by clinical signs yielding 75 categories, which were then grouped into 11 key clusters of signs. Additionally, nine clinical signs were classified as "other signs" (Table S3). Disease-relevant time-to-event measures, including ambulation, were available from 127 cases. As available, biometric data, including length, weight, and head circumference were collected, and normative data from the WHO (https://www.who.int/childgrowth/standards/en) were applied. Development was standardized based on the Denver scales for development.[19,20] Eighty-four published cases included cranial imaging, 54 of which were sufficiently detailed for inclusion. Similar to the clustering approach for clinical features, 95 terms describing cMRI features were grouped into 10 categories (Table S4).

2.3 | Collection and classification of data on sulfatase activities, glycosaminoglycan excretion, and mutations

Sulfatase activities were collected when indicated as measured in leukocytes, fibroblasts, or both. Sulfatase activities were calculated as percent of mean reference when reference ranges were indicated. We grouped individual sulfatase activities in three activity groups for each sulfatase (group 1 [severely reduced]: <10%; group 2 [mildly reduced]: 10-50%; and group 3 [normal]: >50% residual activity).[7,8] Glycosaminoglycan (GAG) excretion was recorded as positive when described as "increased" or "abnormal."

Data on *SUMF1* pathogenic variants were collected and adjusted to Human Genome Variation Society (HGVS) standards. In accordance to a previously published analysis, we classified *SUMF1* pathogenic variants into genetic severity groups based on in vitro residual function, as characterized by residual sulfatase activity and FGE activity and stability.[5,9-11,21-24] This was used to generate a disease severity score for subsequent analysis with three categories. Variant-group 1 included cases with a nonsense variant or severe missense variant in combination with a less severe missense variant, or a combination of two less severe missense variants. Variant-group 2 included cases with any combination of two nonsense variants, combinations of one nonsense variant and one severe missense variant, and patients with two severe missense variants. Variant-group 0 includes all cases with unclassified variants without supportive experimental data (Table S5).

2.4 | Statistical methods

Categorical variables were described by frequencies and percentages; continuous variables were summarized by means and standard deviations. Time-to-event outcomes were collected for clinically relevant events. When a key time to event measure was met, but only ages before and after the event were provided, the mean between available ages was used. Kaplan-Meier curves were produced for survival analyses. Survival between groups was compared using LogRank tests and modeled using the Cox proportional hazard model. To investigate the prognostic effect of clinical events emerging over time, landmark analyses conditioning on survival at certain timepoints were conducted. Results were considered as statistically significant when $P < 0.05$.

2.5 | Software

We used Microsoft Excel (MicroSoft, Redmond, Washington) for data collection and storage, SAS version 9.4 (SAS Institute, Cary, North Carolina) for statistical analysis.

3 | RESULTS

3.1 | Demographics

We identified 143 cases of MSD in the available literature with sufficient clinical and diagnostic information, including 54 females, 69 males, and 20 of unknown sex. The range of ages from first available clinical information to final data collection was 0 to 26 years (Table S2).

Of those with known age at death, the mean survival was 13.0 years (n = 35; 95% CI 9.8-16.2 years, Figure 1). There were no statistically significant differences in the survival by sex (female mean survival: 9.7 years, male mean: 12.4 years, $P = 0.9$). As the method of testing affects the reliability of the diagnosis and testing approaches have evolved over time (biochemical vs molecular), we also characterized the relationship between method of diagnosis and survival. Sixty-three individuals with MSD were diagnosed by genetic and sulfatase activity testing, 55 by sulfatase activity testing and 25 patients by genetic

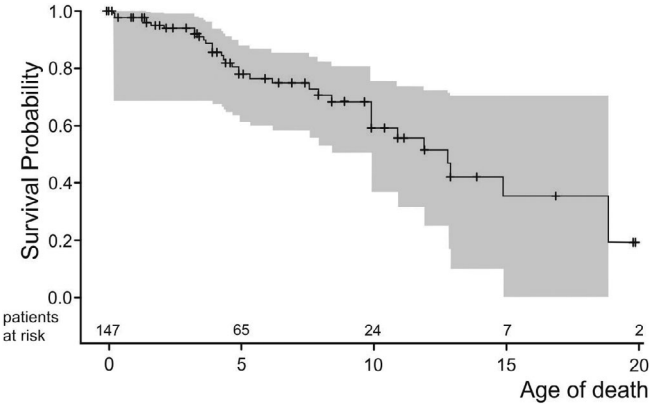

FIGURE 1 Survival statistics of cases with reported age of death in the cohort with number of subjects at risk and 95% Hall-Wellner bands. Mean survival: 13.0 years (n = 35, 95% CI 9.8, 16.2 years)

testing only. We found no statistically significant differences in survival between testing modalities.

