Short Communication

Estimated birth prevalence of Menkes disease and ATP7A-related disorders based on the Genome Aggregation Database (gnomAD)

Stephen G. Kaler⁎, Carlos R. Ferreirab, Lung S. Yamc

⁎ Corresponding author at: Center for Gene Therapy, Nationwide Children’s Hospital, 700 Children’s Drive, Rm WA3021, Columbus, OH 43205, United States.
E-mail address: Stephen.Kaler@NationwideChildrens.org (S.G. Kaler).

ABSTRACT

Background: Previous estimates of the prevalence of Menkes disease, a lethal X-linked recessive disorder of copper metabolism, were based on confirmed clinical cases ascertained from specific populations and varied from 1 in 40,000 to 1 in 354,507. With newly available population-based allelic frequencies of DNA sequence variants, the expected birth prevalence of Menkes disease and other ATP7A-related phenotypes can be reconsidered using Hardy-Weinberg theoretical principles.

Methods: We reviewed the canonical ATP7A transcript in the current version of gnomAD (v2.1.1) to evaluate frequency of complete loss-of-function alleles in a diverse normal control population. As a comparator, we used the DMD locus, associated with Duchenne and Becker Muscular Dystrophy, another X-linked recessive trait. We applied Hardy-Weinberg theory and PolyPhen-2 in silico plus REVEL and CADD ensemble analyses to calculate estimated frequencies of normal and predicted deleterious ATP7A alleles.

Results: We identified 1106 total ATP7A variants out of 205,523 alleles in gnomAD, with missense variants most common (43.4%). Complete loss-of-function variants were found in four ATP7A alleles (frequency = 0.0000194), including three frameshift/nonsense mutations and one canonical splice donor site defect. Assuming Harvey-Weinberg equilibrium, this frequency of pathogenic alleles predicts 1 in 38,810 live male births with Menkes disease or other ATP7A-related disorders each year in the US. The same analysis for DMD loss-of-function variants predicted 1 in 724 newborn males with Duchenne (or Becker) muscular dystrophy. We also identified nine ATP7A missense variants in gnomAD predicted as deleterious by PolyPhen-2 and stringent REVEL/CADD criteria, comprising 12 more disease-causing alleles and raising the estimated birth prevalence to 1 in 8644 and predicting 225 newborns with Menkes disease or other ATP7A-related disorders per year in the US alone.

Conclusions: Assuming Harvey-Weinberg equilibrium, the allelic frequency of deleterious ATP7A variants in a genomic database from a large diverse population predicts a birth prevalence of Menkes disease or ATP7A-related disorders as high as 1 in 8644 live male births. This genome-driven ascertainment of deleterious ATP7A alleles in the population implies a higher birth prevalence of Menkes disease and ATP7A-related conditions than previously appreciated. A population-based newborn screening pilot study for Menkes disease will be instrumental in confirming the prediction.

1. Introduction

Published epidemiologic data for Menkes disease and its phenotypic variants are limited [1–3] and rely on case ascertainment and birth rates, which may each contain considerable sources of bias. Classic Menkes disease is an early-onset (6 to 8 weeks of age) neurodegenerative disorder of copper metabolism that features seizures, hypotonia, failure to thrive, hair and connective tissue abnormalities, and early death, often before three years of age [4–8]. The illness is caused by severe loss-of-function mutations in ATP7A, an evolutionarily conserved copper-transporting ATPase [9]. Occipital horn syndrome and ATP7A-related distal motor neuropathy are allelic variants of Menkes disease that have less distinctive clinical and biochemical signs [10,11] for which reasons diagnostic recognition may be delay or prevented.

We sought an unbiased estimate for the birth prevalence of Menkes disease and related X-linked recessive conditions [9–11] through an
alternative, recently available approach that is considered less subject to bias, i.e., population-based genomic data analysis [12-15]. The Genome Aggregation Database (gnomAD) at Broad Institute, Massachusetts Institute of Technology, Cambridge, MA covers 125,748 exomes and 15,708 genomes from 141,456 unrelated individuals (https://gnomad.broadinstitute.org/) and lists all genetic variants and their allelic frequencies, as well as which variants are predicted to be disease-causing [16].

Untreated Menkes disease is associated with inexorable neurological decline beginning in early infancy; however, early treatment (by 10 to 28 days of age) with subcutaneous injections of Copper Histidinate for three years has been associated with improved clinical outcomes [17,18]. Adeno-associated virus-mediated ATP7A gene addition provides a synergistic complementary effect in a mouse model of this disease [19,20]. While newborn screening (NBS) for this condition is not yet available, the recent therapeutic advances imply the potential importance of early detection.

