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ATP7A-related copper transport disorders: a systematic review and definition of the clinical subtypes

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Abstract:	Purpose To provide differentiating clinical criteria, evaluate genotype-phenotype correlations, and propose management guidelines for the different ATP7A-related copper transport disorders. Methods We performed a systematic literature review by querying the MEDLINE and Embase databases identifying 143 relevant papers, and included data on the phenotype and genotype in 162 individuals with a molecularly confirmed ATP7A-related disorder. Results Early seizures are specific for Classical Menkes Disease (CMD), that is characterized by early-onset neurodegenerative disease with high mortality rates. Ataxia is an independent indicator for Atypical Menkes Disease (AMD), that shows better survival rates than CMD. Bony exostoses, radial head dislocations, herniations and dental abnormalities are specific for occipital Horn syndrome (OHS) that may further present with developmental delay and connective tissue manifestations. Intracranial tortuosity

	and bladder diverticula, both with high risk of complications, are common among all subtypes. Low ceruloplasmin is a more sensitive and discriminating biomarker for ATP7A-related disorders than serum copper. Truncating mutations are more frequently associated with CMD, in contrast with splice site and intronic mutations which are more frequently seen in OHS. Conclusion This systematic review on ATP7A-related disorders sets forth clinical criteria to allocate patients in the different subgroups, identify diagnostic and prognostic markers and propose management guidelines.

To:
Prof. dr. Shamima Rahman and Prof. dr. Matthias Baumgartner
Editors-in-Chief, *Journal of Inherited Metabolic Disease*

Ghent, Belgium
November 27th, 2022

Manuscript submission: "*ATP7A*-related copper transport disorders: a systematic review and definition of the clinical subtypes"

Dear Prof. dr. Rahman, dear Prof. dr. Baumgartner, dear Editor,

We are delighted to submit our manuscript "***ATP7A*-related copper transport disorders: a systematic review and definition of the clinical subtypes**" to Journal of Inherited Metabolic Disease.

Pathogenic variants in the *ATP7A*-gene cause Menkes Disease (MD), Occipital Horn Syndrome (OHS) or X-linked Distal Spinal Muscular Atrophy-4 (SMAX3). Though the molecular basis is similar, the phenotypic spectrum is wide. Whereas Menkes Disease (CMD) shows neurodegeneration with premature death, OHS is considered a milder allelic variant with pathognomonic occipital horns and other exostoses, connective tissue manifestations, a milder neurological presentation, and a significantly longer life expectancy. SMAX3 is considered a distinct phenotype with late-onset symptoms resembling Charcot-Marie-Tooth disease, but an overlap between OHS and SMAX3 was recently reported. Overall, this indicates these *ATP7A*-related disorders form a phenotypic continuum. Nevertheless, in the absence of natural history studies, biochemical biomarkers, and genotype-phenotype correlations, the diagnosis, prognostication, and management of patients with *ATP7A*-related disorders remains extremely challenging.

We are the first to present a systematic review on *ATP7A*-related copper transport disorders, aggregating the phenotypic and genotypic data on 162 reported patients. Based on this, we establish clinical criteria to allocate patients early to the correct *ATP7A*-related phenotype and we propose evidence-based management guidelines. In line with previous reports, a clear genotype-phenotype correlation remains difficult to validate.

We are convinced that our data will facilitate management and follow-up of patients with *ATP7A*-related copper transport disorders and, as such, will attract a broad readership among geneticists, (pediatric) neurologists, and other involved specialists.

All authors have contributed to this work, have approved the manuscript and none have reported a conflict of interest.

We thank you in advance for your valuable time and attention dedicated to our work and hope you will consider publishing our paper in *Journal of Inherited Metabolic Disease*.

On behalf of all authors,

Sincerely,

Prof. Dr. B. Callewaert
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To:
Prof. dr. Shamima Rahman and Prof. dr. Matthias Baumgartner
Editors-in-Chief, *Journal of Inherited Metabolic Disease*

Ghent, Belgium
January 11, 2023

Manuscript resubmission: "*ATP7A*-related copper transport disorders: a systematic review and definition of the clinical subtypes"

Dear Prof. dr. Rahman, dear Prof. dr. Baumgartner, dear Editor,

We are pleased to resubmit for publication the revised version of **"*ATP7A*-related copper transport disorders: a systematic review and definition of the clinical subtypes"**. We appreciated the constructive criticisms of the reviewers. Each of their concerns have been addressed as outlined below.

Reviewer #1:

The manuscript is well-written, the methods are sound, the data is convincing and the interpretations/conclusions are reasonable. This is a study that very much needed to be performed. I cannot detect any major flaws in this work.

We thank the reviewer for assessing our manuscript and this nice comment.

Reviewer #2:

Dr. De Feyter et al. present a systemic literature review of ATP7A gene-related disorders. The methods of the systematic review are well described. The authors do present several interesting new observations related to these disorders that could have clinical utility.

1. Reviewer comment:
   My primary criticism is that this paper does not provide that much new information on this group of disorders compared to already published reviews.

   Author answer:
   The authors are aware of the rather limited amount of new information provided in our study. However, the main purpose of this project was to gather all existing knowledge on *ATP7A*-related disorders in a systematic way in order to provide a comprehensive overview for physicians with less expertise in this disorder and to provide a basis for evidence-based observations and guidelines. After all, a systematic review has not been performed on this topic before. Nonetheless, our review does

report on several interesting observations that are not reported in literature before, or that dispel some previously made assumptions:

- We show that ataxia is an independent indicator for Atypical Menkes Disease (AMD).
- We demonstrate that, in contrast what was been previously published, a quarter of the Occipital Horn Syndrome (OHS) patients present with developmental delay or hypotonia.
- Scalp hematomas do not allow for discrimination between subtypes.
- Intracranial tortuosity and bladder diverticula, both with high risk of complications, are common among all subtypes.
- We could conclude that ceruloplasmin as a more sensitive and discriminating biomarker for *ATP7A*-related disorders than serum copper.
- Hair abnormalities are frequently present and may allow discrimination between Classic Menkes Disease (CMD) and OHS.
- We have put together all existing knowledge on copper therapy.

2. Reviewer comment:

   The authors should consider revising the statement that "early copper histidine treatment has limited value as reports mentioning clinical improvement are scarce". The authors themselves describe a clinical improvement in neurodevelopment in 21% of CMD patients, 20% of AMD patients, and 25% of OHS patients. Clinical improvement in 20-25% of individuals is highly significant, especially since there is no alternate disease modifying therapy for these disorders.

   Author answer:

   Thank you for this attentive comment. Our systematic review identified 56 patients who received copper histidine treatment (38 CMD, 10 AMD, 8 OHS). However, in many cases (total 30, 17 CMD, 8 AMD, 5 OHS), the reports did not mention any effect (positive or negative) of the treatment, which may point to publication bias of those that appreciated some effect. In 9 CMD patients, there was a variable, but rather vague improvement on neurological symptoms, including better muscle tone, improved seizure control and better response to external stimuli. However, 3 out of 9 patients who showed improvement, still died between the ages of 7 months and 6 years, questioning the effect on overall survival. In 2 CMD patients, there was only an improvement in hair color, which is clinically less relevant considering the severity of the neurological phenotype. In addition, side effects such as renal failure have been reported in some patients. However, we do acknowledge the data cannot refute a potential benefit, so we change the statement in the paper:

'The value of early copper histidine treatment is difficult to assess since only a limited number of reports mention, often temporary, clinical improvement which has no effect on overall survival.'

3. Reviewer comment:

   The authors should comment more on additional biochemical studies in ATP7A gene related disorders, such as plasma and CSF catecholamines. As the authors acknowledge, copper and ceruloplasmin have less utility in diagnosing infants with ATP7A related disorders since the normal reference range is lower than in older children and can overlap with these disorders.

