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ORIGINAL ARTICLE

Matrilineal analysis of mutations in the *DMD* gene in a multigenerational South Indian cohort using *DMD* gene panel sequencing

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Abstract

Background: Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder characterised by progressive irreversible muscle weakness, primarily of the skeletal and the cardiac muscles. DMD is characterised by mutations in the dystrophin gene, resulting in the absence or sparse quantities of dystrophin protein. A precise and timely molecular detection of *DMD* mutations encourages interventions such as carrier genetic counselling and in undertaking therapeutic measures for the DMD patients.

Results: In this study, we developed a 2.1 Mb custom *DMD* gene panel that spans the entire *DMD* gene, including the exons and introns. The panel also includes the probes against 80 additional genes known to be mutated in other muscular dystrophies. This custom *DMD* gene panel was used to identify single nucleotide variants (SNVs) and large deletions with precise breakpoints in 77 samples that included 24 DMD patients and their matrilineage across four generations. We used this panel to evaluate the inheritance pattern of *DMD* mutations in maternal subjects representing 24 DMD patients.

Conclusion: Here we report our observations on the inheritance pattern of *DMD* gene mutations in matrilineage samples across four generations. Additionally, our data suggest that the *DMD* gene panel designed by us can be routinely used as a single genetic test to identify all *DMD* gene variants in DMD patients and the carrier mothers.

KEYWORDS

DMD gene inheritance, *DMD* gene mutation profiling, *DMD* gene panel testing, matrilineal inheritance, molecular diagnostics, NGS

Arun Shastry, Sankaramoorthy Aravind, and Meeta Sunil equally contributed to this work.

1 | INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is one of the most common and devastating neuromuscular genetic disorders affecting male children (Moser, 1984). DMD is an X-linked recessive form of genetic disorder primarily characterised by mutations in the *DMD* gene. The symptoms manifest early between 3 and 5 years of age and progress to a more severe muscle weakening condition over the years leading to loss of ambulation (Forst & Forst, 2012). The affected individuals are wheelchair-bound by the age of 7–12 and subsequently develop severe cardiac and respiratory dysfunction in their teens, limiting their life expectancy to about 20–30 years (Passamano et al., 2012).

DMD (OMIM: 300377) spans a length of ~2.5 Mb and comprises 79 exons and 78 introns. The *DMD* gene generates multiple transcripts encoding various dystrophin isoforms (e.g. lymphocyte dystrophin, cortical dystrophin, Purkinje dystrophin, foetal dystrophin, retinal dystrophin, muscle dystrophin, etc.). The muscle-specific isoforms transcribes a ~14-kb long processed RNA that encodes a ~427-kDa protein called 'Dystrophin' (Górecki et al., 1992; Koenig et al., 1988; Monaco et al., 1986). It is primarily expressed in skeletal, cardiac, and smooth muscles where it functions as a structural unit bridging both the internal actin cytoskeleton and the external sarcolemma forming a dystrophin–glycoprotein complex (Gao et al., 2016) that contributes to the structural integrity of the muscle fibre cells.

A recent survey of literature for the global incidences of DMD has estimated the pooled global DMD prevalence at 7.1 cases (95% CI: 5.0–10.1) per 100,000 males and 2.8 cases (95% CI: 1.6–4.6) per 100,000 in the general population, while the pooled global DMD birth prevalence at 19.8 cases (95% CI:16.6–23.6) per 100,000 live male births (Crisafulli et al., 2020). Whereas about 60–65% of the cases result from large deletions of one or more *DMD* exons (Dunnen, 1989; Elhawary et al., 2018; Koenig et al., 1988), 20% are caused by single-nucleotide variations (SNV) including frameshift, nonsense, missense, and indel mutations (Aartsma-Rus et al., 2006; Grimm et al., 2012) and ~11% by duplications (Aartsma-rus et al., 2016; White et al., 2006). Generally in-frame mutations cause a less severe form of muscular dystrophy, known as Becker muscular dystrophy (BMD), whereas frame-shift mutations lead to a more severe DMD phenotype (Muntoni et al., 2003). Majority of the mutations are frame-shift alterations that result in a prematurely truncated non-functional and unstable form of 'Dystrophin' protein product encoded by the *DMD* gene (Monaco et al., 1988).

The mutation rate in *DMD* is estimated to be higher than any other X-linked disorder (Winter & Pembrey, 1982), probably due to the size of the *DMD* gene. The occurrence of *DMD* mutation can be due to maternal inheritance, a *de novo* event, or due to germline mosaicism. Globally several independent studies have been conducted to estimate the exact incidence of inherited and *de novo DMD* mutations and have reported a 16%–35% frequency of *de novo DMD* mutations (Aartsma-Rus et al., 2012; Barbujani et al., 1990; Caskey et al., 1980; Garcia et al., 2014; Haldane, 1935). So far no major genetic studies to understand inheritance patterns of *DMD* in South Asian context have been conducted. Moreover, to understand the inheritance of *DMD* gene variations, it is necessary to identify the exact genomic breakpoint to ascertain if the mutation is inherited or a *de novo* event involving the same exons.

As most common causal mutations for *DMD* are large deletions/duplications (copy number variation-(CNV)), multiplex ligation-dependent probe amplification (MLPA) is the current preferred diagnostic method (Abbs et al., 2010; Bushby et al., 2010; Falzarano et al., 2015). MLPA is a multiplex PCR-based method that can detect deletions and duplications in patients or carriers (Lalic et al., 2005; Verma et al., 2012). Even though MLPA is a cost-effective method for diagnosis of CNV alterations in the *DMD* gene, it cannot give the genomic breakpoints of the deletion or duplication event. Also, MLPA has a higher propensity to miss small indels (<20 bp) and cannot detect single-nucleotide variants (SNVs) and deep intronic mutations (Aartsma-rus et al., 2016; Prodduturi et al., 2018). Patients showing symptoms but negative for *DMD* mutation using MLPA are tested using NGS techniques to detect SNVs or other small indels. Although the current NGS-based methods can detect exonic mutations and deletions, these cannot detect deep intronic variants or the associated genomic breakpoints deep inside the intronic regions.

A single comprehensive genetic test that can detect all mutation types in the *DMD* gene would be an effective molecular diagnostic tool. It will provide a more accurate assessment of *DMD* carrier mutation prevalence in population. Here, we designed a *DMD* gene panel using set of oligonucleotide capture probes that spans the entire *DMD* gene including its 5′ UTR, 3′ UTR, 79 exons, and the corresponding 78 introns. The panel also comprised 80 additional genes associated with different muscular dystrophies.

As a proof of concept for studying the inheritance of *DMD* genes across generations, we collected the matrilineal samples across four generations of 24 clinically confirmed DMD patients. We used the probe set for capture of the genomic region encoding *DMD* and sequenced it to detect mutations in these 24 DMD patients and their matrilineage.

Here we report the observations on the performance of the newly designed comprehensive *DMD* gene panel and inheritance of *DMD* gene variants in the families of the 24 confirmed DMD patients.

2 | MATERIALS AND METHODS

2.1 | Custom *DMD* gene panel

A total 17,409 lockdown probes (2.08 Mb) covering the complete *DMD* gene (NC_000023.11) including introns, exons, 3′ and 5′ UTR regions (12800 probes), and exonic regions from an additional 80 genes (Table S2) associated with myopathy (4,609 probes) were designed and synthesised from Integrated DNA Technologies (IDT), USA.

2.2 | Study design

This study was conducted in accordance with the ICMR National Ethical Guidelines for Biomedical and Health Research. The study was approved by the DART Institutional Ethics committee and informed consent was obtained from the patients as well as the family members (IRB #Ec/DART/002). The study comprises 24 patients from 22 unrelated families (FM-01 to FM-08, FM-10 to FM-18, and FM-20 to FM-24). Overall, our cohort had 77 subjects including the patients/probands (P-1 to P-8, P-10 to P-18, and P-20 to P-24) and siblings (B: brother, S: sister) from the current generation, and members of the maternal lineage (M: mother, GM: grandmother, and GGM: great grandmother; Table S1). All probands in the study were diagnosed with DMD based on established diagnosis criteria that included difficulty in walking, walking on toes, scoliosis, and frequent falls (Archer et al., 2016). Patients in our studies have previously been confirmed positive for a *DMD* mutation by MLPA/mPCR and/or TrueSight gene panel, Illumina (Table 1 and Table S4).

