Preconception carrier screening yield: effect of variants of unknown significance in partners of carriers with clinically significant variants

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Purpose: Expanded preconception carrier screening (ECS) identifies at-risk couples (ARCs) for multiple diseases. ECS reports currently include only pathogenic/likely pathogenic variants (P/LPVs). Variants of unknown significance (VUS) are not reported, unlike genomic or chromosomal array test results in other post/prenatal settings. Couples who are P/LP and VUS carriers (P/LP*VUS) may be at risk, particularly in genes with high P/LP carrier rates. We examined the possible contribution of P/LP*cVUS (coding, nonsynonymous VUS) matings to ECS yield in an Ashkenazi Jewish cohort, a population with well-established preconception screening.

Methods: We analyzed 672 Ashkenazi Jewish genome sequences (225,456 virtual matings) for variants in three different gene sets and calculated the rates of P/LP*P/LP and P/LP*cVUS matings.

Results: Across 180 genes, we identified 4671 variants: 144 (3.1%) P/LP and 1963 (42%) VUS. Across gene sets, the proportion of P/

LP*P/LP and P/LP*cVUS ARCs was 2.7–3.8% and 6.8–7.5%, respectively.

Conclusion: Disregarding VUS in ECS may miss ARCs. Even if only 10% of couples currently classified as P/LP*cVUS are ultimately reclassified as P/LP*P/LP, ECS yield would increase by ≈20%. While current understanding of VUS precludes VUS reporting in ECS, these findings underscore the importance of VUS reclassification. This will crucially depend on enlarging population frequency databases, especially of affected individuals.

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INTRODUCTION

Preconception carrier screening (PCS) aims to identify couples at risk for having offspring with genetic diseases and facilitate informed reproductive choices. PCS was originally limited to a few disorders in specific populations, e.g., Tay–Sachs disease in Ashkenazi Jews (AJ).¹ As recently as 2015, the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics and Genomics (ACMG) recommendations focused on ethnicity-based testing, with only nine diseases indicated for AJ. Pan-ethnic testing was recommended only for hemoglobinopathies, cystic fibrosis, and spinal muscular atrophy.¹ However, next-generation sequencing has led to rapid adoption of expanded carrier screening (ECS), with full sequencing of hundreds of genes.² In 2017, the ACOG indicated ECS as an acceptable strategy.² Simulated and real-world ECS results demonstrate greater yield compared with the 2015 ACOG/ACMG recommendations: a 94-gene panel would double the detection rate of hypothetical affected fetuses in an ethnically diverse population.³ Increased yields are achieved by sequencing entire genes (versus targeted variant testing), adding genes with high carrier rates and milder

phenotypes (e.g., *HFE*), and including an ever-growing number of rare disease genes.^{4–6} The major increment in ECS yield is explained by full sequencing of a modest number of genes with high carrier rates.⁶

Fuller understanding of ECS outcomes should address variants of unknown significance (VUS). As an additional source of what may prove to be pathogenic variants, VUS could significantly affect future ECS yields, particularly in genes with high carrier rates of pathogenic/likely pathogenic variants (P/LPVs). Currently, ECS reports do not include VUS¹ and clinical laboratories do not perform prenatal testing for ECS-detected VUS.

We assessed the effect of VUS on detection rates of at-risk couples (ARCs). We compared the yield of complete gene sequencing versus targeted variant testing in the same gene set, and examined the effect of expanding the number of genes sequenced. We based our analysis on 672 genome sequences of AJ, a population typical of PCS.

MATERIALS AND METHODS

See Supplementary Information for details.

