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Large scale population screening for Duchenne muscular dystrophy—Predictable and unpredictable challenges

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Abstract

Objective: Large deletions and duplications account for 65%–80% of pathogenic Duchenne muscular dystrophy (*DMD*) variants. A nationwide carrier screening for DMD was initiated in Israel in 2020. We assessed the carrier rate and spectrum of variants detected in a cohort of women screened for DMD carrier status and analyzed screening efficacy and challenges related to DMD population screening.

Methods: A cohort of 12,362 women were tested at a single institute using multiplex ligation-dependent probe amplification based copy number analysis of the 79 *DMD* exons. Consecutive sequencing of the primer region was performed when a single exon deletion was suspected.

Results: Deletions involving multiple exons were detected in seven cases and duplications involving multiple exons were found in four. Of these, nine were pathogenic based on previous reports and familial segregation testing, translating to a carrier rate of 1:1374. A family history was reported in three cases. Single exon deletions were suspected in 81 cases; further sequencing detected a single nucleotide variant affecting probe hybridization. These cases clustered according to ethnic origin.

Discussion: Population screening for DMD has a significant yield. Most carriers did not report a family history of dystrophinopathies. Screening should be adjusted for methodological limitations. Some cases may require extensive genetic counseling and work-up.

Key points

What's already known about this topic?

- Deletions and duplications account for 65%–80% of pathogenic Duchenne muscular dystrophy (DMD) variants.

- About two-thirds of DMD pathogenic variants are inherited from a female carrier.
- A nationwide carrier screening for DMD was initiated in Israel in 2020.

What does this study add?
- The carrier rate among 12,362 women tested for deletions and duplications in the *DMD* gene was 1:1374.
- Screening should be adjusted for methodological limitations and may require extensive genetic counseling and work-up.

1 | INTRODUCTION

The criteria for population carrier screening for genetic disorders include phenotype severity that may impact decision-making, high prevalence of carriers, established analytic validity of screening methods, predictable genotype–phenotype correlations, and available prenatal diagnosis and reproductive options.[1,2]

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are progressive muscular dystrophinopathies caused by pathogenic variants in the X chromosome-linked *DMD* gene. The *DMD* gene consists of 79 exons. More than 5000 pathogenic variants in *DMD* have been identified as related to DMD or BMD. Large deletions involving a single exon or multiple exons occur in 60%–70% of cases, duplications in 5%–10%, and sequence variants including single-nucleotide variants (SNVs), small deletions, or insertions in 20%–35%.[3]

Phenotype usually depends on whether the reading frame of the *DMD* gene is maintained.[4,5] However, there are known exceptions to this rule.[6–8] DMD is usually caused by out-of-frame variants, resulting in a severe form of the disease with muscular weakness occurring from 2 to 3 years of age and low survival rates in adulthood.[9] BMD is usually caused by in-frame pathogenic variants, resulting in a milder form of the disease, with a clinical onset ranging from 5 to 60 years and symptoms allowing ambulatory management throughout life, even into old age.[10]

About two-thirds of the pathogenic variants in *DMD* are inherited from a female carrier.[11] Females are asymptomatic in most cases; however, they may express mild symptoms such as muscular weakness, myalgia, and elevated creatinine kinase (CK) levels.[12,13] Females heterozygous for DMD pathogenic variants are also at increased risk for dilated cardiomyopathies.[14,15]

The reported incidence of DMD and BMD among males is about 1/5,000[16] and 1/18,000,[17] respectively. Given the high tendency for de novo pathogenic variants, dystrophinopathies are expected to be prevalent worldwide. To date, population screening for DMD has not been performed; hence, the actual carrier rate is unknown.

According to the American College of Medical Genetics and Genomics practice statement regarding screening for autosomal recessive and X-linked conditions,[1] screening is recommended for DMD. Population carrier screening for deletions and duplications in the *DMD* gene using Multiplex Ligation-dependent Probe Amplification (MLPA) was initiated in Israel, in July 2020.

This study assessed the carrier rate and the spectrum of pathogenic variants in a large cohort of healthy women tested for DMD carrier status in order to evaluate the efficacy and specific challenges related to DMD population screening.

2 | METHODS

2.1 | Data collection

The study cohort included healthy women of child-bearing age tested at the Genetic Institute of Meir Medical Center, Israel, from July 2020 to August 2021. The screening was funded by the Israeli Ministry of Health (MOH). Each woman was required to report her ethnic origin and any family history of genetic diseases. The population referred for screening at our genetic institute is ethnically diverse, and faithfully represents most ethnic groups in Israel. The proportion of different ethnic groups is not uniform among different regions in Israel. The Arab population in our cohort accounts for 33.2% compared to the 21% in the Israeli population.

