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GENETICS IN FETAL MEDICINE

Duchenne Muscular Dystrophy (DMD): Pre-conceptional Counseling

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Abstract Duchenne muscular dystrophy (DMD) is a common X-linked recessive disorder, which is caused by deletion, duplication, or point mutations of the dystrophin gene. This article discusses genetic counseling and prenatal diagnosis in DMD and highlights the need for confirmation of diagnosis by molecular studies in the index case for future prenatal diagnosis. It also addresses how the family can be provided prenatal diagnosis, if the patient is deceased. These principles are illustrated by four case scenarios.

Keywords Duchenne muscular dystrophy · Deletion · Duplication · Prenatal diagnosis · Creatine kinase · MLPA

Introduction

Duchenne muscular dystrophy (DMD) is a common genetic disorder which is inherited as an X-linked recessive disorder. The defective gene is dystrophin, which is the largest gene in humans with 79 exons. About 65 % of mutations responsible for DMD are due to deletion of single/several exons, 5 % are due to duplications, and the remaining 30 % are caused by point mutations in the gene [1].

Pre-conceptional Counseling

The usual scenarios where pre-conceptional counseling is sought in cases of DMD are:

1. Mother of an affected boy planning another pregnancy
2. Sister of an affected boy wanting to know her risk of bearing an affected child
3. Female relatives of the mother of an affected boy
4. Mother who has already lost a boy with DMD and desires another pregnancy.

Inheritance of DMD

DMD is an X-linked recessive disorder. The characteristic features of X-linked pedigree include

- Knight's move in the pedigree (two affected males are connected through a carrier female)
- Only the males are affected
- Uniform severity in males
- No male to male transmission
- All daughters of an affected male patient are carriers and all sons are unaffected
- The sons of a carrier woman have a 50 % chance of being affected and daughters have a 50 % chance of being carriers.

As males have only a single X chromosome, they manifest disease if there is a mutation in the X chromosome. Males are hemizygous for genes, present on the X

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chromosome. Mother of an affected child, and other women who are carriers of mutation in the dystrophin genes have one mutated X chromosome and a normal copy of X chromosome and hence, do not express the disease.

Manifesting Carriers—There are certain rare situations where a carrier woman would express the disease in an X-linked recessive disorder and then she is labeled as a 'manifesting carrier' [2, 3].

1. Nonrandom Lyonization—Normally, X chromosome inactivation occurs randomly in either of the X chromosome in the early embryonic stage, i.e., in 50 % of cells, the normal X chromosome is inactivated or in the remaining 50 % of cells, the mutant X chromosome is inactivated. If the normal X chromosome is inactivated in greater number of cells, then these cells will not make dystrophin and will result in disease, although in a milder form
2. If the woman has *Turner syndrome* (45, X) then a mutation in the dystrophin gene in the single X chromosome will express all features of DMD, as in an affected male.
3. Women with an X autosome translocation—If there is a reciprocal translocation between an autosome and an X chromosome, and if the breakpoint in the X chromosome is disrupting the dystrophin gene, then the woman will manifest the disease. In carrier females with an X autosome translocation, there is a preferential inactivation of the normal X chromosome to prevent the loss of autosomal material as this would adversely affect the survival of the cell if the translocated X chromosome is inactivated.
4. If the woman has mutated dystrophin gene on both the X chromosomes then she will manifest features of DMD.

Gonadal Mosaicism also contributes to recurrence of DMD in a "noncarrier" mother, who has an affected son with DMD and whose DNA sample from the peripheral blood lacks the mutation. Hence, prenatal diagnosis by mutation analysis should be offered to all mothers of an affected child, even if her genomic DNA does not show an abnormality, owing to the risk of underlying gonadal mosaicism.

Pre-conceptional Counseling

This is a stepwise approach by which the couple is informed about the genetics of DMD and the risk of bearing an affected child when the mother is a carrier. When the couple has an affected child, the recurrence risk of 25 %, if the baby is a boy, is explained with the help of diagrams. They are also counseled regarding the option of chorionic villus sampling (CVS) between 10 and 14 weeks or amniocentesis between 15 and 18 weeks for prenatal diagnosis, although CVS is preferred.