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Sequencing data

We used phased genome sequences (without copy-number variant [CNV] analysis) of 672 previously published, unrelated AJ samples.⁷

Genes and variants analyzed

We defined recessive AJ, pan-ethnic, and combined gene sets (Table S1). Genes included were present in at least two of three commercial ECS panels: Baylor,⁸ Mount Sinai,⁹ and Jewish Genetic Disease Consortium (JGDC)¹⁰ for the AJ gene set, and Baylor,⁸ Mount Sinai,⁹ and Counsyl (currently Myriad)¹¹ for the pan-ethnic gene set. The combined gene set is comprised of both AJ and pan-ethnic sets. Variants were classified using InterVar,¹² an automated ACMG classifier, and ClinVar.¹³ Manual reclassification was also performed as follows: (1) variants homozygous in $\geq 5\%$ of our cohort were reclassified as benign, (2) variants homozygous in 1–5% of our cohort were reclassified as benign if the homozygosity rate was $>1\%$ in any population in gnomAD,¹⁴ and (3) variants with discrepant InterVar¹² and ClinVar¹³ classifications (Table S2) were reclassified manually (Supplementary Methods).

Virtual matings

We tested all possible 225,456 ($672 \times 671/2$) theoretical matings, irrespective of gender. When more than one variant in the same gene was present in a sample, the most pathogenic variant was used.

RESULTS

Genes analyzed and variant classification

We analyzed 180 genes underlying autosomal recessive disorders (see “Materials and methods,” Table S1). This combined gene set included 168 genes representing pan-ethnic ECS panels (pan-ethnic gene set) and 55 genes representing AJ ECS panels (AJ gene set). Forty-three genes were included in both pan-ethnic and AJ ECS panels. Following variant classification (see “Materials and methods”) we computed carrier rates and types of ARCs.

In 672 AJ genome sequences, we identified 4671 variants in the exons ± 10 bp of the 180 selected genes. These variants were classified into three categories: P/LP ($N = 144$, 3.1%), VUS ($N = 1963$, 42%), and benign/likely benign (B/LB) ($N = 2564$, 54.9%).

Founder P/LP variants

Founder variants (FVs) were defined as P/LPVs included in AJ targeted variant panels^{15,16} and with combined $>1\%$ carrier frequency per gene.¹⁵ Overall, we identified FVs in 33/55 genes in the AJ gene set. FV carrier rates (Table 1) are similar to previous reports.^{15,16}

Four individuals were FV homozygotes, each in a different gene: *PAH* (phenylketonuria and hyperphenylalaninemia), *GBA* (Gaucher disease), *F11* (factor XI deficiency), and *MEFV* (familial Mediterranean fever) (Table 1). These diseases have

variable phenotypes, and homozygosity for the specific FVs observed can be asymptomatic (Supplementary Results).

Nonfounder P/LP variants in the AJ gene set

We identified 48 nonfounder P/LPVs in the AJ gene set that were heterozygous in 112/672 (16.7%) samples (Table S3). Most of these variants (40/48) are in genes also included in the pan-ethnic gene set, but 20/672 (3%) samples were heterozygous for a nonfounder P/LPV in an AJ-specific gene. There were no homozygotes for nonfounder P/LPVs, nor compound heterozygotes for a nonfounder P/LPV and a FV.

P/LP variants in the pan-ethnic gene set

We identified 84 P/LPVs in the pan-ethnic gene set that were heterozygous in 116/672 (17.3%) samples (Tables S3, S4). Of these, 40/84 were in genes also included in the AJ gene set (Table S3, underlined), and 44/84 were in genes unique to the pan-ethnic gene set (Table S4). One individual was homozygous for a mild phenotype P/LPV in the *BTBD* gene (MIM609019) and one was homozygous for a mild phenotype P/LPV in the *BCSIL* gene (MIM 603647) (Supplementary Results; Table S4).

Overall, for 180 genes, the mean number of P/LPVs per sample was 1.1 (range 0–6). There were 213/672 (31.7%) samples with no P/LPVs.

VUS

As noted above, 1963/4671 (42.1%) variants were classified as VUS. Of these, 733/1963 (37.3%) were coding VUS (cVUS), i.e., exonic, non-UTR, nonsynonymous VUS.