   Author answer:

   Aside from copper and ceruloplasmin, our review appraised different biochemical studies in the diagnosis of the CMD, AMD and OHS, including copper uptake in fibroblasts/lymphocytes, plasma lactate, CSF copper, CSF pyruvate and catecholamine levels. We agree that biochemical analyses of plasma/CSF catecholamines can be valuable in the diagnosis of *ATP7A*-related disorders. Our review includes 8 patients with increased plasma (and CSF) catecholamine levels (6 OHS, 2 AMD), however 1 AMD and 1 CMD patient demonstrated normal plasma catecholamine levels. Due to this limited amount of patients (almost no data in CMD) and the discrepancies in the results, we did not retain the advice to perform catecholamine testing in our manuscript, considering the invasiveness of a lumbar punction in young children.

4. Reviewer comment:

   The authors describe that the "mean age at initial presentation is 1.7 years" in CMD, AMD, and OHS. Symptoms of classic Menkes disease typically begin between 2-3 months of age based on the prior literature. Can the authors comment on this major discrepancy.

   Author answer:

   Thank you for this remark. The age at initial presentation of 1.7 years is the age of presentation for the whole group of *ATP7A*-related disorders (CMD, AMD and OHS). For CMD the range lies between birth-5 years, (mean 5mo, median 3mo), for AMD between birth -1 year 10mo (mean 7mo, median 4mo), for OHS between birth-50 years (mean 5y8mo, med 5mo). Therefore, the high age at diagnosis in OHS patients increases the age in the whole group. To avoid any confusion, we added the specifics for each subgroup to the manuscript.

   'Initial symptoms leading to the diagnosis are presented in Table 1. The mean age at initial presentation is 1.7 years (range: birth – 50 years) and does not differ between the three subgroups (p = 0.614). For CMD the range lies between birth - 5 years, (mean

5 months, median 3 months), for AMD between birth - 1 year 10 months (mean 7 months, median 4 months), for OHS between birth - 50 years (mean 5 year 8 months, median 5 months). More than half of the CMD patients present with seizures. In about a third of OHS patients, symptoms of dysautonomia such as diarrhea, vomiting, temperature instability, and orthostatic hypotension were initially reported. If dysautonomia is reported as an initial symptom in the MD subgroups, it commonly involves temperature stability. In the CMD subgroup cardiocirculatory or respiratory complications at birth occur more frequently: 14.9% (15/101) compared with 0% (0/18, p = 0.123) in AMD and 2.7% (1/37, p = 0.069) in OHS. Other rare perinatal complications include scalp fractures or hematomas.'

5. Reviewer comment:

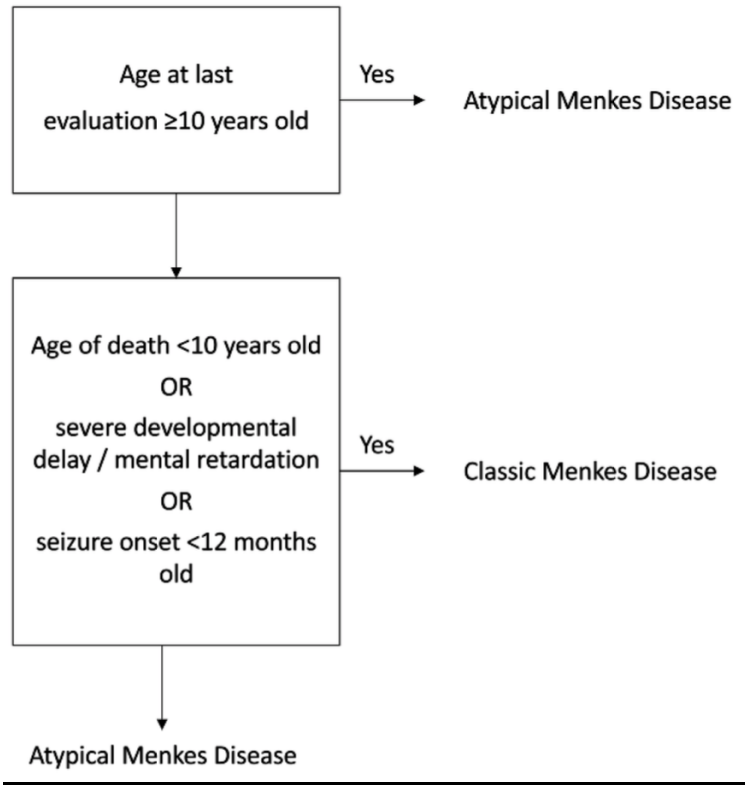
   The authors distinguish between classic (CMD) and atypical Menkes disease (AMD) by "age and severity of the neurological features"; this includes "severe developmental delay/MR" as a distinguishing feature of CMD. How do the authors define "severe" in this case? Further, I would suggest that the distinguishing features between CMD and AMD that are derived from the systematic review and presented as results are inherent to how CMD and AMD are defined in the first place by the authors.

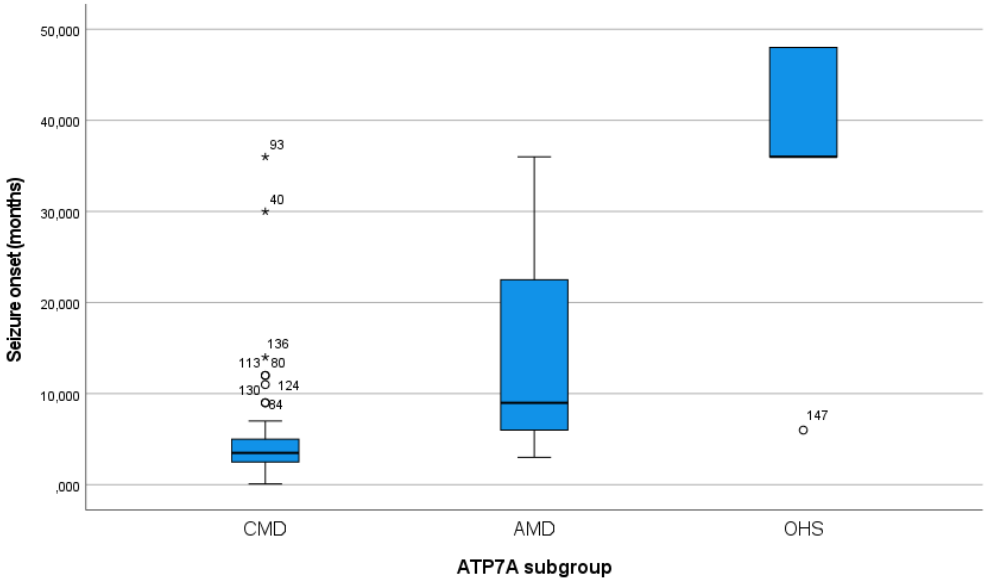
   Author answer:

   This is a valid and incisive comment on the set-up of this study. First of all, it was rather difficult to define severe neurodevelopment/MR since there are no specific recommendations on this subject. We assessed all cases individually (AB and SDF) and scored different aspect of development (possibility to walk independently, speech development, presence of social interactions, …) in order to get a broad view on the functioning of each individual. Some of the distinguishing features between AMD and CMD are inherent to the definition of these entities. This is a major limitation to the study and we tried to reduce its effect as much as possible by making the distinction between CMD and AMD on a very limited amount of features (see supplemental file S3 and below this comment): the age at last evaluation >= 10 years old was chosen deliberately in order to avoid falsely including patients as AMD. If patients were not classified based on this feature, we looked at the severity of neurodevelopmental delay to make further distinction. Finally, to be able to classify very young patients who did not reach developmental milestones just yet, we added seizure onset before the age of one year. The results obtained from this classification could be appreciated as inherent to the aforementioned features at first sight, however, this classification obtained relevant clinical observations as illustrated by the following observation (see second figure below): CMD: seizure onset 3 days – 3 years (med 3.5 months, mean 5 months) vs AMD: seizure onset 3 months – 3 years (med 9 month, mean 1 year 4 months). Even though the cut-off for seizure onset is placed at 1 year, we observed that seizure onset >6 months is very rare in CMD, whereas seizure onset >6 months is

omnipresent in AMD. Furthermore, by using the classification based on neurodevelopmental features, we found discriminating factors not inherent to the selection criteria (ataxia, more females, hair abnormalities).

