PREIMPLANTATION GENETIC DIAGNOSIS FOR HUNTINGTON’S DISEASE - CASE REPORT AND LITERATURE REVIEW

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Abstract

Background: Huntington’s disease is a rare, neurodegenerative, autosomal dominant disorder with adult onset caused by an expansion of 36 or more CAG trinucleotide repeats in the HTT gene. In Brazil and in some other countries, carriers of HTT’s expanded allele of submitted to in vitro fertilization procedure can investigate embryos genetically to identify the ones that have inherited the expanded allele and the ones that inherited only the normal size alleles. In some instances, an individual has a positive familial history for the disease and do not want to know his/her personal carrier status but is willing to conceive children without the disease. Objective: to demonstrate the reproductive possibilities available to patients with Huntington disease through a case report and literature review. Methods: Here, we discussed the application and viability of the genetic screening techniques, based on a non-disclosure case of Preimplantation Genetic Testing (PGT). Results: a couple submitted to a non-disclosure test for Huntington disease was submitted to IVF, embryo biopsy and genetic analysis of the samples due to a familial history of the condition. Chromosome and mutation specific tests were applied, and one embryo was selected for uterine transfer. The couple was not informed why the other embryos were discharged. Conclusion: Although many ethical and practical aspects are involved in this decision, a non-disclosure PGT can be offered as an option for these couples, besides sperm, egg or embryo adoption, or child adoption.

Keywords: blastocyst; neurological disease; genetics; ethics.
Introduction

Huntington's Disease (HD) is an autosomal dominant, neurodegenerative disease with adult onset. Clinical manifestations are usually evident by 35-44 years of age and survival after diagnosis averages 15-18 years. In prodromal stages, some patients might present subtle changes in motor skills, cognition and personality even 15-20 years before first clinical manifestations of disease. However, most patients are diagnosed only when the disease course is up to 2/3 of its clinical evolution [1,2]. Clinical manifestations include anxiety/depression, abnormal eye movement, olfactory dysfunction that posteriorly progress to chorea, dystonia, movement disturbances, generalized weakness, unwanted weight loss, speech difficulties; finally, there is bradykinesia, swallowing problems, inability to speech and to walk, and inability to take care for themself. Neuroimaging frequently shows striatal atrophy.

The mechanism responsible for the disease is the occurrence of a trinucleotide expansion with the minimum of 36 repeats in exon 1 of HTT gene mapped on chromosome 4p16.3. HTT gene codifies huntingtin, a nuclear protein expressed in all tissues that binds to numerous transcription factors [3]. Phenotype severity is proportional to allele expansion size: alleles with less than 26 CAG repeats are considered normal and do not represent any risk for the disease; intermediate alleles have 27-35 repeats and due to instability of this repetition there is risk for expansion, more commonly during spermatogenesis, increasing the odds of having an affected offspring [4]; individuals with 36-55 repeats manifest the adult onset form of the disease; individuals with more than 60 repeats manifest the juvenile form of the disease; The diagnosis is confirmed by demonstration of the expansion by PCR with fragment analysis evaluated by capillary electrophoresis or PCR associated to Southern Blotting techniques [4].

Preimplantation Genetic Diagnosis (PGD), now named PGT-M (preimplantation genetic testing for monogenic diseases) is a technique utilized since 1989 [5] for couples with increased risk of transmitting a determined genetic disease to offspring. It is based on in vitro fertilization and embryo biopsy for genetic analysis [6]. Successful use of PGD for HD was demonstrated in 1998 by Sermon et al, which used the genetic technique to screen embryos from 5 couples at risk for HD [7]. The authors also proposed in a second manuscript in which a proband with a positive familial history has two possibilities: to be genetically tested to know if is carrier of the expanded allele or to perform a nondisclosure test to screen the embryos with no knowledge of the carrier status [8]. In the second option, no information about the embryo status is provided to the couple, so the consultant does not know if there is still risk for being a carrier. Despite the meaning of this achievement, some questions regarding this option should be done: (I) How the perception of the couple towards the consultant carrier
status will change after a possible lack of embryos per transfer after repeated cycles, or on the other hand if there are many embryos to transfer? (II) What will be the implications drawn from this information for the reproductive couple’s life or even in the consultant clinical follow-up?

In this article we intend to discuss these questions, approach ethical concerns about the procedure and other options, and its implications towards the couple’s reproductive life.

Materials and Methods

Case description

An otherwise healthy couple came to the reproductive center willing to conceive a healthy child. There was no history of infertility. Fertility evaluation tests showed normal results. They have never tried to conceive due to the familial history of HD in the mother of the male partner. He was 32 years old, asymptomatic in the moment of the PGT procedure and does not want to perform any predictive testing. His semen analysis is normal as well as peripheral blood karyotyping was 46,XY[20]. The female partner was 31 years old, nulligravida, BMI of 21 Kg/m2; hysteroscopy was normal as well as serology including Zika Virus, and peripheral blood karyotyping was 46,XX[20].

After genetic consultation and discussion of possibilities, the decision was made towards performing assisted reproduction with PGT for HD and aneuploidies with no disclosure embryo status for the clinic and couple.

IVF procedure

Two cycles of ovarian stimulation performed with Alfalfolitropin (r-hFSH 150UI e alfalutropin r-hLH 75UI), (Pergoveris- Merck®) and 150 IU of hp-HMG (Menopur-Ferring®) from day 2 to day 10 of the cycle. Ant-GnRH (Orgalutran-Merck®) with follicle ≥14 (d 8, 9, and 10), and ovulation time was synchronized with r-hCG (Ovidrel-Merck®).

