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BRIEF REPORT

Lessons learned from the first national population-based genetic carrier-screening program for Duchenne muscular dystrophy

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ABSTRACT

Purpose: To summarize the results of first year implementation of pan-ethnic screening testing for Duchenne muscular dystrophy (DMD) and present the ensuing challenges.
Methods: Data acquisition for this study was performed by retrospective search of Ministry of Health registry for reports of all laboratories performing genetic screening tests. DMD testing was performed by multiplex ligation-dependent probe amplification technology. In case of single-exon deletion, sequencing of the specific exon was performed to rule out underlying single-nucleotide variant.
Results: Of overall 85,737 DMD tests, 82 clinically significant findings were noted (0.095%, or 1:1,046 women). In addition, 80 findings with uncertain clinical significance were detected (0.093%, or 1:1072), as well as 373 cases (0.4%, or 1:230) of single-exon deletions subsequently identified as false positives because of underlying single-nucleotide variant, mostly variants in exon 8 in North African Jewish population, and in exon 48 in Arab Muslim population.
Conclusion: Interpretation of population-based DMD carrier screening is complex, occasionally requiring additional genetic testing methods and ethical considerations. Multicenter data registry, including ethnic origin and familial segregation in selected cases, is crucial for optimal definition of the results during genetic counseling and informed decisions regarding prenatal testing.

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Introduction

Population genetic carrier screening for identification of individuals or couples at risk for a child with genetic disorders has been utilized for over 50 years.[1] In Israel, the national genetic carrier-screening program for reproductive purposes was fully launched and covered by the Ministry of Health (MOH) in 2013 for severe and incurable genetic disorders with high rates of infant and childhood morbidity and/or mortality, with carrier frequency of at least 1:60 and/or disease incidence above 1 in 15,000 live births. This program has resulted in a significant decrease in the incidence of several severe diseases, including Cystic Fibrosis, Spinal Muscular Atrophy, Familial dysautonomia, Canavan, and Tay-Sachs diseases.[2]

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy in the pediatric population,[3] with a prevalence of 1:3500 to 1:5000 live births.[4,5] This condition constitutes the severe end of the spectrum of dystrophinopathies, caused by pathogenic variants in the *DMD* gene (OMIM #300377), one of the largest genes, which comprises 79 exons. The most common pathogenic *DMD* variants are deletions (65%-70%) and duplications (≈11%) of 1 or more exons (the remaining 20% to 25% constituting of indels [7%] and sequence variants).[6] According to the "reading frame rule" applicable in about 90% of cases, out-of-frame variants disrupting the reading frame of dystrophin are usually associated with DMD. In-frame variants maintaining the reading frame and allowing the production of partially functional dystrophin cause Becker muscular dystrophy (BMD), a milder dystrophinopathy with later onset of symptoms and slower disease progression.[7,8]

DMD is included in the 2021 American College of Medical Genetics and Genomics list of X-linked conditions appropriate for carrier screening, noting that cross-referencing this gene to ClinGen did not reveal definitive gene-disease association.[1] In July 2020, DMD was included in the national genetic screening program. In this manuscript, we present the results of universal population screening for DMD, as well as the problems and debates arising with its implementation.

Materials and Methods

Population screening for DMD was performed by 13 countrywide genetic laboratories. The test was performed using multiplex ligation-dependent probe amplification (MLPA, P034 and P035 Sauce Kit, MRC-Holland), which was aimed to detect deletions and duplications of the 79 *DMD* exons (~75% of variants). In the case of single-exon deletion, Sanger sequencing of the specific exon was performed to rule out sequence variants affecting MLPA pattern.

The MOH received from the laboratories de-anonymized data, including total number of performed DMD screening tests, characterization of abnormal MLPA results, along with patient's origin, relevant family history, and whether segregation was performed, as well as the specific underlying single-nucleotide variant in case of false-positive single-exon deletion.