At-risk couples: virtual matings

To determine ARCs rates for autosomal recessive conditions, we examined all possible matings between the 672 samples (225,456 theoretical couples). We compared the outcomes of targeted AJ FV testing and full sequencing of the AJ, pan-ethnic, and the combined gene sets. We evaluated the rates of matings where (1) both partners were carriers of a P/LPV in the same gene (P/LP*P/LP) or (2) both partners were carriers of variants in the same gene: one of a P/LPV and the other of a cVUS, (P/LP*cVUS). We did not consider cVUS*cVUS matings to be clinically significant.¹

P/LP*P/LP matings

For AJ FVs, 6454/225,456 (2.9%) of possible matings represent P/LP*P/LP ARCs (Table 2). The fully sequenced AJ gene set (which includes both founder and nonfounder variants) resulted in 7839/225,456 (3.5%) ARCs. This difference is partly driven by nonfounder variants in *CFTR* (MIM 602421) and *MEFV* (MIM 608107) resulting in variable (including mild) phenotypes. Using the pan-ethnic gene set, 6183/225,456 (2.7%) matings were P/LP*P/LP ARCs. The rate of P/LP*P/LP matings was higher in the AJ gene set (3.5%) than in the pan-ethnic gene set (2.7%), even though the AJ gene set includes threefold fewer genes, due to the

Table 1 Ashkenazi Jewish founder variants: observed frequencies and calculated at-risk matings^a

Disease	Gene	Variant	Reported frequency (INGD) ¹⁵	Observed number of carriers/homozygotes		At-risk matings (calculated) ^c
				N/672 (%)		
				Per variant ^b	Per disease	
Factor XI deficiency	<i>F11</i> ^d (MIM 264900)	g.187201412T>C; c.901T>C; p.F301L	4.9%	39, 1 hom (5.9%)	65 (9.7%)	2080 (0.9%)
Familial Mediterranean fever	<i>MEFV</i> (MIM 608107)	g.187195347G>T; c.403G>T; p.E135X	3.5%	25 (3.7%)		
		g.3293310A>G; c.2177T>C; p.V726A	7.7%	52, 1 hom (7.9%)	1378 (0.6%)	
Gaucher disease	<i>GBA</i> (MIM 606463)	g.155205634A>G; c.1226A>G; p.N409S	5.4%	32, 1 hom (4.9%)	36 (5.4%)	630 (0.3%)
Phenylketonuria, hyperphenylalaninemia	<i>PAH</i> (MIM 612349)	g.155210451dupG; c.84dupG; p.L29fs	0.2%	2 (0.3%)		
		g.155205563G>T; c.1297G>T; p.V433L	0.1%	1 (0.1%)		
		g.103260377G>A; c.506G>A; p.R169H	1.3%	8 (1.2%)	24 (3.6%)	276 (0.1%)
		g.103237484G>A; c.1139C>T; p.T380M	0.9%	6 (0.9%)		
		g.103245479C>A; c.898G>T; p.A300S	1.3%	4, 1 hom (0.7%)		
		g.103234285G>A; c.1208C>T; p.A403V	1%	3 (0.4%)		
		g.103246713C>T; c.722G>A; p.R241H	0.6%	1 (0.1%)		
		g.103288582T>A; c.283A>T; p.I95F	0.1%	1 (0.1%)		
Cystic fibrosis	<i>CFTR</i> (MIM 602421)	g.117282620G>A; c.3846G>A; p.W1282X	1.8%	15 (2.2%)	23 (3.4%)	253 (0.1%)
		g.117199644delCTT; c.1521_1523delCTT; p.508delF	1.1%	5 (0.7%)		
		g.117227832; c.1624G>T; p.G542X	0.3%	2 (0.3%)		
		g.117292931C>G; c.3909C>G; p.N1303K	0.1%	1 (0.1%)		
Tay–Sachs	<i>HEXA</i> (MIM 606869)	g.72638920insGATA; c.1277_1281insTATC	2.7%	18 (2.7%)	23 (3.4%)	253 (0.1%)
		g.72638575C>G; c.1421+1G>C; IVS12+1	0.5%	3 (0.4%)		
		g.72642859G>A; c.805G>A; p.G269S	0.1%	2 (0.3%)		
Familial dysautonomia	<i>IKBKAP</i> (<i>ELP1</i>) (MIM 603722)	g.111662096A>G; c.2204+6T>C	2.8%	20 (3%)	190 (0.1%)	
Familial hypercholesterolemia	<i>LDLR</i> ^d (MIM 606945)	g.11240278G>A; c.2479G>A; p.V827I	3.3%	20 (3%)	190 (0.1%)	
Glycogen storage type 1A	<i>G6PC</i> (MIM 613742)	g.41055964C>T; c.247C>T; p.R83C	1.3%	18 (2.7%)	153 (0.07%)	
Hyperoxaluria, type III	<i>HOGA1</i> (MIM 613597)	g.99371368delAGG; c.944_946delAGG	1.8%	11 (1.6%)	18 (2.7%)	153 (0.07%)

