Improvements in the diagnosis of dystrophinopathies: what have we learnt in these last 20 years?

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Introduction. Dystrophinopathies are X-linked genetic disorders caused by mutations in the *DMD* gene. Genetic tests are of utmost importance for management and genetic counseling of these diseases. However, the complexity of the *DMD* gene is a challenge for diagnosis.

Aim. To describe recent advances in the diagnosis of dystrophinopathies, after 20 years since the firsts molecular assays for genetic screening for these diseases.

Development. Currently, a variety of strategies such as automated mutation detection, cell-based methods and high throughput haplotyping have been developed to facilitate diagnosis of dystrophinopathies, carrier detection, prenatal and preimplantation diagnosis.

Conclusion. New technologies have improved early detection and optimal management of dystrophinopathies and have established the basis for future molecular medicine. The most significant advances in dystrophinopathy diagnosis are reviewed herein.

Key words. Carrier. Duchenne. Dystrophin. Preimplantation genetics. Prenatal diagnosis.

Introduction

In the last 20 years, advances in diagnosis of monogenic disorders have not only allowed optimal management of patients and genetic counseling, but also opened promissory therapeutic options in molecular medicine. Since the initial assays to detect mutations in patients with dystrophinopathies in early 90s, several advances have been brought about [1,2]. Dystrophinopathies are a group of diseases that include Duchenne muscular dystrophy (DMD) (OMIM 310200), Becker muscular dystrophy (BMD) (OMIM 300376) and X-linked dilated cardiomyopathy (XLDC) (OMIM 302045) all caused by mutations in the *DMD* gene. Life expectancy in DMD, the most severe of dystrophinopathies, has surprisingly changed from 14 years in the 60s to 25.3 years in 2002 as result of early diagnosis and multidisciplinary management [3]. Despite these advances, some authors have pointed out that currently, there is a delay of 2.5 years between onset of DMD symptoms and definitive diagnosis in nonfamilial cases [4]. To complicate this scenario, recent reports on large patient series have shown that most of them have private mutations (unique mutations in a pedigree) which make diagnosis a challenge [5]. Thus, an efficient system combining optimal diagnostic and treatment algorithms for dystrophinopathies are required [6,7]. Since the development of first multiplex PCR assays to screen most frequently deleted exons in Duchenne patients 20 years ago, considerable progress has been achieved in dystrophinopathy diagnosis. The aim of this review is to provide a current outline of the techniques available for carrier detection (CD), prenatal (PD) and preimplantational genetic diagnosis (PGD) in dystrophinopathies.

Dystrophinopathies

Dystrophin is a structural muscle protein of the dystrophin glycoprotein complex (DGC). Its main function is to form a link between the extracellular matrix and cytoskeleton to protect muscle cells from mechanic damage experienced during contraction [8] [López-Hernandez et al, submitted]. Dystrophinopathy was early defined as 'the expanding phenotype', because besides DMD, a wide spectrum of phenotypes are caused by mutations in the *DMD* gene [9].

BMD patients retain the ability to walk beyond 16 years and show milder disease progression, whereas DMD patients show early onset, walking is achieved

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around at 18 months of age and, as the disease progresses, patients become weak, and lose ambulation between 9-12 years of age. The most simple explanation for the phenotypical difference between DMD and BMD is the 'reading frame hypothesis' which states that mutated dystrophin transcripts maintaining the reading frame result in BMD, while patients with DMD have out-of frame mutations [10], most cases fit this rule. In addition to dystrophin of skeletal muscle, there are other isoforms (e.g Dp260, Dp71) generated by different transcription start sites or alternative splicing. XLDC due to mutations in the *DMD* gene (dilated cardiomyopathy 3B) exhibits almost exclusive cardiac involvement [11]. Phenotypical differences between BMD and XLDC are not completely clear at the molecular level, but mutations at the 5' region of the *DMD* gene altering dystrophin transcription and/or splicing in cardiac muscle, and those affecting the rod-shaped domain are related to XLDC [12].

