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Phenotypic severity in a family with MEND syndrome is directly associated with the accumulation of potentially functional variants of cholesterol homeostasis genes

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Abstract

Background: Male *EBP* disorder with neurologic defects (MEND) syndrome is an X-linked disease caused by hypomorphic mutations in the *EBP* (emopamil-binding protein) gene. Modifier genes may explain the clinical variability among individuals who share a primary mutation.

Methods: We studied four males (Patient 1 to Patient 4) exhibiting a descending degree of phenotypic severity from a family with MEND syndrome. To identify candidate modifier genes that explain the phenotypic variability, variants of homeostasis cholesterol genes identified by whole-exome sequencing (WES) were ranked according to the predicted magnitude of their effect through an in-house scoring system.

Results: Twenty-seven from 105 missense variants found in 45 genes of the four exomes were considered significant (−5 to −9 scores). We found a direct genotype–phenotype association based on the differential accumulation of potentially functional gene variants among males. Patient 1 exhibited 17 variants, both Patients 2 and 3 exhibited nine variants, and Patient 4 exhibited only five variants.

Conclusion: We conclude that *APOA5* (rs3135506), *ABCA1* (rs9282541), and *APOB* (rs679899 and rs12714225) are the most relevant candidate modifier genes in this family. Relative accumulation of the deficiencies associated with variants of these genes along with other lesser deficiencies in other genes appears to explain the variable expressivity in MEND syndrome.

KEYWORDS
ABCA1, *APOA5*, *APOB*, emopamil-binding protein, MEND syndrome, modifier genes

1 | INTRODUCTION

The impact of cholesterol deficiency on human embryonic and fetal development is evident from the occurrence of malformation syndromes due to impaired endogenous sterol biosynthesis (Porter & Herman, 2011). Male EBP disorder with neurologic defects (MEND) syndrome (Arnold, Bruckner-Tuderman, Has, & Happle, 2012) is an X-linked disease that was recently incorporated into the OMIM database with an entry (OMIM #300960) separate from that of

chondrodysplasia punctata 2, X-linked dominant (CDPX2, OMIM #302960). Both of these diseases are due to mutations in the *EBP* (OMIM *300205) gene encoding the enzyme 3-β-hydroxysteroid-Δ8, Δ7-isomerase, which catalyzes the antepenultimate step in cholesterol biosynthesis. Patients with MEND syndrome are born to clinically asymptomatic mothers, whereas male intrauterine lethality occurs for CDPX2 mothers. A total of 19 patients with MEND syndrome have been reported to date. Eight of these MEND cases are sporadic (de Almeida, Has, Rampon, Isaacsson, & de Castro, 2017; Bode et al., 2013; Kelley, Maegawa, Leite, & Kratz, 2005. The male with conradi hunnermann syndrome (CDPX2): A distinct phenotype. In Proceedings greenwood genetic center (pp. 95–96); Milunsky, Maher, & Metzenberg, 2003; Tan et al., 2010. X-linked dominant chondrodysplasia punctata and EBP mutations in males. In College of medical genetics annual clinical genetics meeting. Albuquerque, NM) and the remaining cases represented by members of three families (Barboza-Cerda, Campos-Acevedo, Rangel, Martínez-de-Villarreal, & Déctor, 2013; Furtado et al., 2010; Hartill et al., 2014). Overall, mutations in *EBP* appear to give rise to a continuous spectrum of phenotypes likely related to variations in total Δ8, Δ7 sterol isomerase activity and zygosity (Barboza-Cerda, Wong, Martínez-de-Villarreal, Zhang, & Déctor, 2014). At one end of this spectrum, null mutations (up to 70) give rise to the severe phenotype of CDPX2 females; at the other end, the five hemizygous hypomorphic *EBP* mutations with residual activity lead to the milder phenotypes of patients with MEND syndrome. The most severe cases of MEND syndrome share phenotypic characteristics with CDPX2, including skeletal abnormalities such as chondrodysplasia punctata, skin alterations, and cataracts (Arnold et al., 2012; Barboza-Cerda et al., 2014). Patients with MEND syndrome primarily exhibit neurological phenotypes (Dempsey, Tan, & Herman, 1993) corresponding to abnormalities of the central nervous system, digits, and renal system (Barboza-Cerda et al., 2014). Some features of MEND syndrome potentially overlap with Smith-Lemli-Opitz syndrome (SLOS, OMIM #270400), such as 2–3 toe syndactyly, intellectual disability and severe behavioral problems (Hartill et al., 2014). Sporadic cases of MEND syndrome with the more severe phenotype result in death in early childhood, but familial cases are associated with the longest survival among patients with MEND syndrome; these cases show subtle but significant phenotypic differences despite sharing the same mutation. In one (Barboza-Cerda et al., 2013) of the two families with MEND syndrome exhibiting the largest number (4) of patients, differences in the degree of intellectual disability and expressivity of digital abnormalities were observed; in the other family (Hartill et al., 2014), differences in the presentation of behavioral disturbances and a scarcity of structural anomalies were reported.

During pregnancy, maternal cholesterol is actively transferred to the embryo via the placenta and yolk sac (Yoshida & Wada, 2005). The placenta is able to take up all the available cholesterol from the mother and transfer it proportionally (Rebholz, Burke, Yang, Tso, & Woollett, 2011) to the embryo; thus, the maternal plasma concentration is a rate-limiting factor. Up to 20% of the sterol present in an embryo can be maternal (Shi et al., 1999). As pregnancy proceeds, uptake of exogenous cholesterol predominates until the 19th week of gestation, and then endogenous biosynthesis increases (Amaral et al., 2010; Baardman et al., 2012). The maternal-embryonal transport of cholesterol via the placenta involves diverse lipoproteins. The placenta expresses the low-density lipoprotein receptor (LDLR), APOE receptors, and SR-BI (scavenger receptor class B type I) for the uptake of maternal lipoproteins; the apolipoproteins (APOs) APOB and APOE, and the transporters ATP-binding cassette, subfamily A, member 1 (ABCA1) and ATP-binding cassette, subfamily G, member 1 (ABCG1) for the synthesis of new lipoproteins for fetal use (Woollett, 2011).

SLOS, the most common and well-studied disease among cholesterol biosynthesis disorders, results from mutations in the *DHCR7* (OMIM *602858) gene, which encodes Δ7-sterol reductase. SLOS has wide phenotypic variability (Witsch-Baumgartner et al., 2000). The severity score (Kelley & Hennekam, 2000) for anatomical abnormalities has been applied to a large number of SLOS cases at both molecular and biochemical levels and has allowed the identification of a strong genotype–phenotype relationship. However, patients with SLOS having the same *DHCR7* genotype may exhibit marked differences in severity (Waterham & Hennekam, 2012), even between affected siblings (Solcà, Pandit, Yu, Tint, & Patel, 2007). The total absence of cholesterol synthesis, as in patients with SLOS who are homozygous for null mutations in *DHCR7*, appears to be compatible with life (Witsch-Baumgartner et al., 2004) until the neonatal period, suggesting that exogenous sources of cholesterol support intrauterine survival. Environmental and/or genetic factors (modifier genes), in addition to disease-causing mutation, have the potential to increase or decrease the availability of maternal cholesterol in the embryo to ameliorate or exacerbate the severity of disease presentation (Ciara et al., 2004; Solcà et al., 2007; Witsch-Baumgartner et al., 2004). Overall, the identification of modifier genes that explain the substantial clinical variability observed among patients with Mendelian diseases with the same primary mutation(s) is a challenging task (Génin, Feingold, & Clerget-Darpoux, 2008).