2.3 | DNA isolation, exome library preparation, and sequencing

Genomic DNA was isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen) and quantified using Qubit fluorometry (Thermo Fisher Scientific). For library preparation, 200 ng of the Qubit quantified DNA was fragmented to 180–220 bp inserts. The fragments were then end-repaired, 3′ adenylated, and ligated with the indexed adapters. The adapter-ligated fragments were then amplified with adapter-specific

TABLE 1 Comparison of the MLPA and NGS analyses of the probands

Family ID	Sample ID	Sample name	MLPA result	DMD gene panel result	Exons affected
FM-01	P-1	DMD2	Deletion	Deletion	46–55
FM-02	P-2	DMD22	Deletion	Deletion	33–45
FM-03	P-3	DMD12	Deletion	Deletion	46–50
FM-04	P-4	DMD21	No Variation	SNV	7
FM-05	P-5	DMD3	Deletion	Deletion	46–48
FM-06	P-6	DMD1	Deletion	Deletion	46–49
FM-07	P-7	DMD17	Deletion	SNV	21
FM-08	P-8a	DMD15	Deletion	Deletion	51
FM-08	P-8b	DMD16	Deletion	Deletion	51
FM-10	P-10	DMD36	Deletion	Deletion	1
FM-11	P-11	DMD38	Deletion	Deletion	45–50
FM-12	P-12	DMD40	Deletion	Deletion	35–45
FM-13	P-13	DMD8	Deletion	Deletion	46–47
FM-14	P-14	DMD20	No Variation	SNV	51
FM-15	P-15	DMD6	Deletion	Deletion	18–29
FM-16	P-16	DMD5	Deletion	Deletion	8–9
FM-17	P-17a	DMD4	Deletion	Deletion	45–52
FM-17	P-17b	DMD7	Deletion	Deletion	45–52
FM-18	P-18	DMD14	Deletion	Deletion	51
FM-20	P-20	DMD11	Deletion	Deletion	48–52
FM-21	P-21	DMD9	Deletion	Deletion	49–50
FM-22	P-22	DMD10	Deletion	Deletion	49–50
FM-23	P-23	DMD82	Deletion	Deletion	48–54
FM-24	P-24	DMD18	Deletion	SNV	46

Abbreviation: SNV, single nucleotide variant.

primers followed by size selection and purification to generate gDNA library. Biotin-labelled probes were custom designed (IDT) to capture all the introns and exons of the *DMD* gene. The probes were hybridised with the gDNA library for 4 hours at 65°C. Post incubation, the hybridised libraries were captured using Streptavidin M270 beads (Thermo Fisher Scientific) followed by stringent washes to remove unbound DNA molecules. The enriched libraries were amplified and purified. The final library was assessed for fragment size distribution using TapeStation (Agilent) and was quantified using Qubit (Thermo Fisher Scientific) for sequencing. The quantified libraries were sequenced on Illumina HiSeq 4000 to generate100 bp paired end sequences.

2.4 | Data processing and germline variant calling

Quality check (QC) and adapter trimming for the sequenced reads were performed using fastq-mcf (version 1.04.676; https://expressionanalysis.github.io/ea-utils/). The pre-processed reads were aligned to the reference human genome (GRCh38) downloaded from UCSC Database (Meyer et al., 2013) using BWA-mem (Li & Durbin, 2010). Aligned read processing and variant calling steps were performed using the Sentieon version of GATK (Weber et al., 2016). Briefly, the aligned reads were sorted and duplicate reads were removed. The reads were then realigned around the known indels from 1000 Genome Project (downloaded from ftp://ftp.broadinstitute.org/distribution/gsa/gatk_resources.tgz) for improving the accuracy of indel calls. Base quality score recalibration (BQSR) was done using known variants from dbsnp (version 149). Germline variant calling was performed using the Haplotyper command in Sentieon (Weber et al., 2016).

2.5 | Structural variant identification

The large deletions were predicted using SoftSV (version 1.4; Bartenhagen & Dugas, 2016) based on split reads and discordant reads, and with ExomeDepth (ED, version 1.1.10; Plagnol et al., 2012) based on depth of coverage from targeted sequencing experiments.

2.6 | Variant annotation

Deep annotation of the variants identified above was done using a custom pipeline that utilises VEP (McLaren et al., 2016) programme and gene models downloaded from Ensembl database (release 84; ftp://ftp.ensembl.org/pub/release-84/gtf/homo_sapiens). The pipeline further includes annotation of variants into genes, repeats, genic features (exon, intron, 5′ UTR, 3′ UTR, coding-region, splice-site), transcripts, variant classes (silent, missense, nonsense, stop-loss, start-loss, in-frame, frameshift, etc.), known variants reported in public repositories (ExAC, 1000G, dbSNP, EVS, TwinsUK, JAP1000G, MedGenome-GermlineVariantDatabase), and pathogenicity classes based on known pathogenic variants (from ClinVar, HGMD, SwissVar) and prediction databases (PolyPhen, SIFT, MutationTaster, MutationAssessor, LRT and others). The large deletions were annotated using Bedtools (version 2.17.0) and exon coordinates of the *DMD* gene. All shortlisted variants in the *DMD* gene were inspected using Integrated Genomics Viewer (IGV; version 2.3.92; Robinson et al., 2011) and confirmed.

3 | RESULTS

3.1 | Custom *DMD* gene panel

To identify *DMD* mutations including deletions, duplications, SNVs, and small indels across both exonic and intronic regions, we designed capture probes across the entire gene. Additionally, we included probes to capture exonic regions corresponding to 80 myopathy-related genes in this targeted probe set. The probes directly correspond to 73.34% of the *DMD* gene and have the potential to capture DNA fragments that will cover the entire genomic regions corresponding to the entire *DMD* gene (Figure 1a). We tested the performance of the probe set using DNA from four healthy individuals. We generated ~500 Mb of data for each sample with >85% of bases passing the quality threshold of Q30 (Figure 1b,c). Overall, 79.54% of the *DMD* gene was captured by our panel, of which >94% was sequenced at an average depth of >200X (Figure 1d). Other 80 myopathy-related genes captured by our probe set were also covered on an average of 34.4% (data not shown).

We next tested the *DMD* gene panel on 77 individuals including 24 DMD patients from 22 unrelated families (Figure 2a, Figure S2). Ninety-three percent of the data generated passed the Q30 Phred score (Figure S1a; Table S3) and 99.9% mapped to the genome with a median read duplication rate of 15% (Figure S1b; Table S3). For the ~80% captured *DMD* gene, we obtained a median panel coverage and panel depth of 91.6% and 149.5X, respectively, for the DMD probands. For the remaining samples, we obtained the coverage of 94.5% and depth of 240X (Figure S1c,d; Table S3). The lower *DMD* gene coverage in probands was due to large deletions in the gene (Figure 3d).

