Fish based preimplantation genetic diagnosis to prevent DiGeorge syndrome

Shai Shefi · Gil Raviv · Shlomit Rienstein ·
Gad Barkai · Ayala Aviram-Goldring · Jacob Levron

Received: 18 June 2009 / Accepted: 28 July 2009 / Published online: 13 August 2009
© Springer Science + Business Media, LLC 2009

Abstract

Purpose To report the performance of fluorescence in-situ hybridization in the setting of preimplantation genetic diagnosis in order to diagnose embryos affected by DiGeorge syndrome.

Design Case report.

Setting Academic referral center.

Patient A 32 year-old female affected by DiGeorge syndrome.

Intervention(s) History and physical examination, karyotyping, amniocentesis, preimplantation genetic diagnosis, fluorescence in-situ hybridization.

Capsule Use of fluorescence in-situ hybridization in the setting of preimplantation genetic diagnosis in order to diagnose embryos affected by DiGeorge syndrome.

S. Shefi (✉)
Petach Tikva Andrology Practice,
Petach Tikva, Israel
e-mail: dr.andrology@gmail.com

G. Raviv
Male Infertility Unit, Urology Department,
Chaim Sheba Medical Center, Tel Hashomer,
Affiliated to the Sackler School of Medicine,
Tel Aviv University,
Tel Aviv, Israel

S. Rienstein · A. Aviram-Goldring
The Danek Gertner Institute of Human Genetics,
Chaim Sheba Medical Center, Tel Hashomer,
Affiliated to the Sackler School of Medicine,
Tel Aviv University,
Tel Aviv, Israel

G. Barkai · J. Levron
IVF Unit, Department of Obstetrics and Gynecology,
Chaim Sheba Medical Center, Tel Hashomer,
Affiliated to the Sackler School of Medicine, Tel Aviv University,
Tel Aviv, Israel

Main outcome measure(s) Avoidance of pregnancy with embryo affected by DiGeorge syndrome.

Result(s) Termination of pregnancy with an affected embryo followed by fluorescence in-situ hybridization based preimplantation genetic diagnosis and delivery of healthy offspring.

Conclusion(s) The combination of preimplantation genetic diagnosis with fluorescence in-situ hybridization is recommended to prevent pregnancies with DiGeorge syndrome affected embryos in properly selected patients.

Keywords DiGeorge syndrome · Velocardiofacial syndrome · Microdeletion · 22q11 deletion · Preimplantation genetic diagnosis · Fluorescence in situ hybridization

Introduction

The common approach for prevention of genetic disorders related to chromosomal aberrations is a therapeutic abortion in cases of affected embryos. Usually, the diagnosis of such a disorder is made during the pregnancy, either by invasive procedures to obtain embryonic cells, or by ultrasonographic imaging. However, the ability to perform preimplantation genetic diagnosis (PGD) and to combine genetic tests on single cells with assisted reproductive technology has revolutionized the field of prenatal genetics and now provides a better option for couples in need [1–3]. DiGeorge syndrome (DGS) and Velocardiofacial syndrome are considered to have distinct phenotypic characteristics, both caused by deletion of approximately 3 million DNA bases in the q11 region of chromosome 22 long arm [4, 5]. This microdeletion can be detected today by using a specific fluorescent probe. Over the last decade, implementation of fluorescence in situ hybridization (FISH) has

