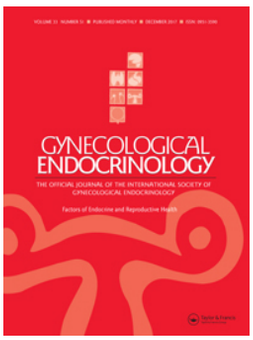

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CASE REPORT

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First preimplantation genetic testing case for monogenic disease in Latvia

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ABSTRACT

Huntington's disease (HD) is fatal neurodegenerative disease caused by a (CAG) triplet repeat expansion in the Huntingtin (*HTT*) gene. Inheritance pattern of the disease is autosomal dominant and onset depending on triplet repeat count. Transgenerational HD transmission can be avoided by preimplantation genetic diagnosis (PGD). Here, we report the first preimplantation genetic testing case for monogenic disease, in Latvia. The result of our work led to the birth of healthy child with normal *HTT* alleles in his genome. We describe a PGD strategy and testing algorithm that can be applied to any couple at risk of transmitting monogenic disease.

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KEYWORDS
Huntington's disease (HD); preimplantation genetic diagnosis (PGD); linkage analysis

Introduction

It is believed that rate of genetic diseases is growing rapidly all over the world. Nevertheless, it is important to know that the number of diseases does not increase, but diagnostic capabilities become much more accessible, as well as new genetic diseases are better recognized.

Confirmed diagnosis for a particular condition provides family an opportunity to choose a specific method of treatment if available, clarify the prognosis and gives estimations regarding reoccurrence risk within a family. Until recent in Latvia even if the parents knew about the increased risk of genetic diseases for their offspring, it was difficult to avoid or change it. The earliest time for diagnosing a genetic disorder was during prenatal testing, and if the diagnosis has been confirmed, the family faced a choice – to terminate the pregnancy or save, knowing that the baby will not be born healthy. Both of these decisions are complex and emotionally difficult to be accept. Currently a method that allows carrying out genetic analysis of embryos before their implantation into the uterine cavity has become available in Latvia, thus avoiding the risk of certain genetic abnormalities in the offspring. This method is called preimplantation genetic diagnosis (PGD) [1].

Two major testing strategies exist for PGD – direct mutation detection and indirect testing of mutated allele commonly known as linkage analysis of single nucleotide polymorphisms (SNPs) or short tandem repeats (STRs) flanking the mutation region. These techniques are used after embryonic (preferentially) trophectoderm cells biopsy following whole genome amplification (WGA) [2]. To increase embryo implantation rates after PGD chromosome analysis (preimplantation genetic screening – PGS) is recommended, it provides useful information about chromosomes of unaffected embryos suitable for transfer. Comparative genomic hybridization (CGH) is a gold standard tool for PGS [3].

Here, we report our PGD strategy for couple carrying *HTT* mutation following birth of a healthy child after PGD/PGS. Developed algorithm further can be applied to any couple at risk of transmitting a monogenic disease.

Materials and methods

Family anamnesis

Family with confirmed Huntington's Chorea mutation willing to undergo preimplantation genetic testing sought for such possibility in an 'IVF Riga' (Riga, Latvia) IVF and reproductive genetics clinic in April 2015. They underwent detailed genetic counseling regarding available options. Informed consent was obtained.

Clinically healthy woman (29 years old) had 38 ± 2 CAG repeats in a *HTT* gene (mild HD, late onset or incomplete penetrance), allele inherited from father, who died of Huntington's Chorea at the age of 64 and had 40 CAG repeats (adult-onset HD, symptoms starting at the age of 41).

Controlled ovarian hyperstimulation

Controlled ovarian hyperstimulation was performed in a short protocol (10 days) with follitropinum alfa (Gonal-F cumulative dose 2000 IU), GnRH antagonist Ganirelixum (Orgalutran 0.25 mg/0.5 ml) was added on day seven, and on the day 10, human choriongonadotropinum Pregnyl 5000 IU was used as an ovulation trigger.