The deletions identified by the *DMD* gene panel were in concordance with the MLPA performed on these patients. Our *DMD* gene panel identified SNVs leading to termination mutation in four patients, where MLPA either identified no variation or a deletion (Table 1; Figure 2b). Twenty out of

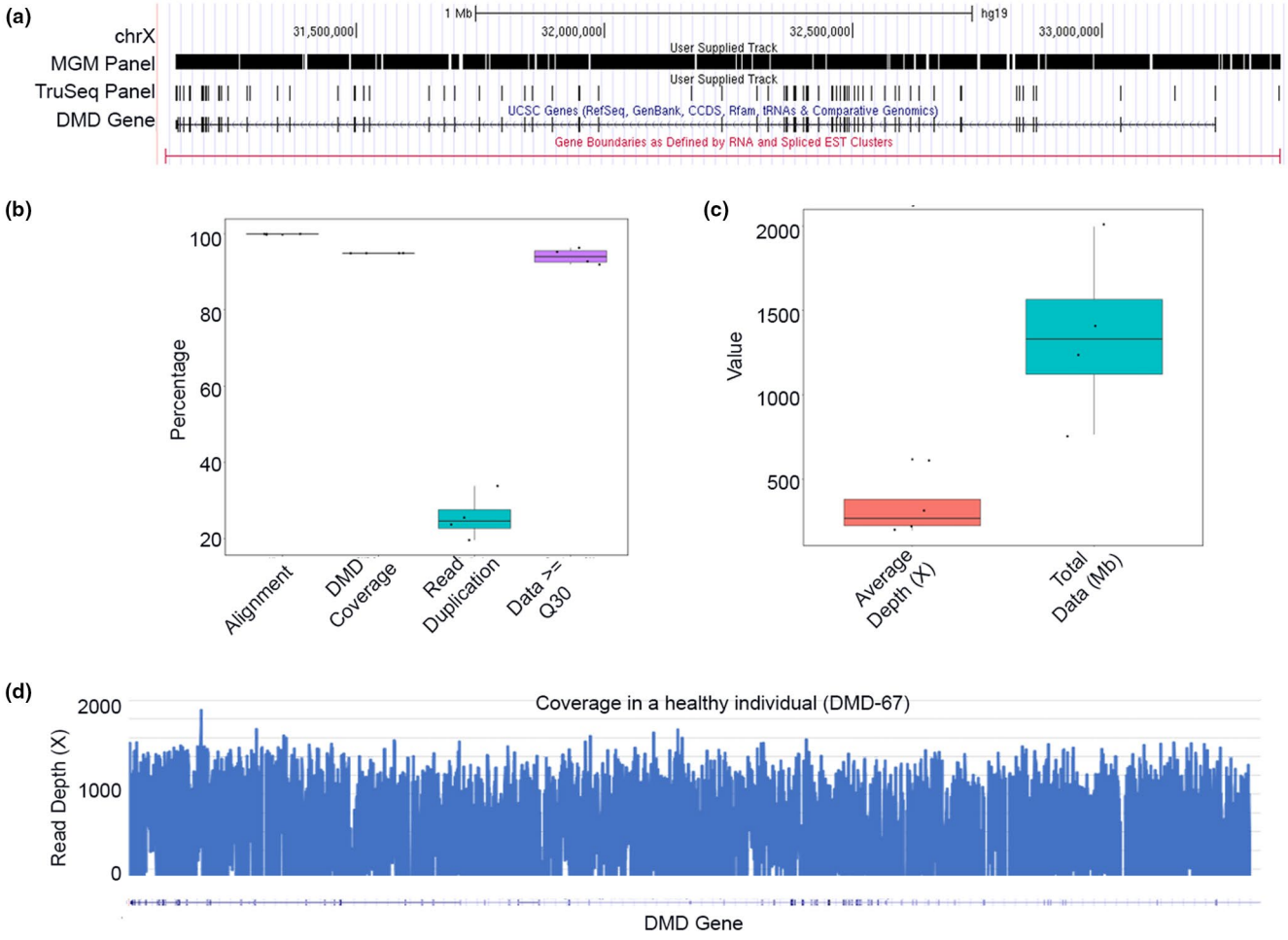

FIGURE 1 Panel design and validation: (a) *DMD* gene coverage of *DMD* gene panel (MGM panel) and Illumina TrueSight panel (TRUPNL). (b) QC metrics including on-target alignment, panel coverage, duplication in sequenced reads, raw sequenced data above Q30, (c) average sequencing depth, and total amount of data obtained from four healthy individuals for panel validation. (d) Uniform depth of sequenced data across the *DMD* gene from a healthy individual

24 patients in this cohort were previously sequenced using Illumina True Sight panel (Aravind et al., 2019). Comparison of the *DMD* mutations identified by our probe set and the Illumina TrueSight panel showed complete concordance in identifying the exon deletions and SNVs. However, the Illumina TrueSight panel was not able to precisely identify the genomic breakpoints of deletions and underestimated the length of large deletions (Figure 3; Table S4) in a majority of the cases as the Illumina TrueSight panel, unlike our probes, did not capture the entire *DMD* gene.

3.2 | *DMD* mutations in the cohort

Analysis of data from family members of the proband samples using our *DMD* probe set allowed us to understand the distribution and maternal inheritance of *DMD* mutations in our cohort. Analysis of the *DMD* gene for pathogenic genetic alterations (SNPs, indels, large deletions) showed both maternally inherited (13 probands, 54%) and *de novo* (11 probands, 46%) mutations (Table S5). The majority of *DMD* gene loss-of-function mutations detected in our cohort was due to large/single-exon deletions (83.3%; Figure 4a). Single-exon deletions were detected in 16.7% and multi-exon deletions in 66.7% of the proband samples. We found point mutation and a single-base indel in four proband samples (16.7%; Figure 4a).

3.3 | Inherited mutations

Eleven out of the 22 analysed families showed maternally inherited *DMD* gene mutations (Table S5). These mutations were hemizygous in the proband and heterozygous in the mother and other maternal subjects from the family. Nine of these inherited mutations were large deletions whereas two families showed single-nucleotide variations (SNV; Figure 4b).

Of the total 11 probands with large, maternally inherited *DMD* deletions, we found 18.2%, 45.4%, 27.3%, and 9.1% to have 1 exon, 2–5 exons, 6–10 exons, and >10 exons

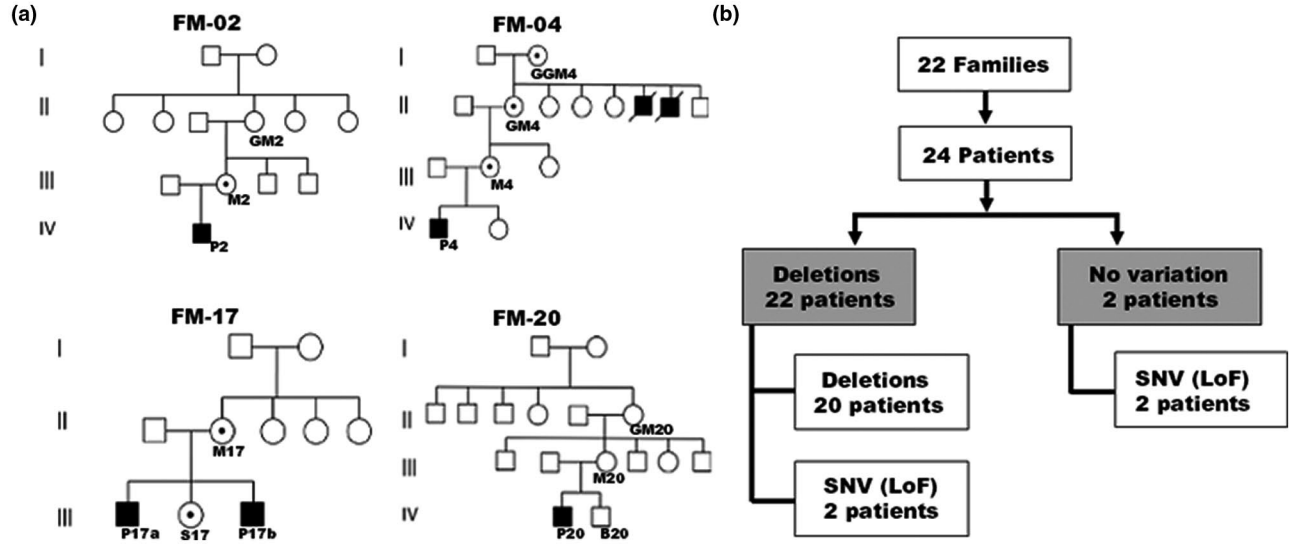

FIGURE 2 Overview of families and *DMD* mutation analysis of DMD patients using MLPA and NGS: (a) Four representative pedigrees (FM-02, FM-04, FM-17, and FM-20) showing the spectrum of samples analysed. Circles represent the female and the squares represent the male. Filled squares represent the affected individuals whereas the circle with black dot represents the disease carrier. Samples collected for genetic analysis are labelled as Proband (P), Brother (B), Sister (S), Mother (M), Grandmother (GM), or Great grandmother (GGM). (b) Comparative analysis of MLPA and NGS results in 24 affected individuals. Grey box shows the results obtained by MLPA, and the white boxes show the results obtained by the *DMD* gene panel

