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ARTICLE

Preimplantation genetic testing in two Danish couples affected by Peutz–Jeghers syndrome

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ABSTRACT

Background: Guidelines from the European Hereditary Tumor Group as well as The Danish National Guidelines for Peutz–Jeghers Syndrome (PJS) state that both prenatal diagnosis and preimplantation genetic testing for monogenic disorders (PGT-M) should be offered to patients with PJS. However, only a few cases resulting in viable pregnancies have been published.

Objective: We present two cases of PJS patients going through PGT-M for PJS. We highlight the awareness of this possibility and discuss the technical and ethical challenges of performing PGT-M for PJS.

Methods and results: Case 1: A 36-year-old male with PJS and his partner were referred for genetic counseling. The patient carried a pathogenic *de novo* variant in *STK11*. After a terminated pregnancy of a fetus carrying the same pathogenic variant, microsatellite polymorphic marker analysis was established, and the patient was offered PGT-M. The female partner of the patient gave birth to a healthy boy after five years of fertility treatment. Case 2: A 35-year-old female with PJS and her partner were referred for genetic counseling. She carried an inherited pathogenic *STK11* variant. The couple was offered PGT-M. Genetic testing of the embryos was performed using microsatellite polymorphic markers. After two rounds of oocyte extraction a blastocyst predicted not to be affected by PJS was identified. The blastocyst was transferred; however, this did not result in a viable pregnancy.

Conclusions: PGT-M can be offered to patients with PJS. The process may be long and filled with ethical dilemmas requiring patients to be motivated and persistent.

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Introduction

Peutz–Jeghers Syndrome (PJS) is a rare tumor predisposition syndrome (TPDS) characterized by the development of hamartomatous polyps in the gastrointestinal (GI) tract (Figure 1), mucocutaneous pigmentations and an increased risk of developing cancer in various organ systems [1]. The major sites of cancer are within the GI tract (esophageal, stomach, duodenum, colon and pancreatic) and breast cancer in females, corresponding to a lifetime cancer risk of 50−60% and 50%, respectively [1]. A pathogenic variant in *STK11* can be detected in up to 90% of cases with clinical PJS [2]. PJS is inherited in an autosomal dominant manner, with 100% penetrance but variable expressivity. Patients with PJS are offered extensive surveillance (starting at the age of eight years in Denmark) to identify cancers at an early stage and allow for minimally invasive treatment options in relation to polyp burden [3].

In Denmark, patients with TPDS are offered genetic counseling in adulthood, including information on reproductive options, e.g., invasive prenatal diagnostics (PNDs) with genetic testing of a chorionic villus sampling (CVS) or amniocentesis, gamete donation, or preimplantation genetic testing for monogenic disease (PGT-M). In Denmark, PGT-M has been offered since 1999 to couples with a known and significant risk of severe genetic disease in future children (Danish law on assisted reproduction https://www.retsinformation.dk/eli/lta/2019/514) and the demand and number of PGT procedures increases. Assisted reproduction technology (ART) including controlled ovarian stimulation, oocyte retrieval, intracytoplasmic sperm injection (ICSI), *in vitro* culture and cleavage stage biopsy or trophectoderm (TE) biopsy from the blastocyst stage is a prerequisite for PGT. Unaffected blastocysts can then be transferred to the uterus in order to achieve pregnancy [4] (Figure 2).

Due to the autosomal dominant inheritance pattern of PJS there is a 50% recurrence risk in each pregnancy, and the couples face potential dilemmas in family planning as reproductive decisions may be affected by ethical, societal, religious and legal issues. There is limited experience of PNDs in PJS patients, but invasive PND performed in patients

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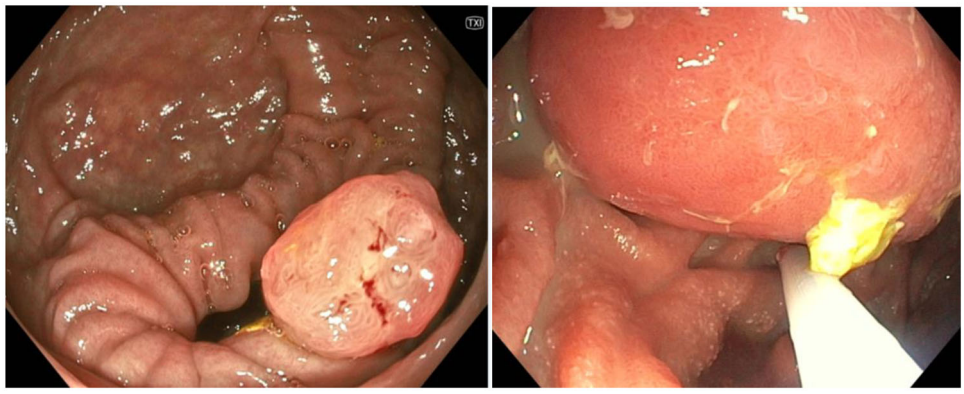

Figure 1. Images from a colonoscopy showing hamartomatous polyps in colon (right) and duodenum (left).

