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Review article

A systematic review and evidence-based guideline for diagnosis and treatment of Menkes disease

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ABSTRACT

Menkes disease is a rare X-linked neurodegenerative disorder caused by defect in copper metabolism. Parenteral copper supplementation has been used as a potential disease-modifying treatment of Menkes disease for decades. However, recent evidence suggests its efficacy only when treatment is started within days after birth, which also has important implications related to the techniques that enable early diagnosis. We aim at proposing a guideline for prenatal and neonatal diagnosis and for disease-modifying treatment of Menkes disease, guided by a systematic review of the literature, and built in conjunction with medical experts, methodologists and patient representatives. Thirteen articles were used for our recommendations that were based on GRADE system. Reviewed evidence suggests that prenatal genetic diagnosis in families with previous diagnosis of Menkes disease is feasible; analysis of plasma catecholamine levels is accurate for neonatal diagnosis of Menkes disease; treatment with copper-histidine is effective to increase survival and reduce neurologic burden of the disease if initiated in the neonatal period; and, treatment indication should not be guided by patient's genotype. In conclusion, our guideline can contribute to standardize some aspects of the clinical care of patients with Menkes disease, especially reducing disease burden and mortality and providers' and families' anxiety.

1. Introduction

Menkes disease (MD) is a lethal infantile neurodegenerative disorder with X-linked inheritance. The disease is caused by pathogenic variants in the *ATP7A*, which encodes a transmembrane copper-transporting P-type ATPase (ATP7A) [1–3]. Pathogenic variants with milder effects in ATP7A cause occipital horn syndrome or a distal form of motor neuropathy [4], entities that will not be covered in the present review.

The incidence of MD at birth is estimated at 1 in 300,000 live births in Europe [5]. In Australia, the incidence can be up to 1 in 40,000 live births [6]. Females are usually asymptomatic, except in rare reports of girls affected with attenuated forms of the disease [7,8].

The phenotype of MD results from decreased activities of enzymes that use copper as cofactor, such as dopamine-β-hydroxylase, cytochrome *c* oxidase, amongst others. The clinical picture is characterized by an early childhood cerebral/cerebellar neurodegenerative and connective tissue disorder, usually perceived around 3 months of age, when patients begin to lose skills, with regression of motor functions, seizures, and hypotonia. Skin laxity, *pili torti*, jowly appearance with sagging cheeks, frontal bossing, and vascular tortuosity are also common features of affected individuals [3,4]. The natural history of the disease is characterized by progressive degeneration of neurological functions leading to death in the first years of life [9].

The diagnosis of MD is classically based on clinical manifestations in association with reduced blood levels of copper and ceruloplasmin [2]. Parenteral copper supplementation is used as a potential disease-modifying treatment since the seventies of 20th century [10]. However, more recent evidence suggests that copper injections might only modify disease progression if initiated within days after birth [2]. Since both

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symptoms and conventional biochemical diagnosis are accurate for diagnostic confirmation of the disease only after 2–3 months of age, and since serum copper and ceruloplasmin levels in healthy newborns overlap with those in infants with MD [2], more reliable diagnostic tests should be available in the neonatal or even prenatal period in order to allow early therapy [3,11].

Possibly due to the rarity of MD, there are no published evidence-based guidelines for the care of these individuals and families. Therefore, we propose an approach for the diagnosis and treatment of MD, guided by a systematic review of the literature. We built our guideline based on questions regarding accuracy of prenatal and neonatal diagnostic methods for MD, and its impact on relevant clinical outcomes. We also tried to assess the efficacy of copper injections for the treatment of the disease and to evaluate potential predictors of treatment response.

2. Methods

The objectives and search strategies were built using the Population, Intervention, Comparator, Outcome format for i) neonatal diagnosis, ii) prenatal diagnosis, iii) treatment efficacy, and iv) prediction of treatment efficacy. For each question, two independent reviewers (FPV and SP) searched Medline, Embase, Cochrane Library and Scielo without restriction of publication date, with the last search carried out in June 03, 2018. We restricted our search to human studies written in English, Portuguese or Spanish. As an example, the Medline search strategy to evaluate efficacy of copper injections compared to placebo or natural history on survival, seizures control and neurodevelopment of boys with Menkes disease was ("Menkes Kinky Hair Syndrome/diet therapy"[Mesh] OR "Menkes Kinky Hair Syndrome/drug therapy"[Mesh] OR "Menkes Kinky Hair Syndrome/therapy"[Mesh] AND "humans"[MeSH Terms]). The full strategies and the flowcharts used in selection of articles for each question and databases are detailed in Appendix 1. We also performed manual citation review to ensure that all relevant studies were found. The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines [12].

2.1. Eligibility criteria for the studies

Studies should have met one criterion from each of the following to be included in the review: 1) population: diagnosis or known risk (25–50%) of MD; 2) study design: systematic review, randomized clinical trial, cohort, case-control, or case series (cross sectional studies were excluded for the therapeutic questions). We excluded studies with any of the following characteristics: 1) population: other *ATP7A*-related disorders; for diagnostic studies: < 10 individuals with MD (we accepted studies with 4–10 at-risk pregnancies for prenatal diagnosis, however if such studies were included it would be graded as very low quality of evidence); for therapeutic studies: < 4 individuals with MD; 2) outcomes: for therapeutic studies: < 48 weeks of follow-up to assess outcomes; 3) study design: case reports or narrative reviews.