'This study may be subject to some limitations. First, the systematic review and predefined search strategy aimed to identify all potentially relevant studies, but does not fully exclude the risk of selection bias. Second, our systematic review assembled data from case reports and case series, which are prone to publication and selection bias, especially since negative data or absent symptoms tend to be underreported. Third, some of the distinguishing features between AMD and CMD are inherent to the definition of these entities. A limitation to the set-up of this study that we tried to reduce as much as possible by making the distinction between AMD and CMD on a very limited and rather objective amount of characteristics (**Supplemental file *S3***). Finally, due to the rarity of *ATP7A*-related disorders, data are limited, especially regarding the AMD and SMAX3 phenotypes. Taken together, our review only approximates a representation of the clinical spectrum of *ATP7A*-related copper transport disorders.'





6. Reviewer comment:

   The authors provide management recommendations, but do not provide evidence for these recommendations based on the systematic review.

   Author answer:

   All advises on management are based on the results of the systematic review on each group of symptoms, initial presentations, causes of death or diagnostic methods, which can be consulted in table 2 and S2.

7. Reviewer comment:

   The authors recommend a 24 hour EEG in all patients with ATP7A related disorders. Surely, this would refer primarily to patients with CMD or AMD and not OHS or SMAX3. Further, what is the purpose of a 24 hour EEG in the absence of clinical concern for seizures? Is there evidence that patients with ATP7A related disorders are at higher risk for subclinical seizures? If there is clinical concern for seizures, why not begin with a standard EEG?

   Author answer:

   Thank you for your attentive remark. Our review did not identify an increased risk for subclinical seizures in either of the subgroups, so in the case of a clinical concern for seizures, we would recommend to start with a standard EEG. This was adjusted in the manuscript. Since a risk for seizures is also present in OHS patients, we also would recommend vigilance in this group.

   'All patients should receive in-depth neurological and developmental evaluation, including an EEG in the case of clinical suspicion of seizures.'

8. Reviewer comment:

   What is the basis for recommending repeat brain MR/MRA every 3 years specifically (i.e. why not annually or every 5 years, etc.)? How does repeating the MRI at regular intervals as screening actually change management?

   Author answer:

   85,1% of patients present with widespread intracranial tortuosity, whereas ischemic events have been reported in 10%. We would recommend performing an MRA in all patients at diagnosis to assess the presence of tortuosity. In case of documented tortuosity, the advice to repeat the MRA every 3 years is, due to the lack of guidelines on this subject, based on (expert) recommendations for other conditions presenting with intracranial tortuosity and risk for ischemic events (arterial tortuosity syndrome, Loeys-Dietz syndrome, autosomal recessive cutis laxa type 1b). We do acknowledge that the actual evidence for these recommendation is low. Considering the young age of the patients, MRA studies implicate general anesthesia which would be difficult to imply on a yearly basis. The goal of this screening modality is to allow for early-on detection of aneurysms or stenosis, which would allow intervention.

   'We recommend performing an MRI and MRA of the brain at diagnosis in order to objectify common but non-specific anomalies such as intracranial tortuosity, atrophy, and myelination problems. Considering the high risk of cerebrovascular incidents in patients with documented tortuosity, imaging could be repeated in this group every three year or earlier in case of new symptoms. In the absence of evidence specific for ATP7A related conditions, this suggestion is based on recommendations for other conditions associating with arterial tortuosity (PMID 36334952, 29323665).'

9. Reviewer comment:

   CVA is listed in the legend for Table 2, but it is not actually included in the table as far as I can see.

   Author answer:

   Thank you for this attentive remark. We deleted CVA from the legend.

We thank you in advance for your valuable time and attention dedicated to our work and hope you will consider publishing our paper in *Journal of Inherited Metabolic Disease*.

On behalf of all authors,

Sincerely,

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ATP7A-related copper transport disorders: a systematic review and definition of the clinical subtypes

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ATP7A-related copper transport disorders: a systematic review and definition of the clinical subtypes

Abstract

Purpose: To provide differentiating clinical criteria, evaluate genotype-phenotype correlations, and propose management guidelines for the different *ATP7A*-related copper transport disorders.

Methods: We performed a systematic literature review by querying the MEDLINE and Embase databases identifying 143 relevant papers, and included data on the phenotype and genotype in 162 individuals with a molecularly confirmed *ATP7A*-related disorder.

Results: Early seizures are specific for Classical Menkes Disease (CMD), that is characterized by early-onset neurodegenerative disease with high mortality rates. Ataxia is an independent indicator for Atypical Menkes Disease (AMD), that shows better survival rates than CMD. Bony exostoses, radial head dislocations, herniations and dental abnormalities are specific for Occipital Horn Syndrome (OHS) that may further present with developmental delay and connective tissue manifestations. Intracranial tortuosity and bladder diverticula, both with high risk of complications, are common among all subtypes. Low ceruloplasmin is a more sensitive and discriminating biomarker for *ATP7A*-related disorders than serum copper. Truncating mutations are frequently associated with CMD, in contrast with splice site and intronic mutations which are more prevalent in OHS.

Conclusion: This systematic review on *ATP7A*-related disorders sets forth clinical criteria to allocate patients in the different subgroups, identify diagnostic and prognostic markers and propose management guidelines.

Synopsis

This systematic review on *ATP7A*-related copper transport disorders provides a practical approach for phenotype differentiation with clinical and prognostic relevance, and proposes management guidelines.

Compliance with Ethics Guidelines

Contribution of individual authors

Conceptualization: A.B., B.C.; Data curation: S.D.F., A.B.; Formal Analysis: S.D.F.; Methodology: S.D.F., A.B.; Supervision: B.C.; Visualization: S.D.F.; Writing – original draft: S.D.F., A.B.; Writing – review & editing: B.C.

Conflict of Interest

Silke De Feyter, Aude Beyens and Bert Callewaert declare that they have no conflict of interest.

Acknowledgements

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Data availability

The submitted manuscript (and supplemental information) includes all data analyzed during this review.

Informed Consent and Animal Rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Key words

ATP7A, Menkes Disease, Occipital Horn Syndrome, ATPase, copper metabolism, neurodegenerative disorder, X-linked, genotype-phenotype

Introduction

Pathogenic variants in *ATP7A* result in copper transport disorders comprising Menkes Disease (MD, OMIM#309400), Occipital Horn Syndrome (OHS, OMIM#304150), and X-linked Distal Spinal Muscular Atrophy-3 (SMAX3, OMIM#300489).

MD is mainly characterized by neurodevelopmental impairment. Other features include connective tissue abnormalities, arterial tortuosity and 'kinky' hair[1-3]. Classical MD (CDM) presents with progressive neurodegeneration and demise in early childhood[4]. OHS comprises a milder phenotype and presents with more pronounced connective tissue features, including cutis laxa, hernias, joint laxity, bladder diverticula, and pathognomonic 'occipital horns'. As in MD, patients with OHS may present with (mild) intellectual disability and dysautonomia. Life expectancy in OHS is substantially longer than in MD[1-3]. Individuals with overlapping features between MD and OHS have been documented[4]. In these instances, the term Atypical Menkes Disease (AMD) is often used. X-linked Distal Spinal Muscular Atrophy-3 (SMAX3), is a late-onset disorder resembling Charcot-Marie-Tooth disease, characterized by distal lower limb weakness and gait instability[5]. Unlike MD and OHS, no cognitive or connective tissue abnormalities are associated[1]. Recently, an overlap between OHS and SMAX3 was reported, indicating that MD, OHS, and SMAX3 are part of a clinical continuum[6].