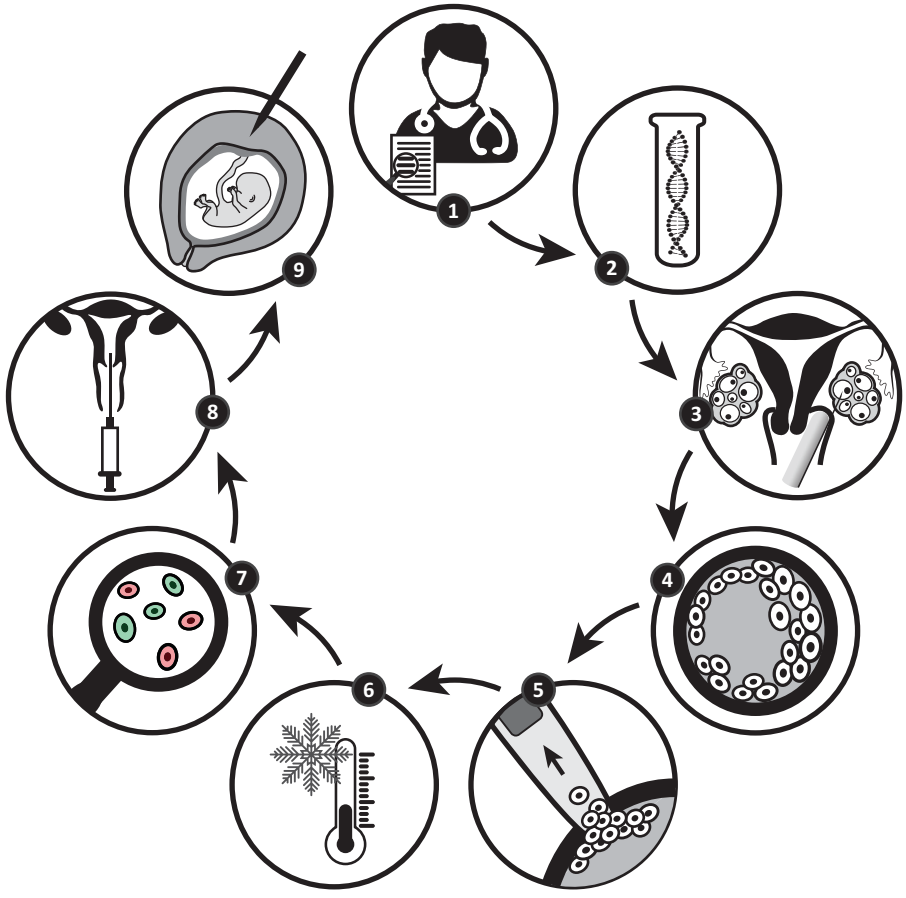

Figure 2. Stepwise presentation of preimplantation genetic testing (PGT). 1) genetic counseling by a clinical geneticist, 2) establishment of the microsatellite polymorphic marker analysis, 3) hormonal stimulation, oocyte extraction and *in-vitro* fertilization, 4) *in-vitro* culturing to reach the blastocyst stage, 5) biopsy from the trophectoderm, 6) cryopreservation of the blastocyst, 7) genetic testing of the trophectoderm biopsy enabling selection between affected and unaffected oocytes, 8) implantation with blastocyst predicted to be unaffected and 9) chorion villus biopsy in week 11 of pregnancy to confirm that fetus is in fact unaffected.

with PJS has been described [5–7], and PJS patients deem reproductive issues important to them [8,9]. We present two cases of PGT-M in PJS patients and discuss the challenges and ethical aspects. Families provided informed written consent prior to publication of this article.