Table 1 continued

Disease	Gene	Variant	Reported frequency (INGD) ¹⁵	Observed number of carriers/homozygotes N/672 (%)		At-risk matings (calculated) ^c
				Per variant ^b	Per disease	
Carnitine palmitoyl transferase deficiency	CPT2 (MIM 600650)	g.99344567C>T; c.107C>T; p.A36V	0.6%	5 (0.7%)		
		g.99371292G>T; c.860G>T; p.G287V	0.3%	2 (0.3%)		
		g.53668099; c.338C>T; p.S113L	1.4%	11 (1.6%)	16 (2.4%)	120 (0.05%)
Persistent hyperinsulinemic hypoglycemia of infancy (PHHI)	ABCC8 (MIM 600509)	g.53676688; c.1342T>C; p.F448L-	0.9% ^f	5 (0.7%)		
		g.53676585_53676586delGA;c.1238_1239delGA; p.Q413fs				
		g.17418602C>T; c.3992-9G>A	1.1%	15 (2.2%)	16 (2.4%)	120 (0.05%)
Deafness	GJB2 (MIM 121011)	g.17417434delAGA; c.4163_4165delTCT; p.1388_1389del	0.1%	1 (0.1%)		
		g.20763553delA; c.167DelT; p.L56fs	3.3%	16 (2.4%)	120 (0.05%)	
Canavan	ASPA (MIM 608034)	g.3402294A>C; c.854A>C; p.E285A	1.8%	14 (2.1%)	91 (0.04%)	
Smith–Lemli–Opitz	DHCR7 (MIM 602858)	g.71146886C>G; c.964-1G>C; IV58-1G-C	2.3%	12 (1.8%)	66 (0.03%)	
Glycogen storage disease type IV	GBE1 ^d (MIM 607839)	g.81691938T>G; c.986A>C; p.Y329S	1.4%	10 (1.5%)	45 (0.02%)	
Congenital amegakaryotic thrombocytopenia	MPL (MIM 159530)	g.43803600T>A; c.79+2T>A	1.6%	10 (1.5%)	45 (0.02%)	
Retinitis pigmentosa	DHDDS (MIM 608172)	g.26764719A>G; c.124A>G; p.K42E	1%	10 (1.5%)	45 (0.02%)	
Joubert	TMEM216 ^e (MIM 613277)	g.61161437G>T; c.218G>T; p.R73L	0.7%	9 (1.3%)	36 (0.01%)	
Usher syndrome type 3A	CLRN1 (MIM 606397)	g.150690352A>C; c.144T>G; p.N48K	1.1%	9 (1.3%)	36 (0.01%)	
Walker–Warburg	FKTN (MIM 607440)	g.108382330insA; c.1167_1168insA	1.6%	8 (1.2%)	28 (0.01%)	
Dihydropyrimidine dehydrogenase deficiency	DLD (MIM 238331)	g.107555951G>T; c.685G>T; p.G229C	1.2%	8 (1.2%)	28 (0.01%)	
Pendred	SLC26A4 (MIM 605646)	g.107312627C>T; c.349C>T; p.L117F	1%	8 (1.2%)	28 (0.01%)	
Wilson disease	ATP7B (MIM 606882)	g.52518297T>G; c.3191A>C; p.E1064A	0.6%	4 (0.6%)	7 (1%)	21 (0.01%)
		g.52518281G>T; c.3207C>A; p.H1069Q	1%	3 (0.4%)		