APOE (OMIM *107741) and *ABCA1* (OMIM +600046) are the only two modifier genes identified for SLOS to date. *APOE* encodes the affinity ligand apolipoprotein for the LDL and LRP receptors (Greenow, Pearce, & Ramji, 2005), which mediates the clearance of very low-density lipoprotein (VLDL) and chylomicron remnants. ABCA1 is a membrane

transporter that mediates the efflux of cholesterol and phospholipids toward apolipoprotein A-I (APOA-I) (Wang, Silver, Costet, & Tall, 2000) as a first requirement for high-density lipoprotein (HDL) synthesis. The first maternal genotypes to be identified as a modifier in SLOS were *APOE* genotypes with the $\varepsilon 2$ allele (Witsch-Baumgartner et al., 2004). These were further confirmed (Lanthaler, Steichen-Gersdorf, Kollerits, Zschocke, & Witsch-Baumgartner, 2013) to be associated with the development of more severe SLOS phenotypes in the sons of these woman. In contrast, the maternal *ABCA1* p.Lys1587 (rs2230808) allele was found to be associated with milder SLOS phenotypes. The product of the *APOE* $\varepsilon 2$ allele shows defective binding to LDLR (Weisgraber, Innerarity, & Mahley, 1982) and is associated with the development of type III hyperlipoproteinemia (Utermann, Jaeschke, & Menzel, 1975). Healthy carriers of the *ABCA1* p.Lys1587 allele exhibit relatively lower plasma HDL cholesterol concentrations and higher plasma cholesterol and LDL levels (Kolovou et al., 2011).

To explain the intrafamilial phenotypic variability in MEND syndrome, we performed whole-exome sequencing (WES) for four related patients, each of whom represented a different degree of phenotypic severity. Gene variant analyses of cholesterol homeostasis genes revealed a suggesting genotype–phenotype association in this family with MEND syndrome. The number of potentially functional gene variants appears to directly correlate with the degree of phenotypic severity among patients with MEND syndrome. Moreover, our genomic analysis suggests that a decrease in the ability to transfer maternal cholesterol to the embryo is the aggravating factor of the phenotype in this family with MEND syndrome.

2 | PATIENTS AND METHODS

2.1 | Editorial policies and ethical considerations

This study represents the follow-up of two previous works. The initial work (Barboza-Cerda et al., 2013) was approved by the ethics committee (GN11-001) of the Facultad de Medicina, Universidad Autónoma de Nuevo León, Mexico. The four mothers of the patients affected with MEND syndrome kindly signed informed consent forms for extraction of genomic DNA from blood.

2.2 | DNA samples

For this study, only genomic DNA of the four mothers and their affected sons from the family with MEND syndrome stored at −80ºC was used.

Patients 1, 2, 3, and 4 exhibited a descending degree of phenotypic severity (Table 1) and were previously known as IV:5, IV:17, V:12, and V:17, respectively. Patients V:12 and V:17 are first cousins, and their mothers are first cousins to IV:17 and second cousins to IV:5.

2.3 | Whole-exome sequencing (WES)

We used WES to comprehensively identify variants in the cholesterol homeostasis genes, which are candidate modifier genes for the MEND phenotype, within the genetic makeup of the four affected individuals of the family with MEND syndrome, each of whom represented a different degree of phenotypic severity. WES was performed at Sistemas Genómicos (Valencia, Spain, www.sistemasgenomicos.com). A database provided the following annotations for every gene variant found: gene symbol, chromosome, strand, chromosome position, RefSeq ID, sequencing depth, var/depth ratio, minor allele frequency (MAF) based on the 1,000 Genomes Project, variant effect and theoretical prediction of damage based on three programs.

2.4 | Selection of candidate modifier genes

Candidate modifier genes for the MEND phenotype were selected based on a search of the scientific literature for genes that play different roles in the cholesterol homeostasis pathway. These include genes associated with lipoprotein synthesis, lipoprotein receptors, and cholesterol transporters, including those involved in reverse cholesterol transport (Boekholdt et al., 2006); APO genes; lipid-related genes (Carlquist et al., 2011); genes associated with mutations responsible for genetic diseases related to cholesterol excluding cholesterol biosynthesis; and gene variants associated with dyslipidemias and phenotypic traits related to levels of high-density lipoprotein cholesterol (HDL-C) (Boes, Coassin, Kollerits, Heid, & Kronenberg, 2009; Chen et al., 2009), plasma lipoprotein cholesterol (Chasman et al., 2009) and plasma triglycerides.

2.5 | Design of the variant scoring system

To determine whether the potential contribution of a variant of a candidate modifier gene was positive (ameliorative, protective) or negative (detrimental, risk increasing) with regard to modifying the severity of MEND syndrome, an in-house scoring system (Table 2) based on the sum of four characteristics was developed. This scoring system was applied to each single variant, regardless of whether two or more variants within the same gene might be in linkage disequilibrium (LD). As shown in Table 2, score levels were established for each characteristic and indicated with increasing consecutive integers (+1 to +4 or −1 to −4), except for the zygosity scoring, where decimals were also used.

TABLE 1 Clinical characteristics of patients with MEND syndrome[†]

Clinical features	Patient 1	Patient 2	Patient 3	Patient 4
Brain				
Intellectual disability	Moderate/Mild	Moderate/Mild	Moderate/Mild	Mild
Microbrachycephaly	+	+	+	+
Development delay	+	+	+	+
Corpus callosum hypoplasia	+	NE	NE	NE
Craniofacial				
Narrow forehead	+	–	–	–
Midface hypoplasia	+	–	–	–
Ptosis	Right	–	–	–
Strabismus	Convergent	Divergent	–	–
Low-set ears	+	+	–	–
Skeletal				
Brachydactyly 2nd, 4th, and 5th fingers	Bilateral	Bilateral	Bilateral	Bilateral
Postaxial polydactyly type B of the hands	Bilateral	–	Bilateral	Bilateral
Postaxial polydactyly type A of the foot	Right	Right	Right	Left
4–5 finger syndactyly (Type III) bilateral	Complete	Complete	Incomplete	Incomplete
2–3 toe syndactyly bilateral	Complete	Complete	Incomplete	Incomplete
Camptodactyly	Bilateral	Bilateral	–	-
Scoliosis	+	+	+	+
Short stature	+	+	+	+
Urogenital				
Renal alterations	+	NE	NE	NE

[†]Data from Barboza-Cerda et al., 2013; NE: non-evaluated.

