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Genetic Diagnosis of Familial Hypercholesterolemia in Residual Newborn Dried Blood Spots

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IMPORTANCE Newborn screening for familial hypercholesterolemia (FH) would dramatically increase the diagnosis of a common, potentially fatal but highly treatable genetic condition in newborns and relatives.

OBJECTIVE To report the results of genetic testing of residual newborn screening dried blood spots (DBS) with biomarkers suggesting high risk for FH as an initial step toward development of multitier newborn screening for FH.

DESIGN, SETTING, AND PARTICIPANTS A cross-sectional study design from July 2021 to July 2022 was used to test residual DBS from newborns with sample collection between 24 and 72 hours of life for total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B. Principal component analysis identified biomarker combinations that accounted for the greatest variance. Mahalanobis distance was calculated to generalize the idea of a standardized *z* score of a single variable to several correlated variables; approximately 8% of samples with the greatest positive Mahalanobis distance were selected for genetic FH testing. The study included a population-based screening for newborns in Wisconsin. Study data were analyzed from July 2022 to June 2024.

EXPOSURES Newborn residual DBS were tested for total cholesterol, low-density lipoprotein cholesterol, and apolipoprotein B, with a subset tested for pathogenic variants in 8 genes associated with FH.

MAIN OUTCOMES AND MEASURES Prevalence of pathogenic variants for FH in a population-based sample of newborn screening DBS.

RESULTS Of 59 927 total newborns, DBS samples were obtained from 10 004 newborns (mean [SD] age, 27.8 [5.6] hours; 5142 male [51.4%]). From 10 004 specimens tested, principal component analysis demonstrated the combination of low-density lipoprotein cholesterol and apolipoprotein B accounted for the greatest variance, and 768 specimens were selected for genetic testing. A pathogenic variant for FH was found in 16 samples yielding a population-based prevalence of 1 in 625 (1.6 per 1000; 95% CI, 0.91-2.60 per 1000) newborns. Pathogenic variants were distributed throughout the entire range of Mahalanobis scores selected for genetic testing.

CONCLUSIONS AND RELEVANCE This cross-sectional study found that screening newborns for FH using first-tier biochemical testing with reflex second-tier genetic testing was feasible and, in this population, identified 1 in 625 newborns with FH. Further refinement and validation are needed before implementation in newborn screening. Routine newborn screening for FH would substantially increase diagnosis of this common, potentially fatal, yet readily treatable condition while providing opportunities for cascade screening.

Invited Commentary

Supplemental content

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Familial hypercholesterolemia (FH) causes lifelong elevated low-density lipoprotein cholesterol (LDL-C), resulting in high risk for premature onset of atherosclerotic cardiovascular disease (ASCVD). Approximately 1 in 300 people across all populations exhibit the FH phenotype.[1] Early treatment can directly prevent premature ASCVD and death,[2] yet FH remains profoundly underdiagnosed.[3] Both universal and selective cholesterol screening strategies during childhood in order to diagnose FH are recommended,[4] but implementation is low.[5]

Newborn screening is a highly successful public health program that provides near-universal population coverage. Screening newborns for FH is an opportunity to significantly increase diagnosis rates for FH at the beginning of life and cascade screen potentially affected relatives who are likely unaware of their diagnosis. Our team evaluated a novel FH screening algorithm measuring total cholesterol (TC), LDL-C, and apolipoprotein B (ApoB) levels in residual deidentified newborn screening dried blood spot (DBS) specimens[6] and described elevations in LDL-C and ApoB levels suggestive of FH.[7] Herein, we report the results of genetic testing of newborn screening DBS with LDL-C and ApoB levels suggesting high risk for FH as an initial step toward development of multitier newborn screening for FH. The ultimate purpose of these and future validation studies is to demonstrate the feasibility of newborn screening for FH using first-tier biochemical testing with reflex second-tier genetic testing of specimens with the highest LDL-C and ApoB levels and determine if this strategy can identify newborns with genetically confirmed FH.

Key Points

Question Can familial hypercholesterolemia be identified through newborn screening?

