INTRODUCTION

Over the past few decades, premarital genetic testing has served as a model preventative measure to prevent the birth of babies affected with serious medical conditions in Jewish communities. Premarital carrier screening programs, such as that in Dor Yeshorim, have contributed to the dramatic reduction of recessive disease incidence in Ashkenazi Jews, especially for Tay Sachs and cystic fibrosis (Ekstein & Katzenstein, 2001; Kornreich et al., 2004). In Israel,
preconception/prenatal carrier screening has been offered to Jewish individuals since 1978 (Zlotogora, 2014). Beginning with Tay Sachs screening, carrier screening has expanded to tens and hundreds of disease-causing alleles for autosomal recessive conditions in Jewish populations around the world (Zlotogora, 2019). In the US, the American College of Obstetricians and Gynecologists (ACOG) established guidelines for genetic testing of Ashkenazi Jewish individuals in 2008 (“Committee Opinion No. 691,” 2017). However, although various screening programs have made efforts to reach other non-Ashkenazi Jewish groups, carrier screening is utilized less frequently in non-Ashkenazi Jewish communities (Akler et al., 2020; Bloch, 2009).

Intramarrriage within parts of the Ashkenazi Jewish population are well documented (Carmi et al., 2014) and is a contributor to a high carrier frequency for certain disease-causing variants for recessive conditions in this ancestral group (Zlotogora, 2014). However, intramarrriage is not unique to Ashkenazi Jewry. To varying extents, Sephardi and Mizrahi Jewish groups have a history of intramarrriage, unique to their geographical origin (Goldschmidt et al., 1960). These population characteristics have led the Israeli Society of Medical Geneticists to establish carrier screening guidelines for all Jews based on country of origin, indicating that all Jewish groups carry pathogenic disease-causing recessive variants and that screening should be tailored based upon genetic ancestry (Zlotogora, 2019).

Syrian and Iranian Jews are genetically quite similar (Atzmon et al., 2010), yet unlike disease-causing variants common to Iranian Jews (Dagan & Gershoni-Baruch, 2010), much information is lacking regarding the frequency of pathogenic variants in Syrian Jews. Hence, we chose Iranian Jews as a comparison group for the assessment of allele frequencies within the Syrian Jewish population. In this study, we characterize the frequency of several pathogenic variants within Syrian and Iranian Jewish cohorts. We establish ancestry-specific frequencies of certain variants in each group, and we describe some variants shared between Syrians and Iranians and between Syrians and Ashkenazis. The latter findings suggest that it may be most practical to apply pan-Jewish variant screening for effective carrier screening within the Syrian Jewish community.

2 MATERIALS AND METHODS

2.1 Ethnic origin definition of study participants

Participants in the screening program provided information regarding the ancestry of all four grandparents. Self-identified ancestry has been previously shown to be accurate (Need et al., 2009). For inclusion in the study, individuals with at least one maternal or one paternal Syrian/Iranian grandparent were selected. For families with 2 or more siblings, 1 sibling was randomly selected for analysis so that the final cohort would include only unrelated Syrian/Iranian individuals. Altogether, 439 unrelated 100% Syrian (with 4 Syrian grandparents), 3401 unrelated mixed Syrian (with less than 4 Syrian grandparents), and 5279 unrelated mixed Iranians were included in the analysis. For comparison, carrier frequencies in Ashkenazi Jews were calculated from roughly 370,000 Ashkenazi Jewish samples in the Dor Yeshorim database.

2.2 Genotyping of sequence variants and the FRDA expansion with high throughput amplicon sequencing

A multiplex PCR assay was used to target the single nucleotide variants (SNVs)/small insertions/small deletions in Tables 1 and 2, as well as the Friedrich’s ataxia (FRDA) GAA expansion locus in intron 1 of the FXN gene. Multiplex PCR products from each sample were barcode indexed before sequencing on an Illumina MiSeq. Resultant sequencing reads were aligned to the reference genome (hg38) and relevant variants were genotyped using GATK UnifiedGenotyper/ haplotypeCaller packages (Broad Institute). Genotyping assays for each SNV/small insertion/small deletion were validated by testing at least 2 or 3 different heterozygote control samples. For FRDA expansion carrier detection, the following extra analytical steps were performed. Sequencing reads were aligned to an “STRdecoys.fasta” reference (obtained from [Dashnow et al., 2018]) which was supplemented with the expected non-GAA FXN intron 1 amplicon sequence from hg38. Using this mapping strategy, it was expected that FRDA repeat-expanded reads would map to both the decoy GAA “chromosome” and the FXN intron 1 “chromosome.” These split-reads were extracted by samblaster (Faust & Hall, 2014) followed by counting the total number of GAA repeats per sample and GAA repeat-bearing reads per sample. Furthermore, within repeat-bearing reads, the number of GAA repeats per read was calculated for each sample. After sorting the number of repeats per read, the 3 highest repeat-per-read frequencies per sample were saved to a final report. True FRDA carriers were detected when the top 2 repeat-per-read frequencies (RPRF) all exceeded 35 GAA repeats. Otherwise, all samples with less than 2 RPRF >35 GAA repeats were reported as non-carriers. All positive FRDA custom amplicon sequencing-based results were validated by an established fluorescent repeat-primed PCR-based method (Ciotti et al., 2004). In addition, the specificity of the sequencing-based method was determined by confirming 367 random non-carrier samples (as determined by high throughput sequencing) using the fluorescent PCR assay.
3 | RESULTS