Applying an evolutionary perspective, a variant was defined as an allele differing in sequence from the ancestral allele, as based on the chimpanzee (*Pan troglodytes*) sequence, rather than from the reference human genome sequence (GRCh37/hg19). In addition, it was assumed that the absence of gene variants (presence of the major allele) was indicative of normal gene function comparable to that found in any individual and not affecting the fitness of the phenotype. In contrast, the presence of a variant might indicate that the allele may be (a) a polymorphic variant with no negative impact on phenotype fitness that became fixed during human evolution and now shows a frequency similar to that of the ancestral allele, (b) a gene variant with a fitness advantage over the ancestral allele that led it to be the major allele with a frequency higher than that of the ancestral allele, or (c) a gene variant with a plausible deleterious effect (e.g., a disease risk variant or a loss-of-function mutation) that led it to become the minor allele or a rare allele transmitted at a lower rate than the ancestral allele and therefore as has been statistically demonstrated (Gorlov, Gorlova, & Amos, 2015), decreasing in frequency in the population and suggesting that it is selected against. This feature of allele frequencies was qualified considering the allele frequencies in the Mexican population rather than global allele frequencies. The impact of a variant on gene function was qualified in the following two ways: via theoretical damage prediction using three computational programs (SIFT (Sorting Intolerant From Tolerant, http://sift.jcvi.org/) (Kumar, Henikoff, & Ng, 2009), PolyPhen-2 (Polymorphism Phenotyping v2, HumDiv-trained predictor, http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al., 2010) and Condel 2.0 (CONsensus DELeteriousness score, http://bg.upf.edu/fannsdb) (González-Pérez & López-Bigas, 2011)) and based on empirical scientific evidence or statistical associations with quantitative traits or phenotypes related to cholesterol homeostasis in genome-wide association studies (GWAS). Finally, as a difficult-to-evaluate factor, we qualified the zygosity of each variant based on its distribution pattern among the four patients with MEND syndrome. Although the distribution of variants is ultimately a factor inherent to familial genetics but subject to population allelic frequency, zygosity scoring was assigned mainly according to a prejudiced expected zygosity bias. This system consisted of a gradual decline in the zygosity of the variant, ranging from homozygosity to heterozygosity to a total absence of the variant, necessarily ranging either from Patient 1 to Patient 4 when the variant could have a more detrimental (negative)

TABLE 2 Scoring system for the gene variants

Score	Damage prediction	Mexican allele frequencies	Zygosity in patients				Associated phenotype
			1	2	3	4	
							Increased uptake of dietary cholesterol or low levels of LDL-C
						hom	
+4				het	het	hom	
		Major allele > 0.75		het	hom	hom	Increased levels of HDL-C
			het	het	hom	hom	
			het	hom	hom	hom	
+3			hom	hom	hom	hom	
		Major allele > 0.5–0.75					
						het	Cholesterol homeostasis protective phenotype
					het	het	
+2				het	het	het	
	No program	Minor allele frequency > 0.25–0.5	het			het	None associated phenotype
				het	het		
+1				het			
					het		
			het	het	het	het	
−1	1 program	Minor allele frequency (low frequency) >0.05–0.25	hom	het	het	het	Associated phenotype unrelated
			hom	het	het		
			hom	het			
−2	2 programs	Minor allele frequency (rare) 0.01–0.05	het	het	het		Impairment of cholesterol homeostasis
			het	het			
−3	3 programs	Mutation like < 0.0/novel variant	het				Increased levels of LDL-C or diminished uptake of dietary cholesterol
−4							Diminished synthesis of lipoproteins

Note: het, patient heterozygous for the gene variant; hom, patient homozygous for the gene variant; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

The scores in each column are indicated with increasing or decreasing consecutive integers (+1 to +4 or -1 to -4), except for those of the zygosity column, where decimals were used according to the number of lines in each scoring range. All the genotypes at the score range of +1 had this same score and were considered being noninformative.

effect, or from Patient 4 to Patient 1 when the variant could have a more beneficial (positive) impact on the MEND phenotype. The grading of this parameter was intended, similar to an association study, to enhance and link the coincidental value to the occurrence of a variant within a determined MEND phenotype (patient). When a variant did not show a biased distribution pattern (i.e., the variant was not present in Patient 1 or Patient 4 or was present in both Patients 1 and 4), the variant was arbitrarily considered a probable neutral allele and scored as +1.

2.6 | Haplotype and genotype analyses

The *ABCA1* and *APOB* (OMIM +107730) haplotypes of the patients with MEND syndrome were inferred using available genomic data (consulted via www.ensembl.org) from 64 individuals with Mexican Ancestry from Los Angeles USA from the 1000GENOMES:phase 3:MXL Project. From the same 64 individuals, available genomic data for 20 out of the 27 total SNPs in Table 3 were retrieved and used for evaluating the usefulness of the "zygosity" parameter. Data for SNPs rs372890455, rs202212506, rs373488861, rs201703316, rs201806273, rs760613534, and rs77815278 were not available.

2.7 | Phenotypes permutation analysis

To determine the contribution of the complementary parameters for Table 3, a total of 26 rankings of genetic variants were generated. A basic ranking included only scores of