The copy number of each of the 79 *DMD* exons was analyzed using the MLPA kit (MRC, Amsterdam, Holland). The MLPA technique relies on sequence-specific probe hybridization to genomic DNA, followed by amplification of the hybridized probe, and semi-quantitative analysis of the resulting polymerase chain reaction products. Relative copy numbers are determined by comparing the relative peak heights of reference probes and target probes in the test samples with those of the reference samples with a known normal copy number.[18,19] MLPA results were interpreted as a deletion when the reading was $0.40 < DQ < 0.65$ compared to the normal copy number and as duplication when it was $1.30 < DQ < 1.65$.

Abnormal results were defined as one copy or more than two copies of each exon analyzed. Each abnormal result was verified by repeating the test with DNA from a different blood sample of the patient. When MLPA suggested a single exon deletion, consecutive testing using Sanger sequencing of the probe region was performed to determine whether the suspected single exon deletion was related to mismatching of the primer, which could lead to a false positive result. If an SNV affecting probe hybridization was detected, we aimed to determine whether it is a normal variation in the population (polymorphism) or a pathogenic variant. We used bioinformatics

tools, such as looking at its prevalence in the normal population using data from gnomAD v2.1.1, and assessed its frequency in the Israeli population based on reports from other Israeli labs. Data regarding DMD testing results are shared between different labs in Israel. Test results are reported by the labs to the MOH and discussed in professional meetings of the Israeli Society of Medical Genetics. These variants were classified according to the American College of Medical Genetics and Genomics (*ACMG*) guidelines.[20]

2.2 | Clinical work-up

All carriers of pathogenic variants and large deletions and duplications received comprehensive genetic counseling. Some cases with deletions or duplications and cases with novel SNVs were further investigated with segregation analysis, including genetic testing (MLPA screening and Sanger sequencing) for first degree relatives, phenotypic assessment by a medical geneticist/neurologist, and referral of relevant family members for echocardiogram and CK testing. In some cases, when a whole gene deletion was detected or when MLPA testing suggested a deletion expanding to regions outside the DMD locus, chromosomal microarray testing was performed. Data regarding segregation analysis and genetic testing are listed in Tables 2 and 3.

2.3 | Ethics declaration

The study was approved by the Meir Medical Center Ethics Committee, Kfar Saba, Israel, in August 2021, approval number 0193-21-MMC. DMD screening was performed as part of a nationwide carrier screening protocol. All patients gave their informed consent for carrier screening. As the study was based on a retrospective analysis of screening results, the Ethics Committee waived the need for additional informed consent.

2.4 | Statistical analysis

Categorical data were compared using Chi-squared or Fisher's exact test (each when appropriate). A probability value of $p < 0.05$ was considered significant. All analyses were performed using SPSS-26 software (IBM, Armonk, NY, USA).

3 | RESULTS

The screening results of the cohort of 12,362 women screened for *DMD* are depicted in Figure 1. The ethnic distribution of the cohort is presented in Table 1.
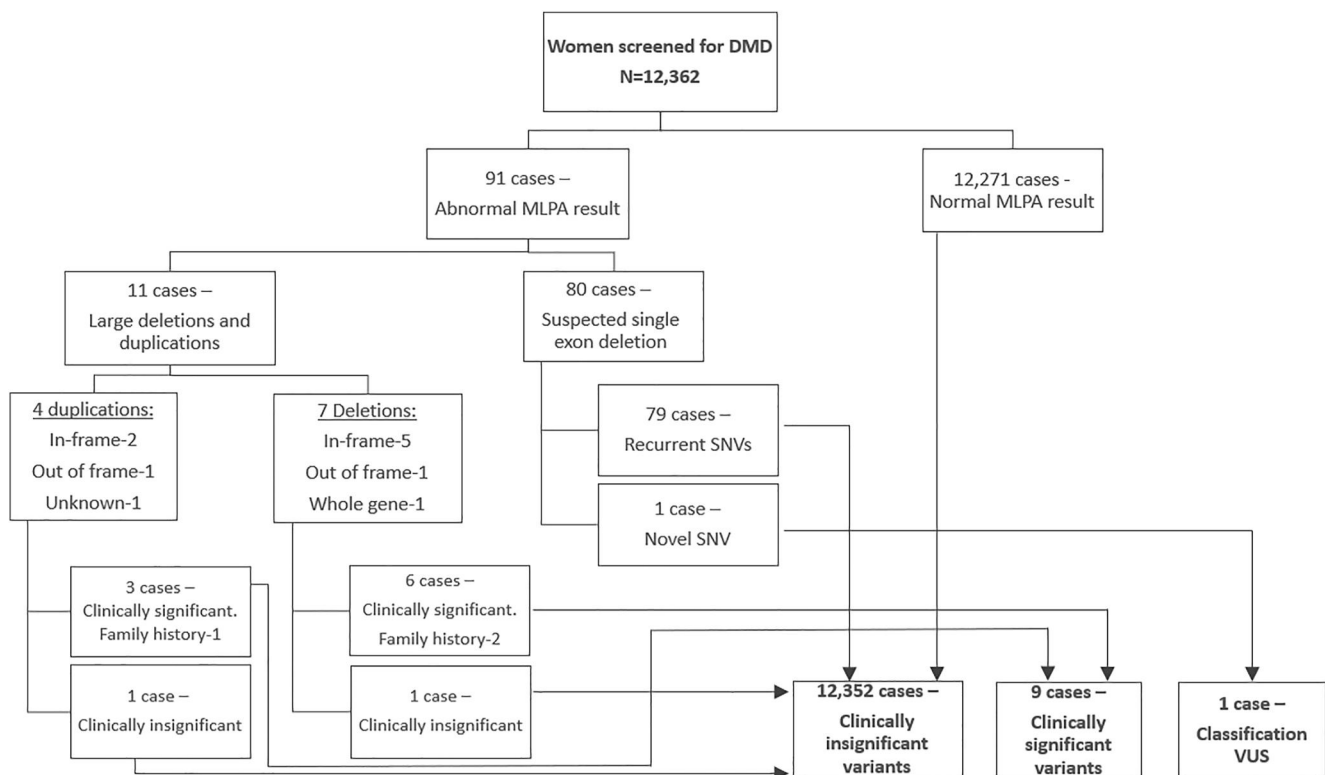

