PGD to select HLA matched embryo for stem cell therapy in epidermolysis bullosa dystrophica

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1 Introduction

Preimplantation genetic diagnosis (PGD) allows families to assure their child's health regarding genetic disorders before pregnancy and also can prevent medical abortion. In this method, diagnoses perform on blastomers biopsied from 8-cell stage embryos which are created by in vitro fertilization method (IVF). PGD combine with HLA typing is a strategy to select healthy HLA-matched embryos as a potential donor for stem cell transplantation of the affected sibling. Here we present application of molecular PGD to select healthy HLA-matched embryo as a donor for Recessive Dystrophic Epidermolysis Bullosa (RDEB). RDEB is a severe inherited skin disorder which blisters affect the whole body.

2 Material & Methods

A family with a 17 years old affected child with RDEB was referred to our laboratory. Peripheral blood samples were collected and genomic DNA was extracted using salting out method. Mutation detection in COL7A1 gene was carried out using direct sequencing method. Fragment analysis and haplotype mapping were performed to track the defective alleles in the family. On day 3 post fertilization one or two blastomers were removed from each embryo. Selected mutation was investigated using direct sequencing and informative STR (short tandem repeat) markers (17 loci for HLA and 8 for Col7A1) were checked using nested PCR method. A healthy HLA-matched embryo was selected and implanted to mother's uterus.

STR markers	Location
D6HLATS232	chr6:23212775+23212929
D6HLATS237	chr6:23715806+23715960
D6HLATS252	chr6:25297075+25297262
D6HLATS265	chr6:26552934+26553134
D6HLATS278	chr6:27808380+27808623
D6HLA1S296	chr6:29698961+29699124
D6HLA1S312	chr6:31288938+31289301
D6HLA3S321	chr6:32179240+32179496
D6HLA2S328	chr6:32853938+32854104
D6HLA2S334.5	chr6:33450556+33450858
D6HLA2S346	chr6:34618123+34618351
D6HLACS359	chr6:35990913+35991063
D6HLACS366.3	chr6:36632954+36633287
D6HLACS380	chr6:38012771+38012932
D6HLACS402	chr6:40211159+40211357
D6HLACS407	chr6:40721911+40722043
D3Col7A1SD4.1	chr3:48183472-48183814
D3Col7A1SU21.7	chr3:46426226-46426512
D3Col7A1SU13.1	chr3:49948558-49948761
D3Col7A1SU25.6	chr3:46039524-46039775
D3Col7A1SD16.4	chr3:46956420-46956773
D3Col7A1SD17.1	chr3:46885128-46885392
D3Col7A1SD19.4	chr3:46648714-46649023
D3Col7A1SD19	chr3:4669553446695793

Table. List of investigated STR markers and their location

3 Results

COL7A1: c.6994 C>G mutation was diagnosed in patient and from 6 blastomers, only one was transferable. Prenatal diagnosis was performed at 16th week of gestational age which confirmed PGD result. The child was born and she was healthy according to the pediatric dermatologist examination.

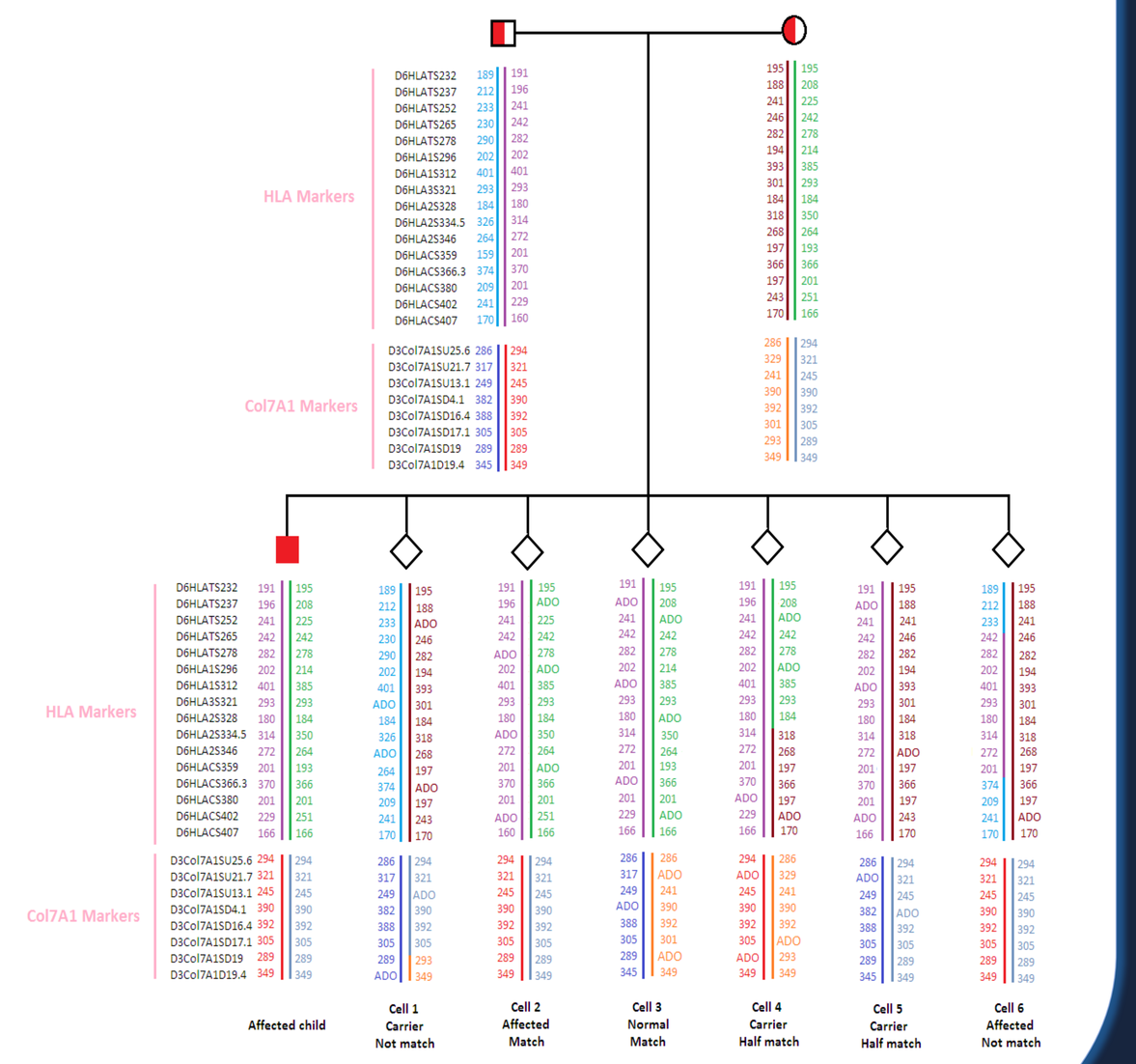

Fig. Haplotype illustrating transferable embryo

4 Conclusion

PGD for a disease in combination with HLA typing is being used to select HLA-matched embryo to have a potential donor for stem cell transplantation when stem cell transplantation is the only choice. In case of successful IVF, using PND can minimize risks of misdiagnosis. There is a controversial point regarding the ethical considrations against having a child only to save a sick sibling. However, it is a morally acceptable decision under conditons that there is no other alternative way because no potential harm against the donor child has been reported.

5 Keywords

Preimplantation genetic diagnosis, Nested PCR, Blastomere, Dystrophic Epidermolysis Bullosa, HLA typing, Iran.