3.2 | Clinical presentation

Most children were described as having neurologic complications (n = 123/137; 90%) with developmental delay (n = 105/137; 77%), as well as skeletal (n = 114/137; 83%) and dermatologic (n = 112/137; 82%) abnormalities. Complications of the cardiovascular system were rarely described (n = 21/137; 15%; Table 1A).

The most frequently reported signs were ichthyosis (n = 97/137; 71%), organomegaly (n = 78/137; 57%), dysostosis multiplex (n = 77/137; 56%), and facial dysmorphism (n = 72/137; 53%). Within the neurologic category signs were diverse (spasticity 72/137; 53%; loss of motor skills 51/137; 37%; epilepsy 46/137; 30%; Table 1B, Table S6). In 56 patients with data on psychomotor development, 32 patients were reported to having achieved independent walking (57%). A subset of cases included biometric information. Growth restriction (n = 57/67; 85%) and malnutrition (n = 49/58; 84%) were frequently noted. Both microcephaly (n = 22/54; 41%) and macrocephaly (n = 17/54; 31%) were described. First reported signs were organomegaly (n = 26/137; 19%) and ichthyosis (n = 24/137; 18%). Most individuals presented with multiple first signs (n = 67/137; 49%, Table S7A). Death was reported in 35 cases, most frequent cause were respiratory complications (n = 17/35; 48%, Table S7B).

3.3 | Course of disease

A burden of disease was calculated by summing the number of key clusters of signs and at a given age into three and 6 months intervals (Table S8, Figure 2A). Over the lifespan of available data, the individual burden of disease increased over time, and the number of patients with no or few reported key cluster of signs decreased (Figure 2A, Table S8). At birth, skeletal (n = 21/127; 17%) and gastroenterologic and nutritional (n = 18/127; 14%) complications were the most frequently reported. Beginning at 9 months of age, developmental delay became the most commonly noted feature (n = 42/127; 33%, Table S8).

We hypothesized that clinical signs correlate with age and disease burden. To address this question, we compared groups based on the number of signs (1-2 verses 3-4 clusters) at birth and 48 months of age. We found that the distribution of reported signs varied based on age and number. In both groups, skeletal complications were the most prevalent key cluster at birth, neurologic and neurodegeneration was most prevalent at 48 months (Figure 2B).

3.4 | Brain imaging abnormalities

Reports of cranial MR imaging from 54 individuals were available for descriptive analysis. Key words were extracted from the case reports to characterize the most common imaging features. The most frequently described brain abnormalities were leukodystrophy (n = 36/53; 70%), cerebral atrophy (n = 22/53; 42%), cerebellar atrophy (n = 18/53; 34%), and hydrocephalus (n = 16/53; 30%). Spinal cord compression was noted in four cases (n = 4/53; 8%; Table 1C).

3.5 | Sulfatase activities, glycosaminoglycan-, and sulfatide-excretion

Enzymatic activities of eight sulfatases in leukocytes and fibroblasts were reported in 79 individuals (n = 328 total measurements). Arylsulfatase A (ASA) was the most frequently reported sulfatase (n = 100 measurements), followed by arylsulfatase B (ASB, n = 70) and arylsulfatase C (ASC, n = 55). ASA activity ranged from 0% to 84.88% of reference activity in fibroblasts and 0% to 52.38% in leukocytes (fibroblasts: mean 12.12%, SD 15.62, median 7.86%, leukocytes: mean 5.71%, SD 8.58, median 3.45%). ASB ranged from 0% to 100.83% in fibroblasts and 0% to 104.71% in leukocytes (fibroblasts: mean 23.1%, SD 26.89, median 12.21%, leukocytes: mean 11.04%, SD 18.15, median 6%) and ASC from 0% to 179.76% in fibroblasts and 2% to 90.74% in leukocytes (fibroblasts: mean 41.98%, SD 49.59, median 20.18%, leukocytes: mean

TABLE 1 Frequency of affection of reported key clusters of signs, most common clinical signs, and most common MRI imaging descriptions in MSD cases

A: Frequency of reported affection of key clusters of signs in MSD cases (n = 137)		
Key cluster	**Number of cases**	**%**
Neurologic and neurodegeneration	123	90
Skeletal	114	83
Dermatologic	112	82
Developmental delay	105	77
Gastroenterologic and nutrition	98	72
Ophthalmic	69	50
Muscular	47	34
Otolaryngologic	45	33
Respiratory	44	32
Oral	26	19
Cardiac and vascular	21	15
Others	25	18
B: Frequency of 10 most common clinical signs in MSD cases (n = 137)		
Sign	**Number of cases**	**%**
Ichthyosis	97	71
Organomegaly	78	60
Dysostosis multiplex	77	56
Spasticity	72	53
Facial dysmorphism	72	53
Growth restriction	57	42
Loss of motor skills	51	37
Malnutrition	49	36
Hypotonia	47	34
Epilepsy	46	33
C: Frequeny of most common MRI imaging descriptions in MSD cases (n = 53)		
MRI feature	**Number of cases**	**%**
Leukodystrophy	36	68
Cerebral atrophy	22	42
Cerebellar atrophy	18	34
Hydrocephalus	16	30
Corpus callosum atrophy	11	21
Delayed myelination	10	19
J shaped sella turcica	8	15
Brain atrophy	7	13
Enlarged cisterna magna	7	13
Cervical cord compresion	4	8