FIGURE 1 DMD screening results of a cohort of 12,362 women tested at a single institute using MLPA based copy number analysis of the 79 *DMD* exons. DMD, Duchenne muscular dystrophy; MLPA, multiplex ligation-dependent probe amplification

TABLE 1 Ethnic distribution of the study population

	Women screened (num, %)	
Ethnic group	N = 12,362	
Ashkenazi Jews	1563	12.6
North African Jews	916	7.4
Iranian and Iraqi Jews	320	2.6
Yemenite Jews	375	3.0
Balkan Jews	117	0.9
Arab Muslims	4054	32.8
Bedouin Muslims	53	0.4
Others[a]	4964	40.2
Total	12,362	100.0

[a]Others includes less common ethnicities as well as all individuals with a mixed ethnicity.

In total, 11 cases of multiple exon deletions or duplications involving multiple exons were detected, of which 9 were defined as pathogenic based on previous reports and familial segregation testing. Hence, the carrier rate detected is 1:1374. The carrier rate of out-of-frame deletions and duplications and whole gene deletions was 1:4120. Of the nine pathogenic variants detected, in three cases, a family history of dystrophinopathies was reported, and in six cases, no family history was reported. There was no difference in carrier rate according to ethnic group. Clinical data regarding large deletions and duplications detected are presented in Table 2.

3.1 | Deletions

Overall, seven large deletions (del) were detected, of which six were pathogenic. Five were in-frame, one was out of frame, and one was a whole gene deletion.

The Exon 16–22 deletion was classified as clinically insignificant based on reports from other Israeli laboratories of four additional cases, including a case of a healthy homozygous female with an unaffected father.

In the patient with deletion of the entire DMD gene, chromosomal microarray testing detected a 16 Mb deletion on Xq [GRCh37/hg19 chrX:24728601–41173009] including 63 genes, of which 30 were OMIM genes.

In one case, no deletion in the DMD gene was detected. However, reference probes detected a suspected large deletion near the DMD gene. CMA testing detected a 23.9 Mb deletion on Xp22.33p22.11 including 160 genes (113 OMIM genes). The deletion was categorized as pathogenic. The carrier reported no abnormal phenotype and her clinical examination revealed no abnormalities. The patient was planning an IVF pregnancy with sperm donation, and preimplantation diagnosis was discussed.

3.2 | Duplications

Four large duplications were detected, of which three were pathogenic. Of the duplications, two were in-frame, one was out of frame, and one included the terminal part of the gene (exons 63–79).

The Exon 1–15 in-frame duplication was classified as clinically insignificant based on MLPA testing results, showing that the father was a healthy carrier.

Duplications of the promotor region were detected in four cases, of which two were in Jews from Bukhara. These were previously reported by other labs, mostly in Jews from Bukhara, and were categorized as clinically insignificant.

3.3 | Single exon deletions related to SNVs affecting hybridization

In 81 cases, a single exon deletion was detected by MLPA (Table 3). In all these cases, sequencing analyses revealed an SNV affecting probe hybridization. This suggests that there was no single exon deletion but rather an artifact created by the SNV (false positive result). Of note, the values in these cases for the specific exon deletions were borderline, with values around 0.67 compared to the expected $0.40 < DQ < 0.65$ value in the seven cases of multiple exon deletions.

