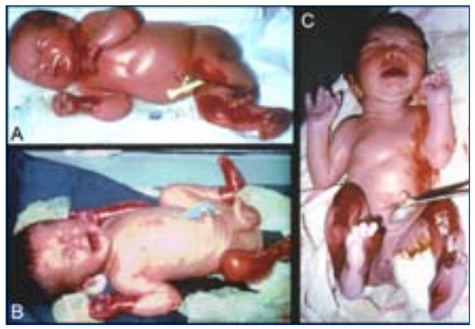
# Epidermolysis bullosa

***Over the last decade, defining the molecular pathology of the inherited blistering condition, epidermolysis bullosa, has led to more accurate diagnoses, better genetic counselling, the feasibility of DNA-based prenatal diagnosis, and the possibility of newer forms of treatment, including somatic gene therapy.***

The term epidermolysis bullosa (EB) refers to a group of inherited blistering skin diseases, some of which are among the most severe conditions in dermatology (Fine et al, 2000). The hallmark of all forms of EB is fragility of the skin such that it blisters following relatively minor trauma. It is estimated that about 10 000 people in the UK suffer from EB. In many cases, it first presents at, or shortly after, birth (*Figure 1*). The mildest and most common form of EB is called EB simplex (Irvine, 2005). Individuals with EB simplex typically have blisters on their feet from rubbing footwear, particularly during the summer when heat and sweating exacerbate the problem. Although usually limited in extent, EB simplex can be extremely painful and debilitating. The junctional and dystrophic forms of EB are generally more severe. Junctional EB can result in very extensive loss of the epidermis, with most such cases failing to survive beyond infancy (McGowan and Marinkovich, 2002). The increased morbidity occurs as a result of overwhelming skin and mucous membrane fragility, which leads to susceptibility to infection and failure to thrive. Dystrophic EB varies in its severity but in all cases blisters are followed by scarring of the skin or mucous membranes. In some cases, particularly more severely affected adults, there is an increased risk of developing skin malignancy, notably squamous cell carcinoma (Mallipeddi, 2002) (*Figure 2*).

***Figure 1. Clinical appearances of neonates with different forms of inherited epidermolysis bullosa (EB). A. EB simplex (Dowling–Meara). The erosions and blisters can be severe at birth but improve during early life and the prognosis is generally good. B. Junctional EB (Herlitz). There is severe mucocutaneous fragility and affected children usually fail to survive beyond the first few months of life. C. Dystrophic EB (Hallopeau–Siemens). This form of EB leads to mutilating scarring and an increased risk of skin cancer.***

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## What causes EB?

EB results from mutations in genes encoding 10 different structural proteins at the junction between the epidermis and the dermis (Uitto and Richard, 2005). Most of these proteins are associated with hemidesmosomes, key cell-matrix adhesion complexes found in skin as well as other epithelia (Mellerio, 1999).Together, these structural proteins form a link from basal epithelial cells, across the basement membrane, to the underlying dermis. When one of these proteins is disrupted by pathogenic mutations, as is the case in EB, this causes a weakness in the structural chain, resulting in mechanical fragility. The distribution of some of these proteins in tissues other than the skin means that certain forms of EB may be associated with extracutaneous features, e.g. patients with dystrophic EB, who have mutations in the type VII collagen gene, may develop corneal erosions and scarring, and oesophageal strictures, reflecting the distribution of type VII collagen in these tissues. Likewise, patients with mutations in plectin have muscular dystrophy as well as skin blisters because plectin is also found in striated muscle.

Collectively, over 400 different mutations have been described in the various types of EB and paradigms for genotype-phenotype correlation have been established

***Figure 2. Forearm of a 42-year-old man with dystrophic epidermolysis bullosa (EB) complicated by squamous cell carcinoma. Individuals with the Hallopeau–Siemens form of recessive dystrophic EB have a 70-fold increased risk of developing skin malignancy, usually in areas of chronic scarring.***





(*Figure 3*). Accordingly, with identification of specific mutations in individuals and families with EB, it has become possible in many cases to translate specific molecular information into predictions of the likely severity of EB and associated clinical features that might be anticipated.

