Reema Alduaiji et al. JBC Genetics. 2023;6(1):036-040
https://doi.org/10.24911/JBCGenetics/183-1676870604

ORIGINAL ARTICLE

Opinion of geneticist regarding performing preimplantation genetic testing for monogenic disorder for variants of unknown significance

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ABSTRACT

Background: Preimplantation genetic testing (PGT) is used to identify a pathogenic variant in embryos created through *in vitro* fertilization. A "variant of uncertain significance" (VOUS) is a genetic variant discovered through genetic testing but with unknown clinical significance. The primary goal is to gauge geneticists' perspectives on performing PGT-M for VOUS in Saudi Arabia, which results in the development of recommendations from higher authorities regarding the criteria of PGT-M in clinical practice.

Methods: After reviewing the literature, a cross-sectional study was conducted employing questionnaire developed using survey monkey. The reliability of the questionnaire was assessed in terms of internal consistency and Cronbach's alpha-assessed test-retest.

Results: In particular, a total of 96 Saudis and non-Saudis, male and female geneticists, agreed to participate in the study. Out of the 96 geneticists, 56 (59.6%) were female. Most participants were of Saudi origin, with a percentage of (76.6%). The most important finding of this study is that 64% of geneticists opposed performing PGT-M for VOUS. The outcome that 94.5% of geneticists concurred that PGT-M is poorly understood was another noteworthy finding.

Conclusion: Future research with a larger sample size is required for performing PGT-M for VOUS, which will help in developing guidelines for PGT-M in Saudi Arabia.

Keywords: PGT-M, VOUS, a variant of uncertain significance, preimplantation genetic testing, variant, Saudi Arabia, geneticists.

Introduction

When one or both of the parents have a known genetic abnormality and are at high risk of inheriting it from their offspring, Preimplantation genetic testing for monogenic disorders (PGT-M) or its previous term preimplantation genetic diagnosis (PGD) is one of the options to prevent the recurrence of the disease in future pregnancies (1). PGT-M is a technique that identifies a pathogenic variant in the early developing embryos created through *in vitro* fertilization before pregnancy (1). The idea behind PGT-M is to prevent those couples from having another affected child with a similar genetic condition, increasing the chance of a successful pregnancy (1). The use of PGT-M is limited worldwide due to a lack of expertise in the field, insufficient guidelines, and ethical dilemmas. A recent study in the USA demonstrated that many laboratories have limitations because of ethical considerations regarding PGT-M (2). In Saudi Arabia, the first report of next-generation sequencing (NGS)-based PGT for aneuploidy was published in March 2021, which indicates that PGT-M is rarely used. To perform

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Received: 20 February 2023 | **Accepted:** 07 March 2023

PGT-M, *in vitro* fertilization, the IVF technique is used to create embryos. Cleavage-stage embryo biopsy is the most common approach to extract a single blastomere from a developing embryo to remove an intact cell with a careful approach not to affect the remaining embryo. Then, DNA will be extracted from this single blastomere and checked for the specific mutation (1-4). Numerous reasons support an indication of PGT-M. For example, a previously affected pregnancy, a genetic condition in the couple, and advanced maternal age (5). A "variant of uncertain significance" (VOUS) is a genetic variant discovered through genetic testing but whose relevance is unknown. According to the American College of Medical Genetics recommendations, genetic variants are classified into five categories based on the quantity and quality of evidence required to categorize the variant as pathogenic, likely pathogenic, VOUS, likely benign, or benign. Suppose a variant is classified as a VOUS. In that case, it signifies insufficient information to identify whether or not the variant is linked to disease at the time of interpretation (5,6). In perinatal genetics, several molecular genetic testing could be used in addition to PGT-M to prevent the recurrence of the diseases in a future pregnancy. For example, chromosomal microarray (CMA) and non-invasive perinatal testing (NIPT), which are based on NGS technology, allow the detection of chromosomal abnormalities either through amniocentesis or chorionic villus sampling in case of CMA or maternal blood in case of NIPT (7,8). Protecting human life is one of the main basic principles in Islam, in other words, encouraging the prevention of any predictable diseases. The government of Saudi Arabia is based on Islamic Sharia. Therefore, the government of Saudi Arabia supports any procedure that helps in starting a healthy family through PGT-M, which aims to prevent any known familial mutation that would affect a fetus (7). There is an ethical dilemma regarding using PGT-M genetic testing in VOUS by clinicians. An ongoing ethical debate about recommending whether to perform PGT-M for VOUS suspected cases has been occurring in the geneticist community (9). This study aims to assess the opinion of geneticists around Saudi Arabia in performing PGT-M for VOUS and help formulate recommendations from high authorities regarding criteria for performing PGT-M in clinical practice for VOUS.