The X-linked *ATP7A* encodes a P-type copper-transporting ATPase (ATP7A). *ATP7A* covers a genomic region of 140 kb and is organized into 23 exons[7,8]. The ATP7A transporter is mainly expressed in the gut, where it mediates copper transport into the bloodstream. In other tissues, except for the liver, ATP7A exerts a dual role through intracellular trafficking. Under basal copper conditions, ATP7A resides in the trans-Golgi network, where it is essential for cupro-enzyme biogenesis[9-13]. When intracellular copper concentrations increase, ATP7A reversibly localizes to the post-Golgi compartments, where it extrudes excess copper from the cell[14-16]. ATP7A consists of 6 amino terminal domains containing a metal-binding CXXC motif, followed by a structural core consisting of 8 transmembrane helices essential for transport, and an N-, P-, and A-domain, important for nucleotide binding, phosphorylation and dephosphorylation, respectively[17] **(Figure *1*)**. No clear genotype-

phenotype correlations have been established. Nevertheless, low to absent functional ATP7A levels have been associated with CMD[18,19], while OHS and AMD are attributed to 'leaky' splice-site and other variants that permit the production of some functional protein levels[20-23]. SMAX3 is caused by rare hypomorphic missense variants. The biochemical phenotype in MD and OHS is often characterized by subnormal copper levels in plasma, liver, and brain due to impaired intestinal absorption, resulting in reduced activities of copper-dependent enzymes, and paradoxical accumulation of copper in other tissues[20,24]. However, serum copper and ceruloplasmin are intrinsically low in healthy infants younger than two months, questioning its use as a reliable diagnostic biomarker[25].

Considering the overlap in clinical characteristics, the lack of natural history studies as well as the absence of predictive biomarkers and a clear genotype-phenotype correlation, counseling and management of patients with *ATP7A*-related disorders is challenging. We conducted a first systematic review on *ATP7A*-related copper transport disorders, and evaluated a possible genotype-phenotype correlation. We provide a practical approach for phenotype differentiation and patient management.

Materials and methods

Study design and search strategy

This systematic review was designed and conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. We aimed to collect the clinical and molecular data of all reported patients with a confirmed *ATP7A*-copper transport disorder. Therefore, we queried the MEDLINE (via Pubmed) and Embase databases using the search string shown in **Supplemental file *S1***. To exclude non-relevant references, queries were restricted to "title" and "abstract" in PubMed and "title", "abstract" and "keywords" in Embase. All references published from inception until November 4th, 2020 were included. EndNote 20 (Thomson Reuters, New York, NY) was used for bibliography management.

Screening process and eligibility criteria

Primary literature screening was performed blinded by two independent investigators (SDF and AB) using the Rayyan web application. Incongruence between investigators was resolved through consensus. Studies written in English, French or German and focusing on clinical and molecular characteristics of *ATP7A*-related disorders, including case reports and case series, were considered eligible. Subjects without a confirmed molecular diagnosis were excluded. Reviews, animal studies, and studies lacking clinical information were excluded. In order to avoid bias and allow comparison among subjects, we set forth criteria for final inclusion depending on the available information. Subjects were included only when information was available on at least four out of seven *ATP7A*-related clinical domains: neurological symptoms, hair or cutaneous abnormalities, connective tissue features, vascular abnormalities, skeletal abnormalities, dysautonomia, and copper biochemistry. Since patients with SMAX3 cannot not fulfill these inclusion criteria, all subjects with SMAX3 were included.

Quality assessment

Included studies were assessed for methodological quality and risk of bias by SDF and AB using the Joanna Briggs Institutes' Critical Appraisal Tools for Case Reports and Case Series[26]. They were classified as of 'good' if al quality criteria were judged as present, 'fair' if one or more criteria were unclear, or 'poor' quality if one or more criteria were absent.

Data acquisition

Data extraction was likewise performed by two independent reviewers (SDF and AB). Of each reported individual, we extracted data on a wide range of clinical characteristics **(Supplemental file *S2*)**. If an individual was described more than once, the most informative reference was used for data collection. Patients were subdivided into four categories: CMD, AMD, OHS and SMAX3. Because the presence of occipital horns is considered pathognomonic for OHS, all patients with occipital hors were included in the OHS subgroup. Two patients were added to the OHS subgroup without evidence for occipital horns. Both had a clinical phenotype primarily determined by connective tissue features and had a relative with proven occipital horns. A distinction between CMD and AMD was made by age and severity of the neurological features **(Supplemental file *S3*)**[4,20]. SMAX3 was distinguished by the presence of distal motor neuropathy and absence of intellectual disability and occipital horns. Molecular data was analyzed separately and supplemented with information concerning the affected domain, *in silico* prediction tools (Align GVGD, SIFT, Mutation Taster), nucleotide and protein conservation, and population databases **(Supplemental file *S4*).**

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics version 27. Continuous data were summarized by mean, median and range. Normality was checked using histograms and QQ plots. The non-parametric Kruskal-Wallis Test was used because of non-normal distribution and low sample size numbers. Categorical data were presented by absolute or relative frequencies. When necessary conditions were met, chi-square test was used to assess heterogeneity in different subgroups,

otherwise, the Fisher-Freeman-Halton exact test was performed. Where appropriate, the Fisher's exact test was applied for post hoc analysis in order to further explore the significant heterogeneity. All statistical analyses were performed two-sided at a significance level of α = 5%. Bonferroni corrections were applied to all post hoc analyses in order to control for the effect of multiple comparison testing.

Results

Search results

A PRISMA flow diagram representing the search strategy is depicted in **Figure *2*.** We identified 3685 unique papers, of which 2849 were excluded by screening title and abstract. From the remaining 836 papers selected for full-text screening, 143 papers were found to meet our final inclusion criteria. Five records were not identified through our search strategy. The included articles were overall of fair to good quality: 53.5% (76/142) was classified as good, 29.6% (42/142) as fair, and 16.9% (24/142) as poor. No records were excluded based on the quality assessment alone. The list of included references and the quality assessment is included as **Supplemental file *S5*.**

The 143 included papers report on 162 patients, of which 13.0% females (21/162) and 87.0% males (141/162), aged between 27 days and 57 years. 101 patients (62.3%, 101/162) were categorized in the CMD, 18 (11.1%, 18/162) in the AMD, 37 (22.6%, 37/162) in the OHS, and 6 (3.7%, 6/162) in the SMAX3 subgroup.

Menkes disease and occipital horn syndrome

Demographic characteristics of MD and OHS patients are presented in **Supplemental file *S6*.** The AMD subgroup consists of significantly more females compared with the CMD and OHS subgroups (p = 0.027 and 0.012, respectively). Age at last evaluation is significantly younger in CMD (mean 2.3 years) than in AMD (mean 14.3 years, p = <0.001) and OHS (mean 18.8 years, p = <0.001). Death is reported in 40.6% (41/101) of CMD patients, which is significantly more compared to OHS (13.5%, 5/37, p =0.006) and tends towards significance compared to AMD (11.1%, 2/18, p = 0.051). Age of death is significantly

younger in CMD (mean 2.3 years) than in OHS (mean 25.3 years) (p = 0.001). Infectious disease is the most common cause of death (31.8%, 7/22) in CMD. In OHS, reported causes of death are post-surgery respiratory insufficiency, gastric ulcer perforation, and rupture of a bladder diverticulum.

