Molecular diagnosis and genetic counseling of EBS and DEB in France: 15 years of experience

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Schematic representation of the epidermis and DEJ with EB types and defective proteins

Fine et al. JAAD 2014
Chromosomes contain the genetic information
Double stranded DNA encodes genetic information
Why is molecular diagnostic important?

- For accurate diagnosis of EB type
- For accurate diagnosis of mode of inheritance
- For prognosis
- For genetic counselling and prenatal diagnosis
- To identify patients with unexpected genotype-phenotype correlations
- To improve understanding of disease mechanisms
- To identify patients suitable for specific therapies
- For epidemiological studies
Postnatal diagnoses for DEB, EBS and JEB since 2000 in our laboratory

All EB = 418
All diseases = 1,148
Splicing mutations can modulate disease severity

- Can allow the production of wild-type type VII collagen
- Can cause RDEB of very different severity
- Can occur deep within introns, where they are amenable to strategies modifying mRNA splicing
- Essential information for therapeutic approaches
NGS is a major technological progress

Classical Sanger Sequencing
(1 run = 96 sequences)

A Technological Breakthrough

Important reduction of the sequencing cost

Next Generation Sequencing
(1 run produces millions of sequences)

Main Drawbacks of NGS:
- Very rapid evolving field, not stabilized yet
- Still complicated to use

From C. Bole
DNA-based prenatal diagnosis

Principle:

To define the disease status of a fetus during a pregnancy at risk by genetic testing of its DNA, most often from the perspective of termination of the pregnancy should the fetus be predicted to be affected.

Restricted to « diseases of a particular severity with no current cure at the time of diagnosis »

The procedure is performed as early as possible to be as ethically acceptable as possible.

Relies on prior identification of the familial mutation(s)
Which EB should benefit from PND?

**EB Simplex**

*Dominant:*  
Generalized severe (Dowling-Meara form) (*KRT5, KRT14*) and specific mutations with particular severity

*Recessives:*  
- with muscular dystrophy (*PLEC*)  
- with pyloric atresia (*PLEC*)  
- very rare forms (*KRT14, PKP1, DSP, EXPH5*)
Prenatal diagnosis procedure

- Genetic counselling: Geneticist and genetic counsellor
- Chorionic villus sampling (10-12 WG) or amniotic fluid (16 WG) under ultrasound guidance
- Genetic testing (Direct, indirect, maternal contamination)
- Prediction of the disease status of the fetus
- Outcome of the pregnancy
Which EB should benefit from PND?

**Junctional EB**
- Generalized severe (*LAM332*)
- JEB with pyloric atresia (*ITGA6, ITGB4*)
- Generalized non lethal (*COL17A1*)

**Dystrophic EB:**
- **Recessive:**
  - Generalized severe
  - Inversed
  - More difficult to assess for generalized intermediate forms because of disease variability
- **Dominant:**
  - Difficult to assess because of disease variability
**Prenatal** diagnoses for DEB, EBS and JEB since 2000 in our laboratory

- **DEB**: 8% (10)
- **EBS**: 6% (7)
- **JEB**: 40% (48)
- **Others**: 46% (56)

**All EB = 65**
**All diseases=121**
Genetic counseling is sometimes difficult because of:

- Non allelic locus heterogeneity (JEB, EBS, …)
- Intra-familial phenotypic variability (EBDR, EBDD, EBS …)
- Mode of inheritance is unusual:
  - Dominant and recessive mutations (DEB)
  - Germline mosaïcism (JEB)
Preimplantation genetic diagnosis (PGD)

• Alternative method to prenatal diagnosis

• Aims at establishing the disease status of the embryos prior to their transfer, thus avoiding termination of an implanted egg

• Highly specialized procedure available only in a limited number of centres

• The overall clinical pregnancy rate is 18% per oocyte retrieval and 25% per embryo transfer in reference Centres in Europe
Preimplantation genetic diagnosis procedure

- Stimulation of follicle production with gonadotrophins
- **Day 0:**
  - Oocyte collection by ultrasound guided aspiration
  - Individual *in vitro* fertilization of oocytes by intracytoplasmic sperm injection
- **Day 3:**
  - Biopsy of the resulting embryos at the 8 cells stage of the blastomere when embryonic cells are still totipotent
  - One or two cells are isolated and used for genetic analysis par PCR (direct and indirect analysis)
- **Day 5:**
  - 1 or 2 unaffected embryos are transferred to the uterus
Preimplantation diagnosis

- Ectodermal dysplasia syndrome with skin fragility (PKP1 deficiency) (Fassihi et al. 2006)

- RDEB generalized severe (haplotype analysis) (Fassihi et al., 2006)

- RDEB inversa (Vendrell et al. 2011)

- JEB with pyloric atresia due to ITGB4 mutations (Ozge et al. 2012)
Non-invasive prenatal diagnostic procedures

Analysis of fetal cells circulating in maternal blood
- Requires cell enrichment and single cell analysis
- Lectin-based method and autoimage analyzing in development

Cell-free circulating fetal DNA
- Has been used for other diseases
- Currently limited to disorders caused by a paternally inherited mutation
Conclusions and perspectives - Diagnosis

• Molecular diagnosis remains essential for accurate diagnosis and genetic counselling

• Can disclose unusual geneotype-phenotype correlations and guide the quest for modifier genes

• Improves understanding of molecular mechanisms involved in disease severity

• Provides critical information for therapeutic approaches (gene editing, exon skipping, nonsense read-through, pre-mRNA modulation…)

• Next generation sequencing significantly accelerates mutation identification but requires heaving investment
Conclusions and perspectives – PND and PGD

• Early DNA-based prenatal diagnosis represents a major clinical benefit of gene identification and mutation analysis

• Pre-implantation diagnostic is likely to be more often used for EB

• Novel non-invasive methods could also be applied to EB in a near future

• Importance of HLA typing in PND and PGD procedures to identify HLA-matched embryos to provide cord blood at birth.
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