All included studies were decided by a consensus between reviewers and two separate assessors (MAPF and JAMS).

2.2. Data extraction and analysis of the quality of the evidence

For data extraction and evaluation of the overall quality of the evidence and the strength of recommendations, we used the Grading of Recommendations Assessment, Development and Evaluation (GRADE system) [13,14]. GRADE system aims to help users of clinical practice guidelines on how much confidence they can place on recommendations by systematically and explicitly informing how judgements were made. The quality of evidence indicates "the extent to which we can be confident that an estimate of effect is correct". Evidence can be downgraded based on study design, risk of bias, inconsistency of results across studies, indirectness of evidence to the practical situation and imprecise data. Quality of evidence can also be rated up due to a large magnitude of effect and the presence of a dose-response gradient. A high quality of evidence means that is unlikely that further research will change the estimate of effect; on the other hand, a very low quality of evidence indicates that the expected effect is very uncertain.

In the absence of published studies addressing a given question, the clinical expertise of the medical team, composed by a clinical geneticist with expertise in inborn errors of metabolism and a neurologist and geneticist with expertise in neurometabolic diseases, was used as Expert Opinion and judged as very low quality of evidence. The strength of a recommendation indicates "the extent to which we can be confident that adherence to the recommendation will do more good than harm". In making this judgment, we gathered the information of quality of evidence with the preferences of patients through representatives and clinicians expert opinions. [13]

2.3. Statistical analysis

Sensitivity and specificity of diagnostic testing, relative risk, the number needed to treat (NNT) of interventions, and its respective 95% confidence intervals were calculated with MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium) when the results were not provided in the study, but there was available data.

3. Results

Thirteen articles were used for building recommendations. The overall quality of evidence [14] for early neonatal diagnosis was considered high. Quality of evidence for prenatal diagnosis was judged as very low. Treatment question reached moderate quality of evidence. In this case, quality of evidence was rated up due to a large magnitude of effect and the presence of a dose-response gradient in the early versus late treatment. Finally, serious risk of bias and indirectness made treatment efficacy prediction evidence classified as very low quality.

3.1. Diagnosis

For the neonatal diagnosis of MD, we considered studies on biochemical or genetic testing performed before 30 days of life. As conventional diagnosis of MD, we considered diagnosis based on clinical suspicion and confirmed by blood analysis of copper and ceruloplasmin after 3 months of age.

3.1.1. Neonatal diagnosis

We retrieved 112 publications through database search for neonatal diagnosis of MD and two studies [2,15] were included in the present review (Appendix 1).

3.1.2. Diagnostic properties

The included studies evaluated the diagnostic properties of plasma catecholamine analysis with the dopamine/norepinephrine ratio and its metabolites dihydroxyphenylacetic acid/dihydroxyphenylglycol ratio. This test reflects the deficiency of dopamine-β-hydroxylase induced by lack of copper. Both studies were done by the same group at a single center in the *National Institute of Health,* USA and evaluated boys under 30 days of life at 50% risk of developing MD and compared those ratios to genetic diagnosis (pathogenic variants in *ATP7A*) or to diagnosis based on copper and ceruloplasmin quantification. Kaler and cols analyzed plasma catecholamines in the neonatal period in 36/81 boys at 50% risk of developing MD and confirmed the diagnosis in 14 individuals. There was no overlap between the affected and unaffected groups for both the ratios of dopamine/norepinephrine and dihydroxyphenylacetic acid/dihydroxyphenylglycol. The provided data allowed us to calculate a sensitivity of 100% (95% CI of 76.84–100%) and specificity of 100% (95% CI of 84.56–100%) for plasma dopamine/

norepinephrine ratio > 0.2 and for dihydroxyphenylacetic acid/dihydroxyphenylglycol ratio > 5 for the diagnosis of MD in the neonatal period. Another case series from the same center [15] evaluated 44 boys (19 affected) at 50% risk of developing MD and had 100% of sensitivity (95% CI of 81.47–100%) and 100% of specificity (95% CI of 86.77–100%) for both ratios using the same cut-offs.

3.1.3. Impact on relevant outcomes

Twelve out of the 14 boys with MD diagnosed in the neonatal period with plasma catecholamine analysis were included in an open-label clinical trial that showed efficacy of copper-histidine treatment before 30 days of life on patients survival.[2]The results of this study will be detailed in the treatment section.

These studies suggest that the analysis of catecholamines in the plasma, with the assessment of dopamine/norepinephrine (values > 0.2) or dihydroxyphenylacetic acid/dihydroxyphenylglycol (values > 5) ratios has adequate accuracy for the diagnosis of MD in the neonatal period and that neonatal diagnosis might be associated with increased survival when early treatment with copper-histidine is initiated. However, plasma catecholamine analysis is not easily available worldwide and the use of this approach as a standard diagnosis for MD might not be feasible in many countries. Genetic analysis has been considered the gold standard for diagnosis of MD [16] and it is becoming more available and cheaper. Therefore, we consider target mutation analysis an alternative to catecholamine analysis for neonatal diagnosis of boys at-risk for MD, if performed in a timely fashion (Expert opinion). We expect the same benefits on clinical outcomes obtained with catecholamine analysis, if a fast result of the genetic study is obtained. However, prenatal or neonatal genetic testing requires the previous identification of the familial mutation.