The Hardy-Weinberg equilibrium law predicts that, in populations with random mating, genotype frequencies are determined by the relative frequencies of alleles at a given locus, as summarized in the equation: p² + 2pq + q² [21]. The term p represents the frequency of normal alleles and q, the frequency of mutant alleles. For X-linked recessive loci such as Menkes/ATP7A, p² represents a combination of healthy females and healthy males with normal alleles, and 2pq constitutes the combination of heterozygous (carrier) females and healthy males with normal alleles and q, the frequency of mutant alleles. For X-linked relative frequencies of alleles at a given locus, as summarized in the equation: p² + 2pq + q² [21]. The term p represents the frequency of normal alleles and q, the frequency of mutant alleles. For X-linked recessive loci such as Menkes/ATP7A, p² represents a combination of healthy females and healthy males with normal alleles, and 2pq constitutes the combination of heterozygous (carrier) females and affected males, each individual with one mutant X-chromosome. The final term (q²) can be dropped for X-linked loci such as ATP7A, since only females have two X-chromosomes, and females with two pathogenic ATP7A variants have never been reported.

Here, we apply Hardy-Weinberg principles [21] to ATP7A genomic data obtained from the largest and most diverse human population sample available [16], to re-evaluate the estimated birth prevalence of Menkes disease.

2. Methods

We reviewed the canonical ATP7A transcript (ENST00000341514) in the current version of gnomAD (Version 2.1.1) (https://gnomad.broadinstitute.org/gene/ENSG00000165240 (accessed December 17, 2019) for predicted severe loss-of-function and evaluated selected missense variants for potential pathogenicity using PolyPhen-2 in silico analyses, as well as REVEL and CADD ensemble analyses [22-24]. We applied the Hardy-Weinberg law [21] to assess the frequencies of normal and mutant ATP7A alleles, and calculated the estimated annual US birth prevalence of classic Menkes disease based on reliable birth statistics [25,26]. A stringent REVEL cut-off value (0.85) was used to determine missense variant pathogenicity [23].

3. Results

The gnomAD database identifies 1106 variants among a maximum of 205,523 ATP7A alleles (Table 1). Most variants noted are missense (43%). To estimate the frequency of pathogenic variants at the ATP7A locus, we analyzed loss-of-function and predicted pathogenic missense variants found in gnomAD. Four ATP7A variants, representing four independent alleles, were predicted as unequivocally loss-of-function alleles (Table 2), out of a total of 205,523 sequenced. All occurred only in females, as expected for a X-linked recessive trait. Two other variants, both canonical splice acceptor site alterations were also reported in gnomAD as loss-of-function alleles, however both variants occurred in apparently healthy males [16].

Using the Hardy-Weinberg law [21], the term q represents the frequency of mutant alleles, which, in this example for ATP7A, is estimated to be 4 in 205,523 or 0.0000194. The allelic frequency of p is equal to 1 minus q, i.e., 1 minus 0.0000194, or 0.9999806. Therefore, the Hardy-Weinberg equation for ATP7A allelic frequencies is:

\[ p^2 + 2pq + q^2 = 1 \]

Using the Hardy-Weinberg law [21], the term q represents the frequency of mutant alleles, which, in this example for ATP7A, is estimated to be 4 in 205,523 or 0.0000194. The allelic frequency of p is equal to 1 minus q, i.e., 1 minus 0.0000194, or 0.9999806. Therefore, the Hardy-Weinberg equation for ATP7A allelic frequencies is:

\[ p^2 + 2pq + q^2 = 1 \]

\[ (0.999806)^2 + 2(0.999806)(0.0000194) + 0 = 1 \]

\[ 0.9999612 + 0.0000387 + 0 = 0.999999 \]

Based on this analysis, unaffected males and non-carrier females with normal ATP7A alleles are predicted to comprise 99.99% of the population under conditions of random mating.

Considering US annual birth rates (3.8 million per year, with slight male birth bias) [25,26], an estimated 1,949,400 males and 1,850,600 females, comprising 5,650,600 ATP7A alleles and X chromosomes, are anticipated to be added to the US population annually. Based on the mutant allele frequency from the gnomAD database (q = 0.0000194), the number of abnormal ATP7A variants predicted each year among US newborns is 110 (0.0000194 × 5,650,600), including both males and females. Given the slight birth bias toward male gender [3,26], the annual number of male newborns with a complete loss-of-function ATP7A allele should approximate 56. This translates to a birth prevalence for classic Menkes disease of one in every 34,810 male births (1,949,400/56), based on the genomic data from gnomAD.