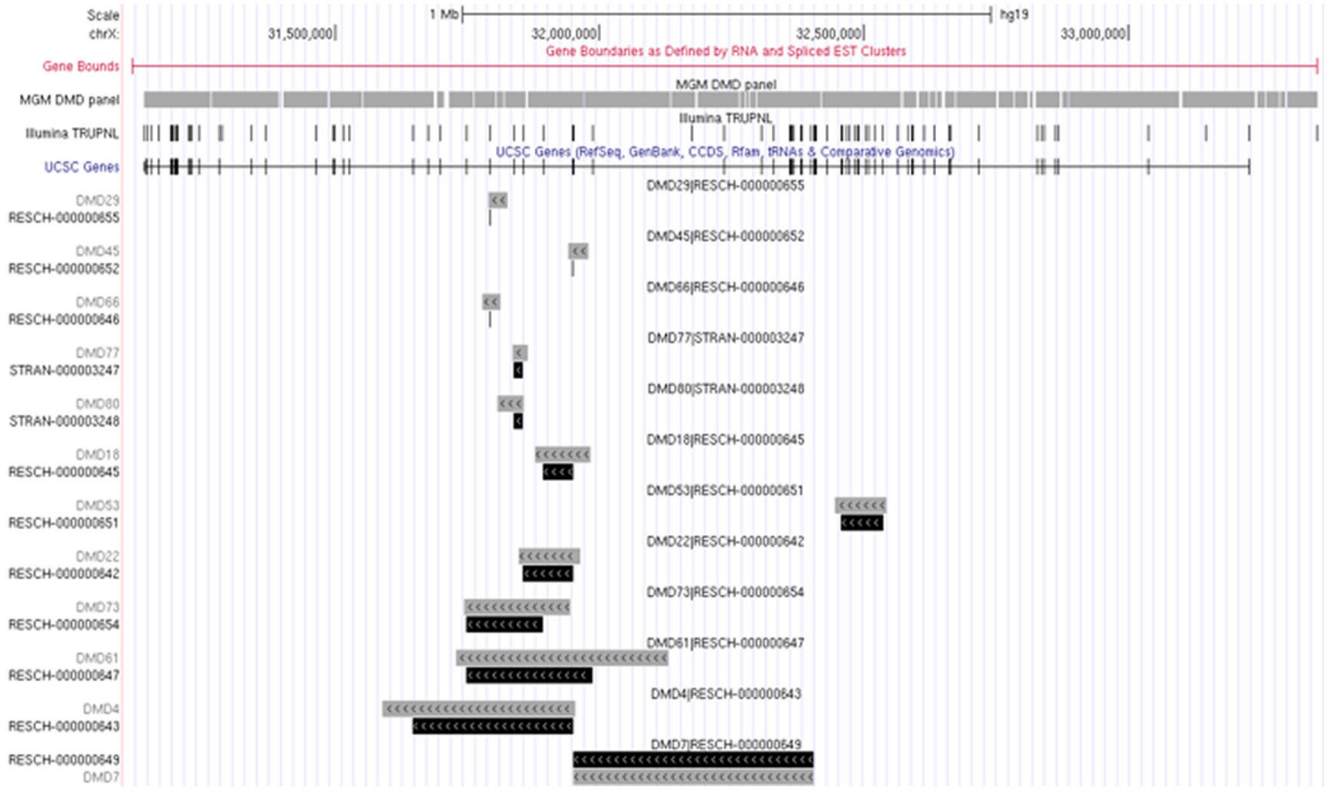

FIGURE 3 Comparison of the *DMD* custom panel with the TrueSight panel: Comparison of *DMD* mutation resolution on 12 proband samples using *DMD* gene panel (MGM *DMD* panel) vs Illumina TrueSight Panel (Illumina TRUPNL). Deletions identified by TrueSight panel are shown in black boxes, and those identified by *DMD* gene panel are shown in grey boxes

long deletions, respectively (Figure 3c,d). Single-exon deletions were detected in two siblings (P-8a and P-8b) who displayed a maternally inherited deletion of exon 51. Multiple-exon deletions were found to be inherited in eight families (FM-02, 03, 05, 06, 11, 17, 22, and 23; Table S5; Figure 3d). These deletions were found between exon 33

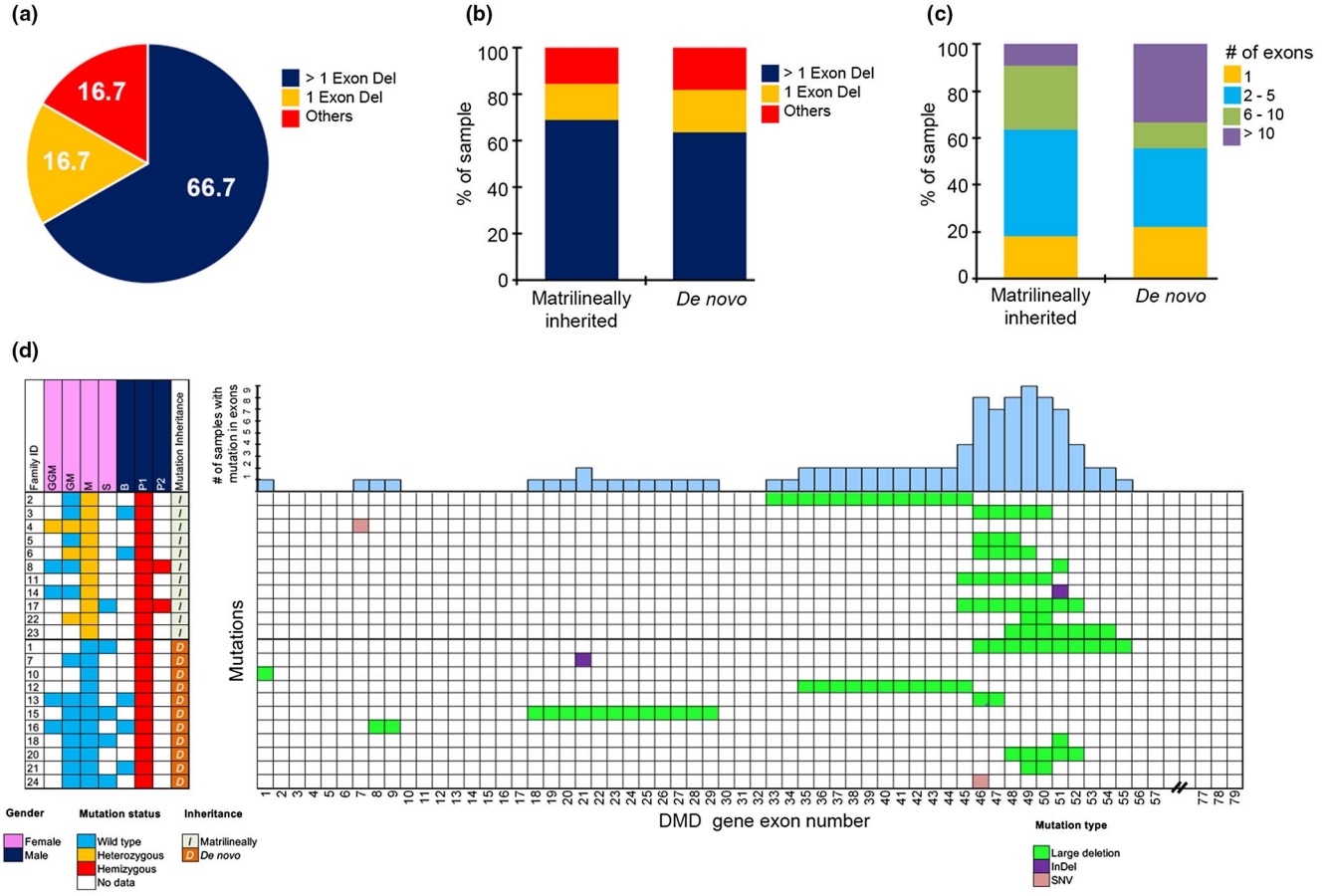

FIGURE 4 Overview of the mutations in the cohort: (a) Percentage of SNVs, single-exon deletions and large deletions identified in the cohort. (b) Percentage of sporadic (*de novo*) and maternally inherited mutations identified in the cohort. (c) Differential number of exons mutated in the *de novo* and inherited cases within the cohort. (d) Heatmaps summarising the different *DMD* mutations (by type, inheritance, length and frequency) across the cohort

and 54. The largest deletion spanning 13 exons from exon 33 to 45 was found in FM-02. Deletions in four (FM-02, 03, 05, and 08) out of nine families were found only in the mother's (M) sample whereas the grandmother (GM) or great grandmother (GGM) did not show any detectable variants.