Preclinical work-up

Before processing this clinical case, preclinical work-up was done:

- Selection of *HTT* gene-linked microsatellites – STR markers (flanking *HTT* gene 2 Mb upstream and downstream) was done using UCSC genomic browser.

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- Design of STR-specific and *HTT* CAG-expansion flanking primers.
- Optimization of multiplex STR markers seminested PCR.
- Family linkage analysis – STR marker informativeness evaluation and haplogroup establishment.
- Confirmation of *HTT* CAG alleles in a family by fluorescent PCR (fPCR) followed by fragment size analysis.

IVF and embryo biopsy

Oocytes were fertilized using ICSI method. Fertilized zygotes were cultivated in Life Global universal medium with 10% protein supplementation in 6% CO_2 and 5% O_2 environment conditions in EmryoScope (Vitrolife) incubator that allows uninterruptable embryo development monitoring using time-lapse system. On day 4 of development, all normally cleaved embryos underwent laser-assisted hatching with RI Saturn laser system to achieve embryo hatching on day 5.

During the punction, 16 oocytes were retrieved, 13 of them were mature, and three had germinal vesicles. After oocytes fertilization with ICSI method and cultivation process, seven embryos become blastocysts and underwent TE biopsy.

Performance of HTT PGD clinical case

Lysis and whole-genome amplification (WGA) of embryo TE biopsies was done using SureMDA kit (Illumina). Embryo haplogroup analysis was carried out using multiplex seminested fPCR for STR-markers and *HTT CAG*-expansion followed by amplified product fragment size analysis on capillary electrophoresis. Embryo chromosome analysis was performed using 24Sure (Illumina) protocol.

Results

All embryos underwent *HTT* adjacent STR marker testing and direct CAG repeat sizing (Figure 1). PGD mother's (P2–2) *HTT* CAG triplet size was shorter for two triplets comparing to her father's allele (P1–1), they CAG repeat sizing was performed at two different laboratories. We included also not fertilized oocyte with three pronuclei (Figure 1, e9) for testing. It had three alleles and some marker allele dropout was observed.

Embryos 7 and 12 (Figure 1, e7 and e12) were tested for chromosome aneuploidies, because they showed better development according to EmbryoScope development evaluation. Embryo 7 showed chromosome 8 mosaic trisomy, and embryo 12 had normal number of chromosomes. These embryos also were tested for CAG repeat mutation expansion in other laboratory, which confirmed our results.

Pedigree includes all family members, informative STR markers are shown. Embryos obtained from PGD family are numerated with 'e' (e9 is fertilized oocyte, which stopped developing on day 1). The numbers indicate STR-marker amplified product sizes. Position of genetic markers is aligned sequentially on the chromosome without distance precision. Red rectangle indicates mutated HTT allele. CAG expansion in HTT is given with resolution of ± 2 triplets.

After the PGD and PGS analyses were done, chosen embryo (e12) thawing and subsequent transfer was performed. Two weeks later, HCG level in mother's blood was 1891 mU/mL,