3.1 Preliminary assessment of the frequency of disease-causing variants in Syrian Jews

To assess carrier frequency in Syrian Jews, we genetically tested a small cohort of 438 unrelated individuals of 100% Syrian Jewish descent (all 4 grandparents of Syrian Jewish ancestry) for at least one disease-causing variant using amplicon sequencing. Table 1 lists 33 pathogenic variants, implicated in 25 different genetic disorders, for which a pathogenic variant was identified in at least one Syrian Jewish individual with 4 Syrian grandparents at Dor Yeshorim. Variant frequencies for Ashkenazi Jews are provided for comparison. We identified relatively high Syrian Jewish carrier frequencies (>0.68%) for well-established Ashkenazi Jewish variants with carrier frequencies over 2% in Ashkenazi Jews (nonsyndromic deafness-associated GJB2:c.167del; congenital stationary night blindness-associated TRPM1:36.8KB DEL; Smith-Lemli-Opitz-associated DHCR7:c.964-1G>C; and cystic fibrosis-associated CFTR:c.3846G>A; Table 1).

In addition, Syrian Jews carried variants that are less frequent in Ashkenazis and implicated in the following autosomal recessive disorders: achromatopsia, congenital adrenal hyperplasia (CYP11B1 gene), Friedreich ataxia, Gaucher disease, inclusion body myopathy, metachromatic leukodystrophy, Niemann–Pick disease, primary hyperoxaluria type 1, retinitis pigmentosa 28, and Ullrich congenital muscular dystrophy type 1.

3.2 Pathogenic variant selection for in-depth investigation of allele frequency in Syrian and Iranian Jews

To further expand the generalizability of the results in Table 1, we assembled a larger cohort of 3401 unrelated “mixed” Syrian Jewish (with at least 1 but less than 4 Syrian Jewish grandparents) and 5279 unrelated “mixed” Iranian Jewish individuals for comparison. For this analysis, a set of 22 different disease-causing pathogenic variants (underlying 20 different diseases) were selected for further investigation (Table 2). For 18 of these variants, at least one family of Syrian and/or Iranian Jewish ancestry approached Dor Yeshorim for assistance with the genetic diagnosis of an affected child or for assistance with premarital screening of a pre-existing disease in the family. Three other variants were selected based on Israel Ministry of Health recommendations for genetic screening of Iranian Jews; and CFTR:c.1521_1523del was included due to its established pan-ethnic prevalence (Palomaki et al., 2004; “Worldwide Survey of the Delta F508 Mutation—Report from the Cystic Fibrosis Genetic Analysis Consortium,” 1990). We note that other disease-causing variants were reported previously in Syrian Jews (Table S1); however, we could not include these variants in our screen because heterozygous control samples for each variant were not available to us for genotyping assay validation. Nonetheless, we summarize these 14 variants underlying 11 different conditions in Table S1 to consolidate established disease-causing variants in Syrian Jews at the time of this publication.

Within the 22 screened variants, 18 family-derived variants were further categorized according to the ethnicity of the affected child as “category 1” (Syrian only), “category 2” (Iranian only), or “category 3” (Syrian and Iranian) variants (Table 2). Category 4 variants were included according to Israel Ministry of Health guidelines, and the category 5 variant is the pan-ethnic CFTR:c.1521_1523del (see Table 2 for categorical variant definitions).