The single-nucleotide variants were classified according to the American College of Medical Genetics and Genomics,[9] using Franklin by Genoox Genomic Community (http://franklin.genoox.com). Specifically, variant prevalence was determined based on gnomAD population databases (https://gnomad.broadinstitute.org/), and prediction tools were used based on the Aggregated Prediction Franklin by Genoox Genomic Community (see Supplemental Table 1). Variant validation was performed using VariantValidator tool.[10]

After omission of false-positive results, for each abnormal MLPA finding, the expected impact on the reading frame was established (ie, in frame, out of frame, or "difficult to predict"), using both Leiden Muscular Dystrophy pages (https://www.dmd.nl/) and the UMD-DMD France database (http://www.umd.be/DMD/W_DMD/index.html). In addition, for each variant, clinical characteristics of previously described cases were sought, based on gene variant database of the Leiden muscular dystrophy pages (https://databases.lovd.nl/shared/view/DMD?search_VariantOnGenome%2FDBID=%3D%22DMD_054555%22) and discussion with Leiden University Medical Center DMD expert (A.A.R.). In case no reports were included in this database, additional search in the UMD-DMD and Pubmed was performed. The variants were defined as "clinically significant" if at least 1 symptomatic patient was reported based on family history or previous publications. Otherwise, the variants were classified as "uncertain significance."

The rates of clinically significant findings, variants of uncertain significance, and single-nucleotide variants were calculated and presented as numbers (percentages).

The study was considered exempt from Ethical Helsinki approval (correspondence number 134-2022-MOH).

Results

Of the 85,737 MLPA tests performed in the scope of DMD carrier screening, 535 cases of deletions/duplications (0.62%) were noted. In 386 of these (72.15%), a single-exon deletion was noted. Sequencing of probe target sequences in these cases showed that the vast majority (373 of 386, or 96.6%) were in fact lowered probe signals associated with single-nucleotide variants (ie, false-positive results).

The 162 abnormal MLPA findings, after omission of false-positive results, were divided into 2 groups: clinically significant findings (Table 1) and findings of uncertain significance (Table 2). Clinically significant findings were found in 82 cases (0.095%, or 1 in 1046 women). These included the 18 out-of-frame variants, 60 in-frame variants

Table 1 Clinically significant findings (also see Supplemental Table 2)