By way of comparison, we applied the same approach to a different X-linked recessive disorder, Duchenne Muscular Dystrophy, for which incidence data are better established due to longer lifespan. Analysis of gnomAD database entries for the DMD locus indicated 19 unequivocally loss-of-function alleles that occurred exclusively in females out of a total of 204,738 sequenced. Using the Hardy-Weinberg law [21], the term q represents the frequency of mutant alleles, which, in this example for DMD, is estimated to be 19 in 204,738 or 0.0000928. The allelic frequency of p is therefore 0.9999072. Therefore, the Hardy-Weinberg equation for DMD allelic frequencies is:

\[ p^2 + 2pq + q^2 = 1 \]

\[ (0.9999072)^2 + 2(0.9999072)(0.0000928) + 0 = 1 \]

\[ 0.9998144 + 0.0001855 + 0 = 0.999999 \]

Based on this analysis, the predicted birth prevalence of DMD equals 1 in 7246 live male births, in reasonable agreement with population-based estimates (1 in 5000 newborn males) [27].
In addition to the four clearly pathogenic ATP7A variants, we identified 28 missense variants predicted by Polyphen-2 as potentially pathogenic and which were not found in males (Table 3). REVEL and CADD ensemble analyses performed for these variants revealed nine with REVEL values > 0.85 (Table 3, see asterisks), which account for 12 additional pathogenic ATP7A alleles. Diagnostic specificity with a REVEL score cut-off of 0.85 is ≥ 99%, i.e., false positive rate ≤ 1% [23]. Addition of these 12 missense alleles to the four complete LOF alleles, increases the predicted birth prevalence of Menkes disease and other ATP7A-related disorders to 1 in 8664 live male births.

4. Discussion

Since 1990, we have enrolled 151 classic Menkes disease subjects from the US in our clinical studies of Menkes disease and ATP7A-related phenotypes (ClinicalTrials.gov NCT00001262, NCT00811785, and NCT04074512). During this timespan, we became aware of at least 30 additional affected subjects in the US. Coupled with results from the present study, this suggests an estimated ascertainment of 10.8% [(151 + 30)/(56 × 30 yr)] by a single diagnostic and treatment referral center.

Classic Menkes disease typically is suspected at birth only in the context of a known family history, and more often is discovered after symptoms of the illness appear, between 6 and 10 weeks of age [4–7]. Later recognition (between two and ten months of age), after considerable diagnostic odysseys, is also common in our experience. Milder neurological phenotypes, such as Occipital horn syndrome and ATP7A-related distal motor neuropathy, present later in childhood, or in adulthood [10,11]. All three conditions currently may be significantly under-diagnosed [1,9]. In addition, subter and as yet unrecognized clinical or biochemical phenotypes may be associated with certain ATP7A variants, including some presumed pathogenic missense alleles detected in gnomAD (Table 3). The expanded estimate of deleterious ATP7A allele frequency based on inclusion of deleterious missense alleles from gnomAD implies as many as 225 newborns with Menkes disease or other ATP7A-related disorders per year in the US alone.

Prior estimates of Menkes disease incidence varied from 1 in 40,000, to 1 in 254,000, to 1 in 354,507 [1–3]. These estimates represented period prevalences based on identified cases of Menkes disease and birth data from Australia, western Europe (Denmark, France, the Netherlands, United Kingdom and West Germany), and Japan, respectively. Several factors may contribute to under-ascertainment of Menkes disease and its variants. These include disparities in access to pediatric medical care and/or tertiary care genetic diagnostic centers, the difficulty for medical professionals in recognizing or suspecting the diagnosis, premature death of affected subjects, and pregnancy losses or elective terminations related to Menkes disease. Miscarriage is a notoriously difficult metric to assess accurately and is estimated to occur in as many as 10–15% of pregnancies in the developed world [28]. We are unaware of an increased rate of miscarriage or stillbirth among known female heterozygotes for Menkes disease and its variants.