Initial presentation

Initial symptoms leading to the diagnosis are presented in **Table *1***. The mean age at initial presentation is 1.7 years (range: birth – 50 years) and does not differ between the three subgroups (p = 0.614). For CMD, the range lies between birth - 5 years (mean 5 months, median 3 months), for AMD between birth - 1 year and 10 months (mean 7 months, median 4 months), for OHS between birth - 50 years (mean 5 year 8 months, median 5 months). More than half of the CMD patients present with seizures. In about a third of OHS patients, symptoms of dysautonomia such as diarrhea, vomiting, temperature instability, and orthostatic hypotension were initially reported. If dysautonomia is reported as an initial symptom in the MD subgroups, it commonly involves temperature stability. In the CMD subgroup cardiocirculatory or respiratory complications at birth occur more frequently: 14.9% (15/101) compared with 0% (0/18, p = 0.123) in AMD and 2.7% (1/37, p = 0.069) in OHS. Other rare perinatal complications include scalp fractures or hematomas.

Neurological disease

Developmental delay is significantly more frequent in CMD (98.6%, 73/74), versus AMD (72.2%, 13/18), and OHS (61.3%, 19/31) (p = <0.001), and also more severe: 95.0% (38/40) of CMD patients present moderate to severe developmental delay, compared to 33.3% (3/9, p = <0.001) in AMD and 28.6% (4/14, p = <0.001) in OHS. Similarly, intellectual disability is reported significantly more in CMD patients (91.3%, 21/23) compared with OHS (53.1%, 17/32, p =0.009), but not compared with AMD (80.0%, 12/15, p = 0.333). Nevertheless, intellectual disability is moderate to severe in 93.3% of the CMD patients (14/15) versus 22.2% (2/9, p = 0.002) in AMD and 57.1% (6/14, p = 0.105) in OHS. Seizures are reported in 85.1% (86/101) of CMD patients, which is significantly more frequent than in

AMD or OHS patients (27.8%, 5/18, p = <0.001 and 16.2%, 6/37, p = <0.001, respectively). An onset of seizures in infancy occurs more often in CMD (mean 0.4 years) than in OHS (mean 2.8 years, p = 0.002). Muscle hypotonia is more frequent in CMD patients (79.2%, 80/101) compared with OHS (12/37, 32.4%, p = <0.001). Ataxia is more frequently described in AMD (27.8%, 5/18), compared to CMD (5.0%, 5/101, p = 0.021). Neurological characteristics are presented in **Table *2***.

Medical imaging confirms intracranial tortuosity in 85.1% of patients (74/87, p = 0.999). Overall, cerebral and cerebellar atrophy are comparable between subtypes, but delayed white matter myelinization and white matter abnormalities are exclusively seen in the MD subgroups, in about a third of them. Similarly, abnormalities of the basal ganglia are only seen in respectively 15% and 10% of the CMD and AMD phenotypes. Cerebrovascular accidents occur in 7.7% (7/91) of patients, of which five with CMD and two with OHS (p = 0.381). MRI findings are presented in **Supplemental file *S7*.**

Other clinical characteristics

Non-discriminatory craniofacial features include sagging cheeks (18.6%, 29/156) and a highly arched palate (10.3%, 15/156). Downslanted palpebral fissures and a long face are more frequently observed in OHS than CMD: 13.5% (5/37) versus 2.0% (2/101, p = 0.045) and 29.7% (11/37) versus 4.0% (4/101, p <0.001), respectively. Hair abnormalities are frequent (79.5%, 124/156) and more prevalent in CMD compared to AMD and OHS. In the MD subgroups, hair is often hypopigmented and kinky, whereas in OHS patients, hair is described as coarse, rather than brittle or fragile. Trichoscopy identifies pili torti in 94.3% (50/53) of patients. Scalp hematomas are relatively frequent in all subtypes (23.7% (23/97)).

Cutis laxa is reported in about half of patients (47.5%, 74/156), and occurs significantly more in OHS compared with CMD, but not compared with AMD. Herniations occur significantly more in OHS compared to CMD and AMD. Inguinal hernia is frequent (85.7%, 30/35), whereas hiatal, umbilical, and femoral hernia are less common (6/35, 4/35 and 3/35, respectively). Bladder diverticula are significantly more frequent in OHS (70.3%, 26/37), although they also occur in CMD (29.7%, 30/101, p

= <0.001) and AMD (38.9%, 7/18, p = 0.120). Likewise, urinary tract infections are reported more often in OHS (24.3%, 9/37) than in CMD (4.0%, 4/101, p = 0.033). Dental abnormalities are highly suggestive for OHS (29.7%, 11/37) and have only been reported in 3 CMD patients. Similarly, joint dislocations are reported in 40.5% (15/37) of the OHS patients, compared to three CMD and one AMD patient. Notably, in the CMD and AMD subgroups, dislocations affect the hips and shoulders, whereas in OHS radial head dislocation is reported in 11/15 patients (73.3%). Joint hyperlaxity is noted less in CMD (8.9%, 9/101), compared with AMD (33.3%, 6/18, p = 0.033) and OHS patients (45.9%, 17/37, p <0.001). Joint contractures are exclusively seen in the OHS subgroup (13.5%, 5/37). AMD and CMD subgroups often show osteopenia (osteoporosis, fractures), whereas OHS presents with bony exostoses. Oculomotor problems (strabismus, nystagmus) are rare in all subgroups. About half of the OHS patients (48.6%, 18/37) have dysautonomia, of which 72.2% (13/18) present with diarrhea, 22.2% (4/18) with nausea or vomiting, 44.4% (8/18) with temperature instability, and 38.9% (7/18) with orthostatic hypotension. Autonomic dysfunction occurs less frequently in MD patients and mainly involves temperature instability (82.4% (14/17) and 50.0% (2/4) in CMD and AMD, respectively). Gastro-intestinal complications involve feeding difficulties in about a third of CMD patients (31.7%, 32/101) and about 15% of AMD and OHS patients (16.7%, 3/18 and 13.5%, 5/37, p = 0.070), and pyloric stenosis which is exclusively seen in OHS patients (10.8%, 4/37, p = 0.004). In addition, gastrointestinal hemorrhages are reported in the CMD subgroup (8.9%, 9/101, p = 0.098). Clinical characteristics are presented in **Table *2.***

Biochemical findings

Serum copper is normal in 16.7% of patients (26/105). There are no significant differences between the number of individuals with normal copper levels between any of the subgroups (p = 0.395). About 90% had low serum ceruloplasmin (89.9%, 116/129). Reportedly low serum ceruloplasmin levels are significantly more common in CMD (96.6%, 85/88) compared with AMD (63.6%, 7/11, p = 0.009) and OHS (80.0%, 24/30, p = 0.024). Other observed biochemical features include hyperbilirubinemia,

increased lactate and catecholamine levels, and increased copper levels in fibroblasts. Copper treatment is reported in 38 CMD patients, 10 AMD patients, and 8 OHS patients, and frequently resulted in a good biochemical response. However, neurodevelopmental improvement is only reported in 8 CMD (21%), 2 AMD (20%) and 2 (25%) OHS patients.

X-linked spinal muscular atrophy-3

Thirteen included papers report on 6 patients from 4 different families with SMAX3, all of them being males. However, Baets et al. also notice a clear phenotype in female relatives. Muscle weakness typically starts from the feet (with absence of the Achilles tendon reflexes) and progresses to the upper limbs. Gait patterns are described as atactic, hackney, steppage or instable. Autonomic system impairment is noted in two SMAX3-relatives and included diarrhea, hyperhidrosis and retrograde ejaculation. Clinical and molecular findings of SMAX3 patients are represented in **Supplemental file *S8***.