Dystrophinopathy in females is an often neglected issue; although it was recently shown that clinical overlapping might occur between limb girdle muscular dystrophies (LGMD) and dystrophinopathy in affected girls [13]. Females with severe DMD phenotype have also been described, originated by different mechanisms:

- Skewed X chromosome inactivation in DMD carriers [14].
- Balanced translocation X:autosome with breakpoints in the *DMD* gene and preferential inactivation of the normal X chromosome [15].
- Monosomy of X-chromosome with mutations in the *DMD* gene [16].
- Maternal isodisomy of X chromosome with mutation in the *DMD* gene [17].
- Simultaneous occurrence of DMD mutations and androgenic receptor gene [18].
- Homozygous mutation in the *DMD* gene (BMD phenotype due to in-frame deletion) [19].

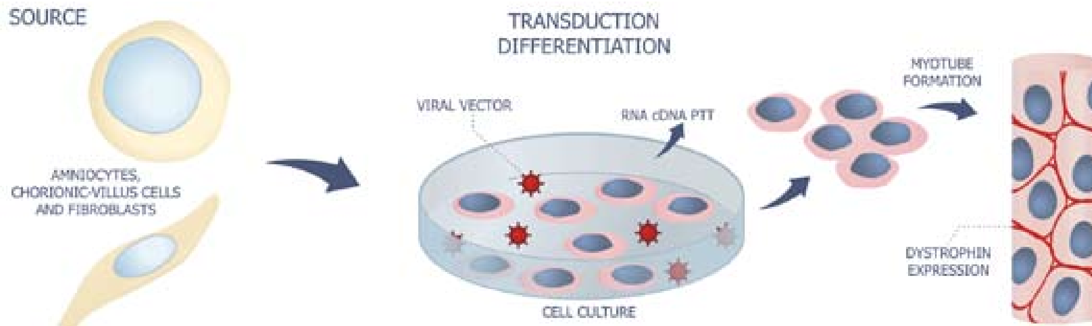
When a dystrophinopathy is suspected, confirmation should be done by genetic testing.

Biological sources for dystrophinopathy diagnosis

The availability of the sample and type of pathogenic variant, play a central role in dystrophinopathy diagnosis (DD) and CD, especially in cases lacking family history (sporadic cases). Given that, the establishment of diagnosis in the proband is the first step towards genetic counseling. To realize this, genomic DNA (gDNA) isolated from blood, is normally used for genetic testing, for both the patient and carrier diagnosis. However, not all the changes at DNA level reveal the defect at RNA level and vice versa. In this regard, it was shown in one report [20] that exons with apparently normal amplification on gDNA, showed a deletion of 61-79 exons when tested from cDNA. In addition, we have previously shown that RT-PCR (using mRNA from lymphocytes) for carrier detection is not feasible, since normal alternative splicing events might cause false positive results [21]. Together these observations imply that diagnosis of dystrophinopathies is not as simple as previously thought.

Recently, some researchers have taken advantage of muscle or skin biopsy for dystrophin protein and gene analysis, especially in sporadic DMD patients [22]. Related to this, muscle biopsy has also been performed for diagnosis of at –risk females. The report stated that the finding of dystrophin-negative fibers in combination with normal spectrin labeling, is indicative of carrier status when encountered in a muscle biopsy of an at-risk female, [23]. Nevertheless, this approach is not accurate enough (overall sensitivity 20 % and 26 % of sensitivity for non manifesting carriers), is only used in experienced hands and often considered an invasive procedure. Instead, *MyoD*-forced myogenesis brings an alternative for CD and PD. Cultured cells of patients and at-risk females are used for mutation detection and protein analysis. Chorionic villi, amniocytes and skin fibroblasts can be used to perform *MyoD* induced myo-differentiation. Muscle specific protein expression, will reveal the molecular defect in the patient [24] (Fig. 1). *MyoD* is a key transcription factor for myogenic differentiation. Its over-expression in non-muscle cells, induced by viral vectors (eg. lentivirus, adenovirus or retrovirus) produces fused multi-nucleated myotubes with an integral sarcomeric structure; after that, protein analysis is performed; Western blot compared to immunofluorescence has the advantage of detecting abnormal size and quantity of dystrophin, (it allows distinguishing BMD cases). Additionally, it can be carried out in a multiplex manner, with other muscular dystrophy related proteins for differential diagnosis [25]. *MyoD* induced myo-differentiation in combination with PTT (protein truncation test) specifically detects nonsense mutations in the coding region by *in vitro* protein synthesis. It would be especially useful when the disease causing mutation is unknown but abolishes or significantly decreases dystrophin expression [25,26] (Table).