3.3 Carrier frequency in the Syrian and Iranian Jewish populations

To determine the frequency of carriers for the variants in Table 2, mixed Syrian, mixed Iranian, and mixed Ashkenazi Jewish individuals were screened using high throughput amplicon sequencing. Only the Syrian cohort was screened for all 22 variants (20 diseases). The Iranian cohort was screened for only 20 of the 22 variants (18 diseases) because the amplicon sequencing assays for COL6A2:c.1402C>T and FXN:GAA expansion genotyping were not available at the time of the Iranian cohort screen. Table 3 shows an increased frequency of category 1 variant frequency in Syrian Jews compared to Iranian and Ashkenazi Jews (not including the two variants for which no Iranian data was generated, and not including GJB2:c.167del which is much more common in Ashkenazis). On the other hand, category 2 and 4 variant frequencies were higher in Iranians than in Syrians and Ashkenazis (Table 3). These results were expected given the variant selection criteria described above. However, notably the category 3 inclusion body myopathy-associated GNE:c.2228T>C variant is present in both Iranians (3.81%) and Syrians (0.71%) and much less so in Ashkenazis (0.09%; Table 3) which might explain why Dor Yeshorim previously identified disease-affected children in both Iranian and Syrian ethnic groups (unlike category 1 variants which were primarily identified in disease-affected children of Syrian Jewish ancestry, and unlike category 2 variants for which affected children were exclusively Iranian Jewish). Regarding the pan-ethnic category 5 CFTR:c.1521_1523del variant, Table 3 shows similar frequency in Syrian (0.56%) and Iranian (0.47%) groups but much higher frequency in Ashkenazis (1.16%). Thus, there are shared alleles between Syrian, Iranian, and Ashkenazi Jews as well as some alleles...
| Variant name | Chromosome (hg38) | rsID | Ref | Alt | Gene | OMIM Gene | Nucleotide changea | Phenotype | Syrian Jewish carrier frequency | Syrian Jewish carrier frequency % | Ashkenazi Jewish carrier frequency | Ashkenazi Jewish carrier frequency % |
|--------------|------------------|------|-----|-----|------|------------|-------------------|-----------|-------------------------------|---------------------------------|---------------------------------|----------------------------------|
| ABCC8:c.3989-9G>A | chr11 | rs151344623 | C | T | ABCC8 | 600509 | NM_000352.6:c.3989-9G>A | HYPERINSULINEMIC HYPOGLYCEMIA, FAMILIAL, 1 | 1/339 | 0.29 | 3969/235602 | 1.68 |
| AGXT:c.731T>C | chr2 | rs121908525 | T | C | AGXT | 604285 | NM_000030.3:c.731T>C | PRIMARY HYPEROXALURIA TYPE 1 | 4/369 | 1.08 | 0/21050 | 0.00 |
| ARSA:c.449C>T | chr22 | rs199476375 | G | A | ARSA | 607574 | NM_000487.6:c.449C>T | METACHROMATIC LEUKODYSTROPHY | 5/376 | 1.33 | 1/51587 | 0.00 |
| ARSA:c.854+3A>G | chr22 | rs1057524566 | T | G | ARSA | 607574 | NM_000487.6:c.854+3A>G | METACHROMATIC LEUKODYSTROPHY | 5/376 | 1.33 | 0/46388 | 0.00 |
| BLM:c.2208T>G | chr15 | rs865899765 | T | G | BLM | 604610 | NM_000057.4:c.2208T>G | BLOOM SYNDROME | 1/404 | 0.25 | 3265/335777 | 0.97 |
| CFTR:c.3846G>A | chr7 | rs77010898 | G | A | CFTR | 602421 | NM_000492.4:c.3846G>A | CYSTIC FIBROSIS | 3/435 | 0.69 | 6721/335635 | 2.00 |
| CFTR:c.254G>A | chr7 | rs75961395 | G | A | CFTR | 602421 | NM_000492.4:c.254G>A | CYSTIC FIBROSIS | 2/381 | 0.52 | 0/97562 | 0.00 |
| CFTR:c.1624G>T | chr7 | rs1521_1523del | ATCT | A | CFTR | 602421 | NM_000492.4:c.1521_1523del | CYSTIC FIBROSIS | 2/435 | 0.46 | 737/335635 | 0.22 |
| CFTR:c.1624G>T | chr7 | rs113993959 | G | T | CFTR | 602421 | NM_000492.4:c.1624G>T | CYSTIC FIBROSIS | 2/438 | 0.46 | 3965/335635 | 1.18 |
| CFTR:c.2989-1G>A | chr7 | rs397508470 | G | A | CFTR | 602421 | NM_000492.4:c.2989-1G>A | CYSTIC FIBROSIS | 1/329 | 0.30 | 0/223335 | 0.00 |
| CNGB3:c.467C>T | chr8 | rs139207764 | G | A | CNGB3 | 605080 | NM_019098.4:c.467C>T | ACHROMATOPSIA | 7/376 | 1.86 | 26/44392 | 0.06 |
| COL6A2:c.1402C>T | chr21 | rs374669775 | C | T | COL6A2 | 120240 | NM_013352.4:c.1402C>T | | 1/369 | 0.27 | 0/21009 | 0.00 |
| CYP11B1:c.992C>T | chr8 | rs1326688256 | G | A | CYP11B1 | 610613 | NM_000497.3:c.992C>T | CONGENITAL ADRENAL HYPERPLASIA | 10/376 | 2.66 | 0/44988 | 0.00 |
| DHCR7:c.964-1G>C | chr11 | rs138659167 | C | G | DHCR7 | 602858 | NM_019098.4:c.964-1G>C | | 2/227 | 0.88 | 2016/88773 | 2.27 |
| DSE:c.387delC | chr6 | N/A | AC | A | DSE | 605942 | NM_013352.4:c.387delC | | | |
| ESCO2:c.1674-2A>G | chr8 | rs80359869 | A | G | ESCO2 | 609353 | NM_001017420.3:c.1674-2A>G | ROBERTS SYNDROME | 2/376 | 0.53 | 0/51519 | 0.00 |
| FAM161A:c.1567C>T | chr2 | rs202193201 | G | A | FAM161A | 613596 | NM_001017420.3:c.1674-2A>G | | 3/154 | 1.95 | 1/49492 | 0.00 |
| G6PC:c.247C>T | chr13 | rs1801175 | C | T | G6PC1 | 613742 | NM_0000497.3:c.992C>T | | 1/104 | 0.96 | 7/32171 | 0.02 |
| FXN:GAA expansion | chr9 | N/A | GAA | GAA | FXN | 606829 | NM_000144:GAA expansion | | 3/331 | 0.91 | 14/5109 | 0.27 |
| GBA:c.1448T>C | chr1 | rs21016 | A | G | GBA | 606463 | NM_000157.4:c.1448T>C | | 155235252 | 0.99 | 88/72333 | 0.12 |
| GJB2:c.167del | chr13 | rs421016 | A | G | GJB2 | 121011 | NM_000157.4:c.1448T>C | | 20189414 | 1.00 | 1/104 | 0.02 |
| GJB2:c.269T>C | chr13 | rs80338945 | A | G | GJB2 | 121011 | NM_000157.4:c.1448T>C | | 20189313 | 1.00 | 1/104 | 0.02 |
| Amino acid change\(^b\) | Phenotype | Syrian Jewish carrier frequency (no. carriers/n) | Syrian Jewish carrier frequency % | Ashkenazi Jewish carrier frequency (no. carriers/n) | Ashkenazi Jewish carrier frequency % | Followed up in Table 2 |