Newborn screening (NBS) for Menkes disease, including DNA-based approaches, is under consideration based on the availability of Copper Histidinate [17,18], an investigational therapy being developed for this condition. Future implementation of NBS is anticipated to identify newborns at-risk for a medically actionable condition during the presymptomatic phase of the illness. All infants with ATP7A variants found at NBS could be evaluated to confirm the presence of Menkes disease by a rapid and reliable assay for plasma catecholamine levels [17,29]. This assay, while not readily convertible to a platform suitable for neureturalmet-based NBS using dried blood spots, will provide a superb secondary test to identify subjects for whom immediate medical treatment is appropriate. Both Menkes disease and Occipital horn syndrome feature distinctively abnormal plasma catechol profiles [10,17,29], whereas patients with ATP7A-related isolated distal motor neuropathy do not [11]. While the latter patients are not candidates for CuHis treatment, other subjects identified with ATP7A variants (Table 3) that alter proper copper metabolism may benefit from it. Follow-up testing for abnormal serum copper and ceruloplasmin levels and/or distinctive plasma neurochemical levels may be relevant for male newborns.

| Variant (hg19/GRCh37) and protein change (NM_000052.7) | Variant Type | Allele Number Detected | REVEL | CADD |
|--------------------------------------------------------|--------------|------------------------|-------|------|
| 1. X-7726679-G-C p.(G626R)*                            | Missense     | 3                      | 0.9629* | 26.0 |
| 2. X-77268415-G-A p.(A738T)                           | Missense     | 1                      | 0.5680 | 25.9 |
| 3. X-77268445-G-A p.(V748I)                           | Missense     | 5                      | 0.7279 | 26.4 |
| 4. X-77268545-G-A p.(V751M)                           | Missense     | 3                      | 0.6949 | 26.5 |
| 5. X-77268516-G-C p.(E771D)                           | Missense     | 1                      | 0.7250 | 22.1 |
| 6. X-77268506-C-G p.(A768G)*                          | Missense     | 2                      | 0.8799* | 26.6 |
| 7. X-77268531-C-A p.(N776K)                           | Missense     | 1                      | 0.6650 | 24.1 |
| 8. X-77268557-C-T p.(P785L)*                          | Missense     | 1                      | 0.8980* | 28.8 |
| 9. X-77268568-G-A p.(V799M)                           | Missense     | 5                      | 0.7329 | 27.0 |
| 10. X-77270205-C-T p.(T818D)*                         | Missense     | 1                      | 0.8640* | 27.2 |
| 11. X-77270244-T-A p.(L831H)                          | Missense     | 1                      | 0.7289 | 26.1 |
| 12. X-77271280-A-G p.(Q843R)                          | Missense     | 1                      | 0.8389 | 26.0 |
| 13. X-77284797-G-A p.(R986Q)                          | Missense     | 2                      | 0.7179 | 26.7 |
| 14. X-77284888-G-T p.(I1020S)                         | Missense     | 1                      | 0.7310 | 27.6 |
| 15. X-77284902-G-A p.(I1024M)                         | Missense     | 1                      | 0.8249 | 24.1 |
| 16. X-77284934-C-T p.(A1035V)                         | Missense     | 1                      | 0.7770 | 29.7 |
| 17. X-77294453-C-T p.(R1211W)                         | Missense     | 2                      | 0.6729 | 25.0 |
| 18. X-77296128-A-C p.(K1237T)*                        | Missense     | 1                      | 0.9449* | 24.4 |
| 19. X-77298998-G-A p.(V1273M)                         | Missense     | 1                      | 0.9350* | 27.9 |
| 20. X-77298284-A-G p.(R1335G)*                        | Missense     | 1                      | 0.8529* | 26.6 |
| 21. X-77298821-C-G p.(L1338V)                         | Missense     | 1                      | 0.8389 | 25.6 |
| 22. X-77300985-G-A p.(G1381D)*                        | Missense     | 1                      | 0.9340* | 30.0 |
| 23. X-77301025-G-T p.(A1394S)                         | Missense     | 1                      | 0.6769 | 28.2 |
| 24. X-77301062-C-A p.(I1407T)                         | Missense     | 1                      | 0.7699 | 25.8 |
| 25. X-77301066-A-C p.(K1408T)                         | Missense     | 1                      | 0.8370 | 27.1 |
| 26. X-77301802-C-T p.(P1413L)*                        | Missense     | 1                      | 0.8859* | 29.1 |
| 27. X-77301927-C-T p.(R1455W)                         | Missense     | 1                      | 0.3160 | 25.8 |
| 28. X-77301990-A-T p.(S1476C)                         | Missense     | 1                      | 0.4709 | 25.7 |
| Total*                                                 |              | 12 alleles             |       |
| Total                                                  |              | 43 alleles             | < 0.85 |
|                                                        |              |                        | > 0.85 |
identified with Table 3 variants.