Figure 1. *MyoD*-forced myogenesis for carrier detection and prenatal diagnosis. Cells (amniocytes or chorionic villi) are induced to express dystrophin through viral delivery of the *MyoD* gene, which allows the assessment of the ability of the patient to express dystrophin. Moreover, cultured cells can be used to isolate RNA to search for mutations by protein truncation test (PTT).



So, until pathogenicity of a particular mutation is proven, an appropriate biological sample of the proband would be necessary for integration of DNA, RNA and protein analyses [27] (e.g Western blot, immunohistochemstry, RT-MLPA). In fact, a recent report stated that 12% of cases determined to be DMD/BMD were found to carry the L276I mutation in the *FKRP* (fukutin-related protein) gene at 19q13.3, which causes LGMD2I, an autosomal recessive form of muscular dystrophy. Diagnosis was performed in sporadic cases with undetected variants and, even after immunostaining analysis in some cases. Taking in account this erroneous diagnosis, PD had already been done in some families [28]. Moreover, deletions in the *DMD* gene are not always disease causing; a healthy man was found to carry a deletion of exon 16 in the *DMD* gene [29].

Methods for genetic testing

Deletions are the most frequent mutations in the *DMD* gene (67.4%) thus; large rearrangements should be analyzed first. Deletions, duplications [30-37] and known point mutations [38] can be analyzed simultaneously by MLPA (Fig. 2). After that, if the mutation is not found, changes at the nucleotide level (e.g base substitutions, insertions or deletions) should be searched [39-43]; nevertheless, due to the size of the *DMD* gene, point mutation screening is challenging. Recently, high resolution melting curve analysis (HR-MCA) has been validated as pre-sequencing scanning method for small variant detection in DMD patients and carriers [44] (Table).

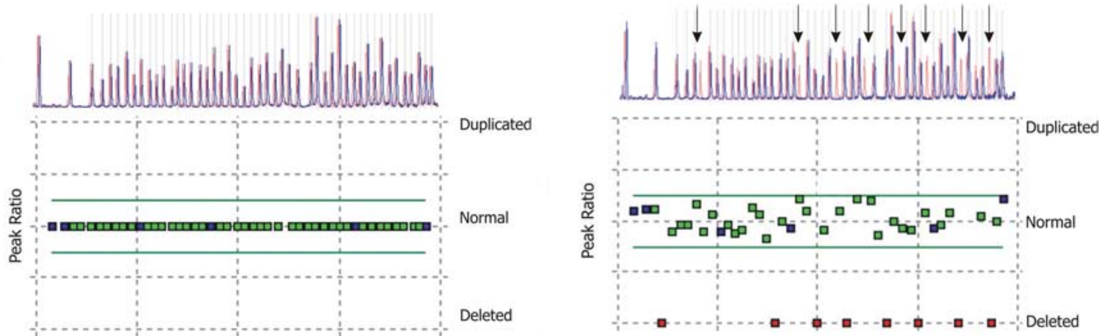
Population-based screening studies

Variation in frequencies and distribution of mutations in the *DMD* gene in different populations have important implications for the establishment of diagnostic strategies and also for future 'customized' gene therapy approaches, (e.g. exon skipping, stop codon read through) which require an exact delimitation of the molecular defect in the patient [45]. Large rearrangements result in the Duchenne phenotype as well as small base changes; thus, frequency of pathogenic variants might be different in distinct populations without altering the incidence of the disease. It was suggested that DMD has lower recurrence in the native black population and non-deletion cases are more prevalent (contrary of what occurs worldwide), but more detailed reports are required to address this hypothesis [46]. Deletion frequency in Singaporeans is relatively small compared to other populations, approximately 40% as opposed to 72% reported in the Leiden Database [5,47]. The small size of families often results in uninformative pedigrees, making segregation analysis difficult to interpretate. Thus, point mutation screening is the most appropriate approach for genetic testing in the above mentioned population [47]. A presumptive high *de novo* deletion frequency was reported in the Mexican population, although extensive and detailed studies confirming this hypothesis are required[48]. It should be noted, that various reports suggested that some families are more prone to '*de novo*' rearrangements in the *DMD* gene [49], this was revealed by several recombination/deletion events in the same pedigree. In addition, a pre-mutated sta-

Table. Methods for dystrophinopathy genetic testing.