|--------------------------|-----------|-----------------------------------------------|---------------------------------|-----------------------------------------------|---------------------------------|------------------------|
| splice acceptor          | HYPERINSULINEMIC HYPOGLYCEMIA, FAMILIAL, 1 | 1/339                           | 0.29                           | 3969/235602                                    | 1.68                            | NO                     |
| NP_000021.1:p. Ile244Thr | PRIMARY HYPEROXALURIA TYPE 1               | 4/369                           | 1.08                           | 0/21050                                       | 0.00                            | YES                    |
| NP_000478.3:p. Pro150Leu | METACHROMATIC LEUKODYSTROPHY               | 5/376                           | 1.33                           | 1/51587                                       | 0.00                            | YES                    |
| splice donor             | METACHROMATIC LEUKODYSTROPHY               | 5/376                           | 1.33                           | 0/46388                                       | 0.00                            | YES                    |
| NP_000048.1:p. Tyr736Ter | BLOOM SYNDROME                             | 1/404                           | 0.25                           | 3265/335777                                    | 0.97                            | NO                     |
| NP_000483.3:p. Trp1282Ter| CYSTIC FIBROSIS                             | 3/435                           | 0.69                           | 6721/335635                                    | 2.00                            | NO                     |
| NP_000483.3:p. Gly542Ter | CYSTIC FIBROSIS                             | 2/435                           | 0.46                           | 737/335635                                     | 0.22                            | NO                     |
| NP_000483.3:p. Phe508del | CYSTIC FIBROSIS                             | 2/438                           | 0.46                           | 3965/335635                                    | 1.18                            | YES                    |
| splice acceptor          | CYSTIC FIBROSIS                             | 1/329                           | 0.30                           | 0/223335                                       | 0.00                            | NO                     |
| NP_061971.3:p. Ser156Phe | ACHROMATOPSIA                              | 7/376                           | 1.86                           | 26/44392                                       | 0.06                            | YES                    |
| NP_001840.3:p. Arg468Ter | ULLRICH CONGENITAL MUSCULAR DYSTROPHY TYPE 1 | 13/331                         | 3.93                           | 0/5922                                        | 0.00                            | YES                    |
| NP_000488.3:p. Ala331Val | CONGENITAL ADRENAL HYPERPLASIA              | 10/376                          | 2.66                           | 0/44988                                        | 0.00                            | YES                    |
| splice acceptor          | SMITH-LEMLI-OPTIZ SYNDROME                  | 2/227                           | 0.88                           | 2016/88773                                     | 2.27                            | NO                     |
| NP_037484.1:p. Tyr129Ter | EHLERS-DANLOS SYNDROME, MUSCULOCONTRACTURAL TYPE2 | 1/369                         | 0.27                           | 0/21009                                       | 0.00                            | YES                    |
| splice acceptor          | ROBERTS SYNDROME                           | 2/376                           | 0.53                           | 0/51519                                       | 0.00                            | YES                    |
| NP_001188472.1:p. Arg523Ter | RETINITIS PIGMENTOSA 28                     | 3/154                           | 1.95                           | 1/49492                                       | 0.00                            | NO                     |
| N/A                      | FRIEDREICH ATAXIA                          | 3/331                           | 0.91                           | 14/5109                                       | 0.27                            | YES                    |
| NP_000142.2:p. Arg83Cys  | GLYCOCEN STORAGE DISEASE TYPE 1A           | 2/397                           | 0.50                           | 4816/335799                                    | 1.43                            | NO                     |
| NP_000148.2:p. Leu483Pro | GAUCHER DISEASE                            | 1/101                           | 0.99                           | 88/72333                                       | 0.12                            | NO                     |
| NP_003995.2:p. Leu56fs   | NONSYNDROMIC DEAFNESS                      | 8/368                           | 2.17                           | 929/32870                                      | 2.83                            | YES                    |
| NP_003995.2:p. Leu90Pro  | NONSYNDROMIC DEAFNESS                      | 1/104                           | 0.96                           | 7/32171                                       | 0.02                            | NO                     |

