Preimplantation Genetic Diagnosis for Cystic Fibrosis

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Introduction

Preimplantation genetic diagnosis (PGD), is a technique used to identify genetic defects in embryos created through in vitro fertilisation before birth. Since only unaffected embryos are transferred to the uterus for implantation, PGD provides an alternative to prenatal procedures. But it was not until 1989 in London that the first unaffected child was born with a successfully PGD cycle. Even though cystic fibrosis (CF) was the first monogenic disorder to accomplish a successful PGD cycle, it was not until 1992 that they succeeded. Nowadays this technique is practiced worldwide, one of the challenges that PGD had to undertake was that only one cell is available for the purpose of this review is to understand cystic fibrosis as well as to comprehend the techniques that made PGD possible, through the years up to the present time.

The disease

Cystic fibrosis is an autosomal recessive chronic disease. It causes thick and sticky mucus to build up in the lungs, digestive tract and other areas of the body.

The symptoms and the severity of the disease can vary widely among patients.

Diagnostic Methods History and Present

1986
The consensus view was that the blastocyst biopsy was the most likely to succeed.

1989
An approach was determined, valuable for couples at risk of transmitting X-linked diseases. The sex was determined with a Y specific probe.

1990
This year were reported the first established pregnancies using PGD, in two couples known to be at risk of transmitting adrenoleukodystrophy an X-linked mental retardation.

1992
Using the polymerase chain reaction (PCR) with nested primers, a normal fragment of 154pb from the CFTR gene was amplified, this fragment included the ΔF508 region (13pb).

First healthy girl (from embryo 2) born free of Cystic Fibrosis through PGD.

1994
First time with PGD for Cystic Fibrosis.

Men with CF usually have CBVD

There was a need to detect more than 1 mutation from one blasterome.

1998
Cell obtaining methodology.

Use of Tyrode’s acid solution

Intracytoplasmatic sperm injection

Use of Tyrode’s acid solution

Diagnosis of Cystic Fibrosis

Common mutations.

Severe phenotypes: WW, ΔA and WD.

Multiplex PCR

Two-round PCR amplification

Nested PCR separately (at each locus)

Intracytoplasmatic sperm injection

Well-defined hole

The access of Tyrode’s acid solution (reduced embryo viability)

2000
Multiplex marker PCR protocol, with four closely linked highly polymorphic markers.

This protocol could be applicable to 87% of the couples carrying a CFTR mutation.

2002
In 91% of the cases at least one partner of the couple carries the ΔF508 mutation.

This strategy was based on a multiplex fluorescent PCR co-amplifying the ΔF508 mutation and two CFTR intragenic polymorphic microsatellites

2009
Four intragenic polymorphic repeats identified: IVS1CA, IVS8CA, IVS17BTA and IVS17BCA.

A procedure was validated for the identification of multiple mutations in a single run, which detects approximately 90% of the mutant alleles.

Consequently, 1 or both causal mutations were unknown in about 20% of the cases.

2013

In the majority of the cases linkage analysis is performed.

Fluorescent in situ hybridization (FISH) is also used in order to assure no chromosomal abnormalities.

Nowadays the PGD sensitivity is 99.2% and its specificity is 80.9%, however the accuracy is statistically higher when PGD is performed on two cells and multiplex protocols are applied.

Conclusions and future developments

Even though the validity, robustness and high diagnostic value of PCR-based PGD has been demonstrated, the wide range of mutations for CF and CFTR – Related Disorders is still a problem for assuring the identification of both embryonic alleles.

Present time: Molecular methods.

Future: Next generation sequencing (NGS) technologies.

Low throughput

Time consuming

Diagnostic rate of 98.91%

Time – 4 CAST

Selected References


9.Use of Tyrode’s acid solution (reduced embryo viability)