Another reliable test for the diagnosis of MD is the analysis of copper uptake and retention in cells [17,18]. We found no study that specifically addressed the diagnostic properties of this method for MD diagnosis in the neonatal period; however, the high accuracy of this method for both prenatal and postnatal diagnosis of MD [19], suggests that this method can be used for MD diagnosis. Copper uptake and retention in cells is laborious, expensive and available in a very limited number of laboratories, making it difficult to be used worldwide.

3.2. Recommendation for neonatal diagnosis of MD

- Plasma dopamine/norepinephrine (values > 0.2) or dihydroxyphenylacetic acid/dihydroxyphenylglycol (values > 5) ratios are recommended for neonatal diagnosis of boys with MD. (Quality of Evidence: High; Strength of Recommendation: Strong in Favor).
- Target *ATP7A* mutation analysis is recommended for neonatal diagnosis of boys with MD in centers without availability of plasma catecholamine analysis. (Expert Opinion; Strength of Recommendation: Strong in Favor).
- Neonatal diagnosis of MD for families in which *ATP7A* mutation remains unidentified should be performed in a reference center that performs plasma catecholamine or copper uptake and retention in cells analysis. (Expert Opinion; Strength of Recommendation: Strong in Favor).

3.2.1. Prenatal diagnosis

Another possibility for early diagnosis of MD is prenatal genetic diagnosis, which might also allow early treatment with copper injections or might allow pregnancy interruption.

Ninety-three studies were identified in our search for prenatal diagnosis of MD (Appendix 1). Three studies were eligible for inclusion in the review [18–20]. Two studies evaluated prenatal diagnosis by genetic testing in families where there was at least one previous case with MD diagnosis (10 pregnancies in total), and one study analyzed copper uptake and retention in amniotic fluid cells and fetal fibroblasts (80 second-trimester pregnancies, 20 of affected males; 53 first-trimester pregnancies, 5 of affected males). However, none of the studies reported the diagnostic properties of neither testing nor relevant clinical outcomes [19,20]. Of note, it was not possible to perform the prenatal molecular diagnosis in one case were the family mutation was not previously identified [19].

These studies suggested that genetic and biochemical (through copper uptake and retention analysis) prenatal diagnosis in families with previous diagnosis of MD is feasible, but there is no well-established evidence to assess its accuracy. Both indirect knowledge of better outcomes for early treatment with copper-histidine and the possibility of pregnancy interruption are expected benefits, whereas undesirable pregnancy loss due to procedure-related complication is a potential harm of prenatal diagnosis of MD. Such decision should be discussed with families in the context of genetic counseling.

3.3. Recommendation for prenatal diagnosis of MD

- Target *ATP7A* mutation analysis and copper uptake and retention in cells analysis might be used for prenatal diagnosis of MD (Quality of Evidence: Very Low; Strength of Recommendation: Weak in Favor). We consider this a weak recommendation, because the decision of performing or not prenatal diagnosis will vary according to families beliefs, and therefore clinicians must ensure that patients' care is in keeping with familial values and preferences.

3.3.1. Neonatal diagnosis versus prenatal diagnosis

We found no study comparing neonatal and prenatal diagnosis of MD. Although the quality of the evidence for neonatal biochemical diagnosis of MD is higher than for prenatal diagnosis, the potential benefit of early treatment with copper-histidine should be the same for both approaches or could be even greater for prenatal diagnosis, because of the great amount of time to organize the logistics of very early treatment. Prenatal diagnosis also has the advantage of offering the possibility of pregnancy interruption; on the other hand, it has the drawback of the risk of undesirable pregnancy loss due to procedure-related complications.

3.4. Recommendation for neonatal diagnosis versus prenatal diagnosis of MD

- The choice between neonatal versus prenatal diagnosis of MD should be individualized (Expert Opinion; Strength of Recommendation: Strong in Favor). This decision should take into account the families values and preferences and the availability of molecular and biochemical diagnosis of MD and the time-frame for obtaining results in the medical center and time-frame to obtain copper-histidine.

Fig. 1 describes the flowchart for early diagnosis and treatment according to our evidence-based guideline.

3.4.1. Treatment

We focused our search on disease-modifying treatment for MD. For symptomatic management and multidisciplinary care (eg. use of antiepileptic drugs, physical therapy, surgery for bladder diverticula, etc.) we suggest the reading of recent reviews on these subjects [21,22].

Our database search resulted in 445 publications (Appendix 1), and nine were included in the review. Most intervention studies compared early (asymptomatic) versus late treatment start (after symptomatic disease) with copper-histidine for boys with MD, without comparing to a placebo or natural history group and presented methodological limitations.

We summarize the findings, which provided the best evidence for the recommendations.