Mutation	Technique	Remarks	Ref.
Large rearrangements (deletions/ duplications)	MLPA Multiple ligation dependent probe amplification	Direct hybridization of exon-specific half probes (length 20-30 nt) and the subsequent amplification of ligated probes by fluorescent multiplex PCR with universal primers in a liquid phase. Allows simultaneous detection of copy number changes and point mutations in a high throughput manner. Single base changes near ligation site would affect product amplification and should be confirmed by other techniques	[30,31]
	MAPH Multiple amplification probe hybridization	Detection of copy number changes with exon specific probes (length 100-200 nt). A membrane is used to fix genomic DNA before hybridization; differences in the relative peak area between healthy controls and patients will determine the rearrangement. Small changes will not affect amplification	[32]
	QF-PCR-CSCE Quantitative fluorescent, polymerase chain reaction and conformation sensitive capillary Electrophoresis	A combined approach to detect copy number changes and small base changes using 12 multiplex PCR assays to screen all 79 exons of the *DMD* gene. Sensitivity close to 100%	[33]
	Multiplex real time PCR	Simultaneous amplification of large number of exons using SYBR Green I dye or TaqMan probes. The number of exons is limited	[34,35]
	Fluorescence in situ hybridization	Hybridization of fluorescent probes on metaphase chromosomes, interphase nuclei or chromatin fibers. It is not as high throughput as other techniques	[36]
	Southern blot	Hybridization with probes in membranes followed by washing steps and autoradiography to detect copy number changes. It is laborious and time consuming when compared to other techniques (7-9 cDNA probes are required to cover the 14 kb coding sequence)	[37]
Small mutations	HRMA High resolution melting curve analysis	Characterizes DNA samples according to their dissociation behavior, as their transition from double stranded DNA to single stranded releases a saturating dye when temperature increases. Fluorescence is continuously collected to display a melting profile. Melting curves are compared between wild type and mutated samples. It is cost-effective	[43]
	SCAIP Single condition amplification/internal primer	Direct sequencing of the *DMD* gene, effective method but specialized equipment is required and the cost limits its applicability	[39]
	PTT Protein truncation test	Detects nonsense mutations from RNA using muscle or lymphocytes as source. It's technically demanding and time-consuming. Tissue is not often available	[26,46]
	DGGE Denaturing gradient gel electrophoresis	Amplicons are electrophoresed with denaturing agents and DNA melting domains dissociate according to their profiles. Subtle changes can be found with nearly 100% sensitivity (95 amplicons are required to screen the *DMD* gene). Suitable for patient and carrier screening	[40]
	DHPLC Denaturing high performance liquid chromatography	Realignment of different sequences after denaturation allows heteroduplex formation; mutated and wilt-type alleles can be distinguished by different retention times (86 amplicons are required to screen the *DMD* gene)	[41]
	cDNA sequencing	After RT-PCR amplicons are directly sequenced to find variants. One disadvantage is the high cost of sequencing	[42]
	SSCP/DOVAM Single strand conformational polymorphism	DNA single strands form secondary structures and, depending on the sequence, different electrophoretic mobilities are expected when a base change is found	[43]
Haplotyping (linkage analysis)	STRs Short tandem repeats	Mostly useful when mutation is unknown and family history is positive, large panel of loci is required for accurate analysis. Multiplexing STRs is high throughput and cost-effective	[59]
	SNPs Single nucleotide polymorphisms/restriction fragment length polymorphisms	Assays with restriction enzymes can be performed for linkage studies. However, RFLPs are not as high throughput as other techniques	[62]

Figure 2. Dosage analysis by MLPA. Copy number changes are detected after peak ratio normalization with control probes and control samples. Deletion of exons 24-41 of dystrophin gene are depicted in the diagram as red squares, whereas in the electropherogram are indicated by arrows.



tus has been suggested in some individuals in which repeated sequences increase the instability of the *DMD* gene and make them prone to genomic rearrangements due to different mechanisms; such as non homologous end joining (NHEJ), replication-dependent fork stalling and template switching (FoSTeS) [50,51].