Genotype-phenotype correlations

We evaluated potential associations between the clinical entity and mutation type or mutated domain (**Supplemental file *S9)***. The OHS subgroup includes significantly more splice site (31.3%, 10/32) and intronic mutations (9.4%, 3/32) compared to the CMD subgroup (10.9%, 10/92, p = 0.033 and 0%, 0/92, p = 0.048, respectively. Truncation mutations (nonsense mutations, frameshift indels, and large structural insertions or deletions), on the other hand, are more frequently in the CMD subgroup (60.4%, 55/91), compared to the AMD and OHS subgroups (28.6%, 4/14, p = 0.041 and 29.0%, 9/31, p = 0.003, respectively). Contrarily, pathogenic variants associated with SMAX3 exclusively concern missense variants in or near the transmembrane helices essential for copper transport.

Discussion

Next-generation sequencing has become state-of-the-art in the workup of nonspecific findings such as neurodevelopmental delay, seizures, or congenital anomalies, increasing the need for early clinical

prognostic markers and management guidelines. In *ATP7A*-related disorders, counseling is challenging due to clinical overlap between ATP7A-related entities, the absence of biomarkers of disease, a clear genotype-phenotype correlation, natural history studies, and validated therapeutic approaches. Nevertheless, a correct diagnosis is necessary to estimate the prognosis and tailor the patient's management. We set forth criteria to differentiate affected individuals into subgroups with clinical and prognostic relevance, based on a systematic review reporting on 162 unique individuals with a molecularly confirmed ATP7A-related diagnosis.

Seizures as initial symptom or seizures occurring before the age of 3 months are highly suggestive for the CMD phenotype. Indeed, early-onset neurological symptoms are key to the diagnosis of CMD[1-3] as more than half of the patients present with seizures, frequently associated with neurodevelopmental delay and hypotonia.

The prevalence of intellectual disability is similar between CMD and AMD, but is more severe in CMD. Ataxia seems specific for AMD. Hence, affected individuals with mild developmental delay, absence of early onset seizures (i.e. before the age of six months) or with ataxia show less severe neurological complications, a low risk for early demise, and can be identified as AMD.

OHS patients can be identified by the presence of occipital horns, as evidenced by laterolateral X-ray imaging of the skull. Before occipital horns can be objectified, the diagnosis of OHS is likely in the presence of at least two of the following criteria: a first-degree family member with OHS, mild developmental delay, radial head dislocations, herniations, or dental abnormalities. Surprisingly, in OHS, still a quarter of patients present with variable neurodevelopmental delay, and/or hypotonia. In contrast, feeding difficulties and dysautonomia as initial symptoms are not as common as previously suggested[3,27]. Scalp hematomas occur frequently but, opposed to previous reports, do not allow discrimination with the more severe subtypes[28,29]. Prominent connective tissue abnormalities and, to a lesser extent, autonomic dysfunction and urological problems such as urinary infections and hydronephrosis, are often a clue to the diagnosis of OHS.

SMAX3 patients are differentiated by neurophysiological examination revealing distal motor neuropathy and show no major neuromotor delay. Criteria for differentiation are represented in **Table *3.***

Causes of death are comparable between subtypes and most often related to respiratory and gastrointestinal complications. In CMD mortality rates surpass 40% in childhood. Mortality rates are significantly lower in AMD and OHS, and rarely observed before adolescence or adulthood, respectively. In contrast to general assumption that intracranial vascular tortuosity is associated with OHS[27], it was present in most patients who underwent brain imaging, regardless of the subtype. Likewise, vigilance for the occurrence of cerebrovascular accidents is primordial, as they occur in up to 10% of affected patients.

Facial features are unspecific and do not contribute to the (differential) diagnosis of *ATP7A*-related disorders. However, hair abnormalities differ between subtypes. There is a significantly higher prevalence of hair abnormalities in CMD, with hypopigmented, brittle and kinky hair, while the hair is usually coarse in OHS. OHS has classically been described to present with ubiquitous connective tissue abnormalities[30]. However, our data indicate that cutis laxa, herniations, and especially bladder diverticula can be found in high numbers of CMD and AMD patients, too. In contrast, dental abnormalities and radial head dislocations seem highly specific for the OHS phenotype.

In congruence with previous genotype-phenotype studies on MD[19,20,23,31-33], CMD is significantly more associated with truncating variants, including frameshift variants, nonsense variants and deletions that lead to very low or absent ATP7A levels. On the other end of the spectrum, in OHS, intronic mutations and splice-site mutations still permit the production of small amounts of *ATP7A* mRNA and some residual protein function. Levels as low as 2-5% are considered sufficient for milder phenotypes[34]. Except for SMAX3, where causal variants exclusively concern missense variants in the transmembrane helices essential for copper transport, no correlation with affected domains is observed[5].

Low serum copper has been suggested as a biomarker and therapeutic target in *ATP7A*-related disorders, assuming that disease severity correlates with the level of copper deficiency[1,10,35,36]. While serum copper is reduced in most patients, no discernable differences exist between subgroups. This observation questions the validity of serum copper level as a biomarker for disease severity and does not seem to reflect remaining transporter function. Moreover, this observation further limits serum copper as a therapeutic readout or target. Indeed, reports on copper supplementation treatment often show increased serum copper values, but did not notably improve neurodevelopment. In contrast, ceruloplasmin level, however, are significantly lower in the CMD group compared with AMD and OHS, indicating that ceruloplasmin might be a more sensitive biochemical marker of disease severity.

Finally, the vast clinical and prognostic information obtained from this cohort may help develop evidence-based management guidelines for *ATP7A*-related disorders. Following the initial diagnosis of an *ATP7A*-related disorder, a thorough clinical examination and genetic counseling should be offered. All patients should receive in-depth neurological and developmental evaluation, including an EEG in the case of clinical suspicion of seizures. We recommend performing an MRI and MRA of the brain at diagnosis in order to objectify common but non-specific anomalies such as intracranial tortuosity, atrophy, and myelination problems. Considering the high risk of cerebrovascular incidents in patients with documented tortuosity, imaging could be repeated in this group every three year or earlier in case of new symptoms. In the absence of evidence specific for ATP7A related conditions, this suggestion is based on recommendations for other conditions associating with arterial tortuosity [37,38]. Laterolateral x-ray imaging of the skull is recommended to objectify the presence of occipital horns. If SMAX3 is suspected, an EMG should be performed to evaluate distal motor neuropathy. All patients should undergo yearly urological evaluation, including ultrasound of the genitourinary tract and video urodynamic studies of bladder function to be able to intervene early on secondary causes of morbidity including urinary tract infections, urinary retention, bladder rupture, or VUR-mediated renal failure. Surgical interventions removing the diverticula often fail, and physiotherapy to increase pelvic floor

control to facilitate complete bladder emptying, self-catheterization, vesicostomy, or continence diversion should be envisaged, in addition to antibiotic prophylaxis. Physiotherapeutic evaluation for early detection of contractures or hypermobility is advised. Early physiotherapy is useful to prevent pain, muscle wasting and contractions. In young adults with a history of fractures, bone densitometry should be advised to rule out osteoporosis. Respiratory infections, the most common cause of death, should be treated early and rigorously. Sufficient hydration is imported considering the risk of autonomic dysfunction with orthostatic hypotension and diarrhea. The value of early copper histidine treatment is difficult to assess since only a limited number of reports mention, often temporary, clinical improvement which has no effect on overall survival.

This study may be subject to some limitations. First, the systematic review and predefined search strategy aimed to identify all potentially relevant studies, but does not fully exclude the risk of selection bias. Second, our systematic review assembled data from case reports and case series, which are prone to publication and selection bias, especially since negative data or absent symptoms tend to be underreported. Third, some of the distinguishing features between AMD and CMD are inherent to the definition of these entities. A limitation to the set-up of this study that we tried to reduce as much as possible by making the distinction between AMD and CMD on a very limited and rather objective amount of characteristics **(Supplemental file *S3*)**. Finally, due to the rarity of *ATP7A*-related disorders, data are limited, especially regarding the AMD and SMAX3 phenotypes. Taken together, our review only approximates a representation of the clinical spectrum of *ATP7A*-related copper transport disorders.