An open-label multicenter clinical trial in Italy described the follow-up of a group of 28 symptomatic boys with MD where 16 were treated

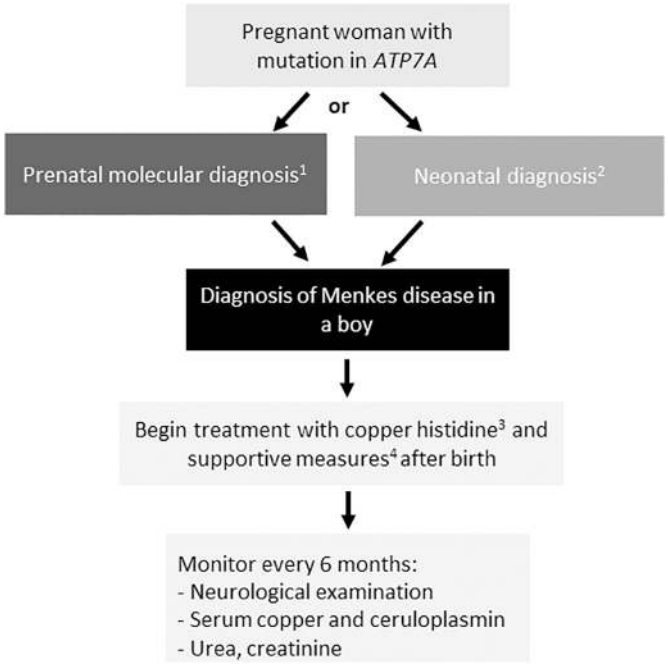

Fig. 1. - Early diagnosis and treatment of Menkes Disease.
[1] Diagnosis by molecular analysis of familial mutation in *ATP7A* by amniocentesis or chorionic villus biopsy; [2] Diagnosis by dopamine/norepinephrine (values > 0.2) or dihydroxyphenylacetic acid/dihydroxyphenylglycol (> 5) ratios or by genetics analysis of the familial mutation in *ATP7A*; [3]Subcutaneous copper histidine: 500 mg/day until the end of the first year of life and 250 mg/day afterwards; [4]Support management: use of anticonvulsants, rehabilitation and stimulation measures, monitoring of bladder diverticula, nutritional support.

with subcutaneous copper-histidine and 12 received no therapy. After 4 years of follow-up, there was no difference in neurological evolution between the groups [21].

An open-label, single-center trial in US [3] evaluated 24 patients with MD treated with subcutaneous copper-histidine in the neonatal period (250-500μg/day, mean age at onset of treatment: 11.8 ± 9.6 days). Seizures were observed in 12.5% treated patients and 46% had at least one abnormal electroencephalogram, after a minimum follow-up of 3 years. The authors compared the data to three previous studies that reported seizures and electroencephalogram abnormalities in 87.5–100% of late-treated or untreated patients. Survival was 62.5% for early-treated versus 8.3–37.5% for MD patients that were late-treated or untreated. Although the study did not perform a statistical comparison of survival between groups, it was possible to calculate the relative risk (RR) and absolute risk reduction with the data provided (see Table 2), and the NNT to avoid one death with early treatment was 2.6 (95% CI 1.59 to 7.25). The same group of investigators performed an open-label clinical trial [2] in which 12 patients with MD were treated with copper-histidine (at same dosage) within 22 days of life. After a mean follow-up of 4.6 years, survival was 92%, compared to 13% in a historical control group of 15 patients with late treatment with copper-histidine. Out of the 12 patients, two had normal neurological development. Although this study did not perform a statistical comparison of survival between groups, it was possible to calculate the RR and absolute risk reduction with the data provided (see Table 2), and the NNT to avoid one death with early treatment was 1.27 (95% CI 0.98 to 1.81). In 2014, Kaler et cols [9] conducted an open-label clinical trial with 57 patients and evaluated the neurological development and growth at age of 3 years (or age of death) of 35 pre-symptomatic patients receiving copper-histidine (same dosage) within less than one month of age and 22 patients who received treatment after symptoms onset. There was improvement in the acquisition of the

Table 1
Key findings and importance on diagnostic questions.

Studies	Method	Menkes disease (n)	Healthy controls (n)	Sensitivity (95% CI)	Specificity (95% CI)	Importance
Neonatal diagnosis						
Goldstein, et al. [15]	Plasmatic ratios of dopamine/norepinephrine and dihydroxyphenylacetic acid/dihydroxyphenylglycol	19	25	100% (81.47–100%)	100% (86.77–100%)	Critical[a]
Kaler, et al. [2]	Plasmatic ratios of dopamine/norepinephrine and dihydroxyphenylacetic acid/dihydroxyphenylglycol	14	22	100% (76.84–100%)	100% (84.56–100%)	
Prenatal diagnosis						
Cao, et al. [20]	Genetic testing	1	4 normal fetus	NA	NA	Critical[a]
Gu, et al. [19]	Genetic testing	1 heterozygous female fetus	4 normal fetus	NA	NA	
Tonnesen & Horn [18]	Copper uptake and retention in amniotic fluid cells and fetal fibroblasts	20 affected males (2nd trimester)	60 normal fetus (2nd trimester)	NA	NA	
		5 affected males (1st trimester)	48 normal fetus (1st trimester)	NA	NA	

NA, not available.
[a] The availability of effective treatment made accuracy a critical outcome.

Table 2
Key findings and importance of treatment with small-molecule copper complexes.