Carrier detection

Women carriers of mutations in the *DMD* gene usually do not display the severe phenotype exhibited by males, since regularly those are heterozygous for the mutation. Nevertheless, about 3% of females are manifesting carriers (females with mild symptoms of DMD) [52]. The prevalence of cardiac abnormalities on echocardiogram and ECG is 18%; approximately 7% develop dilated cardiomyopathy and 12 % show muscle weakness [53]; CK levels are usually elevated about 50-60%, but more evidence is required to establish the diagnosis. In this regard, abnormal electroretinogram (ERG) is associated with heterozygous carriers of mutations in the *DMD* gene when the mutation disrupts the translation of retinal dystrophin isoform (Dp260)**,** although this report was obtained from one family, and a large study is mandatory. Interestingly, it opens the possibility of detecting carrier status in women using a non-invasive method [54]. In most cases, weakness and cardiac involvement do not significantly affect normal activities or life expectancy of carriers [55].

Carrier status and recurrence risk

Females are obligate carriers when they have either one affected son and another affected male relative or when they have two affected sons. In cases of *de novo* mutations, the mother and female relatives of the patient are not carriers and the risk of having another affected son is not increased over population risk. Recurrence risk (RR) estimation can be done in relation to the type of mutation and its location in the *DMD* gene. For germ line mosaicism (mutation is present in germ cells but absent in somatic cells in an individual originated from the same zygote), in cases where the risk haplotype is transmitted, the RR is 8.6% with all types of mutation combined, 8.4% for deletions (15.6% proximal and 6.4% for distal deletions) duplication 12.1% and point mutation 4.4%. When the risk haplotype is unknown, the RR due to germ line mosaicism is the half of the above mentioned values [56]. RISCALW is a software application for estimating RR in DMD families. It is designed to combine different mutation rates depending on sex and mutation type, family structure, CK levels, polymorphic informative content and number of genetic markers analyzed for risk calculation [57].

Genetic markers for haplotyping in the *DMD* gene: direct and indirect analysis

Different genetic markers, useful for CD, PD and PGD have been identified in the *DMD* gene (Fig. 3).

Figure 3. Genetic markers described in the *DMD* gene for linkage analysis. A large panel of genetic markers covering 5', central and 3' regions of the *DMD* gene is shown. In the upper part, the restriction sites for RFLP/SNP are observed and the lower part shows STRs and deletion hotspots (URL: http://www.dmd.nl).

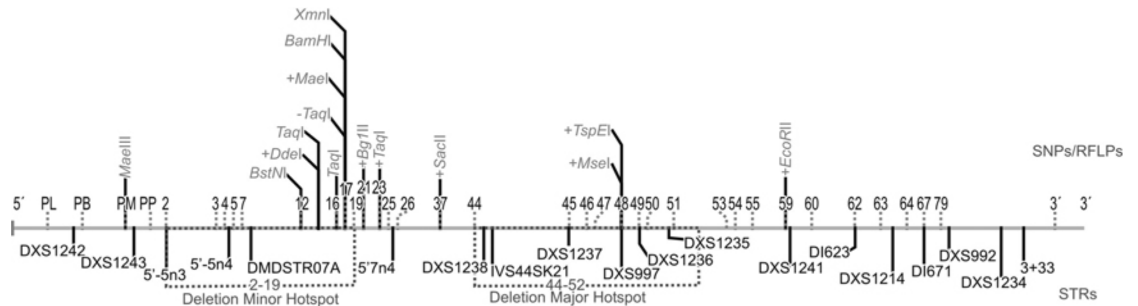
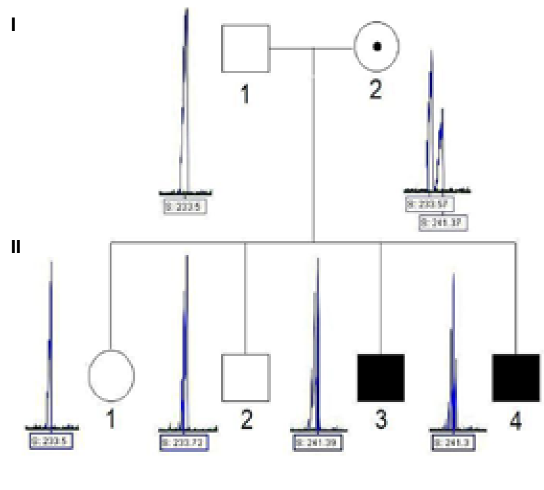