(Continues)
unique to each group. In addition, we also note that 8.2% of the 3401 mixed Syrians were each carriers for at least one of the pathogenic variants in Table 3.

4 | DISCUSSION

We report carrier frequencies for pathogenic variants underlying 25 different autosomal recessive diseases in Syrian Jewish individuals. For 3 of the variants (AIRE:c.254A>G [autoimmune polyendocrinopathy], LIPA:c.260G>T [Wolman’s disease], and GNE:c.2228T>C [inclusion body myopathy (HIBM)]), carrier frequency was assayed previously in Mizrahi Jews and found to be similar to the frequencies reported here (Kaback et al., 2010; The Forward Staff, 2014). Most importantly, we show that at least one pathogenic variant was present in 438 Syrian Jews with four Syrian grandparents, and 20 of these variants (underlying 17 different conditions) are present at a population frequency (>0.6%) for which premarital screening would be recommended. In terms of disease severity and allele frequency, the American College of Medical Genetics (ACMG) guidelines recommend that most of these 20 disease-causing variants should be tested in the Syrian Jewish population (Grody et al., 2013). These criteria include (1) the genetic disorder being tested is severe such that most at-risk couples would pursue prenatal diagnosis if given the option; (2) a clear association exists between the assayed variant and a severe early onset genetic disorder; and (3) relatively high carrier frequency is present in the tested population such that carrier screening would help to reduce disease incidence. Although these criteria have been met for most of the variants in Tables 1 and

| Variant name | Chromosome | Position (hg38) | rsID | Ref | Alt | Gene | OMIM Gene | Nucleotide changea |
|--------------|------------|----------------|------|-----|-----|------|------------|-------------------|
| GNE:c.2228T>C | chr9       | 36217399       | rs28937594 | A   | G   | GNE  | 603824    | NM_001128227.3:c.2228T>C |
| MLC1:c.176G>A | chr22      | 50084727       | rs80358242 | C   | T   | MLC1 | 605908    | NM_015166.3:c.176G>A   |
| MMACHC:c.271dup | chr1     | 45507544       | rs398124292 | T   | TA  | MMACHC | 609831    | NM_015506.3:c.271dup   |
| NDUFS4:c.355G>C | chr5       | 53658555       | rs747359752 | G   | C   | NDUFS4 | 602694    | NM_002495.4:c.355G>C   |
| OTOF:c.5193-1G>A | chr2      | 26462182       | rs111033373 | C   | T   | OTOF  | 603681    | NM_194248.3:c.5193-1G>A |
| OTOF:c.4227+1G>T | chr2      | 26467364       | rs397515601 | C   | A   | OTOF  | 603681    | NM_194248.3:c.4227+1G>T |
| PEX2:c.355C>T | chr8       | 76983824       | rs61752123 | G   | A   | PEX2  | 170993    | NM_000318.3:c.355C>T   |
| SMPD1:c.1829G>A | chr11     | 6394540        | rs140269316 | G   | A   | SMPD1 | 607608    | NM_000543.5:c.1829G>A  |
| SMPD1:c.1826_1828GCC | chr11 | 6394536        | rs120074118 | TGCC | T   | SMPD1 | 607608    | NM_000543.5:c.1826_1828GCC |
| TRPM1:36.8KB DEL | chr15     | 31062999-31099445 | N/A | N/A | N/A | TRPM1 | 603576    | 36.8KB DEL, EX2-7c |
| VAC14:c.2005G>T | chr16     | 70695574       | rs1363536856 | C   | A   | VAC14 | 604632    | NM_018052.5:c.2005G>T  |

Note: Variants are sorted alphabetically according to gene name and then by Syrian Jewish carrier frequency from highest to lowest. Carrier frequencies in individuals with 4 Ashkenazi Jewish grandparents are shown for comparison.
Abbreviations: N/A, not applicable; PMID, Pubmed ID.
aGenbank transcript accession number:nucleotide change.
bGenbank protein accession number:amino acid change (where relevant).
cThis copy number variant is described in PMID: 31645983.
3, for many of these variants (notably COL6A2:c.1402C>T [underlying Ullrich congenital muscular dystrophy type 1] which is present in 1.65% of Syrian Jews) carrier frequency had not been described previously in Syrian Jews.

Testing a small cohort of 100% Syrian Jews (descended from 4 Syrian Jewish grandparents) identified 33 disease-causing variants underlying 25 autosomal recessive conditions. Sixteen of these variants (underlying 13 autosomal recessive conditions) are also present in Ashkenazi Jews. In addition, 22 variants (20 of the 25 conditions) were tested in a larger mixed Syrian Jewish cohort. Predictably, several pathogenic variants were identified with relatively high carrier frequency in both 100% Syrian Jewish and mixed Syrian Jewish cohorts. Thus, the findings of this study suggest that carrier screening could be clinically useful. Based upon our calculated carrier frequencies, 0.82% (82 of 10,000) of Syrian Jewish couples with 4 Syrian grandparents would both be carriers for disease-causing variants in the same gene for one of 33 variants in 25 genes listed in Table 1. Likewise, carrier frequencies in the mixed Syrian Jewish group show that 0.06% of mixed Syrian Jewish couples (6 out of every 10,000) would both be carriers for disease-causing variants for one of the 22 variants in 20 genes in Table 3.