Studies	Intervention group (n)	Control group (n)	Relative effect[a] (95% CI)	Absolute effect (95% CI)	Importance
Survival					
Kaler, et al. [2]	12 (median follow-up of 4.6 y)	15 late-treated (median follow-up of 1.8 y)	10.4 (1.57–68.63)	78.4 (55.1–100)	Critical
Kaler, et al. [3]	24	29 untreated and late-treated	2.02 (1.16–3.52) Pooled data of not early treated series	38.3 (11.6–58.6) Pooled data of not early treated series	
Verrotti, et al. [21]	16 late treatment	12 untreated	NA	NA	
Kaler, et al. [9]	35 (Follow-up for up to 3y)	22 late-treated (Follow-up for up to 3y)	1.75 (0.89–3.42)	21.4 (−4.27–47.13)	
Kim, et al. [8]	12 2 early and 10 late treatment (Follow-up from 1.5 to 4.8y)	–	NA	NA	
Christodoulou, et al. [10]	3 (10-20y of follow-up)	1 treatment started at 7 weeks (18 y of follow up)	NA	NA	
Friedman, et al. [23]	8 late treatment	–	NA	NA	
Yoganathan, et al. [24]	4 late treatment (1 female) (1 year of follow-up, 1 interruption after 6 weeks of treatment)	–	NA	NA	
Seizures					
Kaler, et al. [2]	12 (median follow-up of 4.6 y)	15 late-treated (median follow-up of 1.8 y)	NA	NA	Important
Kaler, et al. [3]	24	29 untreated and late-treated	4.33 (1.44–13.04) Pooled data of not early treated series	80.6 (56.7–90.1) Pooled data of not early treated series	
Verrotti, et al. [21]	16 late treatment	12 untreated	NA	NA	
Kim, et al. [8]	12 (2 early and 10 late treatment with follow-up from 1.5 to 4.8y)	–	NA	NA	
Christodoulou, et al. [10]	3 (10-20y of follow-up)	1 treatment started at 7 weeks (18 y of follow up)	NA	NA	
Friedman, et al. [23]	8 late treatment	–	NA	NA	
Yoganathan, et al. [24]	4 late treatment (1 female) (1 year of follow-up, 1 interruption after 6 weeks of treatment)	–	NA	NA	
Neurodevelopment					
Kaler, et al. [2]	12	15 late-treated	NA	NA	Important
Kaler, et al. [3]	24	29 untreated and late-treated	NA	NA	
Verrotti, et al. [21]	16 late treatment	12 untreated	NA	NA	
Kaler, et al. [9]	35 (Follow-up for up to 3y)	22 late-treated (Follow-up for up to 3y)	Improvement in the acquisition of the four major neurodevelopmental domains ($P < 0.001$)	NA	
Kim, et al. [8]	12 2 early and 10 late treatment (Follow-up from 1.5 to 4.8y)	–	NA	NA	
Christodoulou, et al. [10]	3 (10-20y of follow-up)	1 treatment started at 7 weeks (18 y of follow up)	NA	NA	
Yoganathan, et al. [24]	4 late treatment (1 female) (1 year of follow-up, 1 interruption after 6 weeks of treatment)	–	NA	NA	
Urological complications					
Zaffanello, et al. (2006)	30 copper complex (26 copper histidine). Mean age at start (0.7 ± 1.5 years) (Follow-up from 0 to 16y)	27 untreated (Follow-up from 0 to 16y)	NA	NA	Not important

NA, not available.
[a] Relative risk considered the incidence of the outcome in the control group/incidence of the outcome in the early-treated group.

Table 3
Quality assessment of the overall body of evidence.

N° of Studies	Design	Risk of Bias	Inconsistency	Indirectness	Imprecision	Certainty
Early neonatal diagnosis 2 studies (80 patients)	Observational studies (cohort and cross-sectional)	Not serious[a]	Not serious	Not serious[b]	Not serious	⊕⊕⊕⊕ High[c]
Prenatal genetic diagnosis 3 studies (143 pregnancies)	Observational studies (case series)	Serious[d]	Serious[e]	Serious[f]	Serious[f]	⊕⊖⊖⊖ Very low[g]
Treatment 9 studies (250 patients)	Observational studies (cohort and quasi-experiment)	Serious[h]	Not serious	Not serious	Serious[i]	⊕⊕⊕⊖ Moderate[j]
Prediction of response to treatment 1 study (24 patients)	Observational study (quasi-experiment)	Serious[k]	Not serious	Serious[f]	Not serious	⊕⊖⊖⊖ Very low[l]

[a] Limitations were not considered sufficient to downgrade quality of evidence.
[b] The availability of effective treatment made accuracy a patient-important outcome, instead of a surrogate outcome.
[c] According to Schünemann, et al. (2008), cross sectional or cohort studies can provide high quality evidence of test accuracy.
[d] Consecutive patients with very limited information.
[e] Could not reach available data.
[f] Could not match the research question.
[g] Insufficient information and limited results yielded high uncertainty.
[h] Non-randomized studies, with historical or no control group.
[i] It was not possible to estimate the magnitude of the benefit.
[j] Quality of evidence rated up due to a large magnitude of effect and the presence of a dose-response gradient in the early versus late treatment.
[k] Open-label, non-randomized study with historical control group.
[l] Serious risk of bias and indirectness yielded high uncertainty.

four major neurodevelopmental domains and a non-significant lower mortality (28.5% versus 50%) in the cohort of early-treated patients after comparison to late treated patients Tables 1 and 3.

In 2015, Kim et al. [8] performed an open-label clinical trial in a group of 12 patients with MD, including one girl, and two boys treated in the neonatal period. Mean age at copper-histidine treatment start was 7.3 months (range: 15 days to 27 months). Despite the treatment, 7 patients died before 5 years of age, including 1 patient with neonatal treatment. All others developed serious cognitive impairment.