16.24%, SD 20.53, median 8%; Table S9A,B). When the groups of sulfatase activities were classified by residual activity (see Section 2), most of those available were assigned to the "severely reduced" category (<10% of reference activities, activity group 1, 202 analysis), 101 activities were assigned to "mildly reduced" (10%-50% of reference activities, activity group 2), 25 activities to "normal" (>50% of reference activities, activity group 3) (Table S9A). Reported residual sulfatase activities of different sulfatases were consistently reduced in 37 out of 79 cases (47%; 30 group 1, 38%; seven group 2, 9%). All other cases (n = 42/79; 53%) had residual activities for individual sulfatases spanning all three activity groups. Among all cases, 69/79 had at least one sulfatase in activity group 1 (87%; Figure 2C).

Total GAG excretion was increased in 52 (84%) and normal in 10 out of 62 patients (16%). Urine sulfatides were elevated in 29 (90%) and normal in three out of 32 patients (10%).

3.6 | *SUMF1* variant characteristics

In total, 53 different *SUMF1* pathogenic variants from 99 patients were available (Table S10), including 32 missense and 21 nonsense variants (deletions, insertions, splice-site variants, and stop codons). The testing results revealed both homozygous variants (n = 59) and compound heterozygous variants (n = 40). The most prevalent alleles were c.739G>C, p.G247R and c.1045C>T, p.R349W. Then, 165 out of 195 alleles were recurrent; while 30 were private variants (Figure 2D).

Based on published functional data of previously reported variants, 72 alleles were classified as severe pathogenic missense variants and 36 alleles as less severe missense (Figure 2D, Table S10). Of these, 23 individuals were homozygous for known severe missense variants, and 12 individuals homozygous for known less severe missense variants. Functional data on all other variants were lacking.

3.7 | Disease burden and survival, and clinical sings and survival

MSD-related signs at birth correlated with a reduced survival (mean survival of individuals with signs at birth 9.9 years (SE 1.4) vs 13.2 years (SE 0.6), LogRank test $P = 0.001$, Figure 3A). Individuals presenting with three or more affected key clusters of signs were found to have decreased survival compared to individuals with either 0 or 1 to 2 key sign clusters any at birth (LogRank test $P < 0.001$, Figure S2A). Individuals who acquired their