In conclusion, based on a systematic review of previously reported individuals with *ATP7A*-related disease, we set forth criteria for differentiation into CMD, AMD, and OHS subgroups revealing new diagnostic and prognostic clinical markers. In line with previous reports, we did not identify convincing genotype-phenotype correlations. Finally, we propose some management guidelines for *ATP7A*-related disorders that should be validated in further follow-up studies.

Ethics Declaration

The authors declare no competing interests.

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Tables and figures

Figure *1:* The ATP7A protein

ATP7A consists of six amino terminal metal binding domains (HMBD1-6), a transmembrane domain containing eight transmembrane helices (TM1-8) and an A-, P- and N soluble catalytic domain. Figure created with BioRender.com

Figure *2*: Prisma flow diagram of the selection process

***Table 1*: Symptoms initiating further investigations leading to the diagnosis.**

	Classic MD patients (n = 101)	Atypical MD patients (n = 18)	OHS patients (n = 37)	Total patients (n = 156)	p-values
Seizures	53.9% (41/76)	15.4% (2/13)	0% (0/32)	35.5% (43/121)	<0.001 (0.045, <0.001, 0.237)
Hypotonia	32.9% (25/76)	38.5% (5/13)	15.6% (5/32)	28.9% (35/121)	0.136
Developmental delay	26.3% (20/76)	38.5% (5/13)	25.0% (8/32)	27.3% (33/121)	0.649
Cutaneous, hair	17.1% (13/76)	38.5% (5/13)	28.1% (9/32)	22.3% (27/121)	0.143
FTT, feeding difficulties	19.7% (15/76)	15.4% (2/13)	3.1% (1/32)	14.9% (18/121)	0.076
Autonomic dysfunction	10.5% (8/76)	23.1% (3/13)	34.4% (11/32)	18.2% (22/121)	0.010 (0.597, 0.015, 1.000)
Musculoskeletal	7.9% (6/76)	15.4% (2/13)	28.1% (9/32)	14.0% (17/121)	0.017 (0.993, 0.036, 1.000)
Herniations, bladder diverticula	3.9% (3/76)	0% (0/13)	28.1% (9/32)	9.9% (12/121)	<0.001 (1.000, 0.002, 0.126)
Lethargy	11.8% (9/76)	7.7% (1/13)	6.3% (2/32)	9.9% (12/121)	0.809
Scalp hematomas	6.6% (5/76)	15.4% (2/13)	9.4% (3/32)	8.3% (10/121)	0.358
Urological, renal	3.9% (3/76)	0% (0/13)	31.3% (10/32)	10.7% (13/121)	<0.001 (1.000, 0.001, 0.126)

MD = Menkes disease, OHS = occipital horn syndrome, FTT = failure to thrive. P-values verify whether there is significant heterogeneity between the three subgroups. Post hoc analysis to further explore significant heterogeneity is shown in brackets (classic MD compared with atypical MD, classic MD compared with OHS, atypical MD compared with OHS).

***Table 2*: Overview of the main clinical characteristics in Menkes disease and occipital horn syndrome patients.**

	Classic MD (n = 101)	Atypical MD (n = 18)	OHS (n = 37)	Total (n = 156)	p-values
Hair and scalp					
Hair abnormalities	91.1% (92/101)	61.1% (11/18)	56.8% (21/37)	79.5% (124/156)	<0.001 (0.009, <0.001, 1.000)
Hypopigmented	56.5% (52/92)	18.2% (2/11)	28.6% (6/21)	48.3% (60/124)	0.006 (0.069, 0.087, 1.000)
Kinky, curly	52.2% (48/92)	27.3% (3/11)	9.5% (2/21)	42.7% (53/124)	<0.001 (0.603, 0.001, 0.930)
Sparse	34.8% (32/92)	45.5% (5/11)	28.6% (6/21)	34.7% (43/124)	0.628
Coarse	25.0% (23/92)	18.2% (2/11)	42.9% (9/21)	27.4% (34/124)	0.196
Brittle, easy drop	16.3% (15/92)	27.3% (3/11)	0% (0/21)	14.5% (18/124)	0.041 (1.000, 0.207, 0.099)
Pili torti	97.0% (32/33)	87.5% (7/8)	91.7% (11/12)	94.3% (50/53)	0.316
Scalp hematomas	21.7% (15/69)	33.3% (4/12)	25.0% (4/16)	23.7% (23/97)	0.602
Cutaneous and connective tissue					
Cutis laxa	41.6% (42/101)	38.9% (7/18)	67.6% (25/37)	47.4% (74/156)	0.019 (1.000, 0.024, 0.237)
Herniations	19.8% (20/101)	11.1% (2/18)	54.1% (20/37)	26.9% (42/156)	<0.001 (1.000, <0.001, 0.009)
Bladder diverticula	29.7% (30/101)	38.9% (7/18)	70.3% (26/37)	40.4% (63/156)	<0.001 (1.000, <0.001, 0.120)
Dental abnormalities	3.0% (3/101)	0% (0/18)	29.7% (11/37)	9.0% (14/156)	<0.001 (1.000, <0.001, 0.030)
Skeletal					
Joint dislocations	3.0% (3/101)	5.6% (1/18)	40.5% (15/37)	12.2% (19/156)	<0.001 (1.000, <0.001, 0.030)
Joint laxity	8.9% (9/101)	33.3% (6/18)	45.9% (17/37)	20.5% (32/156)	<0.001 (0.033, <0.001, 1.000)
Pectus deformity	16.8% (17/101)	11.1% (2/18)	29.7% (11/37)	19.2% (30/156)	0.169
Osteoporosis	13.9% (14/101)	11.1% (2/18)	24.3% (9/37)	16.0% (25/156)	0.308
Fractures	12.9% (13/101)	16.7% (3/18)	8.1% (3/37)	12.2% (19/156)	0.571
Wormian bones	11.9% (12/101)	5.6% (1/18)	13.5% (5/37)	11.5% (18/156)	0.751
Bony exostoses	0% (0/101)	0% (0/18)	27.0% (10/37)	6.4% (10/156)	<0.001 (NA, <0.001, 0.063)
Neurological					
Developmental delay	98.6% (73/74)	72.2% (13/18)	61.3% (19/31)	85.3% (105/123)	<0.001 (0.003, <0.001, 1.000)
Intellectual disability	91.3% (21/23)	80.0% (12/15)	53.1% (17/32)	71.4% (50/70)	0.005 (1.000, 0.009, 0.333)
Seizures	85.1% (86/101)	27.8% (5/18)	16.2% (6/37)	62.2% (97/156)	<0.001 (<0.001,<0.001, 1.000)
Muscle hypotonia	79.2% (80/101)	55.6% (10/18)	32.4% (12/37)	65.4% (102/156)	<0.001 (0.120, <0.001,0.432)
Ataxia	5.0% (5/101)	27.8% (5/18)	10.8% (4/37)	9.0% (14/156)	0.008 (0.021, 0.747, 0.405)
Other					
Autonomic dysfunction	16.8% (17/101)	22.2% (4/18)	48.6% (18/37)	25.0% (39/156)	<0.001 (1.000, 0.001, 0.082)
Feeding difficulties	31.7% (32/101)	16.7% (3/18)	13.5% (5/37)	25.6% (40/156)	0.070
Pyloric stenosis	0% (0/101)	0% (0/18)	10.8% (4/37)	2.6% (4/156)	0.004 (NA, 0.015, 0.291)
Biochemical findings					
Low copper	83.3% (75/90)	72.7% (8/11)	73.3% (22/30)	80.2% (105/131)	0.395
Low ceruloplasmin	96.6% (85/88)	63.6% (7/11)	80.0% (24/30)	89.9% (116/129)	<0.001 (0.009, 0.024,1.000)

MD = Menkes disease, OHS = occipital horn syndrome, P-values verify whether there is significant heterogeneity between the three subgroups. Post hoc analysis to further explore significant heterogeneity is shown in brackets (classic MD compared with atypical MD, classic MD compared with OHS, atypical MD compared with OHS).