Figure 4. Segregation analysis. The sister (II-1) of two affected males (II-3 and II-4) and one unaffected male (II-2) is not a carrier, since she inherited the 233 bp allele of the DXS1236 marker (intron 49) from both parents, this allele (233 bp) is not segregated with the DMD phenotype. The mother and both affected males have the 241.3 bp allele of the same locus, which is related to the disease phenotype.



In about 20% of the cases, sample of the affected male is not available [58]; even though, CD, PD and PGD can be performed, by haplotype matching between a healthy male relative and the proband (indirect analysis) [59]. Indirect analysis with informative markers can potentially allow detection of familial XLDC cases in pre symptomatic stage (even when the mutation is unknown), since all affected males in a pedigree are expected to share the same at-risk haplotype. Deletions in the *DMD* gene often involve one or more STR or RFLP loci, which allows direct mutation analysis in the family (Table). In those cases, it is actually possible to exclude carrier status of the mothers, when they are heterozygous for markers encompassing the deletion. Even so, germ line mosaicism cannot be ruled out easily and should be taken into account for risk estimations.

Indirect analysis with genetic markers

Haplotyping or 'linkage studies' (the establishment of the allele in co-inheritance with the disease phenotype through a pedigree analysis using genetic markers) (Fig. 4) is used to detect the at-risk haplotype segregating in the family when the mutation is unknown. Polymorphic informative content of genetic markers for haplotyping is determinant to achieve diagnosis. Variability of such genetic markers should be tested in a population-specific way, to ensure usefulness in genetic testing [60,61]. Estimation of the recombination frequency in a population, as well as mapping recombination events, is essential for genetic counseling based on haplotyping. An advantage of STRs over SNPs is the existence of more alleles and higher heterozygosity, which eventually leads to detection of recombination events, since SNPs/RFLPs are often not informative [61,62]. These could be detected by haplotype segregation studies, in two or three generation families, where informative meioses are representative enough of the group of study. Carsana et al [63] found 24% of recombination events at 3' end of the *DMD* gene in 273 meioses corresponding to 93 non-related DMD families from southern Italy. The extent of allelic association between pairs of loci, also called 'linkage disequilibrium' (LD), is an indirect measure of recombination, related to the physical distance between said pairs, and can be esti-

mated from population data. A study demonstrated significant LD between two STR markers in the *DMD* gene: DXS1235 (intron 50) and DXS1236 (intron 49), from which it can be concluded that recombination events are rare between those markers [64]. No significant LD between other markers in the *DMD* gene has been described thus far.

Prenatal diagnosis

In 1985, for the first time PD on a DMD carrier was performed [65]. Currently, PD is performed by chorionic villus sampling at 10-13 weeks of gestation, or by amniocentesis at 16 weeks of gestation. There are several reports on successful PD using haplotyping for familial cases or specific variant screening for known mutations. Nevertheless, a combination of techniques is regularly required to establish the genetic status of the fetus, when other strategies are not informative. For instance, haplotyping, semi quantitative multiplex analysis and direct sequencing of exons with abnormal migration were reported in a prospective study; the analysis led to the diagnosis of a healthy male and the successful continuation of the pregnancy [66]. Similar to this, a cost-effective assay was developed by using PCR/IP-RP HPLC for fast and accurate PD for copy number alterations analysis using a UV-based detection system [67]. For PD, confirmation by independent methods and post-natal CK levels monitoring is strongly recommended [67]. The first report of PD for XLDC due to a purely intronic deletion abolishing dystrophin expression in cardiac, but not in skeletal muscle, stated the importance of integrating genomic and transcriptional studies when dystrophin splicing pattern plays a crucial role in pathogenesis and could be missed by genomic DNA testing only [12].