Whom to Evaluate

The index child should be evaluated for DMD in the following circumstances [4]:

1. If there is no family history of DMD but the male child, who is less than 5 years old, has a positive Gower's sign or calf hypertrophy, or walks on tiptoes or has inability to walk even at 18 months
2. If there is any suspicion of abnormal muscle function in a family with a positive history of DMD.
3. A male child with an increase in creatine kinase (CK) levels, or an unexplained increase in transaminase enzymes in the blood. Such children are often suspected to have liver disease, and are subjected to liver biopsy.

Evaluation of an Index Case

The first step in genetic counseling is generation of a three-generation pedigree, which gives an important clue towards the mode of inheritance. In sporadic cases, the mother is a carrier in two-third of the cases and the remaining one-third cases are due to 'de novo' mutations. A woman who has an affected son and an affected brother is an obligate carrier. She has 50 % chance of transmitting the defective gene to her sons in each pregnancy, and 50 % of her daughters will be carriers.

In a boy with features consistent with DMD, estimation of serum CK is the first step for evaluation. The normal value of CK in adults is 5–170 U/L. In patients with DMD, the levels of CK will be around 10,000–20,000 U/L.

CK assay is also useful for possible identification of the carrier status. Carrier females have an increased permeability of muscle membranes due to the reduction of dystrophin protein, and this leads to leakage of muscle enzymes into the blood stream. Two-third of the obligate carrier females have an elevated CK when compared with the general female population, but there is an overlap of CK values between normal subjects and obligate carriers. Muscle fibres are multinucleated cells. In a carrier female, some nuclei will inactivate the healthy X chromosome, leading to lack of production of dystrophin. This leads to a situation where the cell wall will be having reduced dystrophin in some areas and normal levels in other areas. CK leaks out from the cells with reduced dystrophin, but the carriers who are able to make enough dystrophin will have

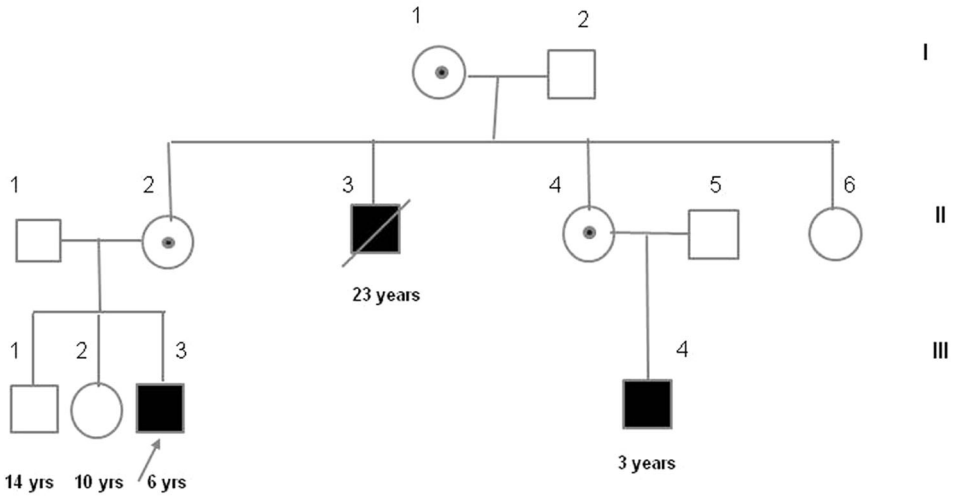

Fig. 1 Pedigree of case 1 with three affected people with DMD (*II 3*, *III 3*, *III 4*). *I 1*, *II 2* and *II 4* are obligate carriers. *III 3* is the proband

normal CK levels. If the CK is high in a woman with a positive family history of DMD, then it suggests that she is a carrier. However, if her CK is normal, it will reduce her chance of being a carrier but does not eliminate it.

Identification of the mutation in every proband is essential for carrier detection and prenatal diagnosis for 'at risk' individuals. If the deletion of exons disturbs the reading frame, then clinical features are that of DMD, while an in-frame deletion gives rise to a milder clinical picture of Becker muscular dystrophy. The exact extent of the deletion is also useful for therapy as exon skipping is being tested for therapy for selected exon deletions. If the patient has a stop codon as a result of a point mutation, therapy is being attempted by administering ataluren, which bypasses stop codons and restores synthesis of dystrophin.