All Jewish groups carry pathogenic disease-causing recessive variants, and Syrian Jews are no exception (Zlotogora, 2014). Moreover, it is becoming increasingly clear that many pathogenic variants previously thought to be exclusive to one Jewish group are present in other Jewish groups. Therefore, these data suggest that more expanded carrier screening panels should be used to address premarital screening in all Jewish groups because what was previously thought to be exclusively an Ashkenazi variant, for example, CFTR:c.3846G>A, is also present in Syrian Jews. Therefore, it is likely that the carrier screening panel will continue to expand as new disease alleles are identified. In addition, it appears that ethnic-specific screens are less effective, especially given that expanding the number of tested alleles adds minimal cost. Rather a comprehensive pan-Jewish panel should include variants identified in Jews of all ancestries, especially now that admixture of Jews of different ancestries occurs more frequently.

| Amino acid change<sup>b</sup> | Phenotype | Syrian Jewish carrier frequency (no. carriers/n) | Syrian Jewish carrier frequency % | Ashkenazi Jewish carrier frequency (no. carriers/n) | Ashkenazi Jewish carrier frequency % | Followed up in Table 2 |
|-----------------------------|-----------|-----------------------------------------------|----------------------------------|-----------------------------------------------|----------------------------------|------------------------|
| NP_001121699.1:p. Met743Thr | INCLUSION BODY MYOPATHY (HIBM) | 8/376 | 2.13 | 1/51647 | 0.00 | YES |
| NP_055981.1:p. Gly59Glu | MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS | 1/273 | 0.37 | 0/53648 | 0.00 | NO |
| NP_056321.2:p. Arg91His | METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA, cbLC TYPE | 1/154 | 0.65 | 372/49724 | 0.75 | NO |
| NP_002486.1:p. Asp119His | LEIGH SYNDROME TYPE 1 | 2/376 | 0.53 | 0/46275 | 0.00 | YES |
| | splice acceptor | | | | | |
| | DEAFNESS, AUTOSOMAL RECESSIVE | 4/101 | 3.96 | 0/30796 | 0.00 | NO |
| | splice donor | | | | | |
| | DEAFNESS, AUTOSOMAL RECESSIVE | 1/102 | 0.98 | 0/30826 | 0.00 | NO |
| NP_000309.2:p. Arg119Ter | PEROXISOME BIOGENESIS DISORDER 5A (ZELLWEGER) | 1/154 | 0.65 | 379/49883 | 0.76 | NO |
| NP_000534.3:p. Arg610His | NIEMANN-PICK DISEASE | 1/93 | 1.08 | 0/25901 | 0.00 | NO |
| NP_000534.3:p. Arg610del | NIEMANN-PICK DISEASE | 2/380 | 0.53 | 66/304382 | 0.02 | NO |
| N/A | CONGENITAL STATIONARY NIGHT BLINDNESS | 2/133 | 1.50 | 946/37796 | 2.50 | NO |
| NP_060522.3:p. Val669Leu | STRIATONIGRAL DEGENERATION | 1/367 | 0.27 | 0/30779 | 0.00 | YES |
Based on the findings in this study, Dor Yeshorim has initiated new educational programming to raise awareness about premarital reproductive planning within Syrian Jewish communities. The disease risk within this group is apparent, and carrier screening is recommended by ACOG for all patients.