Preimplantation genetic diagnosis

PGD is usually performed in couples at risk for genetic diseases to avoid termination of the pregnancy. PGD could be achieved analyzing one or two blastomeres (blastomere biopsy), blastocyst biopsy (BB) or polar body biopsy (PBB) (Fig. 5). Blastomere biopsy is usually done the third day post-fertilization at eight-cell stage. Removal and analysis of one or two blastomeres could result in different test efficiency for the establishment of the diagnosis due to the scarcity of DNA from a single cell. It should be noted that there is a trend of one-cell biopsied embryos to reach higher clinical pregnancy rates compared to two-cell analyzed embryos [68]. This can be avoided using whole genome amplification (WGA) by multiple displacement amplification to generate more DNA template for the analysis. This problem can be also minimized by BB (at day five post-fertilization), nevertheless, reduction in time to perform genetic diagnosis is an emerging issue, because blastocysts need to be transferred no later than day six of *in vitro* development and usually only half of preimplantation embryos reach the blastocyst stage [68]. For DMD, PGD using polymorphic genetic markers was initially achieved by haplotype analysis. The embryo which inherited a different haplotype than the affected brother was the one transferred [69]. The first child to be born after PGD for DMD specific mutation, was the son of a carrier of 3-18 exons deletion [70]. An improved amplification assay to screen four dystrophin exons (6, 8, 18 and 32) and ZFX/ZFY genes for sex determination was done later. However, the small group of analyzed regions limits its applicability to families in which at least one of those exons is deleted [71]. Since then, new tests were developed; the first amplifies 11 loci in the *DMD* gene and SRY gender marker by triple-nested PCR covering exons within the mayor and minor deletion hot spots [72], the second uses MDA for PGD and is designed to screen six exons, eight STRs in the *DMD* gene and amelogenin amplification for sexing; it was implemented with 94.2% success for blastomeres [73]. PGD was also achieved by FISH through accurate screening with probes for specific exons in the *DMD* gene, in combination with probes for X and Y chromosomes for sex determination,

Figure 5. Preimplantation genetic diagnosis. Different screening methods can be used to perform dystrophinopathy diagnosis on blastomeres or blastocyst, like FISH, multiplex PCR (for large rearrangements) or haplotyping (for small or unknown variants). Some of these methods require whole genome amplification (WGA).

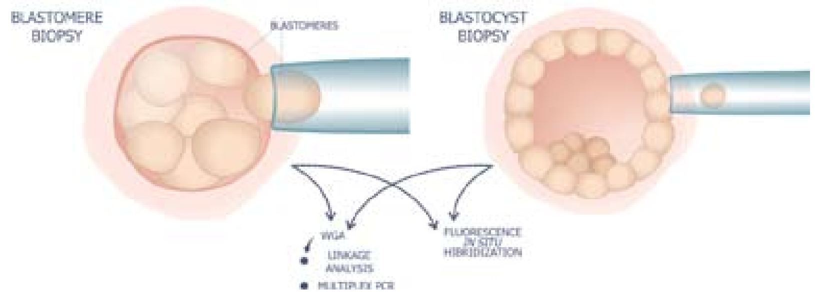
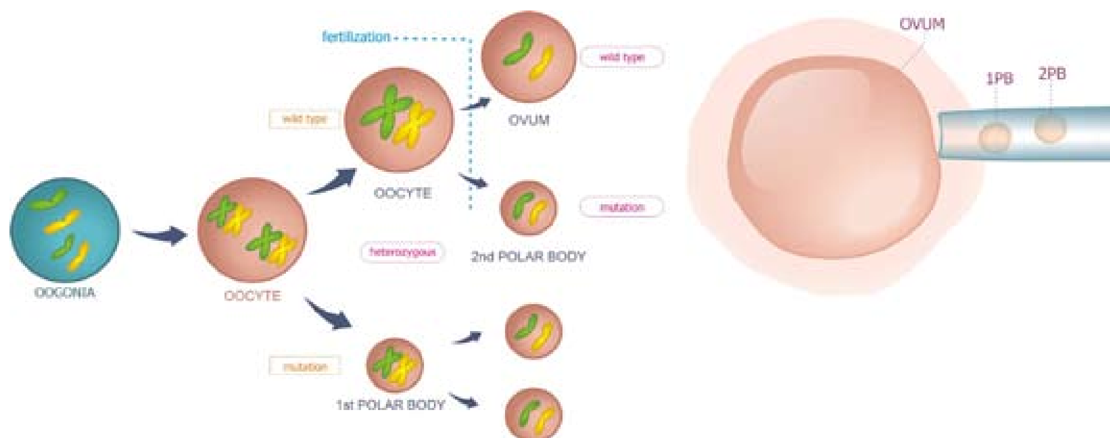