TABLE 3 Gene variants with the most negative scores

Gene	Chr	SNP	Reference sequence	Alleles			Amino acid change	Variant frequency		Zygosity in patients					Associated phenotype	Damage predic-tion	Final score	
				A	R	V		ALL/MXL	Score	1	2	3	4	Score	Score	Score	Neg	Pos
APOA5	11	rs3135506	NM_052968.4:c.56C>G	G	G	A/C	p.Ser19Trp	0.056/0.102	−1.0	het				−3.0	−2.0	−3.0	−9.0	0
ABCA1	9	rs9282541	NM_005502.3:c.688C>T	G	G	A	p.Arg230Cys	0.006/0.070	−1.0	het	het			−2.5	−4.0	−1.0	−8.5	0
NR1H2	19	rs372890455	NM_007121.5:c.425A>C	A	A	C/G	p.Gln142Pro	<0.001(ExAC)	−3.0	het	het			−2.5	+1.0	−3.0	−8.5	+1.0
APOB	2	rs679899	NM_000384.2:c.1853C>T	G	G	A	p.Ala618Val	0.485/0.453	+1.0	het	het	het		−2.0	−4.0	−2.0	−8.0	+1.0
APOB	2	rs12714225	NM_000384.2:c.1223T>C	A	A	G	p.Ile408Thr	0.008/0.000	−3.0			het		+1.0	−4.0	−1.0	−8.0	+1.0
NPC1L1	7	rs138140250	NM_001300967.1:c.817G>T	C	C	A/T	p.Asp273Tyr	0.002/0.039	−2.0	het				−3.0	+1.0	−3.0	−8.0	+1.0
APOE	19	rs429358	NM_000041.3:c.388T>C	C	T	C	p.Arg130Cys	0.151/0.086	−1.0	het				−3.0	−3.0	+1.0	−7.0	+1.0
CTNNA3	10	rs61749223	NM_013266.3:c.478T>A	A	A	T	p.Ser160Thr	0.011/0.008	−3.0	het				−3.0	−1.0	+1.0	−7.0	+1.0
LDLRAD2	1	rs202212506	NM_001013693.2:c.550T>G	T	T	C/G	p.Cys184Gly	0.010(ExAC)	−2.0	het	het	het		−2.0	+1.0	−3.0	−7.0	+1.0
LRP1	12	rs143327344	NM_002332.2:c.11137G>A	G	G	A/C	p.Gly3713Arg	0.001/0.008	−3.0	het				−3.0	+1.0	−1.0	−7.0	+1.0
LRP1B	2	rs150957163	NM_018557.2:c.12047C>T	G	G	A/T	p.Pro4016Leu	0.003/0.008	−3.0	het				−3.0	+1.0	−1.0	−7.0	+1.0
LRP3	19	rs185845419	NM_002333.3:c.1787G>A	G	G	A/T	p.Arg596His	<0.001/0.008	−3.0	het				−3.0	+1.0	−1.0	−7.0	+1.0
SORL1	11	rs140327834	NM_003105.5:c.6194A>T	A	A	T	p.Asp2065Val	0.001/0.0	−3.0		het	het		+1.0	−1.0	−3.0	−7.0	+1.0
LRP8	1	rs5174	NM_004631.4:c.2855G>A	C	C	T	p.Arg952Gln	0.144/0.180	−1.0				het	+2.75	−3.0	−3.0	−7.0	+2.75
LRP5	11	rs80358306	NM_002335.3:c.518C>T	C	C	T	p.Thr173Met	<0.001/0.0	−3.0	het	het			−2.5	−1.0	+1.0	−6.5	+1.0
LRP1B	2	rs35546150	NM_018557.2:c.11200C>A	G	G	T	p.Gln3734Lys	0.045/0.008	−3.0	het				−3.0	+1.0	+1.0	−6.0	+2.0
PLD2	17	rs373488861	NM_002663.4:c.689G>A	G	G	A	p.Cys230Tyr	<0.001 (ESP)	−3.0		het	het		+1.0	+1.0	−3.0	−6.0	+2.0
SORL1	11	rs201703316	NM_003105.5:c.6521A>C	A	A	C	p.Asn2174Thr	0.001(ExAC)	−3.0		het	het		+1.0	+1.0	−3.0	−6.0	+2.0
SPTLC2	14	rs2072672	ENST00000556607.1:c.98G>T	A	C	C/G	p.Gly33Val	0.301/0.359	+1.0	het				−3.0	+1.0	−3.0	−6.0	+2.0
USF1	1	rs201806273	NM_207005.2:c.74A>C	T	T	G	p.Tyr25Ser	0.008(ExAC)	−3.0	het				−3.0	+1.0	+1.0	−6.0	+2.0
LRP5	11	rs760613534	NM_002335.3:c.4480T>C	T	T	C	p.Tyr1494His	<0.001(ExAC)	−3.0				het	+2.75	+1.0	−3.0	−6.0	+3.75
NOXA1	9	rs61748945	NM_006647.1:c.296T>G	T	T	G	p.Leu99Arg	0.015/0.0	−3.0				het	+2.75	+1.0	−3.0	−6.0	+3.75
NOXA1	9	rs112103208	NM_006647.1:c.1396C>T	C	C	G/T	p.Arg466Trp	0.013/0.0	−3.0				het	+2.75	+1.0	−3.0	−6.0	+3.75
APOB	2	rs676210	NM_000384.2:c.8216C>T	G	G	A/T	p.Pro2739Leu	0.366/0.273	+1.0	het				−3.0	+3.0	−3.0	−6.0	+4.0
ABCG5	2	rs6720173	NM_022436.2:c.1810C>G	G	G	C	p.Gln604Glu	0.240/0.328	+1.0	het		het		−2.0	−3.0	+1.0	−5.0	+2.0
LDLR	19	rs11669576	NM_000527.4:c.1171G>A	G	G	A	p.Ala476Thr	0.068/0.016	−2.0			het		+1.0	−3.0	+1.0	−5.0	+2.0
NPC1	18	rs77815278	NM_000271.4:c.1259A>C	T	T	G	p.Tyr420Ser	0.010 (ExAC)	−2.0		het	het	het	+2.0	+1.0	−3.0	−5.0	+3.0

Note: A, ancestral sequence; R, human genome reference sequence; V, variants; het, heterozygous; Neg, negative score; Pos, positive score; ExAC, Exome Aggregation Consortium (http://exac.broadinstitute.org); ESP, Exome Sequencing Project (http://evs.gs.washington.edu/EVS/)

"associated phenotype" and "prediction of theoretical damage," another incomplete ranking that additionally included scores of "variant frequency," a complete ranking and finally 23 rankings including scores of all four parameters, which represent all the permutations between phenotypes of the four patients with MEND syndrome.

2.8 | Genotyping of maternal *APOE* and *ABCA1* (rs2230808)

To determine the maternal contribution to the MEND phenotypic severity of their sons (Patients 1–4), the *APOE* and *ABCA1* genes were genotyped according to Zivelin et al., 1997, with slight modifications to the original sequences of the primers (primer forward, 5′ T deleted and 3′ A inserted; primer reverse, 3′ A deleted), and Kolovou et al., 2011, respectively.

3 | RESULTS

In general, exome sequencing for the four patients in this family with MEND syndrome were of a sufficient depth (20–100×) to achieve reliable genotyping. For some SNPs with the lowest sequencing depth (e.g., 14 reads), the genotype had to be manually determined based on the variant/depth ratio. WES revealed an absence of known coding mutations or splice mutations in 80 genes involved in cholesterol homeostasis; 35 (44%) genes exhibited no variants.

A total of 105 (130 before depuration by linkage disequilibrium) missense variants were found in 45 genes of the four exomes (data not shown). In 85% (89 variants) of these cases, variant calling was ascertained based on the differences in nucleotide sequences from both the chimpanzee sequence taken as the ancestral allele and the human genome reference sequence (GRCh37/hg19). In 15% of the cases, the identified sequence was the same as the ancestral allele, such that the reference sequence of the human genome automatically became the variant. Twenty genes exhibited a single variant, 10 genes two variants, two genes three variants, five genes four variants, four genes five variants, one gene six variants, and three genes either eight, nine, or 21 variants.

As shown in Figure 1, after implementation of the variant scoring system, by which all gene variants were ranked according to their negative and positive scores (Figure 1, left graph), it was observed that the four patients with MEND syndrome (Figure 1, four right graphs) accumulated different numbers of gene variants, mostly variants with higher ratio negative/positive scores (Figure 1 (top) and Table 3). In contrast, among the gene variants exhibiting more positive scores (Figure 1, bottom), a general absence of a distinct distribution pattern was observed. More uniform zygosity, with a trend toward homozygosity (darker bars, Figure 1, left graph), was observed among the four patients with MEND syndrome for at least the eight gene variants with higher positive scores ranging from +7 to +8.25 points. On the basis that the homozygosity of the variants ranged between scores of −4.5 to +8.25 points, the cutoff for significant values to consider gene variants to present greater detrimental potential to modify MEND phenotypic expressivity was fixed at −5.0 (Table 3); at this cut-off point, only 27 (25.7%) of the variants remained significant.