Eighty of the SNVs detected were previously reported in healthy individuals (Table 3) and classified as benign. These included an SNV in Exon 48, an SNV in introns 8, 70, 1, 68, and a single nucleotide deletion in intron 68.

These SNVs clustered according to ethnic origin (Table 3), including 53 cases with exon 48 deletion in Israeli Muslim Arabs and 15 cases of exon 8 deletion in North African Jews.

In one case, a rare SNV was detected in exon 12. Despite extensive segregation studies revealing that the SNP was inherited from a healthy mother and was not present in a male sibling and two maternal uncles, we could not rule out the pathogenicity of the SNV; hence, it was classified as a variant of unknown significance.

4 | DISCUSSION

We report results from the first large-scale population carrier screening for deletions and duplications in the DMD gene. Among 12,362 women, the carrier rate for pathogenic large deletions or duplications, all involving multiple exons, was 1:1374.

Screening for DMD should be assessed according to the established criteria for population screening.[1,2]

The carrier rate of disease-causing deletions/duplications in our cohort is well above the population frequency recommended for screening, as the frequency of affected males with a dystrophinopathy related to a deletion/duplication in the DMD gene can be translated to 1:2,748, similar to the risk of cystic fibrosis (CF) among Caucasians.[29]

TABLE 2 Deletions, duplications, and a whole gene deletion detected by MLPA based population carrier screening for DMD

Test result	Specific variant	Number of cases	Ethnic origin	Previous reports	Personal and family history	Testing additional family members	Classification and significance of variant	Clinical decisions
Multiple exon deletions and duplications	Exon 16–22 Del (in-frame)	1	Arab Muslim	Reported by other Israeli laboratories in four more cases, including a healthy homozygous female with an unaffected father.	No family members with muscular dystrophies or cardiomyopathies.	-	Clinically insignificant	-
	Exon 48–51 Del (in-frame)	2	Arab Muslim; Bedouin Muslim	Reported in patients with a diverse phenotype. Most presenting with BMD also reported in patients with DMD, cognitive impairment, isolated increased CK levels, and asymptomatic patients. [21,22]	1. Nephew with a muscular dystrophy 2. No family members with muscular dystrophies or cardiomyopathies	-	Pathogenic variant with a wide phenotypic spectrum	2. The patient was pregnant with a male fetus. The couple did not proceed with prenatal diagnosis
	Exon 48–50 Del (out of frame)	1	Arab Muslim	Yes	A brother diagnosed with DMD	-	Pathogenic (DMD)	-
	Exon 51–52 Del (in-frame)	2	Arab Muslim	Reported in patients with DMD.[23]	Both patients reported no family members with muscular dystrophies or cardiomyopathies	-	Pathogenic (DMD)	-
Multiple exon duplications	Exon 1–15 duplication (in-frame)	1	Other	Not previously reported	No family members with muscular dystrophies or cardiomyopathies	MLPA testing found the father was a healthy carrier	Clinically insignificant	-
	Exon 3–7 duplication (out of frame)	1	Arab Muslim	Reported in patients with DMD.[24]	Brother diagnosed with DMD, died at age 25	-	Pathogenic (DMD)	-
	Exon 50–59 duplication (in-frame)	1	Other	Reported in patients with DMD.[25]	No family members with muscular dystrophies or cardiomyopathies	-	Pathogenic (DMD)	Prenatal diagnosis revealed a carrier female fetus

TABLE 2 (Continued)

Test result	Specific variant	Number of cases	Ethnic origin	Previous reports	Personal and family history	Testing additional family members	Classification and significance of variant	Clinical decisions
	Exon 63–79 duplication	1	Ashkenazi Jewish	Reported in a single patient with a dystrophinopathy.[26]	No family members with muscular dystrophies or cardiomyopathies	Son with mildly elevated CK (303 mcg/L). Repeat testing and segregation analysis recommended	Pathogenic (dystrophinopathy)	-
Whole gene deletion		1	Other	Whole DMD gene deletions were previously reported (http://umd.be/TREAT_DMD/). Son with normal CK levels.	History of palm numbness. CMA testing detected a 16 Mb deletion on Xq [GRCh37/hg19 chrX:24728601–41173009]. A 7-month-old son diagnosed with hypotonia postpartum, is being followed by physiotherapy with normal motor development, so far. CK levels are within normal limits.		Pathogenic (DMD)	Prenatal diagnosis revealed a non-carrier female fetus.
Promotor duplications		4	2 Jews from Bukhara, 2 Ashkenazi Jewish	Reported by other labs, mostly in Jews from Bukhara	-	-	Clinically insignificant	-

Abbreviations: CK, creatinine kinase; DMD, Duchenne muscular dystrophy; MLPA, multiplex ligation-dependent probe amplification.