Subjects and Methods

A cross-sectional questionnaire-based study was conducted involving hospitals across Saudi Arabia (Figure 1). The research committee of King Abdullah International Medical Research Center (KAIMRC), Riyadh, Saudi Arabia, approved all procedures and were performed in accordance with the ethical standards. Concerning the physical distancing strategy and minimizing face-to-face interaction, we developed an outline questionnaire via Survey Monkey (https://www.surveymonkey.com) that limits one-time participation per unique internet protocol (IP) address. This questionnaire was sent to a sample of Saudi Arabian geneticists via Social media groups. The inclusion criteria were Saudi and non-Saudis, male and female geneticist physicians who agreed to participate in the study. Exclusion criteria were lack of access to the internet, inability to complete an online survey, and non-geneticist physicians. Ninety-six participants agreed to participate and responded with a complete questionnaire. After reviewing the literature, a self-administered questionnaire was designed to have a validated tool (10). The survey was distributed to the participants, who are geneticists from all over Saudi Arabia hospitals. To assess the opinion of geneticists and develop a guideline regarding PGD for VOUS about preimplantation genetic diagnosis, it was a self-administered questionnaire-based survey in English as a soft copy done in Saudi Arabia. Moreover, it would increase awareness about existing problems and ethical issues related to VOUS in the field of PGD. The reliability of the questionnaire was assessed in terms of internal consistency. Cronbach's

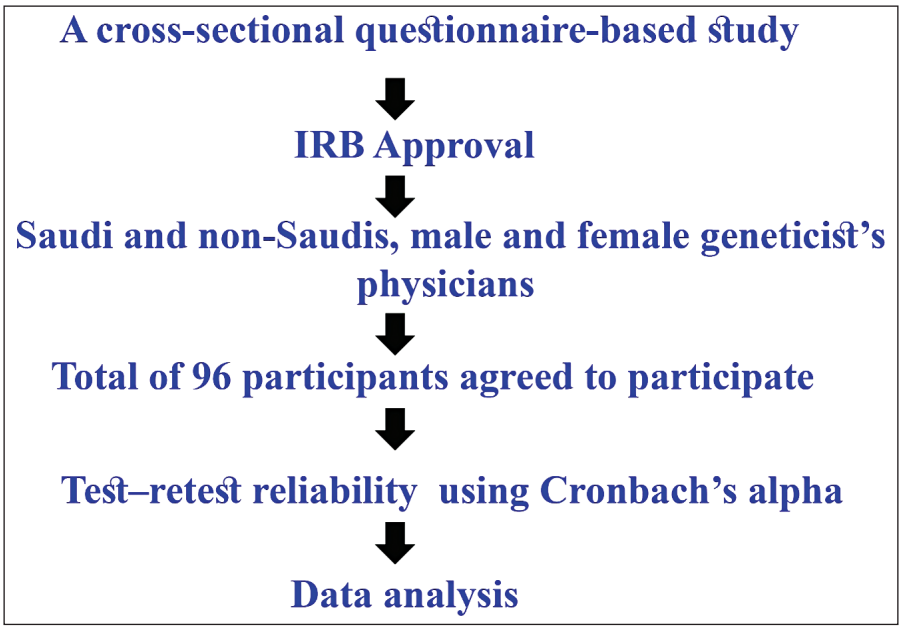

Figure 1. *Flow sheet diagram showing the methodology steps.*

alpha was computed. Test–retest reliability was also assessed using Cronbach's alpha Construct validity of the checklist was assessed using expert opinion, and the final version was approved accordingly after pilot testing. The independent variables were the demographics of the respondents, such as age, experience level, and genetic specialty. Other independent variables were taken to test the geneticist's knowledge level and opinion regarding VOUS and PGT-M. Using a scale of 1 to 3, 1 = Disagree, 2 = Neutral, 3 = Agree. Data entry and analysis were carried out using the statistical program Statistical Package for the Social Sciences version 24. Descriptive analysis was done for categorical variables such as genetic specialty and opinions of geneticists towards PGT-M and VOUS were reported as percentages and frequencies in tables. In addition, tables represented numerical variables such as age and years of experience as percentages and frequencies.