As shown in Table 3, Patient 1 exhibited 17 variants, both Patients 2 and 3 exhibited nine variants, and Patient 4 exhibited only five variants. Patient 1 carried five of the six (rs9282541, rs3135506, rs372890455, rs679899, rs12714225, and rs138140250) variants with the most negative scores ranging from −8 to −9, in stark contrast to the total absence of them in Patient 4. Three (rs9282541, rs372890455, and rs679899) of those six variants were found in Patient 2, and Patient 3 carried two (rs679899 and rs12714225) variants. Patient 1 displayed compound heterozygosity for two genes, *APOB* (rs679899 and rs676210) and *LRP1B* (OMIM *608766) (rs150957163 and rs35546150); Patient 3 showed compound heterozygosity for *APOB* (rs679899 and rs12714224).

Analysis of the genotypes for the SNPs in Table 3 (Figure S1a), including 64 healthy individuals with Mexican ancestry (20/27 SNPs were available from the 1000G project, Phase 3), showed a number (4, 5, or 3, respectively) of gene variants within the mode (3–5) in this population for three of the patients (Patients 2, 3, and 4). Remarkably, Patient 1 carried the largest number (14) of gene variants among all individuals. Overall, the "zygosity" score appears to have contributed to enhancing the final score of certain variants and to the final sum of negative scores *per* individual proportionally (Figure S1b, orange line) to the number of variants carried in comparison with their absence (Figure S1b, grey line).

In addition, phenotype permutation analysis (Figure S2) showed that the top 5 gene variants moved in block among the first 11 positions through the permutations. Interchanges of positions between Patient 2 and Patient 3 (permutations A/D and R/U), considered nearby phenotypically, rendered practically the same rankings. Slightly different rankings were found with interchanges between patients phenotypically related, such as Patient 1 and Patient 2 at positions 2 and 1 (K and L), respectively, or between Patient 3 and Patient 4 at positions 4 and 3 (B and K), respectively. In stark contrast, the interchange of positions between Patients 1 and 4, who express opposite phenotypes, resulted in altered rankings (A and D vs. R and U) in which variants at the third quarter in Table 3 became the top 1–4. Even these altered rankings were differed significantly from the basic ranking (column X, Figure S2b) and from the incomplete ranking (column Y, Figure S2b), which does not include the 'zygosity' score.

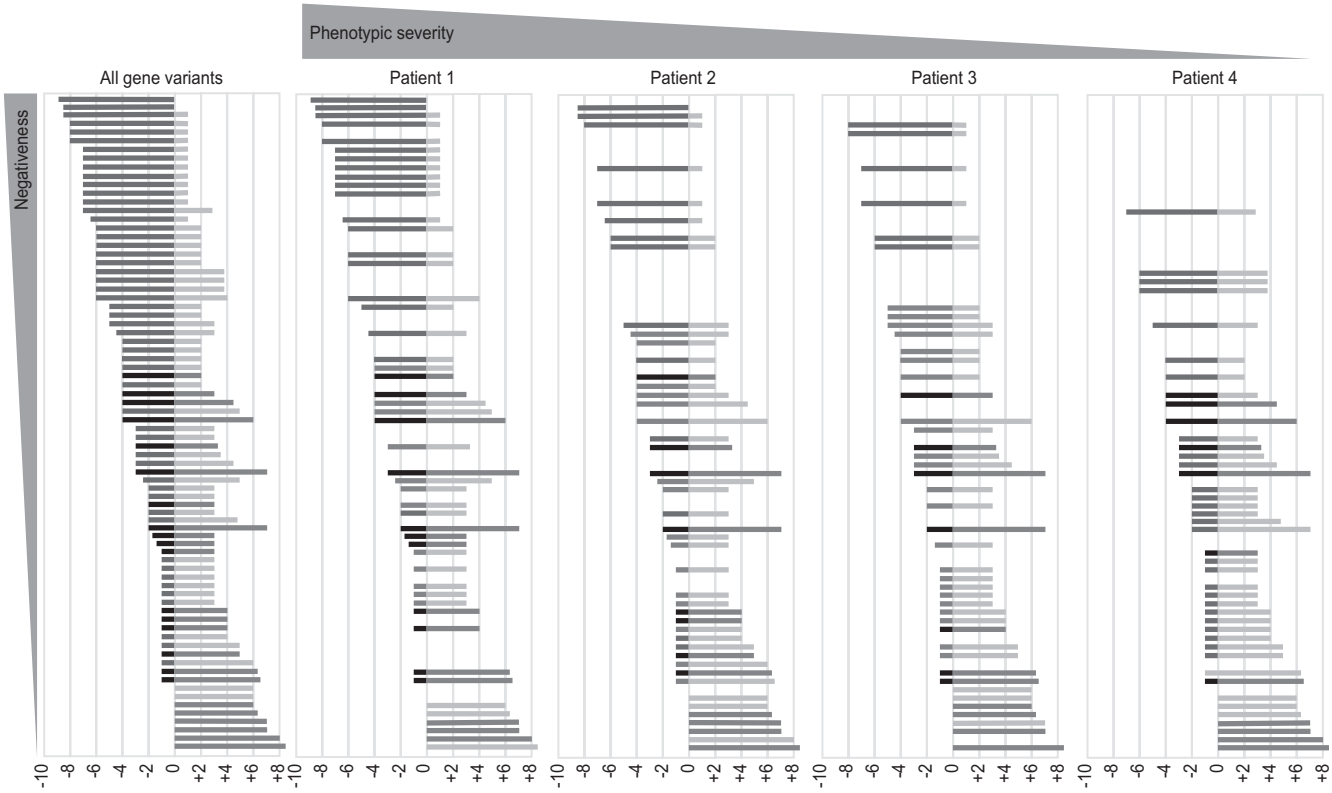

FIGURE 1 Landscape of variants in cholesterol homeostasis genes in patients with MEND syndrome. Seventy-seven gene variants (left graph) from four patients with MEND syndrome (right graphs) who exhibited a descending degree of phenotypic severity (top black triangle) are listed and graphed in decreasing order of negative scoring (left black triangle). Variants on the Y axis are represented as a combination of dark grey or clear grey bars with lengths proportional (*X* axis) to their negative or positive scores, respectively. Gene variants in homozygosity are highlighted (darker bars). The SNP order is as follows: rs3135506, rs9282541, rs372890455, rs679899, rs12714225, rs138140250, rs429358, rs61749223, rs202212506, rs143327344, rs150957163, rs185845419, rs140327834, rs5174, rs80358306, rs35546150, rs373488861, rs201703316, rs2072672, rs201806273, rs760613534, rs61748945, rs112103208, rs676210, rs6720173, rs11669576, rs77815278, rs5882, rs1367117, rs746500859, rs752787876, rs4667596, rs759759368, rs11583680, rs3820198, rs9370867, rs2066718, rs2230808, rs147402550, rs4988321, rs2298813, rs1788799, rs758759368, rs562556, rs2230806, rs4667591, rs3736228, rs1805082, rs185121858, rs2228158, rs3829462, rs897453, rs1144507, rs1801702, rs440446, rs2075252, rs3745974, rs201364912, rs1052748, rs17076657, rs3816614, rs2306029, rs4926972, rs34155071, rs6083, rs6485702, rs371488778, rs6687605, rs4548513, rs2306033, rs12344570, rs3764897, rs6687605, rs8021664, rs55652650, rs2306985, and rs2228171

Up to four *ABCA*1 allelic variants were found among the patients with MEND syndrome. The variant p.Arg230Cys (rs9282541) which was present in both of the patients more strongly affected with MEND syndrome (Patients 1 and 2); and the ancestral allele p.Lys1587 (rs2230808) which was present in Patients 2 and 3, exhibiting the intermediate MEND phenotype, were considered potentially functional based on their associations with decreased levels of HDL-C and higher plasma cholesterol and LDL levels (Clee et al., 2001; Kolovou et al., 2011; Villarreal-Molina et al., 2007; Weissglas-Volkov et al., 2013). Two other *ABCA1* variants, p.Val771Met (rs2066718), present only in Patient 1, and p.Arg219Lys (rs2230806), present in both of the most affected patients (Patients 1 and 2), were considered protective based on their association with increased HDL-C levels and APOA-I (Clee et al., 2001) or decreased triglyceride levels (Evans & Beil, 2003), respectively. Both were associated with a decreased risk of coronary heart disease (Clee et al., 2001; Evans & Beil, 2003).