The Result	*N*	LOVD	UMD	Leiden	Family History	Segregation
del 2	3	OF[a]	OF[a]	6 BMD/DMD (2 - definite DMD)	–	–
del 3-7	1	OF[b]	OF[b]	281 (at least 52 DMD)	Brother	–
del 12-29	1	OF	OF	10 (3 DMD)	Nephew	–
del 46-47	1	OF	OF	361 (at least 109 DMD)	–	–
del 48-50	2	OF	OF	578 (at least 153 DMD)	Brother[1]	–
del 51	3	OF	OF	443 (at least 130 DMD)	–	–
del 52	1	OF	OF	301 (at least 92 DMD)	–	–
dup 3-7	1	OF	OF	83 (32 DMD)	–	–
dup 8-11	1	OF	OF	24 (10 DMD)	Brother	–
dup 18-28	1	OF	OF	3 (2 DMD)	–	–
dup 18-30	1	OF	OF	3 (1 DMD)	–	–
dup 46-57	1	OF	OF	1 BMD/DMD	–	–
dup 61-62	1	OF	OF	1 DMD (+1 heterozygote)	Two brothers + maternal uncle	–
del 3-9	1	IF	IF	281 (at least 52 DMD)	–	–
del 10-29	1	IF	IF	9 (2 BMD, 6 DMD/BMD, 1 MD)	–	–
del 10-41	1	IF	IF	4 (1 BMD, 1 DMD, 2 DMD/BMD)	–	–
del 30	1	IF	IF	9 (2 DMD, 5 BMD/DMD, 2 MD)	–	–
del 44-54	3	IF	IF	1 DMD[c]	Brother[1]	Healthy hemizygous father[1]
del 45-47	3	IF	IF	739 (33 DMD)	Father with BMD,[1] father and nephew with "muscular dystrophy"[1]	–
del 45-49	1	IF	IF	179 (8 DMD)	–	–
del 45-51	20	IF	IF	81 (5 DMD)	–	–
del 45-55	3	IF	IF	202 (11 DMD)	–	–
del 48-49	2	IF	IF	127 (10 DMD)	–	–
del 48-51	5	IF	IF	69 (6 DMD)	Son with DMD (1, 4 y), brother with BMD[1]	–
del 48-55	1	IF	IF	27 (2 DMD)	Son with DMD (1, 10 y)	–
del 49	3	IF	IF	15 (3 DMD)	Son with elevated CPK[1]	–
del 49-51	1	IF	IF	20 (3 DMD)	–	–
del 50-51	2	IF	IF	13 (1 DMD)	–	–
del 50-53	1	IF	IF	6 (4 DMD)	–	Healthy hemizygous father
del 51-52	6	IF	IF	21 (10 DMD)	–	Healthy hemizygous brother[1]
del 52-53	1	IF	IF	12 (1 DMD)	–	–
del 60	2	IF	IF	–[d]	–	–
dup 38	1	IF	IF	[d]	–	–
dup 42-44	1	IF	IF	[d]	–	–
dup 50-59	1	IF	IF	4 (1 DMD, 1 carrier female)	–	–
del all exons	2	DiffToPred	DiffToPred	11 (1 DMD)	Son[1]	–
del 1-2	1	DiffToPred	DiffToPred	7 (4 DMD)	–	–

BMD, Becker muscular dystrophy; *del*, deletion; *DiffToPred*, difficult to predict; *DMD*, Duchenne muscular dystrophy; *dup*, duplication; *IF*, in frame, *LOVD*, Leiden Open Variation Database; *OF*, out of frame, *UMD*, Universal Mutation Database.

[a]Cases where exon 2 deletion is associated with BMD have been reported. Here dystrophin production is facilitated through an internal ribosomal entry site in exon 5.

[b]This variant has been reported for Duchenne, Becker and intermediate muscular dystrophy cases.

[c]Canadian data https://www.dmd.nl/DMD_deldup_Canada.html.

[d]Could result in BMD when the duplication is located within the *DMD* gene (according to discussion with Leiden DMD expert).

reported in DMD/BMD patients or with positive family history, and 4 variants defined by LOVD/UMD as "difficult to predict" but previously reported in DMD/BMD patients. Clinically significant deletions were mainly concentrated between exons 45 and 55, a known mutational hot spot.[11] Family history was reported in 13 cases (15.9%). Segregation analysis found a healthy hemizygous male relative in 3 cases of in-frame deletions (exons 44-54, 50-53, and 51-52).

The 80 findings with uncertain clinical significance included 11 in-frame findings (7 deletions of exons 16-22 and 4 duplications) with negative family history and no

Table 2 Findings with uncertain clinical significance, likely not pathogenic

The Result	*n*	LOVD	UMD	Leiden		
Del 16-22	7	IF	IF	Heterozygous females		1 homozygous healthy female
Dup 1-2	2	DiffToPred	IF	–	–	–
Dup 1-12	1	DiffToPred	IF	–	–	–
Dup 1-15	1	DiffToPred	IF	1 heterozygous female		Healthy hemizygous father
Dup dp427c	58	DiffToPred	DiffToPred	–	–	–
Dup dp427c + exon 1	1	DiffToPred	DiffToPred	–	–	–
Dup dp427c + exon 1-7	2	DiffToPred	DiffToPred	–	–	–
Dup dp427c + exon 1-12	1	DiffToPred	DiffToPred	–	–	–
Dup 1-7	3	DiffToPred	DiffToPred	3 (not relevant)	–	–
Dup 61-79	1	DiffToPred	DiffToPred	–	–	–
Dup 63-79	1	DiffToPred	DiffToPred	1 BMD, 1 healthy female	–	–
Dup 78-79	1	DiffToPred	DiffToPred	–	–	–
Dup all exons	1	DiffToPred	DiffToPred	–	–	–