As well as using information from molecular analyses to refine predictions about phenotype and severity, determination of gene/protein pathology has influenced the classification of EB. Whereas historically subtypes of EB were largely defined by clinical features, for example whether they were scarring or non-scarring, today's understanding of pathogenic mechanisms in the various subtypes has made possible the development of a classification based principally on the underlying genotype.

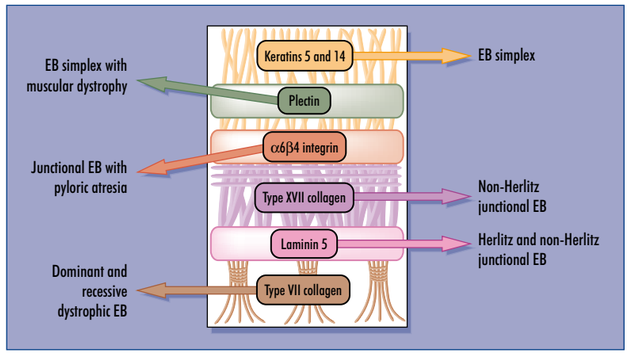



***Figure 3. Schematic representation of the cutaneous basement membrane zone showing the component structural proteins and the inherited forms of epidermolysis bullosa (EB) that result from mutations in the genes encoding them.***

## Rapid and accurate diagnosis of EB

Diagnosis of EB generally begins by taking a skin biopsy from a non-blistered site, which can then be divided for immunohistochemistry and transmission electron microscopy (Petronius et al, 2003). Analysis of these samples allows accurate determination of the subtype of EB, and enables clinicians to anticipate likely severity and other clinical features that might be important. It also gives clues as to which gene(s) should be screened for mutations.

The autosomal recessive forms of EB are usually associated with loss-of-function mutations on both alleles of the relevant gene. This leads to low or absent levels of the corresponding mRNA and protein. Immunolabelling with antibodies to the mutated protein in patients' skin, therefore, typically, shows a marked reduction or complete ablation of staining at the dermal-epidermal junction (*Figure 4*). A panel of antibodies against the various target proteins in EB is readily available and immunostaining of skin sections takes less than 1 day to perform.

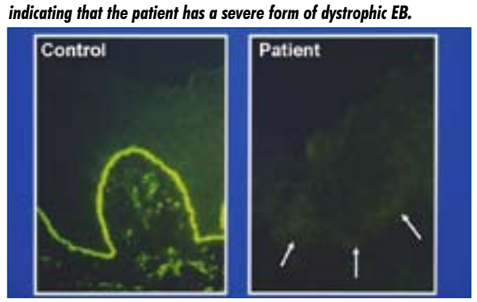
As such, rapid diagnosis of most recessive forms of EB is possible. This is particularly useful in neonates, in whom clinical features may overlap considerably, making a clinical diagnosis in the early stages extremely difficult. This is important because the prognosis of different types of EB may vary significantly. Immunohistochemical analysis of skin samples is also particularly useful in identifying gene/protein pathology in other forms of EB in which there is genetic heterogeneity (McGrath and Eady, 1997).

Ultrastructural analysis of skin samples may yield further information regarding the type of EB. Although laboratory processing takes longer than immunohistochemistry, typically up to several days, it is especially helpful in milder types of EB where protein expression appears normal with the panel of monoclonal antibodies, but splits at the dermal-epidermal junction exist, often with other distinctive morphological changes in specific EB subtypes, such as keratin filament clumping in some forms of EB simplex (Ishida-Yamamoto et al, 1991).