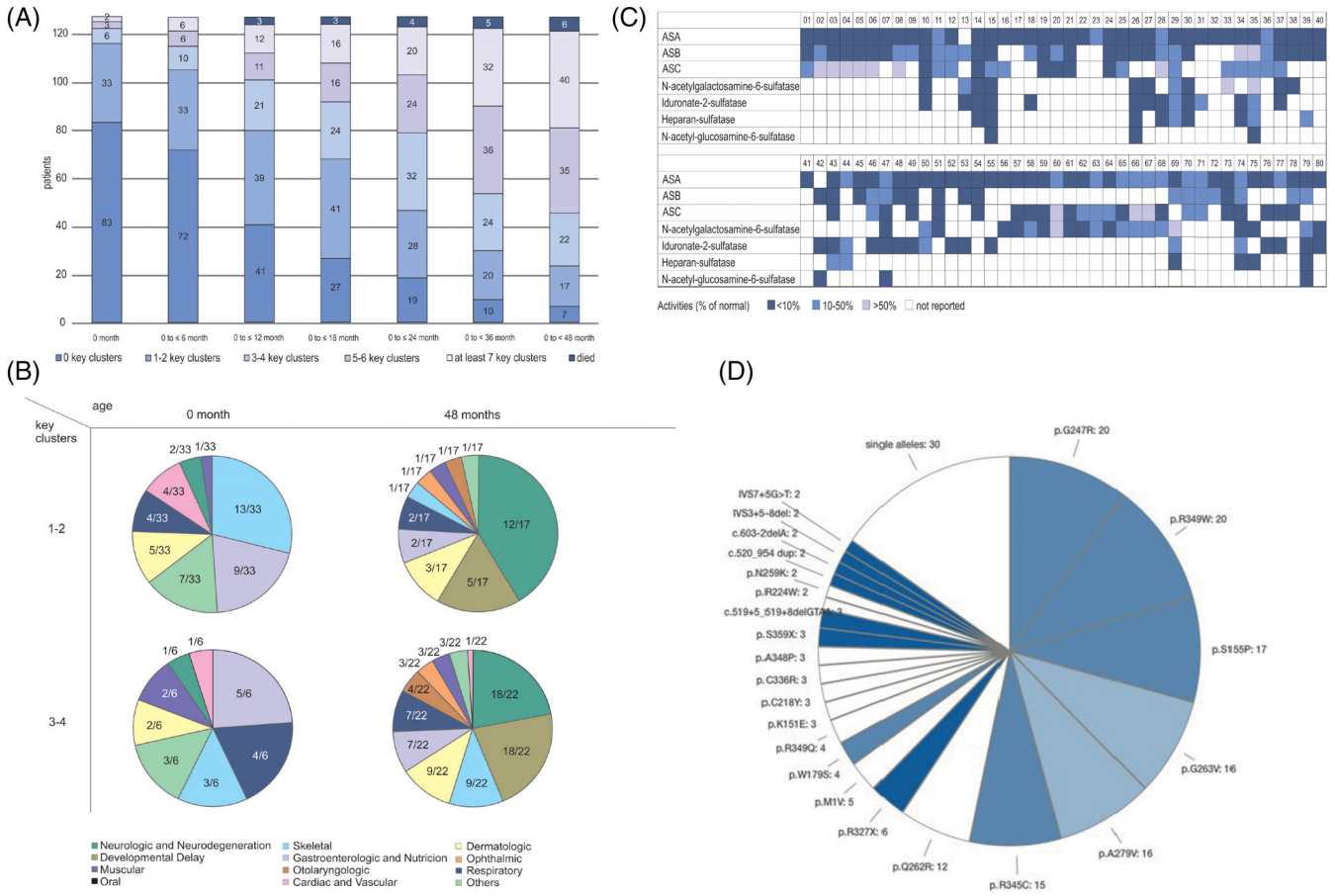

FIGURE 2 A, Disease burden in MSD. The number of cases with reported affection of different key clusters of signs, grouped by number of key clusters and also including deceased patients are displayed at time intervals of 6 months. The number of patients without any reported affection of key clusters decreased over time whereas the number of cases with reported affection and the number of affected key clusters increased. The data demonstrate that MSD is a progressive disease and disease burden increases over time. B, Cross sectional analysis of cases at birth and 48 months with reported features in 1 to 2 and 3 to 4 key clusters of signs respectively. Key clusters of signs were different at birth and at 48 months in both groups indicating that MSD presents with different combinations of signs over time. C, Residual activities of sulfatases in 79 cases in the cohort displayed in a heat map. Dark blue: <10% of reference activity (activity group 1), blue: 10% to 50% (activity group 2), light blue: >50% (activity group 3), white: data not reported. Thirty-seven cases were reported to have all reported sulfatase activities in the same activity group (30 activity group 1, 7 activity group 2). 42 cases had sulfatase activities in different activity groups. Sixty-nine of all cases had at least one sulfatase activity in activity group 1. D, Frequency of 53 *SUMF1* pathogenic variants on 195 alleles in 99 MSD cases. Twenty-one were nonsense, 32 missense variants. The most prevalent alleles were c.739G>C, p.G247R, and c.1045C>T, p.R349W. 165/195 alleles were recurrent; 30 private variants on single alleles. The color code reflects variant severity either assessed on the type of mutation (nonsense or missense) or based on published experimental results on residual stability and activity of variant FGE resulting from *SUMF1* missense alleles. (dark blue: nonsense variants, blue: severe missense variants with reduced activity and stability, light blue: mild variants with either high residual activity or stability, white: no experimental data available)

first clinical signs after birth, but before 12 months of age, had reduced survival compared to individuals without reported signs at 12 months (LogRank test $P = 0.0042$, Figure S2B).

Next, a landmark analysis was conducted to examine the correlation of major key clusters of signs on survival at 12, 24, and 36 months of age. The presence of neurologic signs before 12 months was negatively associated with survival, while neurologic presentation after 36 months was associated with improved survival (landmark analysis at 12 months: LogRank test $P = 0.0012$, 24 months LogRank test, $P = 0.08$, 36 months LogRank test $P = 0.03$; Figure S3A-C). The report of skeletal signs before 24 months was associated with reduced survival (landmark analysis 12 months: LogRank test, $P = 0.002$, 24 months LogRank test, $P = 0.01$; 36 months: LogRank test, $P = 0.1$; Figure S4A-C, 36 months). The presence of isolated skin signs after 36 months correlated with longer survival, skin signs before 36 months did not correlate with survival (landmark analysis at 12 months:

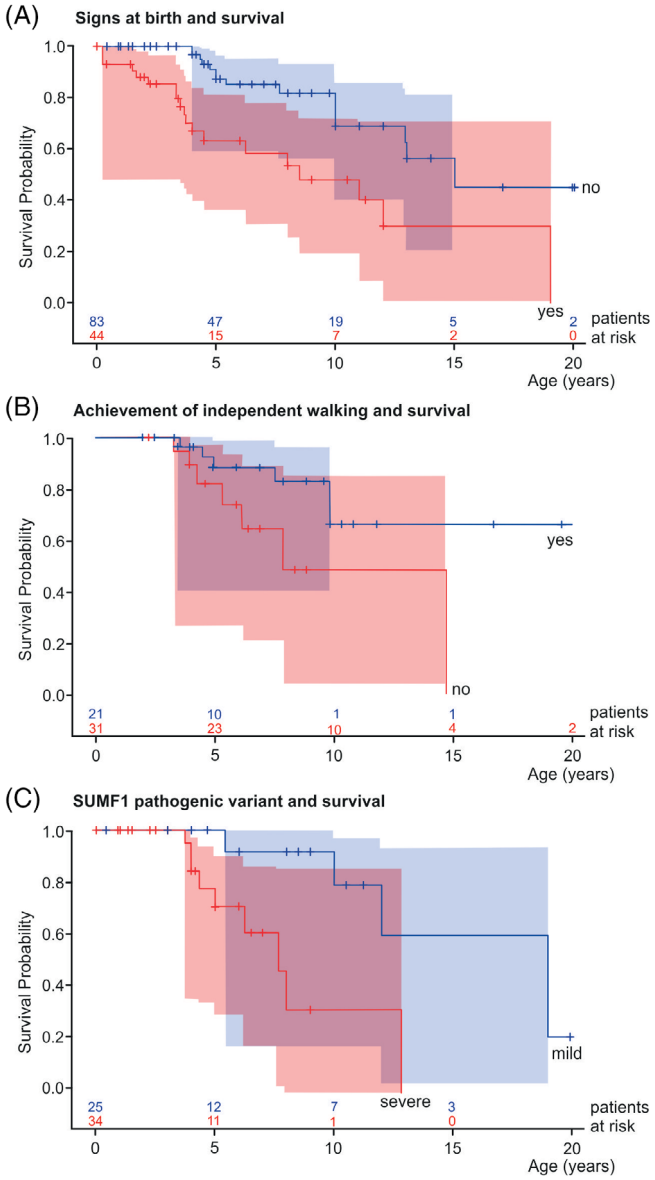

FIGURE 3 A, Correlation of survival of MSD cases and the number of affected key clusters of signs. MSD patients without reported signs at birth (blue) showed longer survival compared to patients with signs at birth (red). B, Correlation of survival with reported walking abilities. Cases with achievement of independent walking (blue) survived longer than cases without achievement of independent walking (red). C, Significant differences in survival of patients with severe (red) compared to patients reported to carry mild *SUMF1* mutations (blue)

LogRank test, $P = 0.4$, 24 months LogRank test, $P = 0.1$, 36 months LogRank test, $P = 0.007$; Figure S5A-C).

Key clusters of signs that were rarely reported, for example, cardiovascular and respiratory involvement, were associated with reduced survival, independent of age of onset (cardiac and vascular: landmark analysis at 12 months: LogRank test, $P = 0.003$; Figure S6A, 24 months LogRank test, $P = 0.026$, 36 months LogRank test, $P = 0.05$; respiratory: 12 months: LogRank test, $P = 0.0013$; Figure S6B, 24 months LogRank test, $P = 0.0013$, 36 months LogRank test, $P = 0.027$). Individuals who never achieved independent walking had reduced survival as compared to patients who achieved independent walking (mean survival no independent walking group 9.2 years (SE 0.4), independent walking group 10.3 years (SE 1.6), LogRank test, $P = 0.0272$; Figure 3B).

3.8 | Genotype, sulfatase activities, and survival

Case genotypes were divided into three categories based on published data on severity (see Section 2.3). Of these, 25 classified as severe (mutation-group 2) and 34 as less-severe (mutation-group 1). Twenty-nine cases could not be classified based on the available data (mutation-group 0).

Decreased survival was associated with the severe mutation group (group 2) compared to individuals in the less severe mutation group (group 1) (mean survival group 1: 15.3 years (SE 1.9), group 2: 8.2 years (SE 1.11), LogRank test, $P = 0.0482$, Figure 3C). There was no significant correlation between sulfatase activity and survival when we analyzed residual activities <10% or >10% of arylsulfatase A, arylsulfatase B, and arylsulfatase C (ASA, LogRank test, $P = 0.06$; ASB, LogRank test, $P = 0.247$; ASC LogRank test, $P = 0.236$).

