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Short Communication

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

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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 Hardy-Weinberg equilibrium, this frequency of pathogenic alleles predicts 1 in 34,810 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 7246 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 8664 and predicting 225 newborns with Menkes disease or other ATP7A-related disorders per year in the US alone.

Conclusions: Assuming Hardy-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 8664 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 clincal 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

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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: $p^2 + 2pq + q^2$ [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*, p^2 represents a combination of healthy females and healthy males with normal alleles, and 2pq connotes the combination of heterozygous (carrier) females and affected males, each individual with one mutant X-chromosome. The final term (q^2) 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 pathogencity [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:

Table 1
Distribution of All *ATP7A* Variants in gnomAD (v2.1.1).

ATP7A Variants	Number	%
Missense Variant	480	43.4%
Intron Variant	398	36.0%
Synonymous Variant	175	15.8%
Splice Region Variant	38	3.4%
3 Prime UTR Variant	7	0.6%
Frameshift Variant	2	0.2%
Splice Acceptor Variant	2	0.2%
5 Prime UTR Variant	1	0.1%
Splice Donor Variant	1	0.1%
Stop Gained	1	0.1%
In-frame Deletion	1	0.1%
Total	1106	100.0%

Source: gnomAD.

Table 2
Predicted Loss-of-Function *ATP7A* Variants Reported in gnomAD (v2.1.1).

Pathogenic variant	Variant TYPE	Allele number detected
1. c.120, +1 g > a	Canonical splice donor site	1
2. Gln200ProfsTer3	Frameshift	1
3. Arg201GlnfsTer23	Frameshift	1
4. Glu264Ter	Nonsense	1
		Total = 4 variant alleles/205,523 total alleles detected (0.0000194)

$$p^2 + 2pq + q^2 = 1$$

$$(0.9999806)^2 + 2(0.9999806)(0.0000194) + 0 = 1$$

$$0.9999612 + 0.0000387 + 0 = 0.9999999$$

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:

$$p^2 + 2pq + q^2 = 1$$

$$(0.9999072)^2 + 2(0.9999072)(0.0000928) + 0 = 1$$

$$0.9998144 + 0.0001855 + 0 = 0.9999999$$

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].

Table 3
Other Potential Disease-Causing *ATP7A* Missense Variants in gnomAD (v2.1.1).

Variant (hg19/GRCh37) and protein change (NM_000052.7)	Variant Type	Allele Number Detected	REVEL	CADD
1. X-77266679-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-77268454-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.(V789M)	Missense	5	0.7329	27.0
10. X-77270205-C-T p.(T818I)*	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-77284787-G-A p.(R986Q)	Missense	2	0.7179	26.7
14. X-77284888-G-T p.(A1020S)	Missense	1	0.7310	27.6
15. X-77284902-A-G 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.(K1233T)*	Missense	1	0.9449*	24.4
19. X-77298098-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-77301023-G-T p.(A1394S)	Missense	1	0.6769	28.2
24. X-77301062-C-A p.(L1407I)	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	43 alleles	<0.85	
	Total*	12 alleles	>0.85	

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, subtler and as yet unrecognized clinical or biochemical phenoytpes 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 neurochemical-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 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.

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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.

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