| Variant Name | Chromosome | Position (hg38) | rsID       | Ref | Alt | Gene       | OMIM Gene |
|--------------|------------|----------------|------------|-----|-----|------------|-----------|
| AGXT:c.731T>C | chr2       | 240875159      | rs121908525| T   | C   | AGXT       | 604285    |
| AIRE:c.254A>G | chr21      | 44286678       | rs179363882| A   | G   | AIRE       | 607358    |
| ARSA:c.449C>T  | chr22      | 50627182       | rs199476375| G   | A   | ARSA       | 607574    |
| ARSA:c.854+3A>G | chr22    | 50626588       | rs105752456| T   | C   | ARSA       | 607574    |
| BLM:c.98+1G>T  | chr15      | 90747491       | rs750293380| G   | T   | BLM        | 604610    |
| CFTR:c.1521_1523del | chr7  | 117559590      | rs113993960| ATCT | A   | CFTR       | 602421    |
| CFTR:c.254G>A  | chr7       | 117509123      | rs75961395 | G   | A   | CFTR       | 602421    |
| CNGA3:c.1585G>A | chr2       | 98396755       | rs104893619| G   | A   | CNGA3      | 600053    |
| CNGB3:c.467C>T  | chr8       | 86670970       | rs139207764| G   | A   | CNGB3      | 605080    |
| COL6A2:c.1402C>T | chr21     | 46121067       | rs37469775  | C   | T   | COL6A2     | 120240    |
| CYP11B1:c.992C>T | chr8      | 142875841      | rs1326688256| G   | A   | CYP11B1    | 610613    |
| DSE:c.387delIC | chr6       | 116399636      | N/A        | AC  | A   | DSE        | 605942    |
| ESCO2:c.1674-2A>G | chr8     | 27803304       | rs80359869  | A   | G   | ESCO2      | 609353    |
| FXN:GAA expansion | chr9    | 69037287       | N/A        | GAA | GAA | FXN        | 606829    |
| GJB2:c.167del  | chr13      | 20189414       | rs80338942  | CA  | C   | GJB2       | 121011    |
| GNE:c.2228T>C  | chr9       | 36217399       | rs28937594  | A   | G   | GNE        | 603824    |
| GPT2:c.159C>G  | chr16      | 46906858       | rs786203999| C   | G   | GPT2       | 138210    |
| LIPA:c.260G>T   | chr10      | 89228368       | rs587778878 | C   | A   | LIPA       | 613497    |
| NDUFS4:c.355G>C  | chr5       | 53658555       | rs747359752 | G   | C   | NDUFS4     | 602694    |
| TYMP:c.433G>A  | chr22      | 50528595       | rs121913037| C   | T   | TYMP       | 131222    |
| USH2A:c.236_239dup | chr1    | 216422097      | rs1553258097| G   | A   | USH2A      | 608400    |
| VAC14:c.2005G>T  | chr16      | 70695574       | rs1363536856| C   | A   | VAC14      | 604632    |

Note: Variants are sorted alphabetically according to gene name. Abbreviations: N/A, not applicable; PMID, Pubmed ID.

*aGenbank transcript accession number:nucleotide change.

*bGenbank protein accession number:amino acid change.

*Variant category definitions: (1) Variant identified in family of Syrian Jewish ethnicity; (2) Variant identified in family of Iranian Jewish ethnicity; (3) Variant identified in families of either Syrian or Iranian Jewish ethnicity; (4) Variants recommended for carrier screening in Iranian Jews; (5) Pan-ethnic variant.

*dReference describing bi-allelic loss-of-function variant/s in the indicated gene that associate with the indicated phenotype. The given variant is novel but predicted to be loss-of-function.
The relatively short list of variants assessed and reported in this study was not designed to be exhaustive for all conditions that may be recommended for Syrian Jewish carrier screening. We acknowledge that there are other disease-causing recessive disorders in Syrians that were not included in this study and, accordingly, we list those that are presently known to us based on literature and the Israeli National Genetic Database (“The Israeli National Genetic Database,” n.d.) in Table S1. Notably, we also did not report the Syrian Jewish carrier frequency for SMN1 (MIM#: 600354) for spinal muscular atrophy (SMA) even though it is the most common autosomal recessive disease across worldwide populations (Verhaart et al., 2017). This is not to imply that SMA and other recessive diseases are not common in Syrian or Iranian Jews as well. On the contrary, we predict that numerous disease-causing alleles not described in this report are likely to be present in Syrian Jews with sufficiently high frequency and severity to justify
TABLE 3  Carrier frequencies in mixed Syrian, Iranian, and Ashkenazi Jewish cohorts