We detected maternally inherited SNV mutations in two families: FM-04 and FM-14 (Table S5; Figure 3d). In FM-04, we identified a nonsense point mutation whereas in FM-14, a frameshift (one base insertion) mutation was detected. In proband P-4, the non-sense mutation arising from base substitution c.583C>T (p. Arg195Ter) in exon7 was found to be inherited across three generations from GGM-4, resulting in a premature stop codon and suggesting a very strong inheritance pattern in FM-04 (Figure 2a). Other male members in this family from the maternal lineage were also reported to be affected by DMD. In P-14, a frameshift mutation with an insertion of 'G' in exon 51 at c.6979dupG (protein change: p. Val2327GlyfsTer3) was confirmed to be inherited from the mother, M-14, whereas the grandmother, GM-14, did not carry any *DMD* mutation (Figure S2).

3.4 | *De novo* mutations

We detected *de novo DMD* mutations in the proband from 11 of the 22 families in this cohort. Probands in nine families had large or single-exon deletions whereas those in two other families had SNVs. All the other maternal subjects in the family did not have any *DMD* mutations (Table S5).

Single-exon deletion was detected in P-10 (5′UTR and exon 1) and P-18 (exon 51) whereas large deletions were identified in P-1 (exons 46–55), P-12 (exons 35–45), P-13 (exons 46–47), P-15 (exons 18–29), P-16 (exons 8–9), P-20 (exons 48–52), and P-21 (exons 49–50). The largest *de novo* deletion of exon 18–29 was detected in P-15, which has not been reported previously. The distribution of the *de novo* large deletions across the exons was random across the two-thirds of the *DMD* gene and did not follow any

TABLE 2 Distribution of mutations among the study group with respect to the exonic regions of the DMD gene

Exonic regions	Total no of cases	%	Familial	%	*de novo*	%
Proximal hot spot (exons 1–20)	4	17.3913	1	4.34783	3	13.04347826
Distal hot spot (exons 45–55)	15	65.2174	9	39.1304	6	26.08695652
Others	4	17.3913	1	4.34783	3	13.04347826

specific pattern that could be explained as *de novo* mutation hotspot.

Only one *de novo* SNV and one single-base deletion in probands from two families were detected. Proband P-7 had a novel indel in exon 21 at c.2293_delG (p. Glu765AsnfsTer3) whereas proband P-24 had a nonsense mutation at c.6598G>T (p. Glu2200Ter).

3.5 | Distribution of mutations in the *DMD* gene

The distribution of the mutations irrespective of their type was analysed based on the mutational hotspot regions (Table 2). Sixty-five percent of the mutations were found to be in the distal hotspot region between exons 45 and 55, 17% in the proximal region between exons 1 and 20, and the remaining 17% in the mid-region between exons 18 and 45. We also checked the distribution of mutations in the different hotspot regions in the inherited and *de novo* cases. In the inherited mutational group, 9% (1/11) were found to occur in the proximal region, 82% (9/11) in the distal region, and 9% (1/11) in the mid-region, whereas in the *de novo* cases, the distribution of mutations was 25% (3/12) in the proximal region, 50% (6/12) in the distal region, and 25% (3/12) in the mid-region. However, distribution of point mutations with respect to the region was random and was found to be scattered among the distal and the proximal hotspot regions. No mutation was detected beyond exon 55 in the *DMD* gene.

4 | DISCUSSION

Recently several novel therapies targeting specific *DMD* mutations (including stop-codon read-through agents, exon-skipping antisense oligonucleotides (AONs) etc.) that target and restore Dystrophin function (Aartsma-Rus et al., 2009; Babbs et al., 2020; Haas et al., 2015; Laing et al., 2011; Ousterout et al., 2015; Reinig et al., 2017) have been developed. An early and accurate molecular diagnosis is critical for most of these therapies (Aartsma-rus et al., 2016). Similarly, prenatal testing and carrier screening for high-risk mothers along with genetic counselling will play an important role in reducing the socioeconomic burden of the DMD disorder. The current protocol for carrier screening and molecular diagnosis involves a two-step process where MLPA and multiplex PCR is first used to identify pathogenic deletions or duplications in the *DMD* gene followed by NGS based methods to identify SNVs and indels in the cases where *DMD* gene mutation is not found by MLPA/mPCR. This increases the cost and diagnosis time. With reduced costs and advances in NGS technology, targeted sequencing of the entire *DMD* gene will be a more efficient method for identifying *DMD* mutations. Previously, others have used *DMD* panels (amplicon/probe based) to detect *DMD* mutations on NGS platforms using the panels that target *DMD* exons primarily (Lim et al., 2011; Wei et al., 2014) whereas some have attempted to look at the whole gene for mutations (Ebrahimzadeh-Vesal et al., 2018; Wang et al., 2014; Zhang, Yang, et al., 2019). Wang et al., (2014) has used the commercially available *DMD* whole gene enrichment kit (MyGenostics Inc.), which, like our probes set, covers all the *DMD* introns, exons, and the promoter region.

In this study, we developed a comprehensive 2.1 Mb target *DMD* gene capture panel that covers the complete *DMD* gene including all the exons and introns. The panel covers 73.3% of whole *DMD* gene with uniform depth. Compared to the *DMD* whole gene enrichment kit from MyGenostics Inc. used by Wang et al., (2014), our *DMD* gene panel also covers the exons of other 80 muscular dystrophy genes and hence provides an additional advantage for its use in any of the muscular dystrophies. Furthermore, the design of this panel allows us to add on additional probes covering other modifier genes like *LTBP4*, which is believed to accelerate muscle regeneration and alleviate muscle fibrosis by reducing TGF-β signalling. Phosphatidylinositol transfer protein α (PITPNA) and Jagged1 that ameliorate the pathology of DMD, *Anxa6* that encodes annexin A6, a calcium-binding protein that regulates the injury pathway, and sarcolemmal resealing, decreased expression of *TCTEX1D1* could be deleterious for the cardiac phenotype in patients with DMD and Osteopontin; encoded by the *SPP1* gene which plays a role in DMD pathology modulating muscle inflammation and regeneration (Chen et al., 2020; Spitali et al., 2020; Vieira et al., 2015). We tested this panel on a set of 24 confirmed DMD patients from 22 families and the members from their matrilineage for up to four generations. We

found that our panel accurately detected the *DMD* mutations. It also identified the mutation types and exact breakpoint involved in *DMD* gene deletions.

Most of the studies undertaken so far, for understanding the carrier status and *DMD* inheritance, have only used the mother and the affected proband samples (Grimm et al., 1994; Helderman-Van Den Enden et al., 2013; Mohammed et al., 2018; Kumar et al., 2020; Zhang, Ma, et al., 2019). With the samples from up to four generations, our cohort provided a unique opportunity to understand the maternal inheritance of *DMD* mutations. Eleven out of 22 families showed maternal inheritance. Five of the inherited *DMD* mutations were only present in the mother and the proband samples, whereas three of these mutations were inherited from maternal grandmother and great grandmother. Mutations in the carrier mothers originate during gametogenesis due to replication errors. Previous studies have shown that the large deletions are predominantly introduced during oogenesis while point mutations/duplications arise during spermatogenesis (Claustres et al., 1990; Essen et al., 1992).