Report of Cases

The counseling for an index patient with DMD is sometimes difficult. To exemplify this, a few case scenarios are presented to understand how each family can be counseled, so that they can choose appropriate investigations for prenatal diagnosis in subsequent pregnancies.

Case 1 (Fig. 1)

A 6-year-old boy (III 3) is brought with features consistent with DMD. There is a history of death of a maternal uncle at the age of 23 years (II 3) with a history consistent with DMD. On evaluation, III 3 has a CK of 8900 U/L and III 4 (3 years old, asymptomatic) has a CK of 3200 U/L.

The pedigree reveals that II 2 and II 4 are obligate carriers as both of them have an affected son and an affected brother (Fig. 1). The maternal grandmother (I 1) is also an obligate carrier (with an affected son and 2 carrier daughters). II 6 has yet not started a family but has a 50 % chance of being a carrier and needs to be evaluated for her carrier status. She should be evaluated with a serum CK analysis. An elevated CK favors the carrier status with her strong positive family history of DMD. MLPA analysis or quantitative PCR analysis is needed for the confirmation of the carrier status in females as they have one normal X chromosome.

The first step towards identification of mutation for III 3 is by using multiple ligand probe amplification (MLPA). MLPA analysis in III 3 showed deletion of exons 8–43 in the dystrophin gene (Fig. 2). This robust test can detect deletions and duplications in all 79 exons, thereby detecting the causative mutations for around 70 % cases of DMD. MLPA is far superior to multiplex polymerase chain reaction analysis (multiplex PCR), in which only groups of 20–25 exons are analyzed (Fig. 2).

The subject II 4 can be offered prenatal diagnosis in subsequent pregnancies as she is expected to have the same mutation. III 1 is already 14 years old and asymptomatic; hence, there is no risk of DMD in him.

III 2 who is 10 year old has a 50 % chance of being a carrier and should be offered assay of CK. Identification of carrier status is not usually offered to a girl less than 18 years in age due to ethical implications. Carrier status should be carried out in her and she should be offered carrier screening after 18 years after obtaining her consent.

Case 2 (Fig. 3)

A 23-year-old woman (III 7) attends the genetic clinic for premarital counseling as her two brothers were suspected to have DMD (Fig. 3). Her elder brother (III 6) expired at 25 years. None of the other family members is affected.

For this family, the evaluation should start from III 8, who is the only living person with probable DMD. The