TABLE 3 Common SNVs found in study population

MLPA reading suggesting a single deletion; specific variant[a]	Number of alleles found out of 24,724 alleles screened (N = 24,724)	Ethnic origin[c]	SNP prevalence in ethnic origin	p-value for SNP prevalence in the specific ethnic origin compared to the rest of the population	Bioinformatics[b]	Reported by other Israeli laboratories/Reported in the literature	Classification	Familial segregation
Exon 1 deletion; SNP in intron 1 (NM_004006.2):c.-188C>T	2	Ashkenazi Jewish	1:1563	-	GnomAD: 10 alleles; benign computational verdicts (BP4).	Yes	Benign	-
Exon 8 deletion; SNP in intron 8 (NM_004006.2): c.831 +10G>A	15	North African Jewish	1:122	-	GnomAD: 1 allele (PM2) ClinVar classification of likely benign, rated 2 stars, with 2 submissions and no conflicts (BP6) and benign computational verdicts (BP4).	Yes, in North African Jews	Benign	-
Exon 12 deletion; SNP in exon 12 (NM_000109.3): c.1340A>C	1	Ashkenazi Jewish	1:1563	-	GnomAD: No alleles (PM2), pathogenic supporting computational verdict (PP3), and benign supporting criteria based on fact that most missense variants are benign (BP1); CADD score[27] of 24.7. Previous classification of a variant in a similar location as VUS	No	VUS	All family members are reported as healthy. SNP inherited from a healthy mother. Not present in a male sibling and 2 maternal uncles tested
Exon 48 deletion; SNP in exon 48 (NM_004006.2): c.7016A>G	53	52 Arab Muslim / 1 Bedouin Muslim	1:156 / 1:106	p < 0.001 / p = 0.204	GnomAD: 3 alleles most missense variants are benign (BP1); with benign computational verdicts (BP4)	Yes, in Muslims/ Reported in the Kuwaiti-Muslim population.[28]	Benign	- / -
Exon 69 deletion; SNP in intron 68 (NM_004006.2): c.9975-24G>A	1	Arab Muslim	1:8108	-	GnomAD: 3 alleles (PM2); benign computations verdict (BP4).	Yes	Benign	-
Intron 71 deletion; SNP in intron 70 (NM_004006.2): c.10224-37G>A	8	5 Bedouin Muslim / 2 Arab Muslim	1:21 / 1:320	p < 0.001 / p = 0.017	GnomAD: 19 alleles (BS1, BS2); benign computational verdicts (BP4).	Yes	Benign	- / -

TABLE 3 (Continued)

MLPA reading suggesting a single deletion; specific variant[a]	Number of alleles found out of 24,724 alleles screened (N = 24,724)	Ethnic origin[c]	SNP prevalence in ethnic origin	p-value for SNP prevalence in the specific ethnic origin compared to the rest of the population	Bioinformatics[b]	Reported by other Israeli laboratories/ Reported in the literature	Classification	Familial segregation
	1	Iranian-Iraqi Jewish	1:640	$p = 0.189$				-
Exon 69 deletion (NM_004006.2): Single nucleotide deletion in intron 68 c.9975-24 del G	1	Arab Muslim	1:8108	-	GnomAD: No alleles; benign computations verdict (BP4).	Yes	Benign	-

Abbreviations: ACMG, American College of Medical Genetics and Genomics; MLPA, multiplex ligation-dependent probe amplification; SNVs, single-nucleotide variants.

[a]For some exons the MLPA probe is located within the exon while for others it is located within the intron, hence the detected SNV can be either exonic or intronic.

[b]According to ACMG guidelines.[20] The number of gnomAD alleles includes the total number of alleles reported in all ethnicities according version 2.1.1.

[c]SNP prevalence in ethnic origin is a calculation of the SNP prevalence based on the number of individuals from each ethnic origin that were screened in our cohort. The number of gnomAD alleles includes the total number of alleles reported in all ethnicities according to gnomAD v2.1.1 (Aggregated).

Moreover, the risk for an affected offspring with a dystrophinopathy is about a fourth compared to the prevalence of Spinal Muscular Atrophy,[30] a disease which is recommended by the ACOG for carrier screening in the population for all ethnic groups.[2] Hence, the carrier rate detected in our cohort supports the effectiveness of a nation-wide carrier screening for *DMD* deletions and duplications.

Currently, there are no data available regarding the carrier rate of DMD/BMD. The figures regarding disease frequency also vary. Based on a disease frequency of 1:5000 males and the fact that two-thirds of the cases are inherited from an affected mother, we can assume a carrier rate of 1:2500. Since MLPA testing does not detect the 20%–35% of sequence variants and small deletions, we can predict that the carrier rate detected by MLPA screening should be about one-third less (hence around 1:3750). Of note, the reported carrier frequency of BMD is much lower.[17]

The carrier rate of out-of-frame deletions and duplications in our cohort, including the case of whole gene deletion is 1:4120; closer to the predicted rate.

