Genetics 101 and how it works for EB

Ellen G. Pfendner Ph.D.
Director, EBDx Program
GeneDx Inc.
Gaithersburg MD USA
Questions EB parents ask

Where did my child’s EB come from?
Can it happen to my next child?
Will my child get better or worse?
Can I have prenatal testing or preimplantation diagnosis for my next pregnancy?
Epidermolysis Bullosa
Clinical Features of the Three Major Forms

Dystrophic: (DEB) : variable severity from severe to very mild, Blistering throughout life, scarring, Tissue cleavage below the basement membrane

Junctional (JEB) : variable severity from neonatal lethal (Herlitz) to mild (non-Herlitz), non-scarring, Tissue cleavage within the lamina lucida

Simplex (EBS) : variable severity from lethal to very mild, non-scarring, Tissue cleavage within the keratinocyte layer
EPIDERMOLYSIS BULLOSA
Clinical Features of Hemidesmosomal Variants

- Generalized Atrophic Benign EB (GABEB) (life-long blistering, hair and nail abnormalities)
- EB with pyloric atresia (EB-PA) (neonatal blistering with gastric malformations)
- EB with muscular dystrophy (EB-MD) (blistering at birth with later onset progressive muscle weakness)

Epidermolysis Bullosa Cutaneous Basement Membrane Zone

Disease
Ddesmosomal
EB SIMPLEX
KiINDLER
EB-MD
JEB –PA
GABEB
JEB
GABEB
JEB

Genes
PKP1,2, PKG1, DSP1
KRT5 KRT14
KIND1
PLECTIN
ITGB4 ITGA6
COL17A1
BP2330 (BPAG1)
LAMB3 LAMA3 LAMC2
COL7A1

Keratinocyte layer
Lamina lucida
Anchoring filaments
Dermis
Anchoring fibrils
Interstitial collagen
Classification of the disease is necessary before mutation detection should be attempted

- There are 3 major classes and up to 5 variant forms
- There are 14 genes encoding the proteins involved in all forms of EB
- Classifications are necessary to narrow down the list of gene candidates before screening can begin
EPIDERMOLYSIS BULLOSA
Type VII Collagen Expression in Skin

Normal

RDEB
## Classification of Major EB subtypes

<table>
<thead>
<tr>
<th>Category</th>
<th>Level of Blistering</th>
<th>Protein</th>
<th>Mutated genes</th>
<th>Inheritance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplex</td>
<td>Basal cell</td>
<td>Keratins 5 &amp; 14</td>
<td>KRT5, KRT14</td>
<td>AD, AR</td>
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<tr>
<td>EBS-O</td>
<td>hemidesmosome</td>
<td>plectin</td>
<td>PLEC1</td>
<td>AD</td>
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<tr>
<td>Junctional</td>
<td>Lamina lucida</td>
<td>laminin A3, B3</td>
<td>LAMA3, LAMB3, LAMC2</td>
<td>AR</td>
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<tr>
<td>Junctional</td>
<td>Lamina lucida</td>
<td>laminin C2</td>
<td></td>
<td>AR</td>
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<tr>
<td>GABEB</td>
<td>hemidesmosome</td>
<td>Collagen XVII</td>
<td>COL17A1(BPAG2)</td>
<td>AR</td>
</tr>
<tr>
<td>EB-PA</td>
<td>hemidesmosome</td>
<td>α6β4 integrin</td>
<td>ITGB4, ITGA6, PLEC1</td>
<td>AR</td>
</tr>
<tr>
<td>EBMD</td>
<td>hemidesmosome</td>
<td>plectin</td>
<td>BPAG1e</td>
<td>AR</td>
</tr>
<tr>
<td>Dystrophic</td>
<td>Sub-lamina densa</td>
<td>Collagen VII</td>
<td>COL7A1</td>
<td>AD, AR</td>
</tr>
</tbody>
</table>

**AD** = autosomal dominant  
**AR** = autosomal recessive
Importance of Checking Family History

Carrier parents have pitted tooth enamel
Indicative of COL17A1 mutations
Or
Parent has dystrophic nails indicative of
a mild dominant DEB mutation

Parents are related in some way
The Family Tree (Pedigree)
Who Benefits Most From Genetic Testing?