It is important to note that even when the mother is not a carrier, it is possible that she may have germline mosaicism for the mutation and is still at a risk of bearing a second male child with DMD (Bakker et al., 1987). Earlier reports have shown that the recurrence risk of DMD in siblings from non-carrier mothers due to germline mosaicism varies between 14% and 20% (Bakker et al., 1989; Essen et al., 1992). However, a large-scale study on 272 families with DMD has estimated the recurrence disease risk due to germline mosaicism to be 8.6%, suggesting the requirement for assessing the carrier risk for the female members of the families with *de novo* mutation (Helderman-van den Enden et al., 2009). We observed that five (FM-02, 03, 05, 08, and 14) out of eleven families with inherited *DMD* variants (deletions or SNV) showed the variants only in the proband and the mother's samples, whereas no detectable *DMD* variants were found on GM or GGM samples. This could be due to the germline mosaicism in the GM or GGM samples or due to paternally derived mutations during spermatogenesis. Furthermore, siblings of probands with de novo mutation from five independent families did not carry the *DMD* mutation. This supports a relatively low risk of recurrence of *DMD* mutations in siblings of affected children from a non-carrier mother as reported previously; however, a larger number of families need to be screened to precisely understand the rate of recurrence due to germline mosaicism.

In our cohort, we did not see any correlation between the carrier status and the type of mutation. This is consistent with previous studies using large study groups where no significant correlation was observed between carrier frequency, type, and the site of mutations (Lee et al., 2014).

In agreement with the previous reports, a majority of exon deletions (65%) in our cohort were detected at the distal region of the *DMD* gene, suggesting a high rate of mutation at distal region as a hotspot for deletion mutations (Passos-Bueno et al., 1992). However, it was interesting to note that three large deletions (FM-15, FM-12, and FM-02) were detected outside the hotspot region. We did not see any hotspot region for SNVs and small indels.

All the inherited *DMD* loss of function mutations detected in our cohort has already been reported earlier. We identified two *de novo* mutations (exons 8–9 in family FM-16 and single-base deletion in exon 21 of family FM-07).

Each brother in 2 of these 11 families, FM-08 and FM-17, carried the same mutation (inherited). Both the brothers in FM-08 (P-8a and P-8b) showed loss of ambulation at the age of 9. In FM-17, one of the affected siblings (P-17a) had loss of ambulation at age 14, whereas the other affected sibling is still able to walk at the age of 11. Except for these two pairs of siblings, we did not see any correlation between the type of mutation and severity of clinical phenotype (early or late loss of ambulation).

It is vital to fully map the intronic breakpoints in *DMD* whole-exon deletion cases, especially cases that show atypical phenotypes as some intronic sequences in the *DMD* gene serve important regulatory roles that come into action during pre- and post-mRNA splicing. Such deletions that affect functional regions could have negative consequences for the patient's phenotype (Keegan, 2019; Muntoni et al., 2003). Precise mapping of intronic mutations, which is often missed by current diagnostic methods, will allow a better understanding of the disease (Keegan, 2019; Neri et al., 2020). It has been shown that intronic variants can lead to novel cryptic exons either by inserting a splice site leading to aberrant splicing or by creating a novel exonic splicing enhancer (ESE) site that will lead to aberrant exons (Neri et al., 2020; Trabelsi et al., 2014). Hence, precise identification of deletion/duplication breakpoints in both introns and exons or SNVs is necessary for designing efficient AON-mediated exon-skipping therapies. Our *DMD* gene panel has the potential to comprehensively detect precise breakpoints and SNVs in *DMD* exons and the introns.

Several clinical trials for AONs targeting single exons are ongoing, and few have been approved by the FDA for treatment. It has been estimated that approximately 70% of patients with deletions can be treated by single-exon skipping (Aartsma-Rus & Corey, 2020). Furthermore, it has been estimated that a larger number of patients carrying *DMD* duplications and nonsense mutations can be treated if multiple exon skipping is achieved (Aslesh et al., 2018; Dzierlega & Yokota, 2020).

Overall, our data presented here show that the panel described in this study can be used clinically for cost-effective,

precise molecular diagnosis and carrier screening of *DMD* gene mutations in suspected or high-risk subjects. This panel also holds potential to identify novel intronic/exonic SNVs and indels. However, given the small size of our patient cohort, we could not test the efficiency of this panel for analysing duplications. Additional validation of this panel on a bigger sample size and comparing the same with the whole-genome sequencing of *DMD* is required.

Additionally, the unique cohort of 22 families with subjects from up to four matrilineal generations shows that DMD inheritance patterns can vary significantly in the populations. Here, we observed an equal occurrence of the de novo and inherited cases. However, any concrete conclusion on this warrants a much larger study cohort. Further studies with more multigenerational families with DMD-affected individuals need to be undertaken to understand precise rates of inherited and *de novo DMD* mutations in South Asian population.

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CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

AUTHOR CONTRIBUTION

SP, AS, UN, and SS conceived and planned the study. SA, BA, and KR sourced the samples and collected all the necessary clinical data. SA, BA, and KR processed the samples for DNA extractions. NT performed the library prep and sequencing. RVL, SP, MS, and RG did the sample data processing and quality control. MS and RG did all the bioinformatics data analyses. AS, SA, MS, RG, and SP did the data interpretations. SP, SA, MS, and AS wrote the manuscript. SS, UN, SP, and AS reviewed and proofread the manuscript.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

Aartsma-Rus, A., & Corey, D. R. (2020). The 10th Oligonucleotide Therapy Approved: Golodirsen for Duchenne Muscular Dystrophy. *Nucleic Acid Ther.*, *30*, 67–70.

Aartsma-Rus, A., Fokkema, I., Verschuuren, J., Ginjaar, I., Van Deutekom, J., van Ommen, G. J., & Den Dunnen, J. T. (2009). Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Human Mutation*, *30*, 293–299.

Aartsma-rus, A., Ginjaar, I. B., & Bushby, K. (2016). The importance of genetic diagnosis for Duchenne muscular dystrophy. *Journal of Medical Genetics*, *10*, 145–151. https://doi.org/10.1136/jmedgenet-2015-103387

Aartsma-Rus, A., Robinow, M., & Roses, A. D. (2012). The genetic status of mothers of isolated cases of Duchenne muscular dystrophy. *Human Genetics*, *31*, 835–847.

Aartsma-Rus, A., Van Deutekom, J. C. T., Fokkema, I. F., Van Ommen, G. J. B., & Den Dunnen, J. T. (2006). Entries in the Leiden Duchenne muscular dystrophy mutation database: An overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle and Nerve*, *34*, 135–144. https://doi.org/10.1002/mus.20586

Abbs, S., Tuffery-Giraud, S., Bakker, E., Ferlini, A., Sejersen, T., & Mueller, C. R. (2010). Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. *Neuromuscular Disorders*, *20*, 422–427.

Aravind, S., Ashley, B., Mannan, A., Ganapathy, A., & Ramesh, K. (2019). Targeted sequencing of the DMD locus: A comprehensive diagnostic tool for all mutations. *Indian Journal of Medical Research*, *50*, 282–289.

Archer, J. E., Gardner, A. C., Roper, H. P., Chikermane, A. A., & Tatman, A. J. (2016). Duchenne muscular dystrophy: the management of scoliosis. *Journal of Spine Surgery*, *2*, 185–194.

Aslesh, T., Maruyama, R., & Yokota, T. (2018). Skipping multiple exons to treat DMD-promises and challenges. *Biomedicines*, *6*, 1–12. https://doi.org/10.3390/biomedicines6010001

Babbs, A., Chatzopoulou, M., Edwards, B., Squire, S. E., Wilkinson, I. V., Wynne, G. M., Russell, A. J., & Davies, K. E. (2020). From diagnosis to therapy in Duchenne muscular dystrophy. *Biochemical Society Transactions*, *48*, 813–821.

Bakker, E., Broeckhoven, C. V., Bonten, E. J., van de Vooren, M. J., Veenema, H., Hul, W. V., Ommen, G. J. B. V., Vandenberghe, A., & Pearson, P. L. (1987). Germline mosaicism and Duchenne muscular dystrophy mutations. *Nature*, *329*(6139), 554–556. https://doi.org/10.1038/329554a0

Bakker, E., Veenema, H., Den Dunnen, J. T., Van Broeckhoven, C., Grootscholten, P. M., Bonten, E. J., Van Ommen, G. J., & Pearson, P. L. (1989). Germinal mosaicism increases the recurrence risk for 'new' Duchenne muscular dystrophy mutations. *Journal of Medical Genetics*, *26*, 553–559.