4 | DISCUSSION

MSD is an ultra-rare, severe systemic disease without therapeutic options. To improve prognosis for families, to establish evidence-based clinical care guidelines and to guide future clinical trial design, it is important to characterize variables that correlate with disease outcomes.[25] As current clinical knowledge on MSD relies on single case reports and a limited number of case series, more comprehensive information is needed. We designed a meta-analysis approach using term-based phenotyping. From the available literature, we collected descriptive data on demographic characteristics, clinical signs, brain imaging features, sulfatase activities, GAG and sulfatide excretion, and *SUMF1* mutations and were able to identify novel factors predicting disease severity in MSD. The 143 cases within this study were within the range of 53 to 248 patients used in previously published meta-analysis and systematic reviews on rare and ultra-rare diseases. These studies led to quantitative information on age of onset, survival, and predictions on disease trajectory in

different lysosomal and metabolic diseases including Krabbe-disease, MPS VII, and Molybdenum Cofactor Deficiency.[14-18,26]

Although published case reports are an easily accessible source of clinical data and often with thorough and detailed descriptions that have previously been shown to expand clinical knowledge systematic reviews using published cases in contrast to prospective observational studies have major limitations. They comprise nonstandardized timing of clinical examinations, test results, and reporting and the same individual could appear in multiple publications.[27] Case reports could be biased toward the presentation of unusual findings and exceptions from common knowledge, which can influence meta-analysis conclusions. The lack of a standardized study protocol, absence of classification scores, differences in denomination of clinical findings and variable time points of reported encounters and follow up encounters impair comparisons. Such factors could introduce errors into calculations and influence the incidence of reported features in the present study. We sought to reduce the duplication of reporting by comparing provided details within publications by the same authors, and removing redundant reports and aimed for minimizing limitations by categorizing reported clinical signs and restricting the analysis of correlations to clearly reported variables such as survival and independent walking. The findings from this work should be further characterized in future natural disease history studies.

This report underscores the clinical variability of MSD, with 75 clinical signs in 11 key clusters. This complexity, in combination with its rarity has limited a full understanding of disease classifications, genotype-phenotype relationships, and biomarker significance. Previously published MSD disease classifications are based on the age of onset and distinguish neonatal very severe forms from severe and milder late infantile and juvenile forms of MSD. Prior classification schemes have included (a) neonatal MSD, the severest form characterized by onset of multiple signs at birth and (b) late infantile MSD characterized by a reduced number of signs presenting after the neonatal period. Late infantile cases are often further subdivided into severe and mild forms based on the onset of signs before or after age 2. Finally, (c) juvenile cases with late onset and slow progression have also been reported.[5,10,11,28]

Despite the limitations of our study as outlined above, our data reveal that MSD is a progressive disease with different clinical signs based on age at onset. Moreover, survival depended on the age of onset and the number of affected key clusters of signs and signs at a given time point, partially confirming historic classifications of neonatal, late infantile and juvenile forms of MSD. However, we found 45 patients with signs in more than one key cluster at birth that did not fit the neonatal severe phenotype. Furthermore, the age of 2 years did not clearly discriminate severe from mild cases.

In terms of survival, we found evidence that early onset of neurologic or skeletal signs (two common categories of signs) correlated with a shorter life-span. However, presence of skin signs, another common feature, was not correlated with life expectancy. Features that discriminated between severe and milder cases in this cohort included the presence of psychomotor delay, especially failure to attain independent ambulation and presence of rare disease features, such as cardiovascular involvement. In summary, in the present cohort, phenotypic variation was not accurately captured by prior disease classification systems. We hypothesize that categorization of MSD as a spectrum from severe to attenuate would more accurately represent this rare and complex disorder. Spectra of disease severity rather than fixed categories have recently been discussed for other lysosomal storage disorders for which actual natural disease history data have been acquired prospectively or from systematic reviews. Therefore MSD is no exemption but in line with diseases like Krabbe disease, MLD, MPS IIIA, and free sialic acid storage disease.[26,29-32] A classification system for MSD that is capable of prognosticating clinical outcomes should We propose that a disease classification for MSD, allowing predictions of disease severity, should include multiple co-variants like age of onset, number of key clusters of signs, involvement of specific organ systems, and psychomotor development.

Genotype-phenotype correlation in MSD has been proposed but is incompletely characterized. Experimental data on enzyme stability and residual activity of pathogenic FGE variants are available for two thirds of known *SUMF1* variants.[5,9-11,21-23] The data available from this study suggest a correlation between survival time and type of *SUMF1* mutation, underscoring the proposed genotype-phenotype correlation. In contrast to *SUMF1* mutation data, we found no differences in survival time in groups of MSD patients with three sulfatase activities either below or above 10% residual activity. Storage material excretion was variable and did also not correlate with survival. Limits of the present dataset prohibited establishing a positive correlation of different activity cut-off values or activities of other deficient sulfatases with survival. More standardized clinical and biochemical data acquired from up-to-date diagnostic analysis are required to fully establish or exclude a correlation between deficient sulfatase activities, storage materials, and clinical signs and disease severity in MSD. However, our results indicated that, despite the obvious pathophysiology of impaired FGE functionality causing deficient sulfatase

activation, many details on the relationship of *SUMF1* mutation, sulfatase activities and clinical presentation remain elusive in MSD. The contribution of single sulfatase deficiencies to clinical signs determining disease burden and survival is unclear. The role of disease modifying molecular factors, differing affinities of sulfatase molecules to FGE variants or yet unknown pathomechanisms resulting from FGE dysfunction could influence the course of disease in MSD. To develop a comprehensive understanding of MSD, future clinical and molecular research is needed.