In the first cycle, 15 follicles larger than 14 mm were visualized by US and twelve MII oocytes were retrieved. ICSI was performed with fresh semen prepared by density gradient preparation. Fertilization occurred in eleven of the twelve oocytes, and embryos were cultured in Vitrolife® media until D6. Four embryos achieved blastocyst stage and were biopsied and cryopreserved. In the second cycle there were eleven MII oocytes in which ICSI was performed with fresh semen prepared by density gradient preparation. Fertilization occurred in seven of the eleven oocytes that were cultured in Vitrolife® media until D6. Two embryos reached blastocyst stage and were biopsied. With the total of 6 embryos cryopreserved,
biopsies were sent to a private laboratory for PCR and NGS analysis of mutation and aneuploidies, respectively.

**PGT methodology**

Direct analysis of the CAG expansion in HTT gene, indirect analysis using polymorphic markers linked to HTT gene (D4S3038, ACHTG, D4S127, D3S3023 and D4D431) by means of fluorescent PCR. Fragment analysis of PCR products was performed by capillary electrophoresis through AB 3130 (ThermoFisher).

Aneuploidy analysis: library preparation with Ion ReproSeqTM PGS kit; Rapid NGS - Ion Reporter software v 5.0 for data analysis; ReproSeq Low-pass whole-genome aneuploidy workflow v 1.0 (minimum coverage 0.01x).

**Results and Discussion**

Huntington’s disease diagnosis is essentially clinical, based on symptoms presentation. However, with diffusion of biotechnology and the decrease of testing prices, the possibility of patients perform genetic tests becomes more accessible and offer new insights about reproductive options for the families.

Once the diagnosis is established in a patient there is possibility to perform predictive testing on the family members older than 18 years old considered at risk of HD development. According to the literature [9,10,11], among the reasons in favor of testing those individuals are the possibility of knowing personal reproductive risk and to allow personal planning of attitudes; in case of a positive test there are the possibility of screening offspring which may as well be in risk for the disease, and eventually minimize clinical follow-up in case of a negative test. Also, it is possible to use a donors’ gamete, excluding the possibility of transmitting the variant allele to the condition. Among the reasons against testing those individuals are the lack of curative therapeutic for the disease; the inability of the patient deals with a positive result with potential suicidal ideation; in case of a negative test the manifest of survival guilt can be also devastating.

Considering family constitution, the choices for an individual at risk for HD are: (I) don’t have children, (II) don’t have other child, (III) have children without perform any genetic testing, (IV) to perform predictive testing and, if positive, test the carrier status of the embryos or (V) don’t perform any predictive testing and test the embryos using a non-disclosure PGT [11].

In the non-disclosure PGT, the embryos are evaluated for the HTT mutation and for aneuploidies. By this technique only non-carriers and euploid will be available for transfer. The complete results found in the embryos are not informed to the couple or the IVF clinic in order to improve confidentiality. The analysis lab will inform to the IVF clinic which embryos are
normal to transfer considering for both conditions. The combined analysis will also improve the pregnancy chances, decreasing the miscarriage rate and increasing implantation rates [12]. It was the option chosen by the asymptomatic consultant of the present case. Here, in this case, only one embryo was considered normal for both conditions (gene mutation and chromosomal abnormalities). Embryo transfer was performed but resulted in no pregnancy.

Despite the large contribution of genetic screening, Braude et al [9] discussed among how confidential is this information. It is noteworthy to mention the process start with gynecologic evaluation, ovarian stimulation, oocyte retrieval, ICSI, embryo culture and evaluation on different days, embryo biopsy, cryopreservation, genetic analysis of cells obtained with proportion of embryos for a posterior transfer. Due to these many steps and many workers involved in the process of medical assistance and reproductive technology, at some point it is not hard to predict the carrier status of the patient and therefore absolute secrecy is practically unfeasible.

Moreover, the couple's perception of the carrier status of the individual at risk is also involved. What if there are no embryos available for transfer? This fact could induce the wrong feeling that the parent is a carrier. In this case, some authors suggest a false transfer (no embryo is transferred in the procedure) [8,9]. We believe this alternative is not sensible and submit the couple to unnecessary medical procedure. On the other hand, what if the couple is up to many embryos for transfer? It could influence the couple's perception on the carrier status of the individual as being negative. Risks of these thoughts to the couple are that even in a situation where there are cryopreserved embryos for future transference; they conceive naturally exposing future offspring to an unnecessary risk. Also, on an individual basis, the patient at risk could also loose medical follow-up and show more intensively depressive feelings in case the disease starts to manifest. All situations are delicate, and the couple should not cease regular medical attendance. The best scenario is the possibility of the couple to be accompanied by a multi professional team, including a gynecologist or urologist, a geneticist, and a psychologist.

Conclusion

Neurodegenerative adult-onset disorders are a challenging group of diseases in terms of clinical follow-up, predictive testing and reproductive choices. Not infrequently, health providers will face situations in which ethical aspects and practical difficulty regarding the right of not to know of an individual at risk willing to conceive a healthy child superimpose technical and financial limitations. More important than the technique choices itself, which will be made
by the couple, it is mandatory to inform exhaustively all the options available and their consequences by an honest dialogue.

REFERENCES