Figure 6. Polar body biopsy. When the first polar body (PB) carries a homozygous mutation, the corresponding oocyte is not affected (orange sign), if the first PB is heterozygous, an additional analysis is required. Upon fertilization but prior to pronuclei fusion, the second polar body (2nd PB) is formed and can also be screened. When this polar body carries the pathogenic variant or the at-risk haplotype, the oocyte is not affected (pink sign).



this approach has the advantage to detect deletions and duplications [36] and avoiding allele drop out (random no-amplification of one allele present in a sample) of PCR based methods [74]. PGD is the most suitable option when germ line mosaicism is detected.

Polar body biopsy

One variant of PGD is PBB; also known as 'preconception genetic diagnosis', it avoids embryo manipulation because only requires the analysis of the genome of one cell of maternal origin. PBB can be achieved in a period of 16-20 hours, before the fusion of the two pronuclei is completed [75]. After ovarian stimulation, oocyte retrieval is achieved, then, intracytoplasmatic sperm injection (ICSI) is performed leading to the first polar body (1st PB) release; it can be removed by laser-assisted dissection for haplotype analysis or screening for pathogenic variants. Recently, PBB has been combined with haplotyping for eight STR markers in the *DMD* gene resulted in the born of a mutation-free child from an affected DMD family in which disease causing mutation was c.1055T> G [76]. This is a valuable option for cases in which female carriers are not willing to carry out termination of the pregnancy and embryo biopsy for PGD is not an option. For instance, in Germany the 'embryo protecting law' prevents PGD on blastomeres, then PBB is the unique alternative to perform PGD [75]. One limitation of PBB would be a high rate of heterozygosity of genetic markers on the first PBs because it prevents any prediction about the status of the oocyte (Fig. 6).

Conclusion

Since 1990, several improvements in dystrophinopathy diagnosis have emerged. Currently, early detection is a priority. Nowadays, high throughput and cost-effective diagnostic strategies improve genetic testing facilitating genetic counseling and increasing our knowledge of this frequent and devastating disease.

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Mejoras en el diagnóstico de distrofinopatías: ¿qué hemos aprendido después de 20 años?

Introducción. Las distrofinopatías son trastornos genéticos ligados al cromosoma X causados por mutaciones en el gen *DMD*. Las pruebas genéticas son de suma importancia para la gestión y el asesoramiento genético de estas enfermedades. Sin embargo, la complejidad del gen *DMD* es un desafío para el diagnóstico.

Objetivo. Describir los avances recientes en el diagnóstico de distrofinopatías, después de 20 años de los primeros ensayos moleculares para la detección genética de estas enfermedades.

Desarrollo. En la actualidad, se han desarrollado una variedad de estrategias, como la detección de mutaciones automatizada, los métodos basados en células y la haplotipificación de alto rendimiento, para facilitar el diagnóstico de distrofinopatías, la detección de portadoras, el diagnóstico prenatal y preimplantacional.

Conclusión. Las nuevas tecnologías han mejorado la detección temprana y el manejo óptimo de distrofinopatías, y han establecido la base para la medicina molecular en el futuro. Los avances más importantes en el diagnóstico de distrofinopatías se revisan en este documento.

Palabras clave. Diagnóstico genético preimplantacional. Diagnóstico prenatal. Distrofina. Duchenne. Portadora.