Barbujani, G., Russo, A., Danieli, G. A., Spiegler, A. W., Borkowska, J., & Petrusewicz, I. H. (1990). Segregation analysis of 1885 DMD families: Significant departure from the expected proportion of sporadic cases. *Human Genetics*, *84*, 522–526.

Bartenhagen, C., & Dugas, M. (2016). Robust and exact structural variation detection with paired-end and soft-clipped alignments: SoftSV compared with eight algorithms. *Briefings in Bioinformatics*, *17*, 51–62.

Bushby, K., Finkel, R., Birnkrant, D. J., Case, L. E., Clemens, P. R., Cripe, L., Kaul, A., Kinnett, K., McDonald, C., Pandya, S., &

Poysky, J. (2010). Diagnosis and management of Duchenne muscular dystrophy, part 1: Diagnosis, and pharmacological and psychosocial management. *The Lancet Neurology*, *9*, 77–93.

Chen, M., Wang, L., Li, Y., Chen, Y., Zhang, H., Zhu, Y., He, R., Li, H., Lin, J., Zhang, Y., & Zhang, C. (2020). Genetic modifiers of duchenne muscular dystrophy in Chinese patients. *Frontiers in Neurology*, *11*, 1–8.

Claustres, M., Kjellberg, P., Desgeorges, M., Bellet, H., & Demaille, J. (1990). Germinal mosaicism from grand-paternal origin in a family with Duchenne muscular dystrophy. *Human Genetics*, *86*, 241–243.

Crisafulli, S. Sultana, J., Fontana, A., Salvo, F., Messina, S., & Trifirò, G. (2020). Global epidemiology of Duchenne muscular dystrophy: An updated systematic review and meta-analysis. *Orphanet Journal of Rare Diseases*. *15*, 1-20.

Dunnen, J. D. (1989). Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. *American Journal of Human Genetics*, *45*, 835–847.

Dzierlega, K., & Yokota, T. (2020). Optimization of antisense-mediated exon skipping for Duchenne muscular dystrophy. *Gene Therapy*, *27*, 407–416.

Ebrahimzadeh-Vesal, R., Teymoori, A., Aziminezhad, M., & Hosseini, F. S. (2018). Next Generation Sequencing approach to molecular diagnosis of Duchenne muscular dystrophy; identification of a novel mutation. *Gene*, *644*, 1–3. https://doi.org/10.1016/j.gene.2017.12.009

Elhawary, N. A., Jiffri, E. H., Jambi, S., Mufti, A. H., Dannoun, A., Kordi, H., Khogeer, A., Jiffri, O. H., Elhawary, A. N., & Tayeb, M. T. (2018). Molecular characterization of exonic rearrangements and frame shifts in the dystrophin gene in Duchenne muscular dystrophy patients in a Saudi community. *Human Genomics*, *12*(1), 18. https://doi.org/10.1186/s40246-018-0152-8

Caskey, C. T., Nussbaum, R. L., Cohan, L. C., & Pollack, L. (1980). Sporadic occurrence of Duchenne Muscular Dystrophy: evidence for new mutation. *Clinical Genetics*, *18*(5), 329–341. https://doi.org/10.1111/j.1399-0004.1980.tb02293.x

Falzarano, M. S., Scotton, C., Passarelli, C., & Ferlini, A. (2015). Duchenne muscular dystrophy: From diagnosis to therapy. *Molecules*, *20*, 18168–18184. https://doi.org/10.3390/molecules201018168

Forst, J., & Forst, R. (2012). Surgical treatment of Duchenne muscular dystrophy patients in Germany: The present situation. *Acta Myologica*, *31*, 21–23.

Gao, Q. Q., & McNally, E. M. (2015). The dystrophin complex: Structure, function, and implications for therapy. *Compr Physiol.*, 1; *5*(3), 1223–1239. https://doi.org/10.1002/cphy.c140048

Garcia, S., De Haro, T., Zafra-Ceres, M., Poyatos, A., Gomez-Capilla, J. A., & Gomez-Llorente, C. (2014). Identification of de novo mutations of Duchénnè/Becker muscular dystrophies in Southern Spain. *Internation Journal of Medical Sciences*, *11*, 988–993.

Górecki, D. C., Monaco, A. P., Derry, J. M., Walker, A. P., Barnard, E. A., & Barnard, P. J. (1992). Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. *Human Molecular Genetics*, *1*, 505–510.

Grimm, T., Kress, W., Meng, G., & Muller, C. R. (2012). Risk assessment and genetic counseling in families with Duchenne muscular dystrophy. *Acta Myologica*, *31*, 179–183.

Grimm, T., Meng, G., Liechti-Gallati, S., Bettecken, T., Muller, C. R., & Muller, B. (1994). On the origin of deletions and point mutations in Duchenne muscular dystrophy: Most deletions arise in oogenesis and most point mutations result from events in spermatogenesis. *Journal of Medical Genetics*, *31*, 183–186. https://doi.org/10.1136/jmg.31.3.183

Haas, M., Vlcek, V., Balabanov, P., Salmonson, T., Bakchine, S., Markey, G., Weise, M., Schlosser-Weber, G., Brohmann, H., Yerro, C. P., & Mendizabal, M. R. (2015). European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscular Disorders*, *25*, 5–13.

Haldane, J. B. S. (1935). The rate of spontaneous mutation of a human gene. *Genet*, *31*, 317–326. https://doi.org/10.1007/BF02982403

Helderman-van den Enden, A. T. De Jong, R., Den Dunnen, J. T., Houwing-Duistermaat, J. J., Kneppers, A. L., Ginjaar, H. B., Breuning, M. H., & Bakker, E. (2009). Helderman-van Den Enden A. T. J. M. et al Recurrence risk due to germ line mosaicism. *Clinical Genetics*, *75*, 465–472.

Helderman-Van Den Enden, A. T. J. M., Madan, K., Breuning, M. H., Van Der Hout, A. H., Bakker, E., de Die-Smulders, C. E., & Ginjaar, H. B. (2013). An urgent need for a change in policy revealed by a study on prenatal testing for Duchenne muscular dystrophy. *European Journal of Human Genetics*, *21*, 21–26.

Keegan, N. P. (2019). Breakpoint junction features of seven DMD deletion mutations. *Human Genome Variation*, *6*, 39.

Koenig, M., Monaco, A. P., & Kunkel, L. M. (1988). The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell*, *53*, 219–228. https://doi.org/10.1016/0092-8674(88)90383-2

Kumar, S. H., Athimoolam, K., Suraj, M., Das Christu Das, M. S., Muralidharan, A., Jeyam, D., Ashokan, J., Karthikeyan, P., Krishna, R., & Khanna-Gupta, A. (2020). Comprehensive genetic analysis of 961 unrelated Duchenne Muscular Dystrophy patients : Focus on diagnosis, prevention and therapeutic possibilities. *PLoS One*, *15*, e0232654.

Laing, N. G., Davis, M. R., Bayley, K., Fletcher, S., & Wilton, S. D. (2011). Molecular diagnosis of duchenne muscular dystrophy: Past, present and future in relation to implementing therapies. *The Clinical Biochemist Reviews*, *32*, 129–134.

Lalic, T., Vossen, R. H., Coffa, J., Schouten, J. P., Guc-Scekic, M., Radivojevic, D., Djurisic, M., Breuning, M. H., White, S. J., & den Dunnen, J. T. (2005). Deletion and duplication screening in the DMD gene using MLPA. *European Journal of Human Genetics*, *13*, 1231–1234.

Lee, T., Takeshima, Y., Kusunoki, N., Awano, H., Yagi, M., Matsuo, M., & Iijima, K. (2014). Differences in carrier frequency between mothers of Duchenne and Becker muscular dystrophy patients. *Journal of Human Genetics*, *59*, 46–50.

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, *26*, 589–595. https://doi.org/10.1093/bioinformatics/btp698

Lim, B. C., Lee, S., Shin, J. Y., Kim, J. I., Hwang, H., Kim, K. J., Hwang, Y. S., Seo, J. S., & Chae, J. H. (2011). Genetic diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: Comprehensive mutational search in a single platform. *Journal of Medical Genetics*, *48*, 731–736.