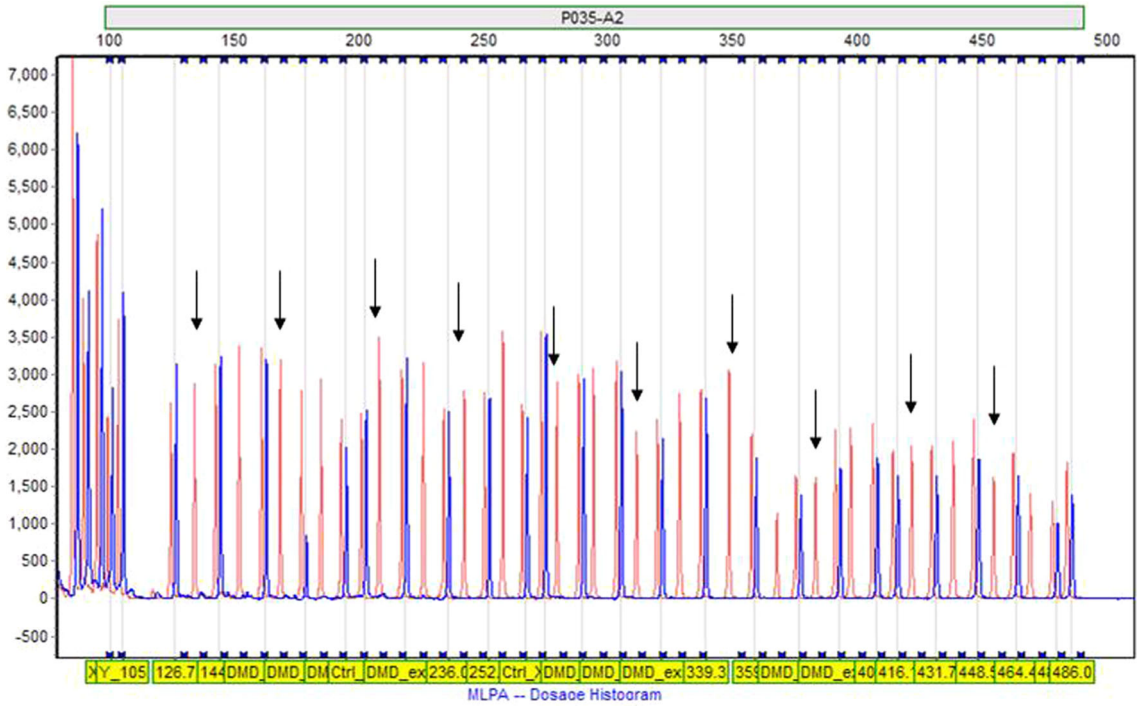

Fig. 2 MLPA analysis in *III 3* (case 1) showing deletion of exons 8–43 in the dystrophin gene depicted by *arrows* where corresponding to the control peak there is a complete absence of peaks for exons 8–43 in *III 3*

serum CK for III 8 was 4280 U/L. MLPA study for deletion and duplication of 79 exons did not show any abnormality. He did not have any calf muscle hypertrophy at 18 years, but the parents did remember that he had mild calf hypertrophy as a child. The next step is to sequence the dystrophin gene, which is time consuming and costly, although next generation sequencing technology has made this cheaper and affordable. Alternately, the way to confirm the diagnosis is by immuno-histochemistry studies for dystrophin protein in a muscle biopsy sample. Muscle biopsy showed complete absence of dystrophin protein, thereby confirming the clinical diagnosis. Sequencing of the dystrophin gene showed a point mutation in exon 70 (c.10141C>T; p.R3381X).

The serum CK in III 7 was 310 U/L, thereby suggesting that she is a carrier. The same mutation was identified in the hemizygous state, which confirmed her carrier status. She has been counseled regarding the option of prenatal diagnosis by CVS at 12 weeks.

Case 3 (Fig. 4)

A 40-year-old woman (II 2) attends the clinic for pre-pregnancy counseling for DMD. Her two sons had expired at

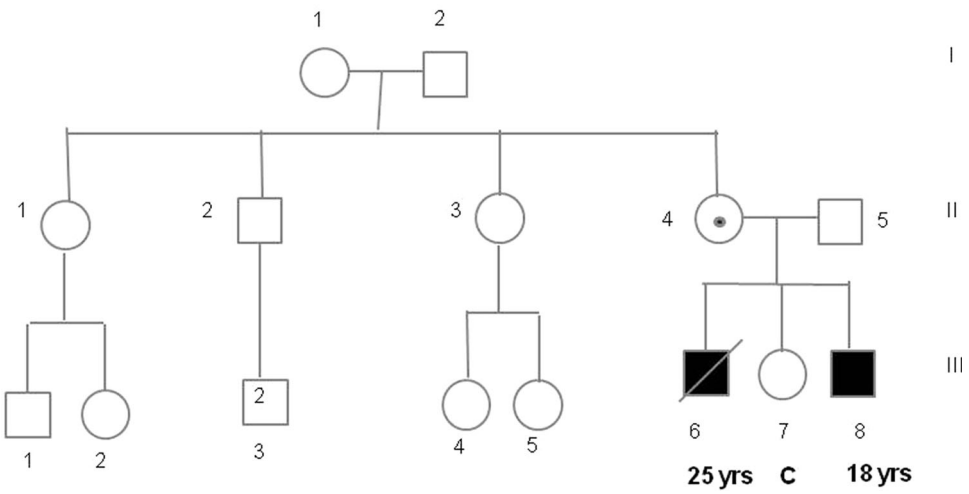

Fig. 3 Pedigree of case 2 showing two affected persons with DMD (*III 6* and *III 8*) and *III 7* is the consultand (*C*)

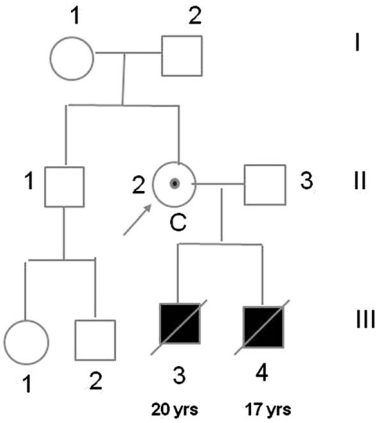

Fig. 4 Pedigree of case 3 showing the two DMD patients who have expired (*III 3* and *III 4*). *II 2* is the consultand

20(III 3) and 17(III 4) years with a history compatible with DMD, but no molecular confirmation was done for either of them (Fig. 4). There are no other affected family members.

