

Preimplantation Genetic Testing (PGT) for breast cancer

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Abstract

Preimplantation genetic testing (PGT) is currently extended to an increasing number of late-onset common disorders with genetic predisposition, including inherited forms of breast and ovarian cancer (HBOC), determined by BRCA1/2 genes. Prevention and treatment of HBOC presents a real challenge, because of incomplete penetrance and variable expressivity of predisposing BRCA1/2 genes. The major problem is that preventive management may not affect penetrance of these genes, which may lead to HBOC even after prophylactic bilateral mastectomy or oophorectomy. So PGT for BRCA1/2 genes is an extremely attractive approach, as it allows not only avoiding the transfer of mutant embryos, but also provides the possibility of having children free from predisposition to HBOC. The present paper summarizes the first systematic experience of 149 PGT cycles for BRCA1/2 gene mutations, which resulted in birth of 68 healthy, disease predisposition free children, demonstrating important clinical implications of PGT as practical means for couples carrying BRCA1/2 predisposing genes.

Introduction

PGT has presently become a part of genetic practices and assisted reproductive technology (ART) [1,2]. Although initially applied to the conditions presented at birth, PGT became similarly useful for late-onset disorders with genetic predisposition, such as hereditary breast and ovarian cancer (HBOC) [3,5]. Because these conditions may manifest despite pre-symptomatic diagnosis and follow up, PGT is becoming an attractive option for the at-risk couples to reproduce avoiding the inheritance of the predisposing genes to their prospective children. The major problem is that pre-clinical diagnosis, prophylactic medication, chemoprevention or preventive management fail to affect penetrance of the predisposing genes, such as in HBOC, which may still be manifested after bilateral prophylactic mastectomy or oophorectomy [6,8].

The first case of PGT for cancer was performed for Lee-Fraumeni disease [9], followed by the report of the first series of PGT for cancers [10], and a few further reports on PGT for different cancers [11-13], including HBOC [14-17], showing feasibility of using PGT as an option for avoiding offspring with predisposition to HBOC. We present here our first systematic experience of 149 PGT cycles for HBOC risk assessment by BRCA1 and BRCA2 mutation analyses, as part of our overall PGT series of approximately five thousand cycles for monogenic disorders, which is the world's largest PGT experience.

Material and methods

A total of 149 PGT cycles for 79 couples at risk for producing an affected progeny with HBOC was performed (list of BRCA1/2 mutations for which PGT was performed is presented in Table 1a and 1b). Of 69 BRCA1 mutations tested, 51 were maternal and 18 paternal, with the most prevalent being 187 del AG mutation (35 of 69 BRCA1 mutations, of which 20 were maternal and 15 paternal in origin). The majority of 55 BRCA2 mutations, were also of maternal origin (42 maternal and 13 paternal), the most prevalent being 6174 Del IT, 22 cases (14 maternal and 8 paternal). Both 187 del AG and 6174 Del IT are founder mutations in individuals of Ashkenazi Jewish ancestry.

All PGT cycles were performed using a standard IVF protocol coupled with micromanipulation procedures of embryo biopsy,

described elsewhere [10-18]. The biopsied blastomeres or blastocyst samples were tested by the multiplex nested PCR analysis, involving the above mutations and linked marker analysis in a multiplex heminested system [10-18]. The majority of cases were performed by blastocyst biopsy procedure [18].

In 88 of 149 PGT cycles, involving an advanced reproductive age, aneuploidy testing was also performed, initially by FISH or PCR analysis [4,18], and then by array-CGH, or next generation technologies (Illumina Inc) (NGS) for 24-chromosome aneuploidy testing. Pregnancy outcome was defined as the presence of a gestational sac with fetal cardiac activity.

As per the informed consent, approved by Institutional Review Board, the embryos free of genetic predisposition to HBOC, based on the mutation and polymorphic marker information, were pre-selected for transfer back to patients, while those with predisposing mutant genes were considered affected, and tested to confirm the diagnosis.

Results and discussion

The results of PGT of 149 cycles performed for 79 at risk couples are presented in Table 2. A total of 155 embryos free of BRCA1/2 mutations and also euploid chromosome set were preselected for transfer in 95 cycles (1.6 embryos per transfer, on the average), yielding 64 clinical pregnancies (67.3% pregnancy rate per transfer), and birth of 68 HBOC predisposition free children. It is of note that the results of PGT were highly accurate with no misdiagnosis observed.

As mentioned, because of advanced reproductive age, concomitant aneuploidy testing was performed in 88 of 149 cycles, of which the majority were tested for 24-chromosome aneuploidy either by array-

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Table 1a. BRCA 1 Mutations for which PGD was performed

BRCA1 MUTATIONS	MATERNAL	PATERNAL	TOTAL
187 del AG	20	15	35
2813 ins A	1	0	1
3100 del GT	0	1	1
3977 del 4bp	1	0	1
5382 ins C	3	0	3
5385 ins C	1	0	1
c5154	1	0	1
c5256 del G	2	0	2
c5407-25T>A	1	0	1
EXON 17del	1	0	1
EXON 8-13 DEL	1	0	1
IVS163 2del 3835	1	0	1
IVS22 (510 bp del)	1	0	1
K679X	1	0	1
Q1313X	1	0	1
287 DEL	1	0	1
3005del	1	0	1
E6-8DEL	1	0	1
R1699W	1	0	1
5360delA	1	0	1
E20 del	1	0	1
2813ins A	1	0	1
3977 del4	1	0	1
IVS17+1 G>A	1	0	1
3100del GT	0	1	1
c.5077_5079delGCT	1	0	1
E 6-8 del	1	0	1
3005del	1	0	1
C61G	3	0	3
886delGT	0	1	1
c3756del4	1	0	1
c.4065-4068del	1	0	1
R1835X	1	0	1
dup ex13	0	1	1
c2679del4	1	0	1
V1736A	1	0	1
W1837R	1	0	1
R1692H	1	0	1
TOTAL(38)	51	18	69

CGH or next generation sequencing (NGS). The transfer of these embryos resulted in 75% pregnancy rate, with corresponding overall reduction of spontaneous abortion rate to as low as 7.6%, being totally absent in cycles tested for 24-chromosome aneuploidy.