A case series of four early-treated patients followed up for over 20 years is the study with the longest follow-up so far [10]. In three of these patients treatment was started within four weeks of age and in one it was started at 7 weeks of age. One patient died (at 10 years of age) and the others were alive at 20, 18, and 10 years of age. The 20-year-old patient had normal cognitive development, but severe bone changes, chronic diarrhea, and bladder diverticula. The 18-year-old patient (treatment started at seven weeks) had mild to moderate cognitive impairment and ataxia. The 10-year-old patient had mild cognitive impairment, bone alterations, and bladder diverticula. All patients had *ATP7A* mutations compatible with the classical MD, but presented a disease course similar to occipital horn disease.

Finally, an English study reported eight cases of MD patients, in which late treatment did not impact on the clinical course and electroencephalogram abnormalities of the disease [23], and an Indian series reported 4 cases of late–treated MD patients [24], in which only a female patient with MD had positive outcomes on growth, hair pigmentation, and milestones after 1 year of cooper histidine treatment (started at 28 months of age).

Therefore, these studies suggest that neonatal treatment with copper complexes (mainly copper-histidine) is effective to promote neurodevelopment, decrease seizures frequency and severity, and increase survival in boys with MD. Noteworthy, some individuals treated before 10 days of life may present normal neurological development.

3.5. Recommendation for disease-modifying treatment of MD

- Neonatal treatment with copper-histidine (before 30 days of life) is recommended for asymptomatic boys with molecular or biochemical diagnosis of MD (Quality of Evidence: Moderate; Strength of Recommendation: Strong in Favor).
- Treatment with copper-histidine after 30 days of life and for symptomatic boys with MD is not recommended (Quality of Evidence: Moderate; Strength of Recommendation: Strong in Favor).

3.5.1. Treatment regimen and follow-up

The dose of copper-histidine with proven efficacy in the reviewed studies and which will be indicated is 250 μg, subcutaneously, twice daily, until the end of the first year of life and 250 μg, subcutaneous, daily, after the first year of life. During treatment, serum copper and ceruloplasmin levels should be monitored for normalization. Treated patients should be followed with neurological examination and analysis of serum copper and ceruloplasmin levels every 6 months [2,3]. Treatment should be continued indefinitely, except in the presence of adverse events that justify its suspension or by clinical judgment of lack of efficacy.

3.6. Prediction of treatment response with copper complexes

We identified 445 publications through database search of which two [2,3], reporting results obtained from a single study, were eligible for full-text review and were included (Appendix 1). Kaler et al. suggested that patients with residual ATP7A activity show better outcomes after early copper-histidine treatment [2]. The authors evaluated 24 individuals with neonatal diagnosis of Menkes disease, which started early treatment with copper-histidine. All patients with missense variants had normal or nearly normal neurological outcomes, including electroencephalogram, whereas 50–60% of patients with large and small deletions, nonsense and canonical splice site mutations had electroencephalographic abnormalities [3]. However, we found no strong evidence to support the decision of prescribing treatment based on the genotype. One study [9] reported no differences in survival rates by the age of 3 years between patients with mild or severe variants treated before 1 month of age. Early treatment rather than the type of the genetic variant seems to be the most important factor for the outcome.

3.7. Recommendation for using treatment-efficacy predictors to indicate copper-histidine

- Type of *ATP7A* mutations might not guide neonatal treatment with copper-histidine (Quality of Evidence: Moderate; Strength of Recommendation: Strong in favor).