Of note, three of the carriers detected reported a positive family history and therefore could have been diagnosed following testing of affected family members.

A single exon deletion was suspected in 81 cases (0.65%). All of them were false positive results emerging from SNVs affecting probe hybridization. Eighty of these SNVs were defined as benign polymorphisms, and in one case, a variant with unknown significance.

Screening for DMD/BMD has several limitations and technical difficulties, some predictable, while others presented only after initiation of population screening.

Principal limitations are that one-third of the cases are de novo and that 20%–30% of variants are coding variants not detectable by MLPA. However, this so-called limitation regarding the inability to detect all carriers during carrier screening is present in most carrier screening tests performed. For example, for CF carrier screening,[31] testing can be performed by either targeted screening for a panel of known mutations, termed classification-based (targeted) testing approach (according to the ACMG guidelines, the ACMG-23 variant panel is the minimal list that should be used), or by sequencing of the entire *CFTR* (MIM 602421) coding region, termed classification-based reporting approach. Each of these methods can theoretically miss certain variants. The targeted approach will only detect the variants included in the panel and will not detect all other variants or any other deletions/duplications, while the classification-based reporting approach may miss noncoding variants, such as deep intronic, or additional specific variants, depending on the testing methodology used. For example, Sanger sequencing will miss deletions and duplications and next generation sequencing might miss variants located in specific regions that are technically challenging for sequencing.

In summary, it seems that the limitation to detect all variants during carrier screening is not unique to DMD. However, in DMD/BMD carrier screening, this limitation, combined with the one-third of cases that result from de novo pathogenic variants, leads to a relatively low detection rate (50%).

In addition, DMD/BMD carrier screening has some significant limitations related to possible inconclusive genotype–phenotype correlations; namely, our inability to predict the clinical phenotype related to some of the detected deletions/duplications. *DMD* gene variants have a wide phenotypic spectrum and since not all variants follow the in-frame/out-of-frame rule, detection of a variant not previously reported may pose a challenge in determining its related phenotype, if any. The detection of a novel variant in women with no family history of the disease may require familial segregation, including clinical assessment and CK levels in family members, which is not always informative. In some cases, unless we can find healthy or affected male carriers, we may not be able to clearly determine the pathogenicity of the variants. Also, some specific deletions/duplications were reported in both DMD and BMD patients and even in very mildly affected individuals.[21,22]

While DMD is considered relevant for population screening, BMD pathogenic variants corresponding to the mild end of the phenotypic spectrum do not unequivocally qualify for screening according to the criteria regarding phenotype severity that may impact decision-making.

Carrier screening is performed for the purpose of offering prenatal diagnosis and preimplantation genetic testing; hence, if the predicted phenotype is not clearly appropriate for such testing, we may be presented with a significant conflict. Overdiagnosis resulting from detection and misclassification of variants that are not pathogenic or related to a mild phenotype may lead to unnecessary prenatal diagnosis and pregnancy termination for an unsubstantiated indication.

With that said, variants associated with variable expressivity are not unique to DMD/BMD. For example, in the case of CF, there are a number of CFTR related phenotypes, some severe enough to justify carrier screening, such as classical CF (MIM 219700), while other nonclassical CF phenotypes, such as congenital bilateral absence of vas deferens (MIM 277180), are not considered relevant for periconceptional carrier screening. It is not always possible to predict the related phenotype for a specific variant and in many cases, the phenotype will be determined by additional factors, mainly the coexistence in cis configuration with another specific variant. The issue of reporting variants of unknown significance is well known in genetic testing. For variants detected for periconceptional carrier screening, the ACMG has recommended that variants associated with variable expressivity that are not currently classified as pathogenic (P) or likely pathogenic (LP) by the laboratory should not be reported unless they are detected in a partner of an individual who carries a P/LP variant.[31]

Another important aspect that presented after initiating the DMD/BMD carrier screening is a technical issue with MLPA, which may yield a false positive result of a single exon deletion, due to an SNV affecting probe hybridization. Though this technical issue is well known and was also reported in CF testing using MLPA,[31] it's scale in the DMD carrier screening was surprising, as the rate of cases with an MLPA result suggestive of a single exon deletion related to an SNV detected using targeted sequencing was

significant. These SNVs require variant evaluation to exclude pathogenicity. Some can be ruled out as pathogenic based on their high prevalence in the population (or in many cases, a specific ethnic group), while others require additional bioinformatics work-up and familial segregation testing. Determining the relevance of these findings requires generating databases and data sharing between laboratories. For example, following data accumulation, we can now determine that the SNPs in exon 8 and exon 48 are considered a common polymorphism in specific ethnic groups and there is no need to perform sequencing or report these findings. Hence, our experience is that there is an ongoing learning curve for managing these findings and we predict that in the future, we will have enough information to determine the clinical insignificance of additional findings in specific populations without the need to perform sequencing for these cases. Of course, this learning curve is population specific; hence, each country that chooses to initiate population screening will need to undergo a process of mapping specific variants in the screened population.