Theoretically, a total of 10 *ABCA1* (SNP order: rs2230808, rs9282541, rs2066718, and rs2230806; p.Lys219Arg, p.Arg230Cys, p.Val771Met, and p.Lys1587Arg) haplotypes, that is, RRVR, RRVK, KRVR, KRVK, KRMR, RRMR, KRMK, RCVR, KCVR, and RCVK, were found to feasibly occur in Mexican individuals. For three *ABCA1* haplotypes, RCVR, KCVR, and RCVK, the likelihood of carrying the p.Arg230Cys variant (rs9282541) was 77%, 55.5%, and 44.4%, respectively. Six *ABCA1* haplotypes were present among the patients with MEND syndrome. Patients 1 and 2 exhibited one *ABCA1* allele, with haplotype RCVR carrying the potentially functional variant p.Arg230Cys. Moreover, Patient 1 carried a second *ABCA1* allele (haplotype KRMR) carrying two linked protective variants, p.Val771Met and p.Arg219Lys; in contrast, Patient 2 carried the second *ABCA1*

allele (haplotype KRVK), with the potentially functional ancestral allele p.Lys1587 linked to the protective variant p.Arg219Lys. Patient 4 was homozygous for the *ABCA1* reference allele (haplotype RRVR), which is the most frequent allele in Mexicans. Patient 3 was heterozygous for the same allele, haplotype RRVR, in compound heterozygosity with the other *ABCA1* allele (haplotype RRVK) carrying the ancestral allele p.Lys1587.

Six probable *APOB* (SNP order: rs17240441, rs1367117, rs12714225, rs679899, rs676210, rs1801702, rs104201, and rs1042034; p.12_14LALdel, p.Thr98Ile, p.Ile408Thr, p.Ala618Val, p.Pro2739Leu, p.Arg4270Thr, p.Glu4181Lys, and p.Ser4338Asn) haplotypes were identified among the patients with MEND syndrome. Patient 1 was heterozygous for the APOB haplotype (LALP)TIVPREN, carrying the p.Ala618Val variant (rs679899), which is within the top 4 in Table 3; the same variant in Patients 2 and 3 is located within haplotype (LALP)TIVPRKN. The second *APOB* allele of Patient 1, haplotype (LALP)TIALTES, carries the variants p.Pro2739Leu (rs676210) and p.Ser4338Asn (rs1042034). In Patient 2, the second *APOB* allele, haplotype (P)IIAPREN, is apparently linked to variants p.12_14LALdel (rs17240441) and p.Thr98Ile (rs13667117). In Patient 3, the haplotype (LALP)TTAPREN carries the variant p.Ile408Thr (rs12714225), which is among the top 5 in Table 3. Finally, Patient 4 was found to be homozygous for the wild-type *APOB* allele, haplotype (LALP)TIAPREN.

Regarding gene expression in the placenta, 14 (66.6%) of the genes in Table 3, accounting for 66.6% (18/27) of the gene variants, are expressed at the protein level, and seven genes (*NPC1L1* (OMIM *608010), *CTNNA3* (OMIM *607667), *LDLRAD2*, *LRP3* (OMIM *603159), *LRP5* (OMIM *603506), *NOXA1* (OMIM *611255), and *ABCG5* (OMIM *605459)) are not expressed.

Additionally, genotyping of maternal *APOE* showed absolute genotypic homogeneity. All four mothers were homozygous for *APOE ε3*, which is the most frequent allele globally. Similarly, genotyping of maternal *ABCA1* (rs2230808) revealed near genotypic homogeneity, with three of the four mothers being homozygous for the variant p.Lys1587Arg, whereas only the mother of the second more severe patient with MEND syndrome (Patient 2) was heterozygous for the ancestral allele p.Lys1587, which is the MAF allele in the Mexican population.

4 | DISCUSSION

We envisioned our familial cases of MEND syndrome to be suitable for the identification of the most relevant modifier genes within the genetic background of each patient, which would first explain the clinical variability in this monogenic disorder and then be extended to all disorders of endogenous biosynthesis of cholesterol. Disregarding the low prevalence of MEND syndrome, it is theoretically more feasible that the hemizygous condition required for the disease-responsible mutation (eliminating sex factors and guaranteeing the same level of deficiency in different individuals) would lead to the establishment of better genotype–phenotype relationships compared with autosomal recessive disorders of cholesterol biosynthesis, in which phenotypic expression may be more variable due to the compound heterozygosity of the associated mutations.

In this study, exome sequencing of patients with MEND syndrome allowed for comprehensive assessment of the nucleotide variations in the coding sequences of candidate modifier genes, which allowed us to rule out the involvement of known or novel disease-causing mutations other than the primary *EBP* (NM_006579.2:c.224T>A, NP_006570.1:p.Ile75Asn, rs797045153, HGMD-PUBLIC CM146526) mutation as genetic factors that modify the MEND phenotype. Up to 70% of the inspected genes carried variants, with most (78%) presenting only a single variant. Therefore, coding SNPs emerge as a genetic factor with the potential to modify the MEND phenotype.

Within the normal context of cholesterol biosynthesis, the phenotype-modifying effects associated with the genomic variants found here would likely be insufficient to cause disease and may go unnoticed. However, regarding the expression of different degrees of phenotypic severity in individuals with MEND syndrome, these effects might be determinant.

We think that the small but cumulative effects associated with these genetic variants are magnified during development due to the high dependence of the maternal cholesterol supplying. Such dependency creates in these individuals a hypersensitivity to any downward fluctuation in the level of this indispensable resource, which ultimately determines the severity of congenital alterations.

Moreover, to identify the most relevant modifier genes for MEND syndrome among the large number of candidate genes, it was important to implement a straightforward SNP scoring system (Table 2), in which gene variants were ranked according to their theoretical relative potential effect of detrimental (Table 3 and Figure 1, left graph, top) or beneficial (Figure 1, left graph, bottom). Given the narrow phenotypic spectrum of MEND syndrome encompassed by the hypomorphic *EBP* mutation p.Ile75Asn in the family in this study, it was desirable to identify a low but differential number of candidate modifier genes carrying relevant variants among the four patients with MEND syndrome. Indeed, we found an apparent genotype–phenotype correlation between the number of gene variants with negative features (negative scores) and the phenotypic severity of MEND syndrome. The success of this genotype–phenotype association likely relied on synergism among all of the criteria of the variant scoring system, which was designed to

identify gene variants with characteristics closer to those of loss-of-function mutations.

