

Preimplantation Genetic Testing (PGT) for cardiac disease.

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Abstract

The application of Preimplantation Genetic Testing (PGT) is no longer limited to conditions presented at birth, but also applied for the adult onset disorders with genetic predisposition, which have never been an indication for prenatal diagnosis. One of such conditions is the inherited predisposition for cardiac disease for which no pre-clinical diagnosis and preventive management exist, with premature or sudden death frequently representing the only clinical realization of the predisposing genes. We have presently performed 75 PGT cycles for 47 couples at risk for producing offspring with predisposition to cardiac diseases, resulting in 37 clinical pregnancies and birth of 32 healthy children, free of genes predisposing to these conditions. This systematic experience demonstrates that PGT may now be a practical means for avoiding the risk of premature or sudden death in the offspring of carriers of a cardiac disease predisposing genes.

Keywords: PGT (Preimplantation Genetic Testing), Inherited cardiac disease, Inherited predisposition to sudden death.

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Introduction

PGT is becoming a useful part of genetic practices and Assisted Reproductive Technology (ART) [1, 2]. In addition to the traditional indications similar to those in prenatal diagnosis, it is gradually being applied not only to the disorders presented at birth, but also to those that are realized later in life, such as genetic predisposition to cancer and cardiac disease [3-5]. A few previous reports demonstrated the usefulness of PGT for inherited cardiac disease, allowing the carriers of predisposing genes to avoid the inheritance of these genes to their offspring [6-8]. The data showed that avoiding the inheritance of the predisposing genes to the offspring may be the only option to prevent the disease, as it may manifest despite pre-symptomatic diagnosis and follow up. This paper presents our systematic work on PGT for inherited cardiac disease, which represents the world's largest series of 75 PGT cycles resulting in the birth of healthy children without the risk of developing an inherited cardiac disease.

Materials and Methods

A total of 75 PGT cycles for 47 couples at risk for producing an affected progeny with inherited cardiac disease were performed, which includes 51 cycles for 30 couples reported earlier [6,7].

To perform PGT the couples at risk underwent a standard IVF cycle, allowing sampling of material from oocytes and/or embryos, described in detail previously [8]. Depending on derivation of predisposing genes from mother or father, the choice of biopsy materials differed, involving Polar Body (PB) sampling, in cases of de novo mutations of maternal origin, and/or embryo biopsy, performed initially

by blastomere sampling at the 8-cell stage, or by blastocyst biopsy at the present time [8]. The biopsied PBs, blastomeres or blastocyst samples were tested by the multiplex nested PCR analysis, directly or after Whole Genome Amplification (WGA), for the mutations predisposing to cardiac disease and linked marker analysis in a multiplex heminested system [8].

In cases of advanced reproductive age, 24-chromosome aneuploidy testing was performed together with mutation analysis, using next generation technologies, initially array CGH and presently NGS (Illumina Inc). Pregnancy outcome was defined as the presence of a gestational sac with foetal cardiac activity.

The embryos derived from the embryos free of genetic predisposition to cardiac disease, 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

Table 1 summarizes the cardiac disorders for which PGT was performed, including dilated cardiomyopathy, determined by 16 predisposing gene mutations (23 cycles for 11 couples), hypertrophic cardiomyopathy, determined by 19 predisposing gene mutations (18 cycles for 12 couples), long QT, determined by different predisposing gene mutations (6 cycles for 6 couples), Holt-Oram syndromes determined by 2 predisposing gene mutations (6 cycles for 3 couples), ACyl-CoA Dehydrogenase, determined by 5 predisposing gene mutations (6 cycles for 5 couples) and a few cycles in couples where cardiac disease was part of other conditions,

Table 1. List of inherited cardiac diseases and their predisposing gene mutations, for which PGT was performed and the clinical outcome of the procedure (AD – Autosomal Dominant; AR - Autosomal Recessive; XLR – X-linked Recessive).

Disease	Gene/ Inheritance	Mutations	Patient	Cycle	Transfers	Embryos transferred	Pregnancy	Delivery	Birth
CARDIOMYOPATHY, DILATED, 1A; CMD1A	LMNA (AD)	S395X, E161K, R189P,K270K, R335T,T528K, R249Q,R482Q (8)	6	15	14	20	9	9	10
CARDIOMYOPATHY, DILATED, 1DD; CMD1DD	RBM20 (AD)	S637G, S395X (2)	1	2	1	1	1	1	1
CARDIOMYOPATHY, DILATED, 1E; CMD1E	SCN5A (AD)	C1004R	1	2	2	2	1	1	1
CARDIOMYOPATHY, DILATED, 1G; CMD1G	TTN (AD)	c.58870G>A,26bpDEL, c.74883-88 delTGTT, c.91792G>T (4)	1	1	1	1	1	1	1
CARDIOMYOPATHY, DILATED, WITH WOOLLY HAIR, KERATODERMA, AND TOOTH AGENESIS; DCWHKTA	DSP (AD)	Q1511X	2	3	2	2	1	1	1
Subtotal CARDIOMYOPATHY, DILATED	-	(16)	11	23	20	26	13	13	14
CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 1; CMH1	MYH7 (AD)	E1142K, V404M, c.533delT, E497D (4)	2	3	2	2	1	1	1
CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 2; CMH2	TNNT2 (AD)	c.487 DEL GAG	1	1	1	2	1	1	2
CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 4; CMH4	MYBPC3 (AD)	D1076fs, W916X; T1028I, 25bp deletion, E441K, c.923-924insAACT, R495G, c.3217delC, R502W, IVS23 -2A>G, c.1504C>T, IVS11-10 C-A (12)	7	11	9	14	6	5	5
CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 7; CMH7	TNNI3(AD)	A157V	1	1	1	1	0	0	0
CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC, 8; CMH8	MYL3 (AD)	M149V	1	2	1	1	0	0	0
Subtotal CARDIOMYOPATHY, FAMILIAL HYPERTROPHIC	-	(19)	12	18	14	20	8	7	8
LONG QT SYNDROME 1; LQT1	KCNQ1 (AD)	A341V, R594P,A344E (3)	2	2	1	1	1	1	1