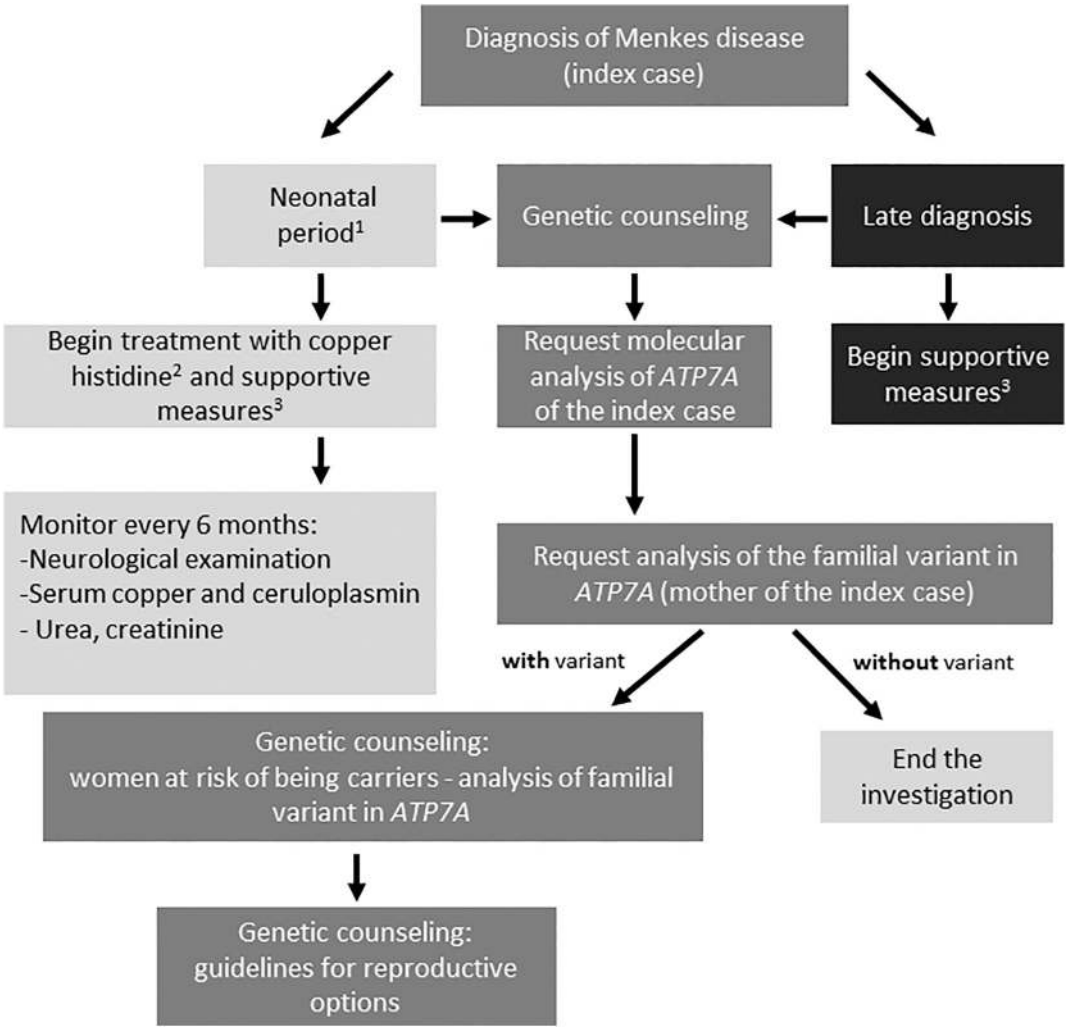

Fig. 2. - Family evaluation of the index case.
[1]Diagnosis by dopamine/norepinephrine (> 0.2) or dihydroxyphenylacetic acid/dihydroxyphenylglycol (> 5) ratios or by genetic diagnosis; [2]Copper histidine subcutaneously: 500 mg/day until the end of the first year of life and 250 mg/day afterwards; [3]Support management: use of anticonvulsants, rehabilitation and stimulation measures, monitoring of bladder diverticula, nutritional support. [4]In cases in which the index case mother is not a carrier of the familial mutation in *ATP7A* the possibility of germline mosaicism should be alerted on a genetic counseling, advising about recurrence risk in future pregnancies.

- Information regarding prognosis accordingly to genotype for boys with MD should be discussed with families before the initiation of the treatment (Quality of Evidence: Very Low; Strength of Recommendation: Strong in Favor).

Fig. 2 presents a flowchart built according to our evidence-based review summarizing the investigation of an index case with Menkes disease and opportunities for genetic counseling, early diagnosis and treatment for novel cases.

4. Discussion

MD is not currently a curable disease. However, it may have a more favorable clinical course if diagnosed early, reducing disease burden for patients and families. The systematic and comprehensive assessment of all available evidence combined with clinicians and patients' perspectives provided a guideline for prenatal and neonatal diagnosis and for disease modifying therapies for MD that might influence and aid in the development of country based guidelines for management of this devastating and neglected disease. Our guideline suggests that (1) prenatal diagnosis in families with previous diagnosis of MD is feasible; (2) the analysis of catecholamines in plasma (and target mutation genetic testing) has adequate accuracy for the diagnosis of MD in the neonatal period; (3) early diagnosis in association with early treatment with copper complexes is associated with increased survival; (4) treatment with copper-histidine is effective to increase survival and reduce neurologic burden of the disease only if initiated in the neonatal period; and (5), up to now, treatment indication should not be guided by patient's genotype, but the information regarding prognosis accordingly to genotype should be discussed with families.

We are aware that some recommendations will rely on each country health resources availability and laws (for pregnancy interruption after prenatal diagnosis, for instance). In addition, insufficient logistic and support systems for early detection, diagnosis, treatment, and follow-up are major barriers to implement this guideline in several countries.

The major limitation of this guideline is the absence of studies with robust designs such as double-blind randomized clinical trials with large sample sizes and low risk of bias or systematic reviews and meta-analyzes of clinical trials regarding MD. Therefore, most of our recommendations were based on case series/cohort studies and open-label clinical trials, which was an expected scenario considering the disease rarity. Moreover, most of the available evidence was produced by a single center, and even deriving from a single center, there was some discrepancies across studies, as the survival rate of 62.5% with

neonatal treatment of 24 MD patients reported in 2010 [3] and the survival rate of 92% with neonatal treatment of 12 patients MD reported in 2008 [2]. The authors explained that this difference reflected the impact of several unexpected deaths from medical complications not generally considered features of MD, which might raise some safety issues of the treatment. Therefore, we highlight the need for further research in the field, and from different research groups.

We believe that this guideline can contribute to standardization of some aspects of the clinical care of patients with MD, especially reducing disease burden and mortality and providers' and families' anxiety. Hence, it can reduce health care costs related to inadequate management of these patients. We expect that this guideline may also lead to improvement in different countries' health systems surpassing the goals for MD and allowing the creation of genetic or biochemical national diagnostic networks, for instance.