- Determination of severity shortly after birth
- Mutation detection facilitates determination of recurrence risk and prenatal testing
The Human Genome

46 chromosomes

22 autosomes
everyone has 2

2 sex chromosomes
males have X & Y
females have 2 X’s

All EB genes are
on non sex
chromosomes
(autosomes)
Banded Human Chromosomes

Bands can be seen by special stains.

Each band may contain tens or hundreds of genes.

A gene contains the information to produce a protein.
EPIDERMOLYSIS BULLOSA
Human Type VII Collagen Gene

- 118 exons
- 9.3 kb cDNA
- on chr 3p
Sequence showing formation of a STOP codon in LAMB3

Normal LAMB3 ex 6

Mutant LAMB3 ex 6

LAMB3 430c->t R144X
Autosomal Recessive Inheritance

For recessive disease the risk is 25% to any further children of that couple.

One recessive mutation is carried by each unaffected parent. The affected child receives one mutation from each parent. Unaffected sibs may have no mutations or only one, making him or her an unaffected carrier.

Risk is 25% there are 4 possible combinations.
Autosomal Recessive Inheritance  
Carrier Risk in an Unaffected Sibling

3 possibilities

Risk of EB

2/3 x 1/2 x 1/350 x 1/2 = 1/2100
Autosomal Dominant Inheritance

No family history of EB

DDEB

2 possibilities
risk is 1/2

Also for EBS (dominant)
Why do mutation screening?

To determine risk of recurrence when inheritance pattern unknown

- If dominant EB risk to fetus is low because neither parent is affected
- If recessive EB risk is 25%
In a family where the affected member has a *de novo* dominant mutation and neither parent is a carrier the risk to next pregnancy is 2-3% due to **gonadal mosaicism**.
Gonadal Mosaicism

- When a subpopulation of reproductive germ cells (sperm or oocytes) carry a *de novo* mutation and the remainder do not.

- When a *de novo* mutation turns up in the family it is difficult to determine from which parent the mutation arose and what proportion of the germ cells remaining also carry the mutation.

- This has implications for recurrence risk in future pregnancies in that family.

- Recurrence risk of a *de novo* mutation has been calculated to be 2-3% for most disorders.
Pitfalls of Assuming Inheritance Pattern

If parent is unaffected assuming autosomal recessive inheritance pattern is risky

DDEB G2043R is the most common dominant mutation and occurs often de novo

EBS most common as dominant disease and can occur de novo but recessive disease has been reported
Pitfalls of Assuming Inheritance Pattern

If parent is mildly affected assuming autosomal dominant inheritance can be misleading. Check to make sure phenotypes are of the same severity.

DEB with one dominant and one recessive mutation
Pitfalls of Assuming Inheritance Pattern

If parent is mildly affected assuming autosomal dominant inheritance can be misleading. Screen for mutations to determine recurrence risk.

R927G/8022-2a→g
wt/wt
Can screen to determine whether this parent is a carrier

R927G/wt
Normal
Pitfalls of Predicting Prognosis from Mutation Data

- In some families phenotype may be variable for the same genotype

- Some dominant mutations are not expressed in carriers (12 cases)
Pitfalls of Predicting Prognosis from Mutation Data

• For some mutations (splicing mutations and amino acid substitutions) it is difficult to predict the severity of the phenotype.

• In some cases where a severe phenotype is predicted (i.e. JEB) we have seen relatively mild disease because the affected exon is spliced out in frame.
PURPOSES OF PRENATAL DIAGNOSIS FOR EB

• To provide a range of informed choices to parents at risk of having a child with EB

• To provide reassurance and reduce anxiety among high-risk families

• To allowing planning for a C-section for delivery of an EB child

• To allow couples at risk for an EB child (who might otherwise not have more children) to begin a pregnancy knowing that a test for EB is available
What happens when prenatal diagnosis is requested?