improved significantly the overall detection rate of this genetic trait, although its use has been very rarely reported [6, 7]. The prevalence of DGS, the most common deletion in newborns, is about 1 in 4000 live births. The majority of cases appear de-novo while the incidence of familial traits may reach up to one third of all new cases [8]. Clinically, this deletion results in a syndrome of abnormalities and symptoms which involve several medical disciplines. Presentation includes typical facial features with variable degrees of cleft palate, cardiovascular anomalies, and cognitive disabilities, abnormal development of the thymus gland associated with immune deficiencies, cerebrovascular anomalies and high incidence of schizophrenia [9, 10]. The main clinical features are represented in the CATCH acronym, which stands for cardiac defects, abnormal facials, Thymic hypoplasia, cleft palate and hypocalcaemia [11]. Adults with 22q11 deletion tend to have a milder form of this syndrome compared to their siblings or the non familial variant. DGS and velocardiofacial syndrome are usually considered to be a single clinical entity, although there may be some phenotypic differences between them [4, 12]. Atypical or mild clinical presentations are common and may go unnoticed until adulthood. As in other conditions which demonstrate variable degrees of phenotypic expression, the phenotype of DGS is hard to predict. It appears that the severity within a given family can range from asymptomatic individuals to severely affected children. 22q11 microdeletion is typically found in the majority (about 90%) of the cases with the phenotypic characteristics of DGS [4, 12]. Since DGS is an autosomal dominant disorder, couples in whom either female or male is affected, have 50% risk for an affected child in each pregnancy. Diagnosis of DGS during pregnancy imposes significant moral dilemmas and emotional stress upon both the parents and the health care providers. The option of PGD may help to eliminate and ease these dilemmas.

Case report

A 32 year-old woman was referred for genetic counseling and evaluation during the first trimester of her second pregnancy. Her 3 year-old daughter has been diagnosed with DGS shortly after birth. Physical examination of the mother demonstrated typical DGS facial features and velopharyngeal anomaly. Diagnosis of DGS was confirmed by karyotyping and FISH in both the mother and her daughter. During genetic counseling the couple was informed about the option of PGD. Amniocentesis was performed, and FISH confirmed the diagnosis of DGS in the fetus. The woman and her spouse elected to terminate the pregnancy, and an elective abortion was carried on. Several months later, preliminary successful FISH trials on

lymphocytes from the mother and the daughter were accomplished, followed by In-vitro fertilization (IVF) attempt. Day-3 embryos underwent blastomere biopsy using a micromanipulation system (Narashiga, Japan) fitted on an inverted microscope (Diaphot 300, Nikon, Japan). The zona pellucida was drilled using a mouth controlled fine bore pipette. Acidified Tyrode's solution (Irvine Scientific, CA, USA) was applied locally and a single blastomere was removed from each embryo using mouth controlled pipette of about 50 μ in diameter. After the manipulated embryos were replaced back for culture the biopsied blastomeres were fixed on a glass slide using 3:1 aceto-methanol solution (Sigma, MO, USA). The fixed nuclei were prepared for hybridization by dehydration in absolute ethanol (Sigma). Direct-labeled dual-color probe mixture made of TUPLE1 probe (HIRA, DGS critical region at 22q11.2) labeled in orange and ARSA probe (MIM 607574 at 22q13.3) labeled in green were used for simultaneous FISH. Chromosome 18 centromer probe in

Fig. 1 **a** A normal embryo displaying 2 green signals of the control 22q13.3 probe, and 2 orange signals of the 22q11.2 probe. **b** An embryo affected by DiGeorge Syndrome displaying 2 green signals of the control 22q13.3 probe, but only 1 orange signal of the 22q11.2 probe

spectrum Aqua (Vysis,) was added for control. Following overnight hybridization the slides were washed for one minute in 0.4 X SSC solution followed by 2X SSC solution at room temperature. The nuclei were analyzed using a fluorescent microscope (Zeiss Axioscope, Germany) with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) controlled by Cytovision automated system (Applied Imaging International, Tyne and Wear, U.K.). All the nuclei were analyzed by two independent observers. The specific FISH signals detected in a given cell nucleus were interpreted as follows: 1) The nucleus was considered to be diploid normal when two green signals, two aqua signals and two orange signals were present; 2) The nucleus was considered to be with the 22q11 deletion when two green signals, two aqua signals and only one spectrum orange signal were present. The patient underwent three IVF treatment cycles. Overall, a total of 14 embryos were suitable for analysis on day-3 of development. Eight embryos were found to be normal (Fig. 1a) and six were found to harbor DGS deletion by FISH based PGD (Fig. 1b). On day 4, five out of the eight normal embryos were suitable for replacement. The other three were arrested and discarded. On the first replacement attempt none of the tested embryos was found to be normal. On the second attempt three normal embryos were replaced but none implanted. On the third IVF attempt two normal embryos, negative for DGS were replaced and the patient conceived. On the 18th week of gestation, an amniocentesis was performed, and embryo negative for DGS was confirmed by FISH and karyotyping. After uneventful full term pregnancy the patient delivered a healthy female baby.