BMD, Becker muscular dystrophy; *del*, deletion; *DiffToPred*, difficult to predict; *DMD*, Duchenne muscular dystrophy; *dup*, duplication; *IF*, in frame, *LOVD*, Leiden Open Variation Database; *OF*, out of frame, *UMD*, Universal Mutation Database.

known previously reported DMD/BMD patients and 69 variants defined by LOVD/UMD as "difficult to predict"—all of these duplications. Prominent findings in this category included 7 deletions of exons 16-22, 1 of these found in homozygous healthy female, as well as 58 duplications of the dp427c promoter.

The single-nucleotide variants in the 373 false-positive single-exon deletion cases are presented in Supplemental Table 1. Of these, 53 variants were novel (ie, not reported in gnomAD or ClinVar). Most prominent was a NM_004006.3:c.831+10G>A polymorphism in exon 8, which was noted in 155 cases, mainly in women of Moroccan Jewish origin, and the 122 cases of NM_004006.3:c.7016A>G polymorphism in exon 48, mainly in Muslim Arab women.

Discussion

According to the results of our study, using a large cohort of screened women, a 1:1046 prevalence of clinically significant variants in the *DMD* gene was noted. Thus, the expected prevalence of DMD/BMD diseases associated with these variants is 1:4182. This is in concordance with the MOH criteria for inclusion in the national genetic carrier-screening program for reproductive purposes, as well with previous literature. It must be noted that the actual prevalence of postnatal DMD/BMD must be higher, considering de novo cases and ~25% of cases carrying small variants within exons that are missed by MLPA.

Our results are roughly congruent with few previous studies describing the results of DMD carrier screening. The first study presenting the results of Israeli DMD screening was published in 2022 by Cohen et al.[12] The authors described a cohort of 12,362 women tested at a single institution, noting 9 pathogenic deletions and duplications encompassing multiple exons (ie, rate of heterozygosity for a pathogenic variant of 1:1374). In addition, in 2020 Westemeyer et al presented the results of 288,268 female *DMD*-related dystrophinopathies carrier screening tests.[13] Of these, pathogenic and likely pathogenic variants were found in 402 cases (0.14%, 1/717), including single-nucleotide variants.

We wish to address the challenges raised during the first year of population screening for DMD using MLPA testing. The majority of former studies presented the results of postnatal DMD testing in symptomatic individuals, important for the affirmation of the diagnosis, adjustment of a tailored follow-up and early intervention services, cessation of further diagnostic investigations (eg, muscle biopsy), discussion of approved therapies, and maternal genetic testing with the possibility of prenatal and/or preimplantation genetic testing. On the contrary, the main aim of healthy population screening is to provide the examined individuals an opportunity to discuss the risk for an affected child and consider reproductive options. The latter include preimplantation genetic testing, use of donor gametes/embryos, adoption, prenatal diagnosis (enabling to either prepare for an affected child, including special postnatal care, or terminate the pregnancy), or a decision not to have children.[1] Thus, one of the basic requirements of genetic counseling in case of positive genetic screening is conveying detailed information and exact risks for the expected morbidity of the offspring. This is especially crucial if the woman heterozygous for a pathogenic variant is pregnant because she may be challenged to perform irrevocable decisions about the pregnancy based on the results of fetal genetic testing and the ensuing genetic counseling.