Other issues with DMD MLPA screening are its high cost, the burden of genetic counseling sessions required to assess findings with uncertain significance,[32] the need to test additional family members, and the anxiety related to these processes.

All these issues should be considered when determining the appropriateness of population screening.

Those in favor would argue that preventing even a few cases of DMD justifies the price of overdiagnosis, resulting in unnecessary testing, counseling, and challenging conflicts regarding preimplantation and prenatal testing.

Another important point to consider is that, due to novel therapeutic options, newborn screening for DMD is considered a beneficial approach by many experts, and a first test for such screening was authorized by the FDA in December 2019.[33–36] Hence, the issue of presymptomatic screening for dystrophinopathies is already an accepted concept.

We conclude that the yield for DMD population screening is significant, with no reported family history dystrophinopathies in most carriers. Screening should be adjusted to methodological limitations and, in some cases, may require extensive genetic counseling and work-up. A thorough review regarding the cost-effectiveness of such screening is needed as well as a thorough discussion regarding the best technology for such screening.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Gregg AR, Aarabi M, Klugman S, et al. Correction to: screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021;23(10): 2015. https://doi.org/10.1038/s41436-021-01300-z

2. American College of Obstetricians and Gynecologists. Committee opinion No. 691: carrier screening for genetic conditions. *Obstet Gynecol.* 2017;129(3):e41-e55. https://doi.org/10.1097/AOG.00000 00000001952

3. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol.* 2003;2(12): 731-740. https://doi.org/10.1016/s1474-4422(03)00585-4

4. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics.* 1988;2(1):90-95. https://doi.org/10.1016/0888-7543(88)90113-9

5. Nicolas A, Raguénès-Nicol C, Ben Yaou R, et al. Becker muscular dystrophy severity is linked to the structure of dystrophin. *Hum Mol Genet.* 2015;24(5):1267-1279. https://doi.org/10.1093/hmg/ddu537

6. Aartsma-Rus A, Van Deutekom JCT, Fokkema IF, Van Ommen G.-JB, Den Dunnen JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve.* 2006;34(2): 135-144. https://doi.org/10.1002/mus.20586

7. Gillard EF, Chamberlain JS, Murphy EG, et al. Molecular and phenotypic analysis of patients with deletions within the deletion-rich region of the Duchenne muscular dystrophy (DMD) gene. *Am J Hum Genet.* 1989;45(4):507-520.

8. Takeshima Y, Yagi M, Okizuka Y, et al. Mutation spectrum of the dystrophin gene in 442 Duchenne/Becker muscular dystrophy cases from one Japanese referral center. *J Hum Genet.* 2010;55(6): 379-388. https://doi.org/10.1038/jhg.2010.49

9. Passamano L, Taglia A, Palladino A, et al. Improvement of survival in Duchenne muscular dystrophy: retrospective analysis of 835 patients. *Acta Myol.* 2012;31(2):121-125. http://www.ncbi.nlm.nih.gov/pubmed/23097603

10. Bradley WG, Jones MZ, Mussini JM, Fawcett PR. Becker-type muscular dystrophy. *Muscle Nerve.* 1978;1(2):111-132. https://doi.org/10.1002/mus.880010204

11. Darras BT, Urion DK, Ghosh PS. *Dystrophinopathies. GeneReviews.* University of Washington, Seattle; 2000. https://www.ncbi.nlm.nih.gov/books/NBK1119/

12. Hoogerwaard EM, Bakker E, Ippel PF, et al. Signs and symptoms of Duchenne muscular dystrophy and Becker muscular dystrophy among carriers in The Netherlands: a cohort study. *Lancet (London, England).* 1999;353(9170):2116-2119. https://doi.org/10.1016/s0140-6736(98)10028-4

13. Hoogerwaard EM, van der Wouw PA, Wilde AA, et al. Cardiac involvement in carriers of Duchenne and Becker muscular dystrophy. *Neuromuscul Disord.* 1999;9(5):347-351. https://doi.org/10.1016/s0960-8966(99)00018-8

14. Kamdar F, Garry DJ. Dystrophin-deficient cardiomyopathy. *J Am Coll Cardiol.* 2016;67(21):2533-2546. https://doi.org/10.1016/j.jacc.2016.02.081

15. Darras BT, Urion DK, Ghosh PS. Dystrophinopathies; 1993. http://www.ncbi.nlm.nih.gov/pubmed/20301298

16. Crisafulli S, Sultana J, Fontana A, Salvo F, Messina S, Trifirò G. Global epidemiology of Duchenne muscular dystrophy: an updated systematic review and meta-analysis. *Orphanet J Rare Dis.* 2020;15(1): 141. https://doi.org/10.1186/s13023-020-01430-8