Using a meta-analysis approach in MSD, we established that meaningful information could be collected and interpreted from the existing medical literature. For future similar studies in different diseases, research on the development of statistical methods that account for heterogeneity between case reports is desired to mitigate the limitations of the approach and improve the validity of results. From case reports available at the time of writing, our analysis provides an approach to a natural disease history data for MSD with identification of novel predictors of survival. This data is important for the detection of MSD, patient counseling and the development of meaningful endpoints in future therapeutic studies. Age at presentation, the presence and timing of specific clinical signs, and genotype are emerging as key variables that influence outcomes. In this report, we demonstrate the utility of systematic review of the medical literature as a cost-effective, robust and clinically meaningful way to characterize an ultra-rate, highly complex, and variable disorder, also applicable in similar disorders.

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CONFLICT OF INTEREST

Lars Schlotawa and Rebecca Ahrens-Nicklas are members of the medical advisory board to United MSD Foundation, USA. TF reports personal fees from Novartis, Bayer, Janssen, SGS, Roche, Boehringer Ingelheim, Daiichi-Sankyo, Galapagos, Penumbra, Parexel, Vifor, BiosenseWebster, CSL Behring, Fresenius Kabi, Coherex Medical, all outside the submitted work. Laura A. Adang is a medical advisor for the MLD Foundation and CureMLD. Joana Preiskorn and Stina Schiller declare no competing interests. Jutta Gärtner reports personal fees from Bayer, Biogen, Novartis and Sanofi. Lars Schlotawa and Jutta Gärtner have a patent for the treatment of multiple sulfatase deficiency (US Patent No. 16591051).

AUTHOR CONTRIBUTIONS

Lars Schlotawa and Tim Friede designed the study. Lars Schlotawa, Jutta Gärtner, and Tim Friede were responsible for the concept of the study. Joana Preiskorn, Lars Schlotawa, and Tim Friede executed the meta-analysis, extracted and calculated all data. Joana Preiskorn, Lars Schlotawa, and Stina Schiller prepared figures and tables. All authors evaluated and discussed the data. Lars Schlotawa and Jutta Gärtner wrote the first manuscript draft. All authors reviewed, edited, and corrected the manuscript. Lars Schlotawa is finally responsible and guarantor.

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REFERENCES

1. Cosma MP, Pepe S, Annunziata I, et al. The multiple sulfatase deficiency gene encodes an essential and limiting factor for the activity of sulfatases. *Cell*. 2003;113(4):445-456.
2. Dierks T, Schmidt B, Borissenko LV, et al. Multiple sulfatase deficiency is caused by mutations in the gene encoding the human C(alpha)-formylglycine generating enzyme. *Cell*. 2003;113(4):435-444.
3. Dierks T, Dickmanns A, Preusser-Kunze A, et al. Molecular basis for multiple sulfatase deficiency and mechanism for formylglycine generation of the human formylglycine-generating enzyme. *Cell*. 2005;121(4):541-552.
4. Dierks T, Schlotawa L, Frese MA, Radhakrishnan K, von Figura K, Schmidt B. Molecular basis of multiple sulfatase deficiency, mucolipidosis II/III and Niemann-pick C1 disease - Lysosomal storage disorders caused by defects of non-lysosomal proteins. *Biochim Biophys Acta*. 2009;1793(4):710-725.
5. Sabourdy F, Mourey L, Le Trionnaire E, et al. Natural disease history and characterisation of SUMF1 molecular defects in ten unrelated patients with multiple sulfatase deficiency. *Orphanet J Rare Dis*. 2015;10:31.
6. Austin JH. Studies in metachromatic leukodystrophy. XII. Multiple sulfatase deficiency. *Arch Neurol*. 1973;28(4):258-264.
7. Eto Y, Gomibuchi I, Umezawa F, Tsuda T. Pathochemistry, pathogenesis and enzyme replacement in multiple-sulfatase deficiency. *Enzyme*. 1987;38(1–4):273-279.