Results

In our study, a survey that asses the opinion of geneticists on performing PGT-M for VOUS was conducted on 96 participants. Most participants were of Saudi ethnicity, with a percentage (76.6%). The participating geneticists consisted of 59.6% females and 40.4% males. As shown in Table 1, 84.8% answered the class of VOUS correctly. The main dilemma is needing more knowledge and evidence regarding performing PGT-M for monogenic diseases for VOUS. Of the 96 geneticists (86), 94.5% agreed with this statement. One of the key findings was that (55) 64.7% of the geneticists were against performing PGT-M for VOUS. However, the majority of the geneticist (75), 82.5%, will perform PGT-M only based on the parents' opinion. In addition, we found (65) 82.3% of the geneticist agreed with the right of patients to choose whether or not to be informed of their PGT-M carrier status.

Moreover, (90) 96.8% of the geneticist agreed on the need for informed consent to perform PGT-M for VOUS. On the other hand, (48) 52.2% agreed that it is only needed if the parents insist on performing it. All geneticists agreed with counseling couples on the ethical issues and psychological stresses of PGT-M. When performing PGT-M for VOUS, (92) 98.9% agreed that they need to include in genetic counseling the risk of having a baby with genetic disorders whether they do PGT-M or not. Furthermore, (85) 96% of the geneticists believed that patients of reproductive age and their families who are at risk should be counseled on IVF-PGT-M as early as possible to maximize their chances of conception. To prevent the transmission of genetic abnormality from a genetic mutation carrier to their offspring, carrier parents can choose to perform IVF-PGT-M, supported by (86) 95.6% of the geneticists. Additionally, (72) 83.7% of the geneticists were in favor that carriers of genetic mutations preferred PGT-M to prenatal testing to decide on terminating the pregnancy or not. Moreover, (69) 88.1% of geneticists supported the claim of children born after PGT-M seem as healthy as children delivered after natural conception or other forms of conception such as IVF or ICSI treatments (Table 2).

Table 1. *Demographic data in numbers and percentages.*

	No	Percentage
Male	38	(40.4)
Female	56	(59.6)
Saudi	72	(76.6)
Non Saudi	22	(23.4)
Experience (in years)		
<10	48	(51.1)
10-<20	30	(31.9)
20-<30	13	(13.8)
>30	3	(3.2)

Discussion

The most remarkable finding in this study is that the opinion of geneticists was significantly against performing PGT-M for VOUS. Ultimately, this led to the geneticists deciding to go with the parents' opinion on whether or not to perform PGT-M. Detailed genetic counseling regarding performing PGT-M for VOUS and its expected psychological stresses is necessary for the parents. Ultimately, this led to the geneticist's decision to go with the parents' opinion on whether or not to perform PGT-M. In addition, geneticists have agreed on the need to mention that whether or not PGT-M is performed, it will not guarantee a genetic disorder-free embryo. A study suggests that the parents should be counseled about the ethical issues, psychosocial stress, procedural limitations, possible results, and its application before performing PGT-M for VOUS (11). Moreover, PGT-M has many advantages over other prenatal testing methods. One is that it helps carriers of genetic mutation avoid the difficult decision of terminating an affected pregnancy or giving birth to a sick child. Another advantage of PGT-M, in the opinion of most geneticists, is that children born using this method seem as healthy as children born to other forms of conception. Another study suggests a great advantage of PGT-M, specifically its ability to detect chromosomal aneuploidy in the embryos and transfer normal chromosomal embryos in order to achieve a healthy and normal pregnancy (3). Furthermore, almost all geneticists agreed on the need for informed consent to perform PGT-M for VOUS. The usage of PGT-M has been widely popular over the past three decades. PGT-M was developed for various genetic conditions and severe disorders, and there are three major disease groups which PGT-M is used for. The first group is sex-linked disorders, such as Rett Syndrome. The second is single gene defects and genetic mutations such as BRCA-1. In addition, the third group PGT-M can help in diagnosing chromosomal disorders (12). Moreover, the PGT-M laboratory must be appropriately insured against the possibility of a misdiagnosis. Even though it is censorious that PGT-M is performed using tests that have been verified and tailored for the couple, there have been multiple incidents of misdiagnosis. Misdiagnosis can occur due to sample-specific factors, such as chromosomal mosaicism in the embryo. It could be a

Table 2. *Responses to perception of geneticists towards PGD in numbers and percentages.*