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R., Thormann, A., Flicek, P., & Cunningham, F. (2016). The ensembl variant effect predictor. *Genome Biology*, *17*, 122.

Meyer, L. R., Zweig, A. S., Hinrichs, A. S., Karolchik, D., Kuhn, R. M., Wong, M., Sloan, C. A., Rosenbloom, K. R., Roe, G., Rhead,

B., & Raney, B. J. (2013). The UCSC Genome Browser database: Extensions and updates 2013. *Nucleic Acids Research*, *41*, 64–69.

Mohammed, F., Elshafey, A., Al-balool, H., Alaboud, H., Al Ben Ali, M., Baqer, A., & Bastaki, L. (2018). Mutation spectrum analysis of Duchenne/Becker muscular dystrophy in 68 families in Kuwait: The era of personalized medicine. *PLoS One*, *13*(5), e0197205. https://doi.org/10.1371/journal.pone.0197205

Monaco, A. P., Bertelson, C. J., Liechti-Gallati, S., Moser, H., & Kunkel, L. M. (1988). An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics*, *2*, 90–95. https://doi.org/10.1016/0888-7543(88)90113-9

Monaco, A. P., Neve, R. L., Colletti-Feener, C., Bertelson, C. J., Kurnit, D. M., & Kunkel, L. M. (1986). Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. *Nature*, *323*(6089), 646–650. https://doi.org/10.1038/323646a0

Moser, H. (1984). Duchenne muscular dystrophy: Pathogenetic aspects and genetic prevention. *Human Genetics*, *66*, 17–40. https://doi.org/10.1007/BF00275183

Muntoni, F., Torelli, S., & Ferlini, A. (2003). Review Dystrophin and mutations: One gene, several proteins, multiple phenotypes. *The Lancet Neurology*, *44*, 731–740.

Neri, M., Rossi, R., Trabanelli, C., Mauro, A., Selvatici, R., Falzarano, M. S., Spedicato, N., Margutti, A., Rimessi, P., Fortunato, F., & Fabris, M. (2020). The genetic landscape of dystrophin mutations in Italy: a nationwide study. *Frontiers in Genetics*, *11*, 131.

Ousterout, D. G., Kabadi, A. M., Thakore, P. I., Majoros, W. H., Reddy, T. E., & Gersbach, C. A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. *Nature Communications*, *6*, 6244. https://doi.org/10.1038/ncomms7244

Passamano, L., Taglia, A., Palladino, A., Viggiano, E., D'Ambrosio, P. A., Scutifero, M., Cecio, M. R., Torre, V., De Luca, F., Picillo, E., & Paciello, O. (2012). Improvement of survival in Duchenne Muscular Dystrophy: Retrospective analysis of 835 patients. *Acta Myologica*, *31*, 121–125.

Passos-Bueno, M. R., Bakker, E., Kneppers, A. L., Takata, R. I., Rapaport, D., Den Dunnen, J. T., & Zatz, M., & van Ommen, G. J. (1992). Different mosaicism frequencies for proximal and distal Duchenne muscular dystrophy (DMD) mutations indicate difference in etiology and recurrence risk. *American Journal of Human Genetics*, *51*, 1150–1155.

Plagnol, V., Curtis, J., Epstein, M., Mok, K. Y., Stebbings, E., Grigoriadou, S., Wood, N. W., Hambleton, S., Burns, S. O., Thrasher, A. J., Kumararatne, D., Doffinger, R., & Nejentsev, S. (2012). A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. *Bioinformatics*, *28*(21), 2747–2754. https://doi.org/10.1093/bioinformatics/bts526

Prodduturi, N., Bhagwate, A., Kocher, J. A., & Sun, Z. (2018). Indel sensitive and comprehensive variant / mutation detection from RNA sequencing data for precision medicine. *BMC Medical Genomics*, *11*, 67.

Reinig, A. M., Mirzaei, S., & Berlau, D. J. (2017). Advances in the treatment of duchenne muscular dystrophy: New and emerging pharmacotherapies. *Pharmacotherapy*, *37*, 492–499.

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26.

Spitali, P., Zaharieva, I., Bohringer, S., Hiller, M., Chaouch, A., Roos, A., Scotton, C., Claustres, M., Bello, L., McDonald, C. M., & Hoffman, E. P. (2020). TCTEX1D1 is a genetic modifier of disease progression in Duchenne muscular dystrophy. *European Journal of Human Genetics*, *28*, 815–825.

Trabelsi, M., Beugnet, C., Deburgrave, N., Commere, V., Orhant, L., Leturcq, F., & Chelly, J. (2014). When a mid-intronic variation of DMD gene creates an ESE site. *Neuromuscular Disorders*, *24*, 1111–1117.

van Essen, A. J., Abbs, S., Baiget, M., Bakker, E., Boileau, C., van Broeckhoven, C., Bushby, K., Clarke, A., Claustres, M., Covone, A. E., & Ferrari, M. (1992). Parental origin and germline mosaicism of deletions and duplications of the dystrophin gene: A European study. *Human Genetics*, *88*, 249–257.

Verma, P. K., Dalal, A., Mittal, B., & Phadke, S. R. (2012). Utility of MLPA in mutation analysis and carrier detection for Duchenne muscular dystrophy. *Indian Journal of Human Genetics*, *18*, 91–94.

Vieira, N. M., Elvers, I., Alexander, M. S., Moreira, Y. B., Eran, A., Gomes, J. P., Marshall, J. L., Karlsson, E. K., Verjovski-Almeida, S., Lindblad-Toh, K., Kunkel, L. M., & Zatz, M. (2015). Jagged 1 Rescues the Duchenne Muscular Dystrophy Phenotype. *Cell*, *163*(5), 1204–1213. https://doi.org/10.1016/j.cell.2015.10.049.

Wang, Y., Yang, Y., Liu, J., Chen, X. C., Liu, X., Wang, C. Z., & He, X. Y. (2014). Whole dystrophin gene analysis by next-generation sequencing: A comprehensive genetic diagnosis of Duchenne and Becker muscular dystrophy. *Molecular Genetics and Genomics*, *289*, 1013–1021.

Weber, J. A., Aldana, R., Gallagher, B. D., & Edwards, J. S. (2016). Sentieon DNA pipeline for variant detection - Software-only solution, over 20 × faster than GATK 3. 3 with identical results Sentieon DNA pipeline for variant detection. *PeerJ PrePrints*, *4*, e1672v2. https://doi.org/10.7287/PEERJ.PREPRINTS.1672V1

Wei, X., Dai, Y., Yu, P., Qu, N., Lan, Z., Hong, X., Sun, Y., Yang, G., Xie, S., Shi, Q., & Zhou, H. (2014). Targeted next-generation sequencing as a comprehensive test for patients with and female carriers of DMD/BMD: A multi-population diagnostic study. *European Journal of Human Genetics*, *22*, 110–118.

White, S. J. A., Aartsma-Rus, K. M., Flanigan, R. B., Weiss, A. L. J., Kneppers, T., Lalic, A. A. M., Janson, H. B., Ginjaar, M. H. B., & Den Dunnen, J. T. (2006). Duplications in the DMD Gene. *Human Mutation*, *27*, 938–945.

Winter, R. M., & Pembrey, M. E. (1982). Does unequal crossing over contribute to the mutation rate in Duchenne muscular dystrophy? *American Journal of Medical Genetics*, *12*, 437–441.

Zhang, J., Ma, D., Liu, G., Wang, Y., Liu, A., Li, L., Luo, C., Hu, P., & Xu, Z. (2019). Geneti analysis of 62 Chinese families with Duchenne muscular dystrophy and strategies of prenatal diagnosis in a single center. *BMC Medical Genetics*, *20*, 180.

Zhang, K., Yang, X., Lin, G., Han, Y., & Li, J. (2019). Molecular genetic testing and diagnosis strategies for dystrophinopathies in the era of next generation sequencing. *Clinica Chimica Acta*, *491*, 66–73.

SUPPORTING INFORMATION

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

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