On the one hand, two widely recognized parameters, "associated phenotype" and "prediction of theoretical damage," provided the basal score. On the other hand, parameters introduced by us, namely "variant frequency" and "zygosity," appeared to be useful for variant ranking by contributing with complementary, congruent scores. For example, the genotypes of the four patients with MEND syndrome and those of 64 other individuals of Mexican ancestry suggested that scores for "variant frequency" were assigned in accordance with allele frequencies. With regard to "zygosity," in accordance with its purpose to attribute weight to the association between the occurrence of genetic variants and the phenotypic severity of the patients, similar to an association study, this measure enhanced the negative scores (orange line, Figure S1b, and column A, Figure S2b) of certain variants, which determined the final ranking (Table 3, and column A, Figure S2b). Such a negative score suggests that these gene variants act as theoretically functional alleles with the potential to represent individual genetic factors that negatively modify the MEND phenotype in aggregate. In support of this assumption, when attempting to identify Mendelian causal mutations in suspected monogenic dyslipidemias through exome sequencing (Stitziel et al., 2015), only certain variants in lipid genes that are likely pathogenic were identified in some families. Moreover, other families displayed significant accumulation of common alleles, suggesting that complex inheritance can be masked as a monogenic disease. Even some common alleles appeared to result in extreme phenotypes in some individuals. It should be noted that, Patient 1, expressing the more severe MEND phenotype, carried the highest number (17) of genetic variants among patients, even when compared to 64 other individuals of Mexican ancestry (Figure S1).

Table 3 proposes a relative significance among gene variants as phenotype-modifying factors; rather than general for disorders of cholesterol biosynthesis, such as the same MEND syndrome, it is particular to the studied family. In support of this finding, the permutation of phenotypes (Figure S1) between patients, especially the interchange between Patient 1 and Patient 4, caused some variants with a minor modifying potential (variants 13, 14, and 21–23 in the basic ranking, column X, Figure S2b) to rise or those top variants (variants top 1–5 in the final ranking, Table 3, and column A, Figure S2b) to fall slightly in position within the new rankings (columns R and U, Figure S2b). In this sense, it is feasible that scoring of these same gene variants in individuals with different phenotypic expressivity may yield other candidate modifier genes to be more significant if this may naturally occur in other families with MEND syndrome.

In this study, *APOA5* (OMIM *606368), *ABCA*1, and *APOB* carried the top negative variants, as shown in Table 3. Indeed, their allelic variants occurred unequally among the four patients with MEND syndrome and thus emerged as the most relevant candidate modifier genes of MEND syndrome.

The most relevant candidate modifier gene of MEND syndrome is *APOA5*. A single allelic variant of this gene, p.Ser19Trp (rs3135506), was observed; notably, this variant, which was ranked first (Table 3), was present only in the most affected patient (Patient 1). This gene variant, which has even been referred to as a frequent mutation (Schaefer et al., 2004), has been associated with hypertriglyceridemia (Pennacchio et al., 2002; Talmud et al., 2002) and likely impairs the process of lipolysis performed by lipoprotein lipases. Although, this variant alone is not sufficient to cause hypertriglyceridemia, even in individuals who are homozygous, it has been suggested to be a crucial cofactor in individuals who are homozygous for the *APOE ε2* allele (Schaefer et al., 2004). Patient 1, similar to all patients with MEND syndrome, lacked *APOE ε2* alleles, although only this patient was heterozygous for the *APOE ε4* allele.

Specifically, the *ABCA1* variant p.Arg230Cys was ranked second in Table 3. This globally rare (0.006, Table 3) variant appears to be restricted to Amerindian-derived populations (Weissglas-Volkov et al., 2013). It was originally reported as a missense mutation, R170C (Wang, Burnett, et al., 2000), in an Oji-Cree individual with familial hypoalphalipoproteinemia (FHA, OMIM #604091) and in two other individuals with low plasma HDL-C concentrations below the 5th percentile for age and sex. Furthermore, this functional variant has been found in up to 20.1% of Mexican mestizos, in association with decreased HDL-C and APOA-I levels and with obesity, metabolic syndrome, and type 2 diabetes (Villarreal-Molina et al., 2007). This family with MEND syndrome apparently carries another *ABCA1* allele that is potentially functional, the ancestral allele p.Lys1587. In healthy individuals, this allele has been shown to confer a 59% increase relative to that conferred by variant p.Lys1587Arg in risk of exhibiting plasma low-density-lipoprotein cholesterol (LDL-C) levels above normal limits (Marvaki et al., 2014) and is overrepresented in low-HDL individuals (Slatter, Jones, Williams, Van Rij, & McCormick, 2008). Importantly, the presence of the allele p.Lys1587 in the mothers of SLOS children correlates with the development of mild phenotypes in their sons (Lanthaler et al., 2013).

APOB is considered the third most relevant candidate modifier gene for MEND syndrome because up to seven of its allelic variants have been reported to be significantly associated with increased levels of LDL-C or hyperlipidemia. Among these variants, p.Ala618Val (rs679899) (Gu et al., 2017) and p.Ile408Thr (rs12714225) (Teslovich et al., 2010) were the fourth and fifth top variants in Table 3, respectively. The presence of p.Ala618Val in compound heterozygosity with p.Ile408Thr in Patients 1, 2, and 3 and the absence of

both in Patient 4, who had the mildest MEND phenotype, supports its contribution to the genotype–phenotype association in MEND syndrome.

4.1 | Gene deficiencies during pregnancy

Previous studies have suggested that the effects of modifier genes in SLOS are more significant during the developmental stage than during later stages (Lanthaler et al., 2013; Witsch-Baumgartner et al., 2004). The congenital nature of the alterations in MEND syndrome and the roles of genes carrying potentially functional variants in the maternal–fetal transfer of cholesterol prompted us to search for genotype–phenotype relationships during development.

Evidence of the negative effects on gene function of the potentially functional gene variants found here (Table 3) derives from association studies on related metabolic traits. These studies may suggest that alterations in the functions of placentally expressed genes are likely associated with the degree of phenotypic severity. However, the lack of experimental functional assessments limited us to speculation regarding how the presence of such gene variants may result in the deficient transfer of maternal cholesterol to the embryo and ultimately aggravate the phenotype in this family with MEND syndrome.