LONG QT SYNDROME 2; LQT2	KCNH2 (AD)	W1001X,A561T,G604S (3)	3	3	1	1	1	1	1
LONG QT SYNDROME 8; LQT8	CACNA1C (AD)	G406R	1	1	1	1	1	1	1
MYOPATHY, MYOFIBRILLAR, 1; MFM1	DES (AD)	N342D	1	2	2	3	1	1	1
HOLT-ORAM SYNDROME; HOS	TBX5 (AD)	T223M, Y114X (2)	3	6	6	7	2	2	3
NOONAN SYNDROME 1; NS1	PTPN11 (AD)	N308D,M504V, I309V,Q79R,T468M,Y63C (6)	3	5	2	3	2	1	2
EMERY-DREIFUSS MUSCULAR DYSTROPHY 1, X-LINKED; EDMD1	EMD (XLR)	W200X, c49_82+6	3	4	4	6	3	3	3
CARDIOSKELETAL MYOPATHY WITH NEUTROPENIA AND ABNORMAL MITOCHONDRIA	TAZ (XLR)	c.492insC	1	1	1	1	1	1	1
CARDIOENCEPHALOMYOPATHY, FATAL INFANTILE, DUE TO CYTOCHROME c OXIDASE DEFICIENCY 1; CEMCOX1	SCO2 (AR)	R262delCA, E140K, Y241X (3)	2	4	5	10	2	2	2
ACYL-CoA DEHYDROGENASE, VERY LONG-CHAIN, DEFICIENCY OF; ACADVL	ACADVL (AR)	Δ257,R469Q,R453Q,C1837 C-T;14bp del; S22X,A397P,T393A	5	6	8	12	2	2	2
TOTAL	20 Genes	65 Mutations	47	75	65	91 (1.4 On the average)	37 (57%)	35	39

such as cardioencephalomyopathy (4 cycles for 2 couples), and Noonan syndrome 1 (6 cycles for 3 couples). All but two couples tested for cardiac disease were carriers of autosomal dominant mutations, one having no previous affected progeny, but had a family history of premature or sudden death.

Of 75 PGT cycles performed, 65 resulted in transfer of 91 cardiac disease predisposition free embryos, yielding 37 clinical pregnancies (57% pregnancy rate per transfer) and birth of 39 predisposition free children. Overall the testing was done for 65 different predisposing gene mutations, which was extremely accurate and reliable, with the results obtained in over 90% of the tested samples, with the information including also euploidy status. On the basis of mutation and aneuploidy testing, 91 genetic predisposition free embryos were transferred in 65 cycles (1.4 mutation free embryos per transfer cycle on the average). As a result, 37 patients became pregnant after the first or second PGT cycles and delivered 39 healthy children free of predisposing gene mutations. No misdiagnosis was observed, suggesting

that procedure is highly accurate and acceptable for a wider clinical application.

Presented results show that PGT may be offered as a realistic option for couples at high risk for producing offspring with cardiac disease, to avoid inheritance of the predisposing genes from parents. It is obvious that information about such option can be extremely useful for couples at risk, as if inheritance of these genes is not avoided, their offspring will be at risk for the cardiac disease development, which may manifest at the childhood, or later in adult life, with the main clinical realization of premature or sudden death.

To offer the procedure to those who may actually benefit from the procedure, family history may appear important as part of the clinical documentation, including not only cardiac disease, such of heart attack and sudden death at young ages, but also information about pacemakers or internal cardiac defibrillators, arrhythmia or heart surgery in the family members. No doubt that the penetrance of predisposing genes depends on the mode of inheritance and the lifestyle,

such as an excessive exercise, but there is always risk of carriers of predisposing genes to contract a cardiac arrest or sudden death, which is therefore an obvious indication for PGT. It is also understood that symptoms of inherited cardiac disease may be easily overlooked, so again the family history may be the reason for undertaking an exclusion testing of predisposing gene mutations, which may justify offering PGT. So it may be expected that cardiac disease predisposing genes will be an important candidate for preconception screening program packagers, currently being implemented in many patient groups, such as in ART practice. With such a prospective identification of carries of genes predisposing to inherited cardiac disease, PGT might appear a useful tool for avoiding the risk for producing offspring with a high probability of premature or sudden death at their lifespan.

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