***Table 3*: Differentiation of ATP7A-related disorders**

Suspect for OHS: X-ray imaging skull	
Occipital horns	OHS
No occipital horns but ≥2 of the following: - First degree family member with OHS - Mild developmental delay - Radial head dislocations - Herniations - Dental abnormalities - Diarrhea	
Suspect for MD	
≥1 of the following: - >5 years old - Mild developmental delay - Seizure onset >6 months - Ataxia	AMD
Seizures - Occurring <3 months - As initial symptom	CMD
Suspect for SMAX3: electromyography limbs	
Distal motor neuropathy	SMAX3

MD = Menkes disease, OHS = occipital horn syndrome, SMAX3 = X-linked spinal muscular atrophy-3

Figure 1

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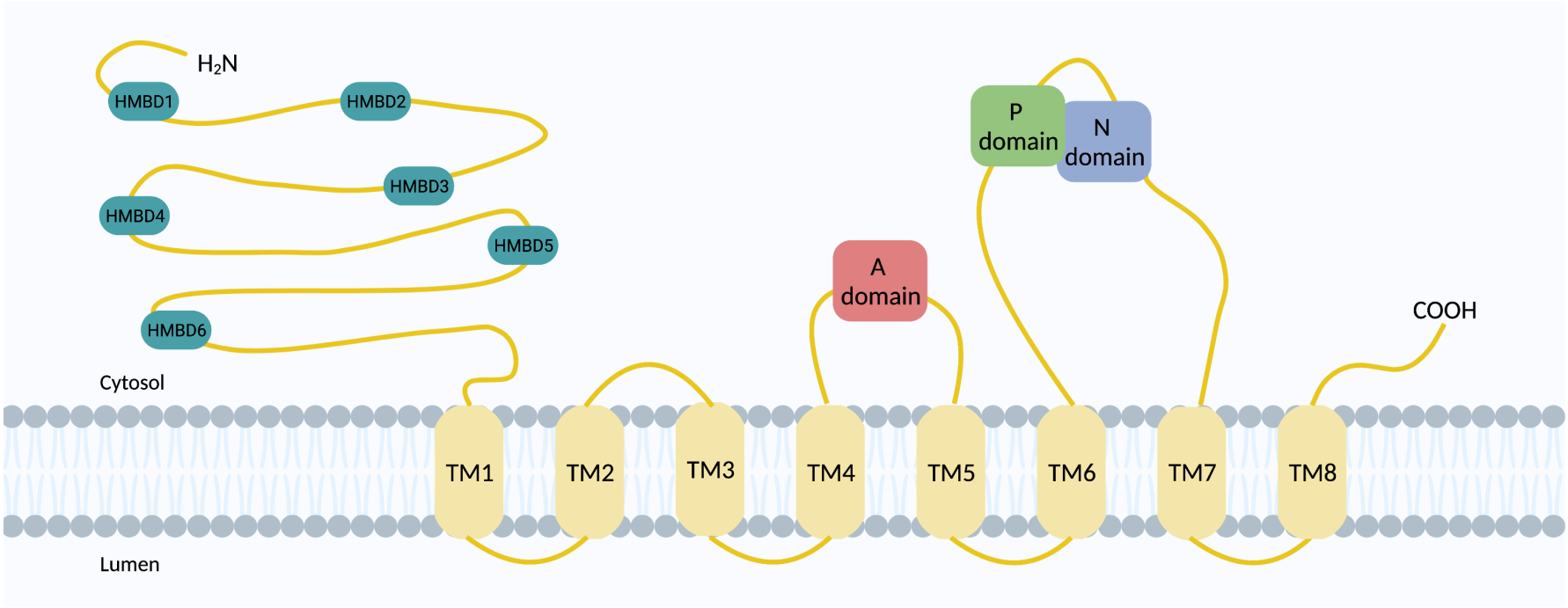

Figure 2

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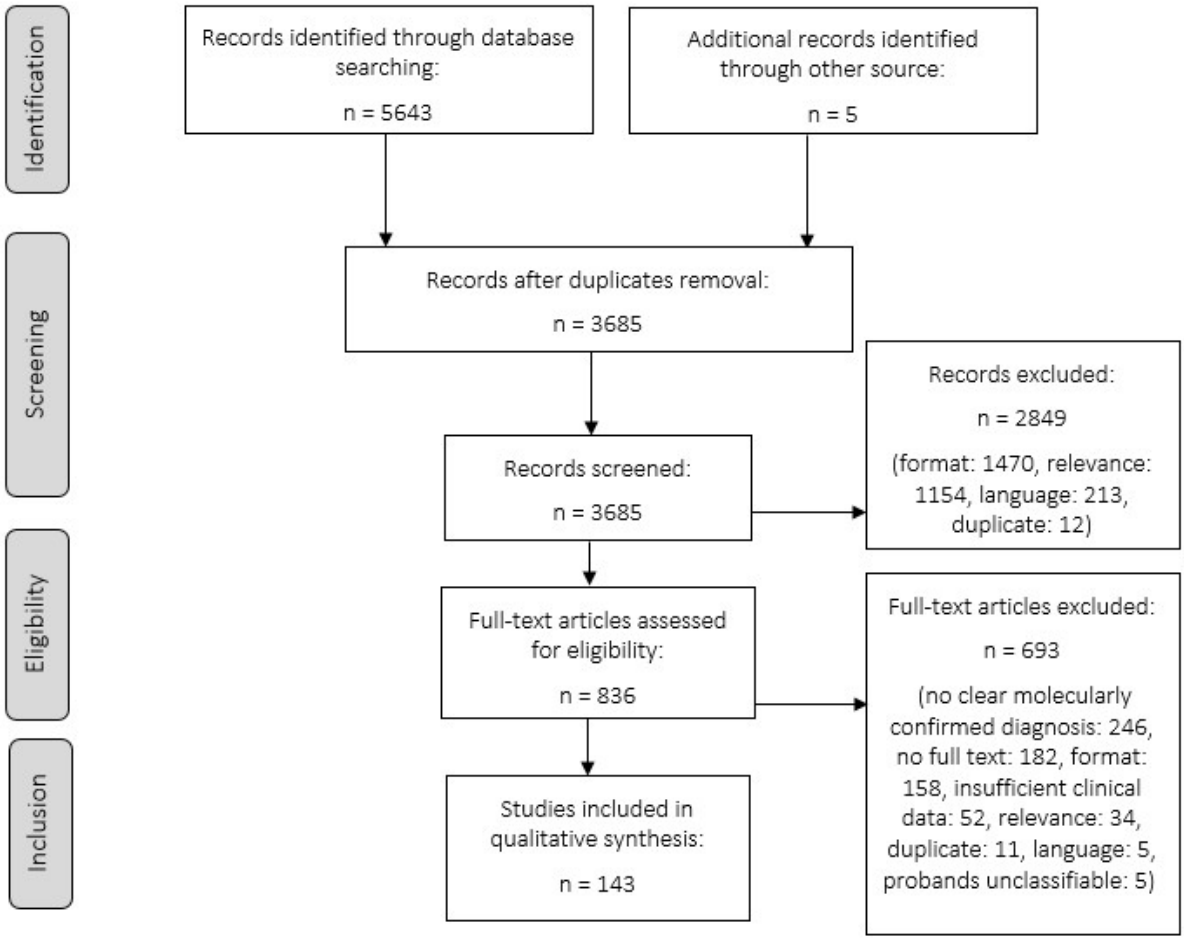

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