For this family, there were no index cases for evaluation and the diagnosis of DMD was not proven by molecular methods. The consultand (II 2) was evaluated with serum CK, which showed a value of 254 U/L, thereby suggesting that she is a carrier. MLPA analysis in her showed the heterozygous deletion of exons 46 and 47, thereby confirming her carrier status (Fig. 5). She was informed about the option of CVS at 12 weeks for prenatal diagnosis. Due to her advanced age, she was told about screening for trisomies if the fetus was unaffected with DMD.

Case 4 (Fig. 6)

A nonconsanguineous family came for counseling as their 2-year-old son (III 2) with normal motor milestones, while being evaluated for vomiting and loose stools, was found to have elevated serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) enzymes, 354 U/L and 250 U/L, respectively. Detailed workup had ruled out an underlying liver disorder, but his serum CK was 19,342 U/L. There is no family history of DMD. Mother has an unaffected younger brother (Fig. 6).

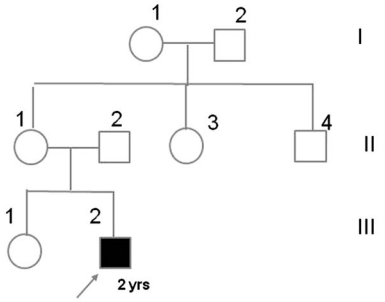

Fig. 6 Pedigree of case 4 showing the confirmed diagnosis in a single member (*III 2*) with no positive family history

The second commonest mode of diagnosis of DMD is following incidental detection of elevated transglutaminase enzymes in male children. On careful evaluation, the boy had mild calf muscle hypertrophy and hypertrophy of the brachioradialis, bilaterally. MLPA analysis showed duplication of exons 18 and 19, thereby confirming the diagnosis of DMD (Fig. 7). The mother's CK was 94 U/L and the MLPA analysis did not show duplication for these exons. For a carrier female with duplication, the peak for the affected exons will be 1.5 times taller than the control peak. Even though she is not a carrier for DMD, there is a risk of gonadal mosaicism so that some ova might be containing the mutated gene.

Detailed clinical evaluation in the index case is the first step to be undertaken when a family presents with a male