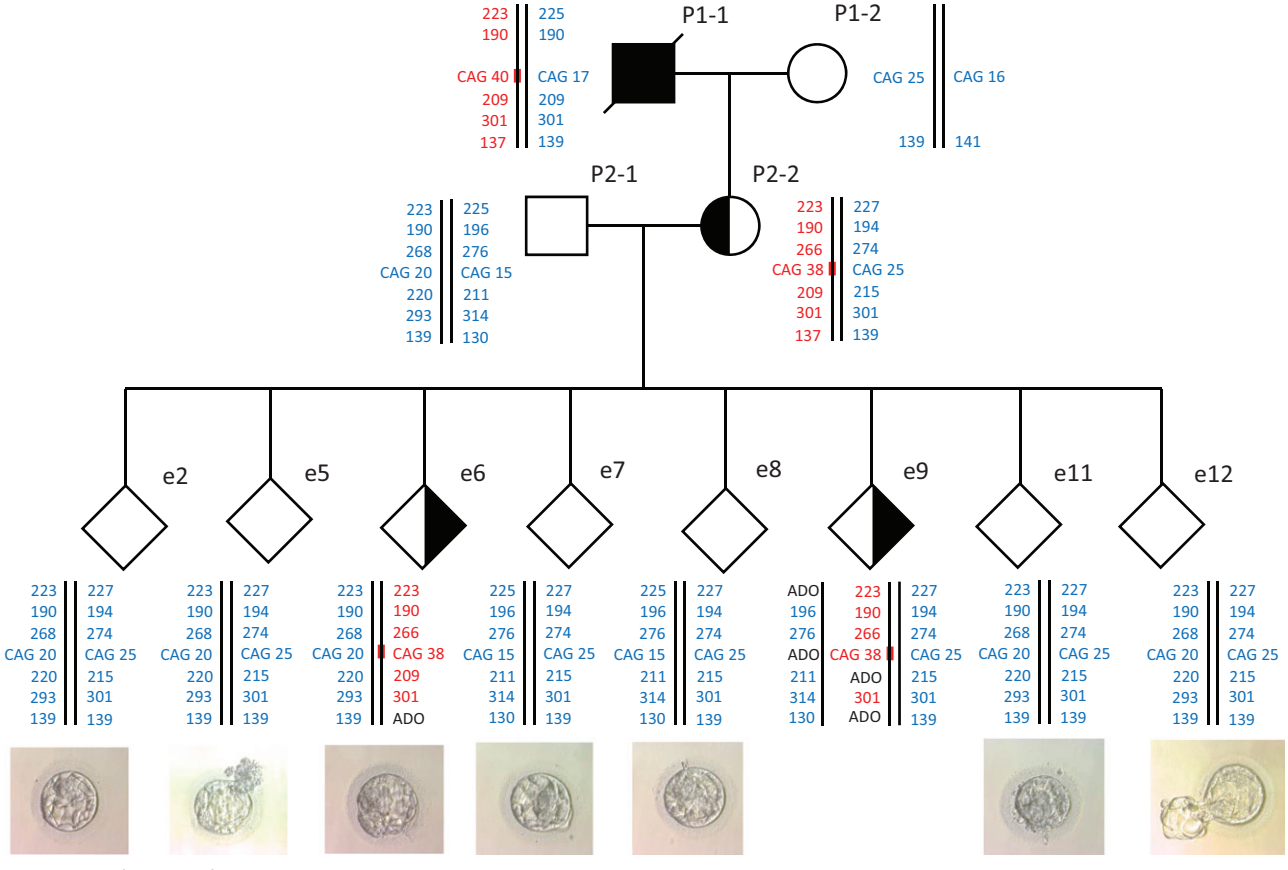

Figure 1. Pedigree of the PGD family with linkage and embryo images.

progesterone level 103 nmol/L, later 70 h after that HCG level was 5297 mU/mL, progesterone level – 104 nmol/L.

The pregnancy was uneventful. A healthy child was born on the week 41. The labor complication was shoulder dystocia. Baby's weight was 4400 g, height 57 cm. Apgar score rate was 6 after first minute and 9 after five minutes.

After birth, sample of umbilical cord blood was obtained and DNA was extracted. CAG expansion was tested and results confirmed normal paternal and maternal alleles with CAG expansion 20/25 ± 2 triplets in a child, what fully confirms our methodology precision.

Conclusions

- For embryo preimplantation genetic testing, we used following algorithm:
  - To access loci of interest, we performed adjacent STR marker haplotype testing and direct mutation testing (*HTT* gene CAG sizing).
  - To exclude embryo aneuploidies, we performed aCGH in mutation-free embryos suitable for transfer.
  - We confirmed HTT (CAG) allele genotype of born child in cord blood.
- According to statistics for autosomal dominant type of inheritance, half of the acquired embryos should be mutated. Regardless of statistical data, our results show only 25% mutated embryos. This can be explained by the small cohort for which the analysis was carried out.
- Our PGD testing algorithm was successful and is further applicable for other PGD cases with some modifications depending on inheritance pattern of genetic disorder.

Disclosure statement

All authors assure that they have no conflicts of interest and the work has not been presented in any meeting.

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