| Variant Name | Variant category | Syrian Jewish carrier frequency (no. carriers/n) | Syrian Jewish carrier frequency % | Iranian Jewish carrier frequency (no. carriers/n) | Iranian Jewish carrier frequency % | Ashkenazi Jewish carrier frequency (no. carriers/n) | Ashkenazi Jewish carrier frequency % |
|--------------|------------------|-----------------------------------------------|-----------------------------------|-----------------------------------------------|-----------------------------------|-----------------------------------------------|-----------------------------------|
| COL6A2:c.1402C>T | 1                | 56/3401                                       | 1.65                              | ND                                            | ND                                | 29/10125                                      | 0.29                              |
| GJB2:c.167del | 1                | 41/3401                                       | 1.21                              | 3/1112                                        | 0.27                              | 1078/41414                                    | 2.60                              |
| FXN:GAA expansion | 1              | 19/3401                                       | 0.56                              | ND                                            | ND                                | 26/9208                                       | 0.28                              |
| CYP11B1:c.992C>T | 1                | 9/3401                                        | 0.26                              | 2/3010                                        | 0.07                              | 6/60345                                       | 0.01                              |
| NDUFS4:c.355G>C | 1                | 6/3401                                        | 0.18                              | 2/3132                                        | 0.06                              | 1667454                                      | 0.02                              |
| VAC14:c.2005G>T | 1                | 5/3401                                        | 0.15                              | 0/1087                                        | 0.00                              | 0/39212                                      | 0.00                              |
| ESCO2:c.1674-2A>G | 1               | 4/3401                                        | 0.12                              | 0/3010                                        | 0.00                              | 7/61630                                       | 0.01                              |
| CNGB3:c.467C>T | 1                | 4/3401                                        | 0.12                              | 3/2904                                        | 0.10                              | 37/59336                                      | 0.06                              |
| BLM:c.98+1G>T  | 1                | 1/3401                                        | 0.03                              | 0/1088                                        | 0.00                              | 139219                                       | 0.00                              |
| ARSA:c.854+3A>G | 1                | 1/3401                                        | 0.03                              | 0/3010                                        | 0.00                              | 139219                                       | 0.00                              |
| ARSA:c.449C>T  | 1                | 1/3401                                        | 0.03                              | 0/3236                                        | 0.00                              | 8/67953                                       | 0.01                              |
| AGXT:c.731T>C   | 1                | 1/3401                                        | 0.03                              | 0/347                                         | 0.00                              | 2/27885                                       | 0.01                              |
| CFTR:c.254G>A   | 1                | 0/3401                                        | 0.00                              | 1/3576                                        | 0.03                              | 4/117781                                      | 0.00                              |
| GPT2:c.159C>G   | 1                | 0/3401                                        | 0.00                              | 0/3007                                        | 0.00                              | 1/61586                                       | 0.00                              |
| DSE:c.387delI   | 1                | 0/3401                                        | 0.00                              | 0/342                                         | 0.00                              | 0/27799                                       | 0.00                              |
| AIRE:c.254A>G   | 2                | 9/3401                                        | 0.26                              | 55/3761                                       | 1.46                              | 1873327                                       | 0.02                              |
| LIPA:c.260G>T   | 2                | 1/3401                                        | 0.03                              | 15/1435                                       | 1.05                              | 8/40987                                       | 0.02                              |
| GNE:c.2228T>C   | 3                | 24/3401                                       | 0.71                              | 120/3147                                      | 3.81                              | 61/67615                                      | 0.09                              |
| TYMP:c.433G>A   | 4                | 4/3401                                        | 0.12                              | 34/3747                                       | 0.91                              | 1073272                                      | 0.01                              |
| USH2A:c.236_239dup | 4             | 2/3401                                        | 0.06                              | 18/3588                                       | 0.50                              | 10/65282                                      | 0.02                              |
| CNGA3:c.1585G>A | 4                | 1/3401                                        | 0.03                              | 11/3761                                       | 0.29                              | 9/73408                                       | 0.01                              |
| CFTR:c.1521_1523del | 5              | 19/3401                                       | 0.56                              | 25/5279                                       | 0.47                              | 4303/370954                                   | 1.16                              |

Note: Data is shown for unrelated individuals with at least one Syrian Jewish grandparent or at least one Iranian Jewish grandparent or at least one Ashkenazi Jewish grandparent, as indicated. Abbreviations: N/A, not applicable; ND, no data.
inclusion in premarital screening. However, the goal of this study was not to establish definitive carrier frequencies for all pathogenic variants in Syrian Jews. Instead, we chose a diverse group of variants which were obtained primarily through our work with Syrian families that were affected by genetic diseases for which they had no prior awareness. As we identify these recessive conditions in families in the community, it will be important to increase awareness and to have the infrastructure to disseminate these findings to premarital couples who are likely to be carriers for one or more conditions given the degree of shared ancestry in these small populations.

ACKNOWLEDGMENTS
This work was funded by Dor Yeshorim, The Committee for Prevention of Jewish Genetic Diseases.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
Conceptualization: D.A.Z., S.Y.S., Y.H., J.E.; Data curation: D.A.Z., C.L., Y.H., T.W.; Formal Analysis: D.A.Z., C.L., Y.H.; Investigation: D.A.Z., W.K.C., C.L., S.Y.S., Y.H., T.W., A.E.; Methodology: D.A.Z., R.B., Y.K., H.M., R.B.; Resources: A.E., J.E.; Supervision: D.A.Z., Y.H., T.W., A.E., J.E.; Validation: D.A.Z., W.K.C., C.L., S.Y.S., Y.H., T.W.; Writing—review & editing: D.A.Z., W.K.C., S.Y.S., Y.H., T.W.; Writing – original draft: D.A.Z.; Writing–original draft: D.A.Z., C.L., S.Y.S., Y.H., T.W.; Formal Analysis: D.A.Z., W.K.C., C.L., Y.H.; Conceptualization: D.A.Z., S.Y.S., Y.H., J.E.; Data cura-

EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS
Ethical approval for this study was obtained from the Dor Yeshorim institutional review board according to guidelines of the Declaration of Helsinki. DNA samples were obtained with written informed consent from self-identified Syrian, Iranian, or Ashkenazi Jewish individuals enrolled in the Dor Yeshorim carrier testing program.

DATA AVAILABILITY STATEMENT
The source data for this study is available upon reasonable request.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

How to cite this article: Zeevi, D. A., Chung, W. K., Levi, C., Scher, S. Y., Bringer, R., Kahan, Y., Muallem, H., Benel, R., Hirsch, Y., Weiden, T., Ekstein, A., & Ekstein, J. (2021). Recommendation of premarital genetic screening in the Syrian Jewish community based on mutation carrier frequencies within Syrian Jewish cohorts. Molecular Genetics & Genomic Medicine, 9 e1756. https://doi.org/10.1002/mgg3.1756