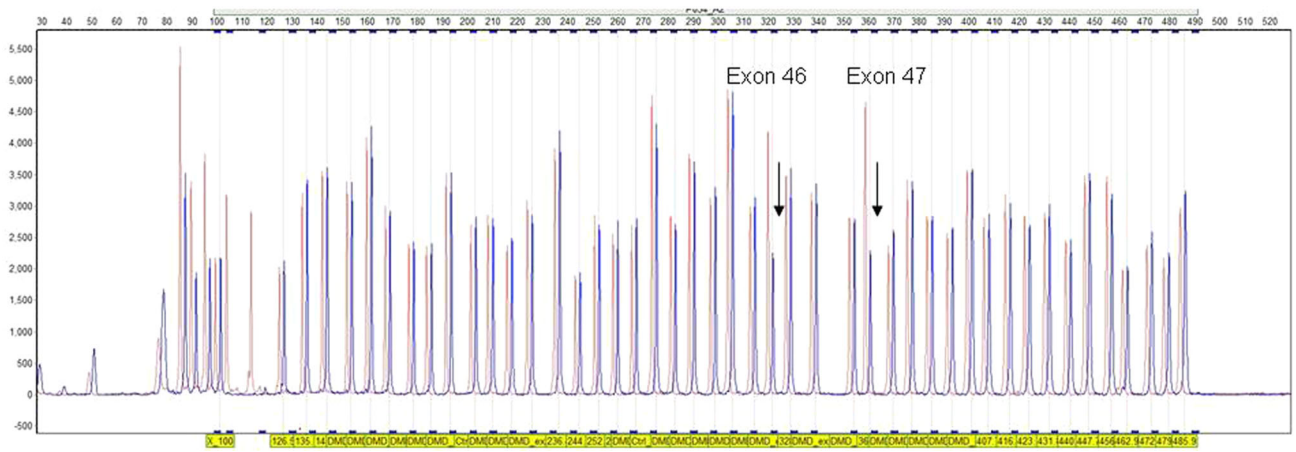

Fig. 5 Hemizygous deletion for exons 46 and 47 confirms the carrier status in *II 2*. The size of peaks corresponding to exons 46 and 47 depicted by *arrows* are half when compared with the controls which denote her carrier status

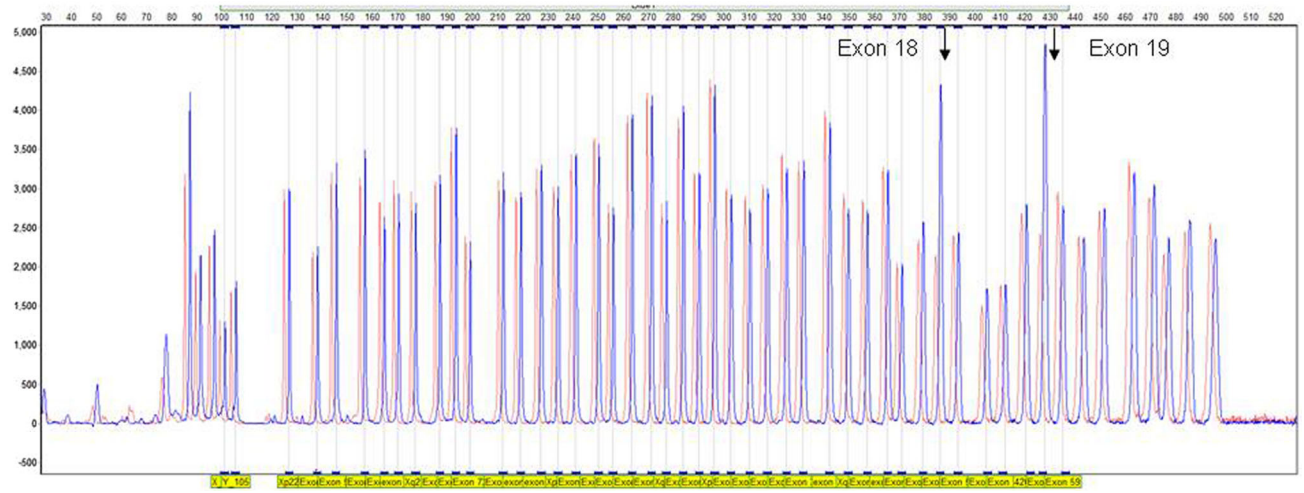

Fig. 7 MLPA analysis showing duplication of exons 18 and 19 in dystrophin gene in *II 2*. The peaks for exons 18 and 19 are two times taller than the control peaks thereby confirming the duplication for these exons

child with history suggestive of DMD. If the parents are consanguineous, the possibility of autosomal recessive muscle disease, such as limb girdle muscular dystrophy (LGMD), should also be considered as a differential diagnosis, as these children also present with elevated CK and calf muscle hypertrophy. In the latter instance, evaluation by MLPA analysis will be normal and muscle biopsy with immunostaining with specific antibodies may confirm the diagnosis for LGMD. Muscle biopsy is not advisable as a first tier diagnostic modality for DMD. Even though muscle biopsy can confirm the diagnosis, the family cannot be offered prenatal diagnosis without confirming the mutation analysis. Moreover, a muscle biopsy should always be accompanied by immunostaining to diagnose the specific type of muscle dystrophy.

It is prudent to identify the mutation in the index child at the time of diagnosis as the analysis of point mutation is labor-intensive and this is the underlying cause for around 30 % cases of DMD. It would be difficult to offer prenatal diagnosis if the mother comes with an ongoing pregnancy and the MLPA of the index child is normal, as sequencing of the gene is mandatory for further molecular analysis and this takes time.

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Compliance with Ethical Standards

Conflict of interest None.

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