Findings In this cross-sectional study of 59 927 newborns, 10 004 residual newborn dried blood spot (DBS) specimens were tested for low-density lipoprotein cholesterol and apolipoprotein B followed by familial hypercholesterolemia genetic testing for specimens with the highest levels of the 2 biomarkers, with 1 in 625 newborns genetically confirmed with familial hypercholesterolemia.

Meaning This study found that newborn screening for familial hypercholesterolemia was feasible with first-tier biochemical testing followed by reflex genetic testing, although further refinement and validation are needed.

Methods

This cross-sectional study was granted a waiver of informed consent from the institutional review board, which permitted use of deidentified, residual newborn screening DBS specimens collected in Wisconsin between July 2021 and July 2022. Simulations indicated that a sample size of 10 000 specimens would provide adequate precision for detection of FH pathogenic variants. A minimal demographic dataset was acquired from each DBS card including birth weight, gestational age, and sex. Race and ethnicity data were not analyzed in this study. Only samples collected between 24 and 72 hours after birth were included. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

TC, LDL-C, and ApoB levels were measured using previously described methods.[6] We previously demonstrated both significant seasonal variability in TC, LDL-C, and ApoB levels throughout the calendar year and variability across different 96-well plates on which specimens were run.[7] Therefore, use of absolute threshold values (in original units of milligrams per deciliter) for genetic testing was not appropriate. Concentrations for each biomarker were normalized to the median within each 96-well plate to produce multiples of the median, exhibiting less seasonal and interplate variation.

Statistical Analysis

As TC, LDL-C, and ApoB are interrelated variables, principal component analysis was performed to identify combinations of the 3 biomarkers that accounted for the greatest variance. Mahalanobis distance, a multivariate distance metric that measures the distance between a point and any defined measure of center for a distribution (median), was calculated to generalize the idea of a standardized z score (which would be for a single variable) to a collection of interrelated variables. Specimens were ranked by Mahalanobis distance with the largest positive values (ie, the highest LDL-C and ApoB levels) selected for genetic testing.

DNA was isolated from DBS using the Maxwell Rapid Sample Concentrator DNA Formalin-Fixed Paraffin-Embedded kit (Promega). Targeted next-generation sequencing was performed using a panel that included *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE*, *LIPA*, *ABCG5*, and *ABCG8*. Sanger sequencing confirmation was carried out, and Infinium Global Diversity Array with Cytogenetics-8 (Illumina) confirmed large deletion and duplication events in *LDLR*. Variant interpretation and classification followed guidelines from the American College of Medical Genetics and Genomics and the Association of Molecular Pathologists.[8] Study data were analyzed from July 2022 to June 2024.

Results

Specimens were collected from 10 004 residual newborn screening DBS samples obtained from 10 004 infants (mean [SD] age, 27.8 [5.6] hours; 4862 female [48.6%]; 5142 male [51.4%]) from a total of 59 927 newborns, with demographic and biochemical results reported previously.[7] Principal component analysis indicated approximately 93% of variability in TC, LDL-C, and ApoB levels and was captured by LDL-C and ApoB, with TC having a relatively small contribution to total variability. Therefore, TC was not used in any further analyses, and a composite Mahalanobis distance from LDL-C and ApoB was calculated for specimens in each batch, with the most extreme 192 distances in each batch (192 × 4 = 768; 7.68% of all specimens) selected for genetic testing.

A total of 16 pathogenic or likely pathogenic variants were identified (**Table 1**); thus, the prevalence of pathogenic variants for our cohort of 10 004 newborns was 1 in 625 (1.6 per

Table 1. Pathogenic and Likely Pathogenic Variants in Familial Hypercholesterolemia Genes Found in 10 004 Newborns

