# Preimplantation Genetic Testing (PGT) for cardiac disease.

**Anver Kuliev*, Svetlana Rechitsky**

Reproductive Genetic Innovations, Northbrook, IL

**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 hose that are realized later in life, such as genetic predisposition to cancer and cardiac disease [3-5]. A few previous reposts 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 30 cycles for 51 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 blatomere sampling at the 8-cell stage, or by blsastocyst 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 in 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 |

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|---|---|---|---|---|---|---|---|---|---|
| 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 |
| CARDIOENCEPHA-LOMYOPATHY, FATAL INFANTILE, DUE TO CYTOCHROME c OXI-DASE DEFICIENCY 1; CEMCOX1 | SCO2 (AR) | R262delCA, E140K, Y241X (3) | 2 | 4 | 5 | 10 | 2 | 2 | 2 |
| ACYL-CoA DEHYDROGENASE, VERY LONG-CHAIN, DEFICIENCY OF; ACADVLD | 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 carries 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 32 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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***Correspondence to:**

Anver Kuliev
Reproductive Genetic Innovations, Northbrook, IL
Tel no.: 847 400-1515
E-mail: anverkuliev@hotmail.com