17. Bushby KM, Thambyayah M, Gardner-Medwin D. Prevalence and incidence of Becker muscular dystrophy. *Lancet (London, England).* 1991;337(8748):1022-1024. https://doi.org/10.1016/0140-6736(91)92671-n

18. Sellner LN, Taylor GR. MLPA and MAPH: new techniques for detection of gene deletions. *Hum Mutat.* 2004;23(5):413-419. https://doi.org/10.1002/humu.20035

19. Lai KKS, Lo IFM, Tong TMF, Cheng LYL, Lam STS. Detecting exon deletions and duplications of the DMD gene using multiplex ligation-dependent probe amplification (MLPA). *Clin Biochem.* 2006;39(4): 367-372. https://doi.org/10.1016/j.clinbiochem.2005.11.019

20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5): 405-424. https://doi.org/10.1038/gim.2015.30

21. Uwineza A, Hitayezu J, Murorunkwere S, et al. Genetic diagnosis of Duchenne and Becker muscular dystrophy using multiplex ligation-dependent probe amplification in Rwandan patients. *J Trop Pediatr.* 2014;60(2):112-117. https://doi.org/10.1093/tropej/fmt090

22. Waldrop MA, Yaou RB, Lucas KK, et al. Clinical phenotypes of DMD exon 51 skip equivalent deletions: a systematic review. *J Neuromuscul Dis.* 2020;7(3):217-229. https://doi.org/10.3233/JND-200483

23. Sertić J, Barišić N, Sostarko M, Brzović Z, Stavljenić-Rukavina A. Deletion screening of the Duchenne/Becker muscular dystrophy gene in Croatian population. *Coll Antropol.* 1997;21(1):151-156. http://www.ncbi.nlm.nih.gov/pubmed/9225508

24. Punetha J, Mansoor S, Bertorini TE, Kesari A, Brown KJ, Hoffman EP. Somatic mosaicism due to a reversion variant causing hemi-atrophy: a novel variant of dystrophinopathy. *Eur J Hum Genet.* 2016;24(10):1511-1514. https://doi.org/10.1038/ejhg.2016.22

25. Zimowski J, Fidziańska E, Holding M, Zaremba J. Two mutations in one dystrophin gene. *Neurol Neurochir Pol.* 2013;47(2):131-137. https://doi.org/10.5114/ninp.2013.34586

26. Flanigan KM, Dunn DM, Von Niederhausern A, et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat.* 2009;30(12):1657-1666. https://doi.org/10.1002/humu.21114

27. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet.* 2014;46(3):310-315. https://doi.org/10.1038/ng.2892

28. Mohammed F, Elshafey A, Al-Balool H, et al. Mutation spectrum analysis of Duchenne/Becker muscular dystrophy in 68 families in Kuwait: the era of personalized medicine. *PLoS One.* 2018;13(5): e0197205. https://doi.org/10.1371/journal.pone.0197205

29. O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet (London, England).* 2009;373(9678):1891-1904. https://doi.org/10.1016/S0140-6736(09)60327-5

30. Prior TW. Carrier screening for spinal muscular atrophy. *Genet Med.* 2008;10(11):840-842. https://doi.org/10.1097/GIM.0b013e318188d069

31. Deignan JL, Astbury C, Cutting GR, et al. CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2020;22(8):1288-1295. https://doi.org/10.1038/s41436-020-0822-5

32. Sukenik-Halevy R, Ludman MD, Ben-Shachar S, Raas-Rothschild A. The time-consuming demands of the practice of medical genetics in the era of advanced genomic testing. *Genet Med.* 2016;18(4): 372-377. https://doi.org/10.1038/gim.2015.96

33. Beckers P, Caberg J.-H, Dideberg V, et al. Newborn screening of Duchenne muscular dystrophy specifically targeting deletions amenable to exon-skipping therapy. *Sci Rep.* 2021;11(1):3011. https://doi.org/10.1038/s41598-021-82725-z

34. Chrzanowski SM, McAnally MM, Kang PB. An opportune time for newborn screening in Duchenne muscular dystrophy. *JAMA Neurol.* 2021;78(8):901-902. https://doi.org/10.1001/jamaneurol.2021.1782

35. Vita GL, Vita G. Is it the right time for an infant screening for Duchenne muscular dystrophy? *Neurol Sci.* 2020;41(7):1677-1683. https://doi.org/10.1007/s10072-020-04307-7

36. Wood MF, Hughes SC, Hache LP, et al. Parental attitudes toward newborn screening for Duchenne/Becker muscular dystrophy and spinal muscular atrophy. *Muscle Nerve.* 2014;49(6):822-828. https://doi.org/10.1002/mus.24100

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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