Table 1 continued

Disease	Gene	Variant	Reported frequency (INGD) ¹⁵	Observed number of carriers/homozygotes N/672 (%)		At-risk matings (calculated) ^c
				Per variant ^b	Per disease	
Fanconi anemia C	FANCC (MIM 613899)	g.97934315T>A; c.456+4A>T; IVS4+4	1.2%	7 (1%)	21 (0.01%)	
Usher syndrome 1F	PCDH15 ^e (MIM 605514)	g.56077174G>A; c.733C>T; p.R245X	0.8%	6 (0.9%)	15 (0.01%)	
Polycystic kidney disease	PKHD1 (MIM 606702)	g.51890841delCCinsG; c.3761_3762delCCins; p. Gln1256ArgfsTer47	1.3%	6 (0.9%)	15 (0.01%)	
Mucopolipidosis IV	MCOLN1 ^e (MIM 605248)	g.7591645A>G; c.406-2A>G; IVS3-2	0.8%	5 (0.7%)	10 (0.004%)	
Bloom	RECQ3 ^e (MIM 604610)	g.91310156delGins7; c.2207_2212delATCTGAINsTAGATTC	0.8%	4 (0.6%)	6 (0.003%)	
Niemann–Pick A	SMPD1 ^e (MIM 607608)	g.6413206T>C; c.911T>C; p.S304P	0.1%	1 (0.1%)	2 (0.3%)	1 (0.0004%)
Maple syrup urine disease (MSUD) type 1b	BCKDHB (MIM 248611)	g.6413285delC; c.996delC; p.F333S fs	0.3%	1 (0.1%)		
		g.80878662G>C; c.548G>C; p.R183P	1%	2 (0.3%)	1 (0.0004%)	
Enhanced S-cone syndrome	NR2E3 ^d (MIM 604485)	g.72103821A>C; c.119-2A>C	0.2%	1 (0.1%)	0	
Glycogenosis type VII	PFKM ^d (MIM 610681)	g.48525177G>A; c.450+1G>A	0.5%	1 (0.1%)	0	
Sum:				6454	6454 (2.9%)	

^aGenes are listed by total carrier frequency; we excluded X-linked genes, *SMN1* (MIM 600354), and the *NEB* (MIM 161650) exon 55 deletion (NM_001271208:g.152502272_152504773del).
^bnorm = homozygote.
^c $n =$ Observed number of carriers/homozygotes $(n(n-1)/2 \text{ [\%]})$. The proportion is calculated as $(n(n-1)/[672 \times 671]) \times 100$.
^dGenes that are included in only one or in none of the commercial pan-ethnic expanded carrier screening (ECS) panels.
^eGenes in which variants are considered to be founder variants (FVs) despite a <1% carrier frequency because they are included in all commercial AJ panels.
^fThese two FVs (g.53676585_53676586delGA;c.1238_1239delGA;p.Q413fs and g.53676688T>C;c.1342T>C;p.F448L) were considered in the analysis as a single variant, since they consistently occur in cis on the same haplotype, both in all five carriers in our cohort, and as previously reported.²⁰
Bold indicates the percentages for results from this study.

Table 2 Rates of at-risk matings: P/LP*P/LP and P/LP*cVUS

Test type ^a	Outcome ^b	P/LP*P/LP ^c N (%) ^e	P/LP*cVUS ^d N (%) ^e
AJ founder variants	Total	6465 (2.9%)	
AJ sequencing set (55 genes)	Single	7347 (3.3%)	14784 (6.6%)
	Double	492 (0.2%)	
Pan-ethnic sequencing set (168 genes)	Total	7839 (3.5%)	15276 (6.8%)
	Single	5783 (2.5%)	15056 (6.7%)
	Double	400 (0.2%)	
Combined sequencing set (180 genes)	Total	6183 (2.7%)	15456 (6.9%)
	Single	8069 (3.5%)	16305 (7.2%)
	Double	625 (0.3%)	
	Total	8694 (3.8%)	16930 (7.5%)

AJ Ashkenazi Jewish, cVUS coding variant of unknown significance, LP likely pathogenic, P pathogenic.

