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Single cell PCR amplification of microsatellites flanking the *COL7A1* gene and suitability for preimplantation genetic diagnosis of Hallopeau—Siemens recessive dystrophic epidermolysis bullosa

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Summary

Background: Hallopeau—Siemens recessive dystrophic epidermolysis bullosa (HS-RDEB) is a severe inherited blistering skin disorder caused by mutations in the anchoring fibril type VII collagen gene, *COL7A1*. There is currently no effective treatment but DNA-based prenatal testing in families at risk of recurrence is possible, mostly involving chorionic villus sampling at 10—11 weeks' gestation.
Objectives: An alternative method, for avoiding recurrence of HS-RDEB, is preimplantation genetic diagnosis (PGD). This involves DNA analysis of single blastomeres extracted from late cleavage stage embryos following in vitro fertilisation.
Methods: To establish PGD for HS-RDEB, we designed and optimised a sensitive single cell semi-duplex polymerase chain reaction (PCR) assay for two highly polymorphic dinucleotide repeat microsatellite markers, D3S1581 (telomeric) and D3S1289 (centromeric), close to the *COL7A1* gene.
Results: We demonstrated high PCR efficiency, low allele drop out rates and no contamination in testing this assay on 50 single buccal cells of known heterozygous genotype and 13 research blastomeres from donated embryos.
Conclusions: This semi-duplex PCR method provides robust, reproducible and informative amplification results for single cells. Moreover, this test has now been approved for clinical application by the UK Human Fertilisation and Embryology

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Authority (HFEA). As such, the development of PGD for HS-RDEB broadens the range of prenatal testing options and personal choice for couples at reproductive risk of this severe genetic skin disease.

1. Introduction

Epidermolysis bullosa (EB) is the name given to a group of inherited skin disorders characterised by fragility of the skin and mucous membranes [1]. The individual diseases vary in their impact from relatively minor disability to death during infancy [2]. Hallopeau—Siemens recessive dystrophic epidermolysis bullosa (HS-RDEB) (OMIM 226600) is one of the most severe subtypes and is associated with high morbidity (widespread skin and mucous membrane erosions) and premature mortality (aggressive early-onset squamous cell carcinoma) [3]. All cases involve pathogenic loss-of-function mutations on both alleles of the *COL7A1* gene, which encodes for type VII collagen, the major component of anchoring fibrils at the dermal—epidermal junction [4,5]. At present, there is no cure for this condition and many couples who have had one affected child are keen to consider prenatal diagnosis in subsequent pregnancies [6].

In the 1980s, prenatal diagnosis for HS-RDEB was performed by analysing fetal skin biopsy samples at ~16—18 weeks of gestation. Diagnosis was based on the ultrastructural plane of blister formation and on particular morphological abnormalities using transmission electron microscopy as well as skin immunohistochemistry using specific basement membrane antibodies [7,8]. In the 1990s, HS-RDEB was shown to result from *COL7A1* gene mutations, leading to the feasibility of DNA-based diagnosis, usually involving DNA extracted from chorionic villi at 11—12 weeks' gestation. Since 1994, therefore, DNA-based prenatal tests have mostly been performed either through direct assessment of pathogenic *COL7A1* mutations or using indirect linkage markers given that there is no known genetic heterogeneity in HS-RDEB [6,9—11].

Analysis of fetal skin biopsies and DNA-based prenatal tests, however, only allows the diagnosis of an affected fetus to be made once pregnancy is established, and the prospect of termination may be associated with considerable emotional and physical distress for the parents. An alternative approach that obviates the need for termination of pregnancy is preimplantation genetic diagnosis (PGD). This is a highly specialised and rapidly evolving field, involving in vitro fertilisation (IVF) techniques, that allows embryos to be tested for genetic disorders before they are transferred to the uterus and before pregnancy has begun [12,13]. The genetic diagnosis is performed on single blastomeres obtained from 3-day-old IVF embryos [14]. Only embryos free of the disorder are available for transfer to the mother.

Since its first clinical application in 1990, PGD has become a well-established treatment option for couples at risk of transmission of a genetic disease [15]. Although only practised in relatively few centres worldwide, application of new technology has seen an exponential leap in the number of diseases that can now be treated using PGD [16]. PGD for severe inherited dermatological conditions, however, has rarely been described. Two cases of PGD for Herlitz junctional EB have been reported, although only a biochemical pregnancy was achieved in each case [17]; and more recently, there has been a successful case of PGD for skin fragility-ectodermal dysplasia syndrome with birth of a healthy baby 4 years after embryo diagnosis, and following two frozen embryo replacement cycles [18].

The aim of this study, therefore, was to develop a suitable generic test which could be used in PGD of HS-RDEB, thus broadening the range of prenatal testing options and choice for couples at reproductive risk of this severe inherited skin disorder.

2. Materials and methods

2.1. Rationale for design of PGD test

Over 200 different mutations have been reported in HS-RDEB [5,19], most of which are family-specific and only a few recurrent mutations have been identified [20,21]. Adding to the molecular diversity of pathogenic mutations is the structural complexity of the type VII collagen gene. Although the gene is relatively compact (~36 kb), the coding region is composed of 118 distinct exons spanning ~9 kb of cDNA. Collectively, therefore, it is not feasible to design a generic mutation-based PGD test. Instead, selected polymorphic microsatellite markers were identified to design and develop a semi-duplex polymerase chain reaction (PCR) method.