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Introduction

Pre-implantation genetic diagnosis (PGD) is a technic that has been applied for couples with known genetic disorders to prevent the birth of children affected by monogenic defects.

In molecular PGD, the blastomeres are biopsied from 8cell stage embryos, via in vitro fertilization. Therefor abnormalities in embryos are detectable before transferring them in to the mother's uterus.

PGD make the opportunity to diagnose the disease status, chromosomal aneuploidies, HLA typing and sex selection simultaneously.

Fanconi anemia is a genetically heterogeneous disease with autosomal recessive inheritance. It is characterized by physical abnormalities, bone marrow failure and increased risk for malignancy.

This article presents experience of molecular PGD to select unaffected and HLA-matched blastomere/s for the purpose of transplanting to his affected sibling.

Keywords PGD, HLA, Fanconi anemia, IVF

Molecular PGD for finding HLA match embryo for stem cell therapy in Fanconi anemia

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Material & Method

After genetic consolation, peripheral blood samples were collected in tubes containing EDTA and genomic DNA was extracted using salting out method.

Homozygosity mapping with help of polymorphic STR markers were performed on each blastomere for FANC G gene to track the defective alleles in the family. Simultaneously, haplotype analysis was done to find the HLA matched embryo for all members of the family. Additionally, the possibility of the presence of the causative mutation in FANC G gene was tested by Sanger sequencing.

Result From 8 investigated blastomeres, 3 were normal (1 halfmatched HLA, 2 not matched HLA). 1 carrier with halfmatched HLA. 4 affected (2 full matched HLA, 2 halfmatched HLA).

Conclusion PGD prepare a chance to have a healthy child who can donate stem cells for the treatment of affected child. This method is a reliable technique with 99.9% accuracy, Results obtained from haplotype mapping in parallel with direct mutation detection.

				Affeo chi		cted Id	Cell 1 Normal Not match		Cell 2 Normal Half match		Cell 3 Affected Full match		Cell Carri Half m	
				D6HLACS407	166	170	170	170	170	170	166	170	170	:
				D6HLACS402	252	248	236	236	236	248	252	248	236	
				D6HLACS383	207	207	223	215	223	207	207	207	223	
					370	378	370	366	370	378	370	378	370	
					189	204	103	193	193	204	189	204	102	
					231	227	231	247	231	227	231	227	231	
					221	322	210	247	210	322	210	322	210	
					210	222	210	218	210	202	210	222	210	ľ
				DOHLA35321	289	289	293	185	293	181	289	181	293	
			-		198	194	194	190	194	194	198	194	194	
	D6HLACS407	170	166	D6HLATS273	209	201	201	197	201	201	209	201	201	
	D6HLACS402	236	252	D6HLATS262	416	420	412	412	412	ADO	416	420	412	
	D6HLACS383	223	207	D6HLATS252	241	237	233	237	233	237	241	237	233	
	D6HLACS366.3	370	370	D6HLATS237	196	188	208	200	208	188	196	188	208	
	DOFLA25340 D6HLΔCS359	193	189					-						
		231	231	D9FANCGSD9.1	267	267	263	263	263	263	267	267	263	
	D6HLA2S334.5	318	318	D9FANCGSD9.06	285	285	283	295	283	295	285	285	283	
HLA Markers	D6HLA3S328	185	181	D9FANCGSD6.7	275	275	259	275	259	275	275	275	259	
	D6HLA3S321	293	289	D9FANCGSD1.8	279	279	287	279	287	279	279	279	287	
	D6HLA1S296	194	198	D9FANCGSU7 8	198	198	206	198	206	198	198	198		
	D6HLATS273	201	209		204 138	138	278	156	278	156	284	138	278 138	
	D6HLATS262	412	416		204	204	270	270	270	270	204	204	270	
	D6HLATS252	233	241							/	•			
	D6HLATS237	208	196					>	<	>			\langle	
•	DIANCOJDJ.1	203	207				/			\backslash				
		205	265											L
	D9FANCGSD6.7	259	2/5											L
	D9FANCGSD1.8	287	279											I
Fanc G Markers	D9FANCGSU7.8	206	198									_		Ļ
	D9FANCGSU13.3	138	138											L
1	D9FANCGSU15.4	278	284											L

Haplotype illustrating embryos