^aGene set sequencing tests are based on full sequencing of all genes in the set.

^bSingle outcome = occurrence of only one type of outcome (either P/LP*P/LP or P/LP*cVUS, even if in multiple genes); double outcome = occurrence of both types of outcomes.

^cP/LP*P/LP = at least one occurrence of both partners carrying a P/LPV in the same gene.

^dP/LP*cVUS = at least one occurrence of one partner carrying a P/LPV and the other partner carrying a cVUS (exonic non-UTR, nonsynonymous VUS) in the same gene.

^eN = number of virtual matings, of 225,456 possible matings.

Bold indicates the percentages for results from this study.

contribution of frequent FVs in genes unique to the AJ gene set (2080 ARCs for *F11*, 190 for *LDLR*, 45 for *GBE1*; Table 1).

P/LP*cVUS matings

P/LP*cVUS matings may represent an ARC if the cVUS is ultimately classified as P/LP. Depending on the gene set used, 6.8–7.5% of matings were P/LP*cVUS (Table 2). The proportion of P/LP*cVUS couples was 1.9–2.6 times greater than that of P/LP*P/LP couples, depending on the gene set.

Modeling the effect of cVUS reclassification

We evaluated the effects of cVUS reclassification on P/LP*cVUS frequency. Information on allele frequencies in affected and unaffected individuals is likely to accrue rapidly. Also, allelic data can be assessed by determining homozygosity in unaffected individuals and by using phased data, to determine if a cVUS occurs in *trans* to a P/LPV in unaffected individuals (in which case it would be considered LB).

Overall, 106/1963 (5.4%) VUS were homozygous in one or more individuals (Table S5), of which 21/106 (19.8%) were homozygous cVUS. Of 1963 VUS, 33 (1.7%) were found in *trans* to a P/LPV, either a FV or nonfounder variant, including 15/33 (45.4%) which were in *trans* cVUS (Table S6). Of these 33 variants, 3 were also homozygous in one or more individuals. Overall, 4.9% of cVUS were thus reclassified as LB/B.

Hypothetical fulfillment of ACMG criteria¹⁷ PM2 (variant absent in unaffected controls) or PS4 (variant significantly more prevalent in affected individuals versus controls) would respectively change 19/733 (2.6%) and 712/733 (97.1%) of cVUS classifications to P/LP. The much greater potential impact of PS4 fulfillment indicates that obtaining variant frequency data in affected individuals is particularly important for VUS reclassification.

DISCUSSION

We assessed PCS yield using different strategies: AJ FV testing or full sequencing of different gene sets. We focused on the possible contribution of P/LP*cVUS matings to testing yield. Rates of at-risk P/LP*P/LP matings were 2.9% for targeted AJ FV testing, versus 3.5%, 2.7%, and 3.8% for sequencing of AJ, pan-ethnic, and combined gene sets, respectively. This is consistent with previous estimates of 3–4% ARC yield with ECS in AJ.¹⁶ Rates of P/LP*cVUS matings were 6.8%, 6.9%, and 7.5% in the AJ, pan-ethnic, and combined gene sets, respectively. The rate of P/LP*cVUS matings is ≈1.9–2.7-fold greater than that of P/LP*P/LP matings. As a screening test, ECS is not designed to detect 100% of ARCs. However, our results indicate that even if only a small proportion of cVUS are ultimately determined to be P/LP, variants currently classified as cVUS could significantly impact ECS yield.

Compared with targeted variant PCS, greater ECS yield has been achieved, as expected, by fully sequencing genes and by increasing the number of genes sequenced. We found that even in a well-characterized population like AJ, where the variant spectrum is well established, full sequencing (of an AJ gene set) increased ARC detection by 21%. This reflects the contribution of rare P/LPVs in genes with high carrier rates for common variants, and is consistent with previous studies demonstrating greater yield by expanding the variant repertoire in AJ.¹⁶ In contrast, adding genes (the combined gene set) increased ARC detection by only 8.6%.