Discussion

Over the past few years significant improvement was achieved in our understanding of the molecular basis of DGS. It appears that large, chromosome-specific low copy repeats (LCRs) are common within the deletion interval and are probably responsible for chromosome DNA misalignment with aberrant recombination during meiosis, resulting in the formation of this relatively common genetic trait [8]. The typical 3 MB deletion may lead to diverse clinical manifestations involving the cardiovascular, immune, endocrine and neural systems. It is not uncommon, therefore that diagnosis of such cases are made during patient encounters with health care providers from various medical disciplines. Due to the unpredicted phenotype in DGS,

most of the couples with this trait choose to terminate an affected pregnancy, like our patient. Thus, it is important for pediatricians as well as other specialist care givers to be familiar with modern strategies. Although both PGD and FISH are quite common in referral centers, their combination, as in the case reported, is rare. In order to prevent pregnancy and birth of affected babies, this strategy has clear advantage over the traditional prenatal testing. Although assisted reproductive technology is an expensive treatment modality and PGD is practiced only at referral IVF centers, we feel that counseling patients with this deletion to pursue PGD outweighs the risks and emotional stress which is imposed by pregnancy terminations. We hope our experience will encourage both physicians and DGS patients to use PGD rather than facing the dilemma of pregnancy termination in order to eliminate this genetic trait.

References

1. Handyside A. Sex and the single cell. *New Sci.* 1990;126:34–5.
2. Edwards RG, Handyside AH. Future developments in IVF. *Br Med Bull.* 1990;46:823–41.
3. Coonen E. Pre-implantation diagnosis of genetic disease. *Eur J Obstet Gynecol Reprod Biol.* 1996;67:81–3.
4. Scambler PJ, Kelly D, Lindsay E, Williamson R, Goldberg R, Shprintzen R, et al. Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. *Lancet.* 1992;339:1138–9.
5. McDonald-McGinn DM, Tonnesen MK, Laufer-Cahana A, Finucane B, Driscoll DA, Emanuel BS, et al. Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: cast a wide FISHing net!. *Genet Med.* 2001;3:23–9.
6. Iwarsson E, Ahrlund-Richter L, Inzunza J, Fridström M, Rosenlund B, Hillensjö T, et al. Preimplantation genetic diagnosis of DiGeorge syndrome. *Mol Hum Reprod.* 1998;4:871–5.
7. McDonald-McGinn DM, Zackai EH. Genetic counseling for the 22q11.2 deletion. *Dev Disabil Res Rev.* 2008;14:69–74.
8. Cuneo BF. 22q11.2 deletion syndrome: DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes. *Curr Opin Pediatr.* 2001;13:465–72.
9. Shprintzen RJ, Goldberg RB, Lewin ML, Sidoti EJ, Berkman MD, Argamaso RV, et al. A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. *Cleft Palate J.* 1978;15:56–62.
10. Gothelf D, Presburger G, Zohar AH, Burg M, Nahmani A, Frydman M, et al. Obsessive-compulsive disorder in patients with velocardiofacial (22q11 deletion) Syndrome. *Am J Med Genet.* 2004;126B:99–105.
11. Wilson DI, Burn J, Scambler P, Goodship J. DiGeorge syndrome: part of CATCH 22. *J Med Genet.* 1993;30:852–6.
12. Goldberg R, Motzkin B, Marion R, Scambler PJ, Shprintzen RJ. Velo-cardio-facial syndrome: a review of 120 patients. *Am J Med Genet.* 1993;45:313–9.