In silico programs such as PolyPhen-2 have an estimated predictive accuracy of 65–80%, resulting in overestimation of missense changes as deleterious, and may not be as reliable at predicting missense variants with a milder effects [30]. In contrast, the REVEL and CADD ensemble approaches to assessing pathogenicity are considered far more reliable [23,24]. Since missense mutations are rarely responsible for Duchenne (or Becker) muscular dystrophy, we did not extend our gnomAD analysis to possible pathogenic missense alleles at the DMD locus [31].

The current analysis reinforces the value of population-based genomics in assessing the true incidence of rare inherited disorders that may be difficult to ascertain for various reasons. We hypothesize that a newborn screening pilot study for Menkes disease will confirm a higher than previously estimated prevalence, as noted for Pompe disease and other rare inherited disorders following implementation of newborn screening [12–15,32]. Earlier detection of ATP7A variants by newborn screening would contribute to considerably reduced morbidity and mortality from classic Menkes disease and related conditions [17,18] and alleviate parent suffering.

Details of the contributions of individual authors

S.G.K. conceived the project, analyzed data, created tables, and co-wrote the article. C.R.F. analyzed data and co-wrote the article. L.S.Y. analyzed data, co-wrote the article, and created Tables. S.G.K. is the guarantor and corresponding author for this work.

Details of funding

This work was supported by the Center for Gene Therapy, Abigail Wexner Research Institute, Nationwide Children’s Hospital, Columbus, OH and the intramural research programs of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institute of Neurological Disorders and Stroke, and the National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

Details of ethics approval

Not applicable.

Patient consent statement

Not applicable.

Documented approval from institutional committee for animal care and use

Not applicable.

Declaration of Competing Interest

Dr. Kaler’s NIH laboratory received research funding from a Collaborative Research and Development Award (CRADA) between NIH and Cyprium Therapeutics, Inc., New York, NY in 2017. Cyprium is focused on development of novel therapies for the treatment of Menkes disease and related copper metabolism disorders. Dr. Yam is an officer and employee of Cyprium.