In cancer predisposition genes, studies found that 10–25% of VUS will ultimately be reclassified as P/LP.¹⁸ There are no comparable data in the preconception field. However, even considering only cVUS, and assuming only 10% of cVUS are reclassified as P/LPVs, the added yield would be ≈19% for the AJ gene set, and ≈20% for the combined gene set. These estimates disregard the possibility that some noncoding VUS could prove to be P/LP.

Variant classification is a major challenge. ACMG classification categories include population data, computational predictions, functional evidence, segregation data, allelic information, and data from disease databases.¹⁷ Functional analyses of variant effects are difficult to perform at large scale. More obtainable are cosegregation and allelic data, which inform six ACMG criteria, and frequency in healthy and affected individuals, which inform three ACMG criteria. Our results demonstrate the power of using allelic data in VUS reclassification: we reclassified 4.9% of cVUS as B/LB by analyzing unaffected individuals for homozygosity or for occurrence of a VUS *in trans* to a P/LPV. Modeling the effect of two frequency criteria showed that hypothetical fulfillment of PS4 (greater prevalence in affecteds versus controls) would reclassify 97.1% of cVUS to P/LP. In contrast, hypothetical fulfillment of PM2 (variant absent in controls) would reclassify only 2.6% of cVUS to P/LP. This underscores the importance of efforts such as ClinVar¹³ to aggregate variant data in affected individuals.

We note that VUS are reported in other settings, both postnatally (e.g., cancer predisposition in adults, exome sequencing in children) and prenatally (e.g., chromosomal microarrays). We believe ECS results should not, and currently do not, include VUS, which cannot be used for prenatal diagnosis or pregestational testing. Results of this study should not be interpreted as a call to report VUS in ECS, but only as evidence for the importance of further research. Clinically, reanalysis of ECS results prior to each pregnancy may be warranted, similarly to periodic reanalysis of negative exome results in pediatric disease.¹⁹

Limitations of this study include omission of X-linked conditions, including fragile X, and large deletions/duplications (e.g., the common *SMN1* deletion). AJ samples may be particularly amenable to using homozygosity and occurrence *in trans* to P/LPVs as methods for VUS reclassification.

Conclusions

ECS detects P/LP*cVUS in $\approx 7\%$ of couples. Even if only 10% of cVUS are ultimately reclassified as P/LPVs, ARC detection rates would increase by $\approx 20\%$. Enlarging population frequency variant databases, particularly for affected individuals, would facilitate VUS reclassification for ECS. Reanalysis of ECS results prior to each pregnancy may also be warranted.

SUPPLEMENTARY INFORMATION

The online version of this article (<https://doi.org/10.1038/s41436-019-0676-x>) contains supplementary material, which is available to authorized users.

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DISCLOSURE

S.C. is a paid consultant to MyHeritage. D.M.B. declares stock ownership and compensation from Igenity, where he serves as the Chief Executive Officer. The other authors declare no conflicts of interest.

ETHICAL APPROVAL

Access to the data was provided by The Ashkenazi Genome Consortium (TAGC).⁷ Written informed consent was obtained from all participants and institutional review board (IRB) approval was obtained from all participating institutions. In the United States: Albert Einstein College of Medicine, New York, NY; Columbia University, New York, NY; Mount Sinai School of Medicine, New York, NY; New York University, New York, NY; North Shore University Hospital–Long Island Jewish Medical Center, NY; Yale University, New Haven, CT; University of Pittsburgh, Pittsburgh, PA; Johns Hopkins University, Baltimore, MD; Cedars-Sinai Medical Center, Los Angeles, CA. In Canada: The University of Toronto, Toronto. In Israel: The Hebrew University of Jerusalem, Jerusalem; Tel Aviv Sourasky Medical Center, Tel Aviv; Rambam Medical Center, Haifa; The National Genetics Ethics Committee of the Israeli Ministry of Health. (See Supplementary materials and methods for references.)

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