8. Chang PL, Rosa NE, Ballantyne SR, Davidson RG. Biochemical variability of arylsulphatases -a, -B and -C in cultured fibroblasts from patients with multiple sulphatase deficiency. *J Inherit Metab Dis*. 1983;6(4):167-172.
9. Cosma MP, Pepe S, Parenti G, et al. Molecular and functional analysis of SUMF1 mutations in multiple sulfatase deficiency. *Hum Mutat*. 2004;23(6):576-581.
10. Schlotawa L, Steinfeld R, von Figura K, Dierks T, Gartner J. Molecular analysis of SUMF1 mutations: stability and residual activity of mutant formylglycine-generating enzyme determine disease severity in multiple sulfatase deficiency. *Hum Mutat*. 2008;29(1):205.
11. Schlotawa L, Ennemann EC, Radhakrishnan K, et al. SUMF1 mutations affecting stability and activity of formylglycine generating enzyme predict clinical outcome in multiple sulfatase deficiency. *Eur J Human Genetics: EJHG*. 2011;19(3):253-261.
12. Hijazi L, Kashgari A, Alfadhel M. Multiple sulfatase deficiency: a case series with a novel mutation. *J Child Neurol*. 2018;33 (13):820-824.
13. Ahrens-Nicklas R, Schlotawa L, Ballabio A, et al. Complex care of individuals with multiple sulfatase deficiency: clinical cases and consensus statement. *Mol Genet Metab*. 2018;123(3): 337-346.
14. Slama T, Garbade SF, Kolker S, Hoffmann GF, Ries M. Quantitative natural history characterization in a cohort of 142 published cases of patients with galactosialidosis-a cross-sectional study. *J Inherit Metab Dis*. 2019;42(2):295-302.
15. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M. Quantitative clinical characteristics of 53 patients with MPS VII: a cross-sectional analysis. *Genet Med*. 2017;19(9):983-988.
16. Mechler K, Mountford WK, Hoffmann GF, Ries M. Ultra-orphan diseases: a quantitative analysis of the natural history of molybdenum cofactor deficiency. *Genet Med*. 2015;17(12): 965-970.
17. Komatsuzaki S, Zielonka M, Mountford WK, et al. Clinical characteristics of 248 patients with Krabbe disease: quantitative natural history modeling based on published cases. *Genet Med*. 2019;21:2208-2215.
18. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M. A cross-sectional quantitative analysis of the natural history of Farber disease: an ultra-orphan condition with rheumatologic and neurological cardinal disease features. *Genet Med*. 2018;20 (5):524-530.
19. Frankenburg WK, Dodds J, Archer P, Shapiro H, Bresnick B. The Denver II: a major revision and restandardization of the Denver developmental screening test. *Pediatrics*. 1992;89(1): 91-97.
20. Frankenburg WK, Dodds JB. The Denver developmental screening test. *J Pediatr*. 1967;71(2):181-191.
21. Annunziata I, Bouche V, Lombardi A, Settembre C, Ballabio A. Multiple sulfatase deficiency is due to hypomorphic mutations of the SUMF1 gene. *Hum Mutat*. 2007;28(9):928.
22. Schlotawa L, Radhakrishnan K, Baumgartner M, et al. Rapid degradation of an active formylglycine generating enzyme variant leads to a late infantile severe form of multiple sulfatase deficiency. *Eur J Human Genetics: EJHG*. 2013;21(9):1020-1023.
23. Jaszczuk I, Schlotawa L, Dierks T, et al. Expanding the genetic cause of multiple sulfatase deficiency: a novel SUMF1 variant in a patient displaying a severe late infantile form of the disease. *Mol Genet Metab*. 2017;121:252-258.
24. Hinderhofer K, Mechler K, Hoffmann GF, Lampert A, Mountford WK, Ries M. Critical appraisal of genotype assessment in molybdenum cofactor deficiency. *J Inherit Metab Dis*. 2017;40(6):801-811.
25. Day S, Jonker AH, Lau LPL, et al. Recommendations for the design of small population clinical trials. *Orphanet J Rare Dis*. 2018;13(1):195.
26. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M. A cross-sectional quantitative analysis of the natural history of free sialic acid storage disease-an ultra-orphan multisystemic lysosomal storage disorder. *Genet Med*. 2019;21(2):347-352.
27. Sandu N, Chowdhury T, Schaller BJ. Trigemino-cardiac reflex examination G. How to apply case reports in clinical practice using surrogate models via example of the trigeminocardiac reflex. *J Med Case Reports*. 2016;10:84.
28. Adam MP, Ardinger HH, Pagon RA. Multiple sulfatase deficiency, et al., eds. *Genetic Counseling*. GeneReviews® Seattle, WA: University of Washington; 1993-2020.
29. Bascou N, DeRenzo A, Poe MD, Escolar ML. A prospective natural history study of Krabbe disease in a patient cohort with onset between 6 months and 3 years of life. *Orphanet J Rare Dis*. 2018;13(1):126.
30. Harrington M, Whalley D, Twiss J, et al. Insights into the natural history of metachromatic leukodystrophy from interviews with caregivers. *Orphanet J Rare Dis*. 2019;14(1):89.
31. Nijmeijer SCM, van den Born LI, Kievit AJA, et al. The attenuated end of the phenotypic spectrum in MPS III: from late-onset stable cognitive impairment to a non-neuronopathic phenotype. *Orphanet J Rare Dis*. 2019;14(1):249.
32. Shapiro EG, Nestrasil I, Delaney KA, et al. A prospective natural history study of Mucopolysaccharidosis type IIIA. *J Pediatr*. 2016;170:278-287.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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