References

[1] D.M. Danko, E. Cartwright, P.E. Campbell, V. Mayne, Is Menkes’ syndrome a heritable disorder of connective tissue? Lancet 2 (7733) (1971) 1089 Nov 13.
[2] T. Tooneissen, W.J. Kleijer, N. Horn, Incidence of Menkes disease, Hum. Genet. 86 (1991) 48–410.
[3] V.H. Gu, H. Kodama, K. Shiga, et al., A survey of Japanese patients with Menkes disease from 1990 to 2003: incidence and early signs before typical symptomatic onset, pointing the way to earlier diagnosis, J. Inherit. Metab. Dis. 28 (4) (2005) 473–478.
[4] J.H. Menkes, M. Alter, G.K. Steigleder, D.R. Weakley, J.H. Sung, A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration, Pediatr 29 (1962) 764–779.
[5] S.G. Kaler, Menkes disease, Adv. Pediatr. 20 (1994) 263–304.
[6] S.G. Kaler, C.J. Liew, A. Donsante, J.D. Hicks, S. Satu, J.C. Greenfield, Molecular correlates of epilepsy in early diagnosed and treated Menkes disease, J. Inherit. Metab. Dis. 33 (2010) 583–589.
[7] A. Verrotti, A. Carelli, G. Coppolla, Epilepsy in children with Menkes disease: a systematic review of literature, Child Neurol. 29 (12) (2014 Dec) 1757–1764.
[8] K.E. Stevens, J.E. Price, J. Marko, S.G. Kaler, Neck masses due to internal jugular vein phlebitascia: frequency in Menkes disease and literature review of 85 pediatric cases, Am. J. Med. Genet. Part A 15 (2010 Apr 20), https://doi.org/10.1002/ajmg.a.61072.
[9] S.G. Kaler, ATP7A-related copper transport diseases-emerging concepts and future trends, Nat. Rev. Neurol. 7 (2011) 15–29.
[10] S.G. Kaler, L.K. Gallo, V.K. Proud, et al., Occipital horn syndrome and a mild Menkes phenotype associated with splice site mutations at the MNK locus, Nat. Genet. 8 (1994) 195–202.
[11] M.J. Kerenzer, G.A. Nicholson, S.G. Kaler, et al., Missense mutations in the copper transporter gene ATP7A cause X-linked distal hereditary motor neuropathy, Am. J. Hum. Genet. 86 (2010) 343–352.
[12] A.J. Coffey, M. Durkie, S. Hague, et al., A genetic study of Wilson’s disease in the United Kingdom, Brain 136 (Pt 5) (2013) 1487–1497.
[13] M. DeCastro, J. Johnston, L. Biesecker, Determining the prevalence of McArdle disease from gene frequency by analysis of next-generation sequencing data, Genet. Med. 17 (2015) 1002–1006.
[14] C.R. Ferreira, Prevalence of adenylosuccinase liase deficiency based on aggregated exome data, Mol. Genet. Metab. Rep. 10 (2015) 81–82, https://doi.org/10.1016/j.ymgmr.2015.12.009.
[15] J. Gao, S. Brackley, J.P. Mann, The global prevalence of Wilson disease from next-generation sequencing data, Genet. Med. 21 (2019) 1155–1163.
[16] K.J. Karczewski, L.C. Francioli, G. Tiao, et al., Variation across 141,456 human protein-coding genes, bioRxiv (Preprint) (2019), https://doi.org/10.1101/531216.
[17] 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.
[18] 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.
[19] A. Donsante, L. Yi, P. Zerfas, et al., ATP7A gene addition to the choroid plexus results in long-term rescue of the lethal copper transport defect in a Menkes disease mouse model, Mol. Th. 141 (2011) 214–2125.
[20] M.R. Haddad, E.Y. Choi, P.M. Zerfas, et al., Cerebrospinal fluid-directed rAAV9 ratPTPA plus subcutaneous copper histidinate advance survival and outcomes in a Menkes disease mouse model, Mol. Ther. Methods Clin. Dev. 10 (2018) 165–178.
[21] G.H. Hardy, Mendelian proportions in the mixed population, Science (1928) 49–50.
[22] http://genetics.wbhl.harvard.edu/php2/.
[23] N.M. Ioannidou, J.H. Rothstein, V. Pejaver, S. Siddhha, S.K. McDonnell, S. Baheti, M.R. Haddad, E.Y. Choi, P.M. Zerfas, et al., A systematic review of literature, J. Hum. Genet. 99 (4) (2016 Oct 6) 877–895, https://doi.org/10.1016/j.jhug.2016.08.016 (Epub 2016 Sep 22).
[24] P. Benitez, D. Witten, G.M. Cooper, J. Shendure, M. Kircher, CADD: predicting the deleteriousness of variants throughout the human genome, Nucleic Acids Res. 47 (D1) (2019 Jan 8) D886–D894, https://doi.org/10.1093/nar/gky1016.
[25] J.A. Martin, B.E. Hamilton, M.J.K. Osterman, Births in the United States, NCHS Data Brief. (318) (2018) 1–8.
[26] S.N. Austad, The human prenatal sex ratio: a major surprise, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 4839–4840.
[27] J.R. Mandell, C. Hilling, N.D. Leslie, et al., Evidence based path to newborn screening for Duchenne muscular dystrophy, Ann. Neurol. 71 (2012) 304–313 PubMed: 22451200.
[28] M. Fallah, L.A. Skip, B. Dahl, T.G. Nyenswah, H. Flomo, M. Glayweon, T.L. Lorsh, S.G. Kaler, E.S. Higgs, A.P. Galvani, Pregnancy outcomes in Liberian women who conceived after recovery from Ebola virus disease, Lancet Glob. Health 4 (10) (October 2016) e678–e679.
[29] S.G. Kaler, D.S. Goldstein, C. Holmes, J.A. Salerno, W.A. Gahl, Plasma and cerebrospinal fluid neurochemical pattern in Menkes disease, Ann. Neurol. 33 (1993) 171–175.
[30] S. Ichihara, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (2015) 405–424.
[31] J. Juan-Mateu, L. Gonzalez-Queveda, M.J. Rodriguez, M. Baena, E. Verdura, A. Nascimento, C. Ortez, M. Baiget, DMD mutations in 576 dystrophinopathy families: a step forward in genotype-phenotype correlations, PLoS One 10 (8) (2015 Aug 18) e0135189, https://doi.org/10.1371/journal.pone.0135189.
[32] B.R. Burton, J. Charron, G.E. Hoganson, et al., Newborn screening for lysosomal storage disorders in Illinois: the initial 15-month experience, J. Pediatr. 190 (2017) 130–135.