Sample	LDL-C, mg/dL	ApoB, mg/dL	Gene (transcript)	Variant info	Classification
22-0311	119	88	*LDLR* (NM_000527.5)	c.420G>C (p.Glu140Asp)	Pathogenic
22-0271	132	95		c.662A>G (p.Asp221Gly)	Pathogenic
22-0250	126	79		c.1359-1G>A	Pathogenic
22-0251	70	73			
22-0670	83	45		c.1775G>A (p.Gly592Glu)	Pathogenic
22-0300	154	38		c.343C>T (p.Arg115Cys)	Likely pathogenic
22-0254	136	34		c.542C>G (p.Pro181Arg)	Likely pathogenic
22-0252	122	58		c.907C>T (p.Arg303Trp)	Likely pathogenic
22-0439	127	46		c.2026G>C (p.Gly676Arg)	Likely pathogenic
23-0042	136	13		Duplication of exons 9-10	Likely pathogenic
22-0047	145	19	*APOB* (NM_000384.3)	c.10580G>A (p.Arg3527Gln)	Pathogenic
22-0267	126	98			
22-0361	125	74			
22-0368	141	79			
22-0524	77	68			
22-0699	64	30			

Abbreviations: ApoB, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol.

SI conversion factor: To convert ApoB to grams per liter, multiply by 0.01; LDL-C to millimoles per liter, multiply by 0.0259.

Table 2. Ranking of Specimens With Pathogenic or Likely Pathogenic Variants for Familial Hypercholesterolemia[a]

		Only[c]		
Sample	ApoB/LDL-C[b]	ApoB, mg/dL	LDL-C, mg/dL	TC, mg/dL
22-0047	115	109	214	346
22-0250	20	21	66	547
22-0251	77	47	2413	506
22-0252	156	200	110	760
22-0254	70	1512	28	710
22-0267	2	1	10	799
22-0271	24	23	107	17
22-0300	191	1476	77	140
22-0311	68	41	561	1186
22-0361	23	12	396	355
22-0368	14	7	328	810
22-0439	186	451	82	1559
22-0524	56	22	542	1756
22-0670	51	25	1662	2362
22-0699	33	21	429	31
23-0042	41	91	23	103

Abbreviations: ApoB, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

SI conversion factor: To convert ApoB to grams per liter, multiply by 0.01; LDL-C to millimoles per liter, multiply by 0.0259; TC to millimoles per liter, multiply by 0.0259.

[a] Specimens with a low rank number reflect higher levels of ApoB, LDL-C, and TC. Specimens with ranks 1 to 192 were selected for genetic testing.

[b] Rank number of combined ApoB/LDL-C is based on Mahalanobis distance (which generalizes the idea of a standardized *z* score to a collection of interrelated variables).

[c] Rank number of a specimen based on its individual rank within a batch.

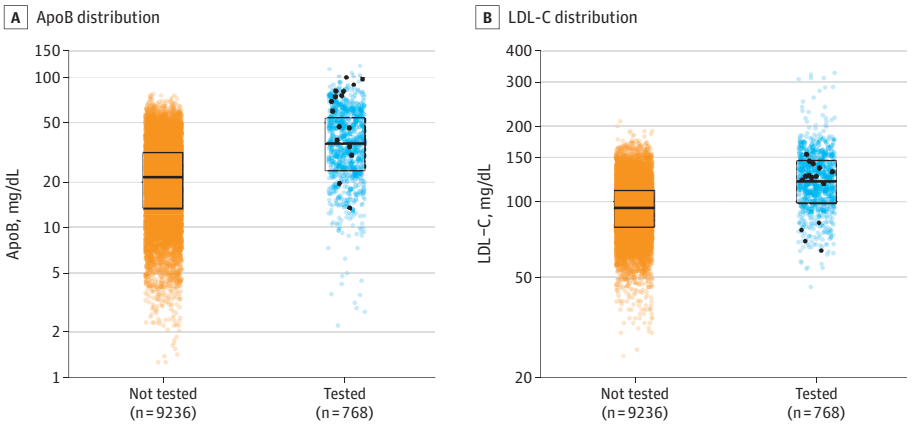
1000; 95% CI, 0.91-2.60 per 1000). Mahalanobis distance from the combination of LDL-C and ApoB identified the most variants, followed by individual ranking for ApoB alone, and then LDL-C alone (**Table 2**). There were 48 variants of uncertain significance (VUS) identified, and they are shown in the eTable in Supplement 1. The **Figure** shows the distribution of absolute values of LDL-C and ApoB by status of genetic testing and the 16 samples with pathogenic or likely pathogenic variants overlaid.