Methods and results

Case 1: A male patient, now 37 years old, was diagnosed with PJS at age 12 years due to mucocutaneous pigmentation. At the age of 18, he was found to have hamartomatous polyps in the small intestine, and at age 20 the diagnosis of PJS was verified through genetic testing identifying a germline variant in *STK11*, c.783C > G, p.(Tyr261Ter) (NM_000455). The patient's parents did not carry the variant, which subsequently was interpreted as having occurred *de novo*. By the age of 26, the patient sought genetic counseling as he wanted to know his family planning options. He was determined not to pass on a pathogenic variant in *STK11* to a future child; however, PGT-M could not be performed at that time because he did not have affected

family members for linkage analysis. Invasive PND was offered. In 2016, his partner became pregnant. The pregnancy was terminated for other reasons and the fetus was found to carry the pathogenic variant in *STK11*. With the use of DNA from the fetus, an informative microsatellite polymorphic marker was identified (D19S886) as prognostic for carrier status of the pathogenic variant in *STK11*, and the couple was referred to one of the two Danish public PGT centers for PGT-M. In 2017, the couple underwent two PGT-M procedures with cleavage stage biopsies resulting in two blastocysts without the pathogenic variant that was transferred consecutively, resulting in one blighted ovum but no child. In 2018, the couple had another two PGT-M procedures with primary blastocyst cultivation, as the laboratory procedures had changed. A total of three blastocysts developed and TE biopsies were performed. Only one blastocyst was without the pathogenic variant, and was transferred without achieving a pregnancy. After this, the female partner was diagnosed with diabetes type 2 and the fertility treatment was paused until the diabetes was well regulated. By the end of 2019, a fifth PGT-M procedure was performed, resulting in one blastocyst without the pathogenic variant and a biochemical pregnancy. Finally, in May 2020, a last PGT-M procedure was performed, resulting in three blastocysts, two without the pathogenic variant, and the transfer of the first blastocyst resulted in an ongoing pregnancy. The patient had moderately decreased sperm quality. The patient and his partner were both 35 years old at the time. A follow up CVS in week 11 confirmed that the fetus did not carry the variant in *STK11*. The pregnancy progressed without complications, and in week 39 + 0 a healthy boy was born *via* vaginal delivery.

Case 2: A female patient, now 36 years old, was diagnosed with PJS at age 3 years, due to GI bleeding caused by a hamartomatous polyp in the stomach, which subsequently resulted in a partial gastrectomy. During her adolescence and early adult years, she had several polyps removed from the stomach, small intestine and colon. In adulthood, the diagnosis was genetically confirmed when a germline variant (*STK11*, c.297_298insT, p.(Gln100Serfs*63), NM_000455.4) was identified. The variant proved to be inherited from an affected parent. At age 31, the patient and her male partner sought genetic counseling for family planning options, and subsequently opted for PGT-M. The PGT treatment was initiated in one of the two public PGT Centers in Denmark, where Multiplex PCR was established, combining SNaPshot minisequencing for the pathogenic variant and microsatellite polymorphic marker analysis with the D19S427 marker, which was identified as prognostic for carrier status. In 2019, the couple underwent two PGT-M procedures resulting in a total of eight blastocysts and TE biopsies, but none were suitable for transfer after the genetic analysis. Subsequently, the couple moved to another part of Denmark and treatment was moved to the other public Danish PGT Center. Here, an analysis was established using the closely linked marker D19S427_B in combination with the marker STK11_c.297dup, the latter as a direct detector of the variant in *STK11*. The first PGT procedure in the second center resulted in four blastocysts and TE biopsies. Two blastocysts were predicted to carry the variant in *STK11*, one blastocyst did not yield a PCR response even after re-biopsy, and one blastocyst did not carry the pathogenic variant and was transferred. Unfortunately, this did not result in a pregnancy. The couple was encouraged to seek genetic counseling prior to potential use of the blastocyst without PCR response, but has so far not opted for further treatment.

Discussion

Reproductive issues and family planning are considered important by many patients with an increased risk of passing on an inherited condition [8,10], but there is limited experience applying PGT in patients with inherited TPDSs. Studies have shown that many patients with TPDS are unaware of PND and PGT-M, or are unaware that these techniques are available to them [10,11]. In Rich et al., only 24% of 370 TPDS patients had knowledge of PGT, and higher awareness was associated with childhood TPDS and higher socioeconomic status [10].

PJS affects patients' decisions to have children [8,9]: A questionnaire survey among 38 PJS patients showed that 40% had altered reproductive choices because of PJS and that 33% were reluctant to have children due to PJS [9]. Van Lier et al. found that 19% of 52 patients did not want children because of PJS. Patients were overall positive toward the use of PND and PGT-M [8]. This corresponds with findings by Rich et al. who found that 72% of their patients thought PGT should be offered and 43% would consider using PGT [10].