Unfortunately, in many cases of true-positive abnormal MLPA results during implementation of Israeli DMD population screening, the information regarding the exact risk for severe early onset disability and other health outcomes was suboptimal because of the significant paucity of evidence. For instance, during first months of screening, findings of uncertain significance were included in laboratory reports and communicated to the women. Subsequently, with accumulating data, some of these findings, such as the dp427c duplication noted in numerous women of Bucharian

Jewish origin or the in-frame deletion of exons 16-22 described in a healthy homozygous woman, were excluded from the laboratory report.

In addition, limited data were found for clinical consequences associated with several clinically significant findings, such as deletions of exons 44-54, 52-53, or 60 or duplications of exons 1-2, 50-59, or 62-79 (Table 1). Genetic counseling in such cases was carried out under conditions of noticeable uncertainty, causing significant psychological distress to women heterozygous for a pathogenic variant.[14] This anxiety was further aggravated when dealing with findings of uncertain significance, mostly DMD duplications, which have less predictable effects on the reading frame because the duplications may not be located in the *DMD* gene but in other genomic locations, leaving the *DMD* gene intact.[15] Furthermore, even if the duplication is located in the *DMD* gene, it may be in an opposite orientation (inverted), and the location may differ, affecting the phenotype.[15] Finally, if the duplication contains the first or the last exon of the *DMD* gene, it is likely that a normal transcript can still be formed.

Finally, the high frequency of false-positive single-exon deletions caused by underlying single-nucleotide variant, shown in 0.4% of all DMD tests (ie, 1 in 230), was unexpected, as previous studies examining this subject yielded much lower numbers. For instance, Kim et al showed that, of the 290 MLPA results yielding deletions or duplications, a single exon was involved in only 75 (25.9%) of these cases (as opposed to 79.4% in our study).[16] Among these single-exon MLPA variants, false-positive events accounted for approximately 14.7% (11/75), in contrast to 96.6% in our study. Similarly, Dama et al have demonstrated single-exon deletions in 25.1% of DMD/BMD patients, 6.1% of them caused by sequence variants near the probe-binding site.[17] These differences may probably be related to diagnostic genetic testing of symptomatic males in previous publications vs screening of healthy females in our study. The high rates of false-positive results have initially caused the laboratories to perform single-exon sequencing in each case of MLPA single-exon deletion. Initially, many of these results were reported to the patients, causing unnecessary anxiety. Therefore, a countrywide database was established, showing specific single-nucleotide variants in specific populations.

The accumulating data enabled to shed light on the challenges associated with DMD MLPA testing. In a recent meeting of Israeli Society of Medical Geneticists, it was suggested not to report the following findings in MLPA DMD testing (with an appropriate disclaimer in the laboratory report): variants of uncertain significance, single-exon deletions associated with an underlying non-pathogenic single-nucleotide variant, or findings without any known clinical consequences (such as deletion of exons 16-22). In addition, it was suggested not to perform exon sequencing in cases of a seemingly false-positive single-exon deletion, in which a single-nucleotide variant has been reported several times in the specific origin. A dedicated committee was established to further explore these suggestions.

In summary, the first national population-based genetic carrier-screening program for DMD/BMD yielded noticeable prevalence of clinically significant *DMD* variants (1 in 1046). However, for some variants, the information regarding the clinical implications was inadequate. We suggest that accumulating data should be continuously stored in countrywide databases and periodically summarized to facilitate the interpretation of the results and the concomitant genetic counseling. Non-disclosure of findings of uncertain significance or of variants not reported to be associated with DMD should be considered. Finally, the additional complexity related to population screening for X-linked disorders, as opposed to autosomal recessive diseases, should be taken into account.

Data Availability

The data that support the findings of this study are available from the corresponding author (L.S.).

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Ethics Declaration

The study was considered exempt from Ethical Helsinki approval (correspondence number 134-2022-MOH). Informed consent was not required (not applicable for a retrospective study), and individual data were de-identified.

Conflict of Interest

The authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2023.100981) contains supplemental material, which is available to authorized users.

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