Discussion

Results of this cross-sectional study suggest that screening DBS specimens for FH using first-tier biochemical testing for LDL-C and ApoB followed by second-tier genetic testing identified newborns with genetically confirmed FH at rates consistent with studies of FH screening in older children. Our prevalence (1 in 625 or 1.6 per 1000; 95% CI, 0.91-2.60) was lower than 1 in 300 people but is consistent with the prevalence of monogenic FH variants reported in universally screened pediatric populations in Slovenia (1.4 per 1000; 95% CI, 1.2-1.6)[9] and Japan (2.6 per 1000; 95% CI, 1.9-3.5).[10]

Our approach offers several advantages over alternative methods for FH screening. First, this approach should detect all newborns with homozygous FH, which causes cardiac events beginning in childhood when left untreated,[11,12] as those newborns will have the most extreme elevations in LDL-C and ApoB levels. Second, some individuals with FH variants have LDL-C levels within the normal range[13,14] resulting in a false-

Figure. Distribution of Apolipoprotein B (ApoB) and Low-Density Lipoprotein Cholesterol (LDL-C) by Status of Genetic Testing



SI conversion factor: To convert ApoB to grams per liter, multiply by 0.01; LDL-C to millimoles per liter, multiply by 0.0259.

A, ApoB. B, LDL-C. Sixteen specimens submitted for testing found to have pathogenic/likely pathogenic variants are shown as solid black circles. The box within each distribution shows the median (solid black horizontal line) and 25th/75th percentiles as the lower/upper box edges.

negative test result. These individuals likely have variants with lower expressivity and/or a polygenic etiology, with lower ASCVD risk. As morbidity from FH is directly related to LDL-C levels, the approach we use would avoid identifying such individuals as newborns and the potential introduction of harm, although all children with an LDL-C level above the threshold may benefit from ongoing monitoring of their LDL-C levels. Even if our approach only identifies 50% of individuals with FH, this will raise current FH diagnosis rates (currently 5%-10%) tremendously. Third, biochemical testing has substantially lower cost than genetic testing.

Limitations

Limitations to this study include the use of deidentified specimens, which does not permit longitudinal prospective study of LDL-C and ApoB levels. Although use of a single biochemical marker for screening would be simpler, using ApoB or LDL-C alone would have missed 4 of 16 (ApoB) or 8 of 16 (LDL-C) of the pathogenic variants. In newborn screening, it is common practice to use multiple biomarkers (eg, screening for phenylketonuria includes both phenylalanine and tyrosine, and congenital hypothyroidism uses both thyrotropin and thyroxine assayed by separate methods). We did identify 1 potentially pathogenic copy number variant that could not be confirmed due to insufficient residual sample, and no genetic testing was performed on specimens with Mahalanobis distances below the established cutoff. Wisconsin has a high prevalence of individuals who claim Northern European ancestry, potentially limiting generalizability of these results to more diverse populations. Additionally, this study focuses on detection of genotypically confirmed FH, and thus, our diagnostic criteria are more stringent than phenotypic FH definitions. If newborn screening for FH is implemented clinically, a multipronged approach would likely be adopted to ensure that newborns with high LDL-C and ApoB levels but negative genetic testing results are still investigated and monitored as high risk. If any VUS are later reclassified as pathogenic or if a larger proportion of specimens underwent genetic testing, this would increase our genotypically confirmed FH prevalence. Finally, this study tested specimens in batches, which limits translatability in the real world. In order to validate our approach, a prospective study is needed to test DBS in real time, with follow-up LDL-C and ApoB values in those above a predetermined threshold being reflexed for genetic testing. This is consistent with general newborn screening practice, where confirmatory testing is routinely performed.

Conclusions

In conclusion, results of this cross-sectional study suggest that newborn screening for FH using first-tier LDL-C and ApoB testing with reflex second-tier genetic testing was feasible and, in this cohort, identified 1 in 625 newborns with FH. Routine newborn screening for FH would substantially increase diagnosis of this common, potentially fatal, yet readily treatable condition while providing opportunities for cascade screening. It is noteworthy that newborn screening for FH is intended to complement, rather than replace, universal pediatric lipid screening as this algorithm may miss potentially half of cases.

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Author Contributions: Dr Peterson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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