Statements	AG	NS	DA
1. PGD is an early form of genetic testing and, when combined with IVF, enables gestation of only unaffected embryos.	83 (91.2)	5 (5.5)	3 (3.3)
2. There is lack of knowledge and evidence regarding performing PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	86 (94.5)	4 (4.4)	1 (1.1)
3. I will not perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	55 (64.7)	25 (29.4)	5 (5.9)
4. Informed consent is needed to perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	90 (96.8)	2 (2.2)	1 (1.1)
5. I will go with the opinion of the parents, after detailed genetic counseling to perform PGD for monogenic diseases (PGT-M) for variants of unknown significance (VOUS).	75 (82.5)	10 (11.0)	6 (6.6)
6. Patients at risk of a genetic disorder have the right to choose not to know of their carrier status. Non-disclosure PGD or exclusion PGD can enable this while offering the option of conceiving mutation-free children who will not go through similar emotional turmoil.	65 (82.3)	10 (12.7)	4 (5.1)
7. Informed consent is needed only in case the parents insist on doing PGT-M.	48 (52.8)	21 (23.1)	22 (24.2)
8. Couples should be counseled on ethical issues relevant to their PGD and the expected psychological stresses during the decision-making process and the IVF–PGD treatment	92 (100)		
9. When performing PGT-M for VOUS you need to include in detailed genetic counseling there is a risk of having a baby with genetic disorders whether you do PGT-M or not	92 (98.9)	1 (1.1)	
10. Patients who are at risk and/or family members at reproductive age should be counseled on IVF–PGD as an option to conceive healthy children, as early as possible to maximize their chances of conception	85 (96)	2 (2.3)	1 (1.1)
11. Most carriers of genetic mutations opt for PGD over prenatal testing to avoid facing the difficult decision of whether or not to terminate an affected pregnancy or to give birth to a sick child	72 (83.7)	9 (10.5)	5 (5.8)
12. A patient carrying a known genetic mutation or chromosomal abnormality can choose to use IVF–PGD to prevent transmission of the genetic abnormality to their offspring and future generations	86 (95.6)	4 (4.4)	
13. Thousands of children born after PGD seem as healthy as those delivered after natural conception or after IVF and/or ICSI treatments only for infertility	69 (88.1)	7 (9.0)	2 (2.6)

technique-specific issue, such as maternal or paternal contamination or allele dropout (13,14). On the other hand, many are unaffected by the method. System failures could include mistakes in labeling and misidentification of tagged samples. The most crucial factor is abstaining from and eliminating human error or system failure. Proper genetic counseling for the affected family is essential for rare hereditary diseases. In addition, the best approach for treating such a condition, which has no treatment, is parenteral genetic screening/diagnosis (15,16). Similarly, in Saudi Arabia, newborn screening (NBS) of infants between 24 and 72 hours of birth can avoid disability and possibly even death by checking for conditions advised by the national Newborn Screening Committee. The NBS program aims to identify infants born with specific genetic, metabolic, and functional abnormalities (17). Future treatment studies may be aided by identifying genes-variants linked to a given condition from a particular population (18,19). The main limitation of this study was the literature gap, particularly in PGT-M for VOUS. Therefore, this presents the need for further development in research about PGT-M for VOUS in the field of genetics.

Conclusion

This study showed that 64% of geneticists were against performing PGT-M for VOUS. Moreover, most agreed that there is a lack of knowledge about PGT-M due to a lack of guild lines and a lack of research regarding PGT-M. Therefore, future research is needed regarding ethical considerations of PGT-M and its implication. It will help higher authorities develop the guild lines for PGT-M regarding VOUS usage in clinical practice in Saudi Arabia. Furthermore, improving the psychosocial impact on the couple performing PGT-M for VOUS is crucial.

List of Abbreviation

CMA	Chromosomal microarray
IVF	In vitro fertilization
KAIMRC	King Abdullah International Medical Research Center
NIPT	Non-invasive perinatal testing
PGD	Preimplantation genetic diagnosis
PGT	Preimplantation genetic testing
PGT-M	Preimplantation genetic testing for monogenic disorders
VOUS	Variant of uncertain significance

Acknowledgments
We thank all participants who took the time and contributed valuable contributions to this project.

Funding
None.

Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
The study was approved by the Institutional Review Board of KAIMRC, Riyadh, Saudi Arabia (Memo Ref. No. IRBC/0999/ 21).

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