- Affected individual should be biopsied to determine type of EB.
- Sample from affected individual should be sent for mutation detection to identify mutations before a pregnancy is started.
- Once the mother becomes pregnant the obstetrician determines how many weeks old the fetus is by ultrasound.
- Chorionic villus sampling (CVS) is scheduled for 10-12 weeks.

OR

- Amniocentesis is scheduled for 16-19 weeks.
CHORIONIC VILLUS SAMPLING

ultrasound locator used to monitor correct placement

villi of chorion

villus of chorion
grow cells in culture

remove sample of chorionic villi

fetal cells

grow cells in culture

flexible catheter

uterus wall

placenta

flexible catheter

DNA analysis

Figure 3-11b Biology Today, 3/e © 2004 Garland Science
(A) AMNIOCENTESIS

- Extract amniotic fluid
- Fetal cells in amniotic fluid
- Grow cells in culture
- Genetic analysis
Prenatal Diagnosis continued

- Samples is taken by obstetrician under ultrasound guidance.
- Sample is sent to hospital lab where a portion of the sample may be retained for chromosome studies.
- CVS samples are dissected to remove maternal tissue.
- Sample is sent to diagnostic lab overnight.
- DNA is extracted from sample.
- Maternal contamination is evaluated.
- Assays for specific mutations in that family are begun.
- Assays may involve sequencing as well as other methods.
- Assays are performed completely in duplicate. Turnaround time is usually 2 weeks.
- Results are read at laboratory.
- Oral results given to physician.
- Written results faxed to physician.
Prenatal diagnosis is requested for a twin pregnancy

An example of a family which presented for mutation detection and subsequent prenatal testing:

Prenatal diagnosis is requested for a twin pregnancy
Prenatal Diagnosis for R635X in Twin Pregnancy

markers  JEB Pt  Fetus A  Fetus B

NORMAL  Mutant R635X  Mutant R635X
Prenatal diagnosis performed in 180 pregnancies

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Affected</th>
<th>Carrier</th>
<th>Inconclusive</th>
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<tbody>
<tr>
<td>JEB</td>
<td>19</td>
<td>20</td>
<td>55</td>
<td>3</td>
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<tr>
<td>DEB</td>
<td>17</td>
<td>18</td>
<td>45</td>
<td>4</td>
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<tr>
<td>EBS</td>
<td>6</td>
<td>2</td>
<td></td>
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</tbody>
</table>

Correct genotype/phenotype prediction in 160 cases
Results inconclusive in 7 cases
10 cases lost to follow up
Collaborators:

The Staff at GeneDx

Jouni Uitto MD PhD

Aoi Nakano MD PhD
Karl Nielsen BS
Roslyn Varki MD
Monjir Bakshi

Sarah Fratta BS
Sara Sadowski BS
Sarah Cash MD

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DebRA of America
and
Families of EB Patients
EPIDERMOLYSIS BULLOSA
MOLECULAR DIAGNOSTICS
Summary Data
TJU and GeneDx

Samples analyzed

• Dystrophic EB: 542
  • COL7A1 465 now > 95%
  • DOMINANT 77 14.2%

• Junctional EB: 252
  • LAMA3 31 12.3%
  • LAMB3 179 71%
  • LAMC2 21 8.3%
  • BPAG2 21 8.3%
### Summary Data

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>EB with pyloric atresia</td>
<td>71</td>
<td>76.4%</td>
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<tr>
<td>ITGB4</td>
<td>54</td>
<td>76.4%</td>
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<tr>
<td>ITGA6</td>
<td>5</td>
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<tr>
<td>PLEC1</td>
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<tr>
<td>EB with muscular dystrophy</td>
<td>22</td>
<td>17.0%</td>
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<tr>
<td>PLEC1</td>
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<tr>
<td>EB simplex</td>
<td>129</td>
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<tr>
<td>KRT5</td>
<td>67</td>
<td>52.2%</td>
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<tr>
<td>KRT14</td>
<td>62</td>
<td>48.0%</td>
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