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Unfortunately, the final document of this initiative for Menkes disease, which had similar conclusions to the present guideline, was not considered by the Brazilian Ministry of Health, because copper-histidine is not registered in the Brazilian Health Regulatory Agency (ANVISA). As there is no pharmaceutical company producing copper-histidine and therefore no financial interest, there is no prediction on when this drug will became available in Brazil. In other words, the country who demanded such protocol will be the first one that will not implement it, because of regulatory issues that do not favor the use of real orphan drugs. Therefore, Brazilian families with Menkes disease will not be able to benefit from the best care of disease according to a careful evidence-based recommendation. On the other hand, we truly hope that the present guideline and the efforts for its construction might benefit other families with Menkes disease across the globe.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2018.12.005.

References

[1] S.G. Kaler, W.A. Gahl, Application of a copper blotting method to the study of Menkes disease, Biol. Trace Elem. Res. 38 (1993) 73–81.
[2] S.G. Kaler, C.S. Holmes, D.S. Goldstein, et al., Neonatal diagnosis and treatment of Menkes disease, N. Engl. J. Med. 358 (2008) 605–614.
[3] S.G. Kaler, C.J. Liew, A. Donsante, J.D. Hicks, S. Sato, J.C. Greenfield, Molecular correlates of epilepsy in early diagnosed and treated Menkes disease, J. Inherit. Metab. Dis. 33 (2010) 583–589.
[4] S.G. Kaler, ATP7A-related copper transport diseases-emerging concepts and future trends, Nat. Rev. Neurol. 7 (2011) 15–29.
[5] T. Tonnesen, W.J. Kleijer, N. Horn, Incidence of Menkes disease, Hum. Genet. 86 (1991) 408–410.
[6] D.M. Danks, E. Cartwright, P.E. Campbell, V. Mayne, Is Menkes' syndrome a heritable disorder of connective tissue? Lancet 2 (1971) 1089.
[7] L.B. Moller, M. Lenartowicz, M.T. Zabot, et al., Clinical expression of Menkes disease in females with normal karyotype, Orphanet J. Rare Dis. 7 (2012) 6.
[8] J.H. Kim, B.H. Lee, Y.M. Kim, et al., Novel mutations and clinical outcomes of copper-histidine therapy in Menkes disease patients, Metab. Brain Dis. 30 (2015) 75–81.
[9] S.G. Kaler, Neurodevelopment and brain growth in classic Menkes disease is influenced by age and symptomatology at initiation of copper treatment, J. Trace Elem. Med. Biol. 28 (2014) 427–430.
[10] J. Christodoulou, D.M. Danks, B. Sarkar, et al., Early treatment of Menkes disease with parenteral copper-histidine: long-term follow-up of four treated patients, Am. J. Med. Genet. 76 (1998) 154–164.
[11] H. Kodama, C. Fujisawa, W. Bhadhprasit, Inherited copper transport disorders: biochemical mechanisms, diagnosis, and treatment, Curr. Drug Metab. 13 (2012) 237–250.
[12] A. Liberati, D.G. Altman, J. Tetzlaff, et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration, BMJ 339 (2009) b2700.
[13] D. Atkins, D. Best, P.A. Briss, et al., Grading quality of evidence and strength of recommendations, BMJ 328 (2004) 1490.
[14] H.J. Schunemann, A.D. Oxman, J. Brozek, et al., GRADE: assessing the quality of evidence for diagnostic recommendations, ACP J. Club 149 (2008) 2.
[15] D.S. Goldstein, C.S. Holmes, S.G. Kaler, Relative efficiencies of plasma catechol levels and ratios for neonatal diagnosis of menkes disease, Neurochem. Res. 34 (2009) 1464–1468.
[16] S.G. Kaler, M.P. Adam, H.H. Ardinger, R.A. Pagon, et al. (Eds.), ATP7A-Related Copper Transport Disorders, GeneReviews((R)), Seattle (WA), 1993.
[17] L.B. Moller, J.D. Hicks, C.S. Holmes, et al., Diagnosis of copper transport disorders, Curr. Protoc. Hum. Genet. (2011), https://doi.org/10.1002/0471142905.hg1709s70 (Chapter 17:Unit17 19, PubMed PMID: 21735378; PubMed Central PMCID: PMC3156244).
[18] T. Tonnesen, N. Horn, Prenatal and postnatal diagnosis of Menkes disease, an inherited disorder of copper metabolism, J. Inherit. Metab. Dis. 12 (Suppl. 1) (1989) 207–214.
[19] Y.H. Gu, H. Kodama, E. Sato, et al., Prenatal diagnosis of Menkes disease by genetic analysis and copper measurement, Brain Dev. 24 (2002) 715–718.
[20] B. Cao, X. Yang, Y. Chen, et al., Identification of novel ATP7A mutations and prenatal diagnosis in Chinese patients with Menkes disease, Metab. Brain Dis. 32 (2017) 1123–1131.
[21] A. Verrotti, A. Carelli, G. Coppola, Epilepsy in children with Menkes disease: a systematic review of literature, J. Child Neurol. 29 (2014) 1757–1764.
[22] R. Ojha, A.N. Prasad, Menkes disease: what a multidisciplinary approach can do, J. Multidiscip. Healthc. 9 (2016) 371–385.
[23] E. Friedman, A. Harden, M. Koivikko, G. Pampiglione, Menkes' disease: neurophysiological aspects, J. Neurol. Neurosurg. Psychiatry 41 (1978) 505–510.
[24] S. Yoganathan, S.V. Sudhakar, G. Arunachal, et al., Menkes disease and response to copper histidine: an Indian case series, Ann. Indian Acad. Neurol. 20 (2017) 62–68.