The placenta expresses APOE receptors that mediate receptor-mediated endocytosis of APOE-containing lipoproteins as an additional source (Woollett, 2011) of exogenous cholesterol. This observation suggests that placental deficiency in the uptake of maternal cholesterol inside of APOE-containing lipoproteins might be involved in the phenotypes presented herein, especially in Patients 1 and 4, who have variants in APOE receptor genes. This possibility is supported by the observation that more severe SLOS phenotypes in sons are exhibited when their mothers are heterozygous or homozygous for the *APOE ε2* allele (Lanthaler et al., 2013; Witsch-Baumgartner et al., 2004). Patient 1 accumulated more variants with negative scores in the same number of APOE receptor genes (up to five, Table 3) in a nearly exclusive manner. Among these variants, p.Gly3713Arg (rs143327344), which is in the multi-ligand receptor-encoding *LRP1* (OMIM *107770) gene, is perhaps the most relevant. Patient 4 was a carrier of the *LRP8* (OMIM *602600, APOER2) variant p.Arg952Gln (rs5174), which ranked 14th (Table 3) and is a risk factor for myocardial infarction and coronary artery disease (Shen et al., 2007).

Additionally, a deficiency in direct uptake of cholesterol by the embryo/fetus was due either to the failed assembly of VLDL-like lipoproteins in the placenta or, more likely, to impaired LDLR binding of placental VLDL-like lipoproteins carrying a potentially less efficient APOB-100 ligand. Interestingly, all patients with MEND syndrome appear to have a basal deficiency in APOB function because they carry *APOB* alleles carrying ancestral sequences of the SNPs rs1042034 and rs676210. These SNPs are hypothesized to predispose middle-aged and elderly members of the Chinese Yugur population to hyperlipidemia in combination with other genetic or nutritional factors (Gu et al., 2017). Patient 2 and especially Patient 3 may exhibit somewhat higher degrees of deficiency in the synthesis of lipoproteins because of the presence of compound heterozygosity for potentially functional *APOB* alleles. Thus, similar to Patients 2 and 3, Patient 1 can theoretically synthesize only 50% of lipoproteins with the deficient APOB lipoprotein. However, the remaining 50% may be synthesized with superior efficiency for lipoprotein-triacylglycerols lipolysis based on the decreased levels of total triacylglycerols, total cholesterol and LDL associated with the presence of the protective *APOB* variant p.Pro2739Leu (rs676210) (Mäkelä et al., 2013; Teslovich et al., 2010), which is linked to the ancestral allele p.Ser4338 (rs1042034) in a haplotype free of potentially functional variants. Notably, Patient 1 is also a carrier of the variants p.Ser19Trp (rs3135506) and ε4 (rs429358) in the genes *APOA5* and *APOE*, respectively, the products of which are integral components of lipoproteins. In combination with *APOB* variants, these two *APOs* might yield lipoproteins that are defective for subsequent catabolism, either in binding to their receptors, upstream lipolysis processes or both. Thus, globally, 87.5% of the lipoproteins in Patient 1 may incorporate at least one APO-defective isoform, 37.5% may incorporate two defective isoforms, 12.5% may incorporate up to three defective isoforms, and 12.5% may incorporate no defective isoforms.

Impairment of the supply of placental cholesterol to the embryo/fetus through reverse cholesterol transport mediated by ABCA1 is a factor that may increase the phenotypic severity of MEND syndrome, which is supported by the decrease (~30%) in cholesterol transfer to the fetus observed in a mouse model of SLOS with a disruption of *Abca1* (Lindegaard et al., 2008). It has been proposed that the deficiency of cholesterol efflux toward APOA-I for HDL formation in the placenta conferred by the p.Lys1587 allele causes an overload of cholesterol in the syncytiotrophoblast, resulting in increased cholesterol efflux toward the fetus mediated by ABCG1 (Lanthaler et al., 2013). This change would explain the association of the maternal *ABCA1* genotypes, including the potentially functional p.Lys1587 allele, with the development of milder SLOS phenotypes in their children. Conversely, the patients with MEND syndrome with the least favorable *ABCA1* genotypes (Patients 1 and 2) exhibited the most severe MEND phenotypes. The presence/absence of the p.Arg230Cys variant and, to a minor degree, the ancestral allele p.Lys1587 supports the findings regarding MEND phenotypic severity. Regarding the overall functionality of ABCA1, the variant p.Arg230Cys may exert a dominant-negative effect over any other allele, as

suggested by the lack of a proportional gene dosage effect on HDL-C levels in homozygous individuals (Villarreal-Molina et al., 2007).

Interestingly, another aggravating factor of HDL synthesis on the fetal side of the placenta in Patients 1 and 2 might be the presence of the variant p.Gln142Pro (rs372890455) of the *NR1H2* (OMIM *600380) gene encoding nuclear receptor subfamily I group H member 2 or liver X receptor beta (LXRβ). LXRβ is a cholesterol sensor that regulates ABCA1-mediated cholesterol efflux at both transcriptional and post-translational levels (Hozoji-Inada, Munehira, Nagao, Kioka, & Ueda, 2011). Although it has not been associated with any phenotypic trait, this variant theoretically ranked third in Table 3 (−8.5 points), which suggests that it may impair transcription of *ABCA1* when the exogenous cholesterol cell concentration increases. It is feasible that the amino acid change Gln142Pro in the LXRβ DNA-binding domain (DBD) may alter the DBD structure and its interaction with the retinoid X receptor (RXRα) in the RXRα-LXRβ heterodimer. In addition, Gln142 precedes the Gln143 residue (Lou et al., 2014), which contacts the side chains of His288 and Glu291 in the RXRα ligand-binding domain. We suggest that the deficiencies of ABCA1 and LXRβ may ultimately be redundant. Thus, *ABCA1* appears to be a candidate modifier gene that exerts a protective effect in disorders of endogenous cholesterol synthesis.

When attempting to evaluate the genetic potential of mothers of sons with MEND syndrome to supply cholesterol within lipoproteins to the placenta, we found an absence of allelic variants of *APOE* (ε2 allele) and *ABCA1* (p.Lys1587 allele), for which significant associations with contrasting phenotypic severities in SLOS were demonstrated (Lanthaler et al., 2013; Witsch-Baumgartner et al., 2004). Our findings suggest that all four mothers were competent in supplying cholesterol within chylomicron remnants or HDL to the placentas of their sons with MEND syndrome. However, it remains to be determined whether the variants in *APOA5*, *ABCA1*, and *APOB* found in Patients 1 and 2 were inherited from their mothers; such inheritance would suggest an unanticipated additional deficiency in the supply of cholesterol to the placenta through chylomicrons, VLDL and HDL. Such a deficiency would be consistent with the abovementioned genotype–phenotype association.

We conclude that *APOA5*, *ABCA1*, and *APOB* are the most relevant candidate modifier genes in this family with MEND syndrome. Relative accumulation of the deficiencies associated with variants of these genes along with other lesser deficiencies in other genes appears to explain the variable expressivity in MEND syndrome.

The application of our research approach to patients with SLOS might promote the validation of our findings and expansion of the current list of gene variants of candidate modifier genes for disorders of cholesterol synthesis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

AUTHORS' CONTRIBUTIONS

M.C.B-C and M.A.D conceived and designed the study, analyzed the results, and wrote the manuscript; G.M-A performed the RFLPs analyses; R.G-G and O.B-Q contributed to the interpretation of the results and discussion. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT

WES data and the analysis of 105 missense variants are available upon request to the corresponding author.

PATIENT CONSENT

Obtained.

ETHICS APPROVAL

This study (GN11-001) was approved by the ethics committee of the Facultad de Medicina, Universidad Autónoma de Nuevo León, Mexico.

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