Patients with a TPDS have several options regarding family planning. They can choose: 1) not to have their own biological children but adopt or not to have children at all, 2) to have children and accept a 50% risk of this child inheriting the pathogenic variant in *STK11*, 3) gamete donation and 4) invasive PND or PGT-M.

If couples opt for 1) and 2) there are no ethical biomedical-related dilemmas, whereas 3) opting for gamete donation confers dilemmas related to fertility treatment. Sperm donation is non-invasive (in contrast to oocyte donation) and is much more accessible in the public reimbursed Danish system with almost no waiting time compared to one – two years waiting time for donated oocytes. Therefore, sperm donation may seem the better option if the potential father is the carrier of a variant in *STK11*. In the private Danish Fertility Clinics, both sperm and oocyte donation are easily accessible, but oocyte donation has a much higher price. Concerning 4) the arguments in support of PND and PGT-M are reproductive autonomy, avoiding life-long concern that a child might develop severe disease, cost of life-long treatments (*versus* cost of PND and PGT-M), and psychosocial burdens of having a TPDS and concerns related to surveillance and symptoms [12]. In addition, there is the argument to spare future generations of reproductive dilemmas. Arguments against PND and PGT-M are comprised the possibility that better treatment options are available when the child becomes an adult, the possibility of a more benign clinical course, the risk of devaluing the lives of people with TPDS, medicalization of

reproduction (hormone stimulation and risk of hyperstimulation), and the risk of having to choose whether to terminate a wanted pregnancy (for PND) and for PGT-M the exhaustive process of fertility treatment with no guarantees of achieving a healthy pregnancy. Legally, PGT-M can be offered in Denmark if a future child has a significant risk of severe disease but with no definition of what constitutes 'severe disease' (Danish law on assisted reproduction https://www.retsinformation.dk/eli/lta/2019/514). Thus, the offer of PGT-M in Denmark is usually decided at multidisciplinary team conferences and based on a consensus decision among clinical geneticists, fertility doctors and molecular biologists.

Opting for PGT-M can confer technical challenges; PGT-M in Denmark is based on either genetic marker analysis, direct testing for the pathogenic variant, or a combination of both. A genetic marker analysis is a microsatellite polymorphic marker analysis, which essentially is a screening for the allele on which the pathogenic variant is located. In order to establish a microsatellite polymorphic marker analysis, DNA from a minimum of two affected relatives are needed to ensure identification of the specific allele carrying the pathogenic variant. Additionally, it is not always technically possible to establish the markers, despite having DNA from two affected family members. The chance of achieving a pregnancy through PGT-M has increased in recent years, going from a 20 – 25% chance for each blastocyst transfer to roughly 40% today [13], but PGT-M is time consuming, making the option of PGT less attractive for patients who are eager to achieve a pregnancy within a short time frame.

In addition, patients may have ethical and social concerns related to opting for PGT-M. These can be based on religious beliefs, access to healthcare, education and medical history [10,14]. In some religions, PGT is considered to be unacceptable, and some consider PGT comparable with abortion [10]. Other religions consider PGT-M to be an acceptable option compared to donation of gametes or termination of a pregnancy. There can be concerns about the risk of a 'slippery slope', i.e., the advent of PGT will lead to unethical use (selection based on gender, IQ, or germline gene editing). In addition, there may be concerns about cost, both in countries with insurance based and universal healthcare systems. However, comparing the cost of PGT-M to lifelong surveillance and treatment of PJS, PGT-M may very well be cost-effective [15]. Despite surveillance being available for PJS, most PJS patients develop cancer during their life and the general life-expectancy is reduced compared to the general population.

The perceived balance between benefits of PGT-M and disadvantages is highly individual, depending on the couples ethical, religious and socioeconomic status, and is also influenced by the framework of healthcare systems. Information about all reproductive options is relevant and welcomed by PJS patients as it allows them the opportunity to choose the reproductive strategy most suitable in their lives.

Conclusion

We report two cases of patients with PJS syndrome undergoing PGT-M to achieve pregnancy with an unaffected fetus. The two cases described in this article demonstrate that PJS patients want PGT-M, and highlight why the option should be presented to these patients when they are planning to start a family.

Ethical approval

Both patients provided written consent to this publication.

Author contributions

AMJ had the initial idea for the article. AB wrote the first draft. LR, JGK, TD, KL and AMJ commented on the manuscript. AB submitted the manuscript.

Code availability

Not applicable.

Disclosure statement

To the best of our knowledge, no conflict of interest, financial or other, exists.

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Data availability statement

All data is submitted.

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