The results support a practical value of PGT for HBOC risk reduction in offspring at risk for inheriting parental mutations in cancer predisposition genes by profoundly reducing the likelihood of inheritance of the pathogenic variant and thus reducing the lifetime risk for developing HBOC and other solid tumours in children of parents with pathogenic variants in cancer predisposition genes. PGT is increasingly accepted by at risk couples as a realistic option to avoid producing offspring with predisposition to HBOC, determined by mutations in *BRCA 1/2* genes. So, the at-risk couples will clearly benefit from the information about such option, as if inheritance of these genes is not avoided, their offspring will be predisposed to HBOC, that may manifest at any time of their lifespan.

To ensure that this approach is utilized by those at need, it may be useful to incorporate the family history into the clinical settings to obtain information about family members with HBOC that may

help to identify candidate couples requiring PGT. While chances that their offspring develop the disease will differ depending on the specific mutations involved, mode of inheritance, genetic background and environmental risk factors, the presence of *BRCA1/2* mutations alone justifies the parents' requests for PGT. One of the immediate at risk groups to benefit from a family history may be those couples undergoing IVF for fertility treatment, within the framework of which PGT is provided, to ensure avoiding the inheritance of genetic susceptibility factors.

It should be also mentioned, that the information about the extended family history may not always be available, so the future implementation of preconception screening programs for identification of carriers of genes predisposing to HBOC might be of great utility for applying PGD, as useful tool for avoiding the risk of producing offspring with HBOC at their lifespan.

As in other common disorders with genetic predisposition, PGT for HBOC has also important ethical implications, as most of these conditions are not present at birth and may not be realized even during the lifetime. So, the couples at risk could be reluctant to use prenatal diagnosis for cancer, as pregnancy termination cannot be justified for this purpose. On the other hand, PGT seems to be ethically more acceptable, allowing couples to reproduce, establishing only pregnancies free of predisposing genes. This makes it important to provide genetic counselling services to inform patients at risk of having children with a strong genetic predisposition to HBOC about the availability of PGT.

Table 1b. BRCA 2 Mutations for which PGD was performed

BRCA2 MUTATIONS	MATERNAL	PATERNAL	TOTAL
1417 ins 4 bp	1	0	1
2776 del C	1	0	1
2942 ins 4 bp	1	0	1
3036-4 bp del	3	0	3
6174 Del T	14	8	22
9686 del G	1	0	1
c.5849	0	1	1
c.9097 dup	1	0	1
Q583X	1	0	1
3398del	1	0	1
5385insC	1	0	1
2942ins4	0	1	1
5578delAA	1	0	1
DUP Exon 20	1	0	1
c.8673_74delAA	1	1	2
c.4359ins6	1	0	1
c.5946delT	1	0	1
955delCA	2	0	2
S1955X	1	0	1
2041 delA	1	0	1
886delGT	0	1	1
IVS13-2A>G	1	0	1
IVS17del3ins2	1	0	1
3398delAAAAG	1	0	1
4355del4	1	0	1
c.5946delT	1	0	1
c.4359ins6	1	0	1
c.8673_74delAA	0	1	1
c6486_6489delACAA	1	0	1
5578delAA	1	0	1
TOTAL (30)	42	13	55

Table 2. Clinical Outcome of PGT for Breast Cancer predisposition

TEST TYPE	PATIENT	CYCLE	ET	# EMBRYOS TRANSFERRED (Average per cycle)	PREGNANCY (%)	SAB (%)	DELIVERY (%)	BABY
<i>BRCA 1, 2</i>	28	61	40	69 (1.73)	25 (57%)	4 (16%)	21 (84.1%)	27
<i>BRCA 1,2 +24AT</i>	51	88	55	81 (1.2)	39 (75%)	3 (7.6%)	36 (92.3%)	41
TOTAL	79	149	95	150	64	7	57	68

Without such information these couples may even remain childless because of their fear to opt prenatal diagnosis and possible pregnancy termination.

As can be seen from Table 2, the majority of PGT cycles resulted in birth of children free of predisposing genes. With current progress in the study of the molecular basis of HBOC, and sequencing of the genes involved, predisposition to HBOC will soon become one of the emerging PGT indications, representing already significant proportion of our PGT experience for Mendelian disorders. Also, despite still existing ethical and legal issues involved in PGT for late onset disorders with genetic predisposition, an increasing number of patients regard the procedure as their favourable option to have an offspring free of mutation predisposing to HBOC. Of course, the patients should be aware of the previously raised concerns that PGT with ART may increase their own risk for developing HBOC due to their carrier status, but it has been reported that there is actually no difference in ovarian response of these patients compared to the matched control [19].

It should be also mentioned, that PGT for HBOC is still highly controversial, because these cancers present beyond early childhood and even later may not be expressed in 100% of the cases. However, the above systematic experience in offering PGT for this indication shows that the availability of PGT allows some couples forgoing pregnancy, which otherwise may not be attempted because of their concern that their children could be at risk for HBOC. In conclusion, the presented PGT experience for HBOC shows that PGT for this indication is highly accurate, reliable and safe, and may be recommended for wider application in primary prevention of predisposition to HBOC.

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