## Better genetic counselling of EB

Making a rapid and accurate diagnosis of EB is helpful in determining prognosis and anticipating or preventing disease complications. Although a skin biopsy can be useful in this regard, molecular analysis of EB genes has further helped improve genetic counselling. For example, in many cases of mild–moderate severity dystrophic EB, born to clinically unaffected unrelated parents, it is often difficult to decide whether inheritance is autosomal recessive or de novo dominant (*Figure 5*). Determining the inheritance pattern clearly has important implications for the family concerned: if autosomal recessive, the parents have a 25% risk of recurrence in each subsequent pregnancy, but the proband is highly unlikely to have affected offspring of his/her own; whereas a de novo dominant mutation means that the parents are unlikely to have further affected children themselves, but the proband has a 50% risk of passing the disease on to each of his/her children. Screening for mutations in the type VII collagen gene, however, now helps differentiate genotype and therefore makes discussion of inheritance risk with individual families much more accurate (Horn and Tidman, 2002).

***Figure 4. Immunohistochemical labelling of skin biopsies can be useful in making a rapid diagnosis of epidermolysis bullosa (EB). Control skin labelling with an antibody to type VII collagen shows bright linear fluorescence at the dermal–epidermal junction. In contrast, the patient's skin shows a complete absence of type VII collagen immunoreactivity (arrows show unstained dermal-epidermal junction), indicating that the patient has a severe form of dystrophic EB.***



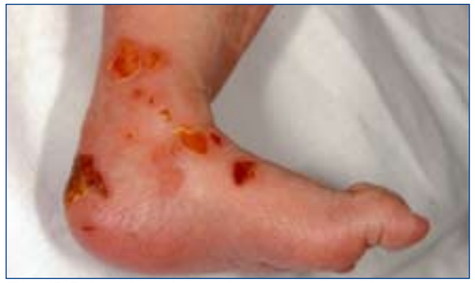


***Figure 5. Patient with an indeterminate type of dystrophic epidermolysis bullosa. There are trauma-induced blisters and erosions around the ankle. Clinically, it is not possible to say whether this is dominant or recessive disease, but molecular analysis of the type VII collagen gene shows that this patient has a dominant type of mutation.***

## Prenatal diagnosis of EB

Prenatal diagnosis of severe forms of EB by fetal skin biopsy was first reported 25 years ago. Biopsies were taken at 16–20 weeks' gestation and the skin samples were analysed by transmission electron microscopy and immunohistochemistry (Eady, 2001). During the 1990s, however, as the molecular basis underlying different forms of EB was elucidated, it became feasible to screen for gene mutations in the fetus, usually using DNA extracted from chorionic villi (*Figure 6*). Initial work-up of a family wishing to have prenatal diagnosis in an at-risk pregnancy involves molecular analysis of parents and previously affected children to determine pathogenic mutations or informative genetic markers (Christiano et al, 1996, 1997). Subsequently, when a pregnancy is established, DNA-based prenatal testing can be performed at 10–12 weeks' gestation. Apart from the obvious advantage of being performed in the first trimester, prenatal testing from chorionic villi can be also be applied to more subtypes of EB than fetal skin biopsy testing.

***Figure 6. Chorionic villi. Determining the molecular basis of epidermolysis bullosa (EB) has led to the feasibility of DNA-based prenatal diagnosis. Most prenatal tests for EB are now based on examination of fetal DNA extracted from chorionic villi at 10–12 weeks' gestation.***

Knowledge of the molecular basis of EB has also led to the development of preimplantation genetic diagnosis for some forms of EB (McGrath and Handyside, 1998). This uses in-vitro fertilization techniques with analysis of DNA from a single cell at around the 8-cell stage of embryonic development. Affected embryos are discarded but those unaffected by EB can be implanted in the uterus. Preimplantation genetic diagnosis extends the choice of prenatal options for couples at reproductive risk of recurrent disease, particularly for those not wishing to have termination of an affected established pregnancy for religious, cultural or personal reasons.

## Improving clinical care for people with EB

The optimal management of individuals with EB is a major challenge. Comprehensive and effective health care requires input from a multidisciplinary team that includes dermatologists, specialist nurses, plastic surgeons, dentists, gastroenterologists, interventional radiologists, paediatricians, otolaryngologists, oncologists, ophthalmologists, physiotherapists, occupational therapists, carers and others, reflecting the multisystem nature of this disease. In the UK, the complexity of providing the best possible care for people with EB has been realized and in 2003, a framework for national centres for EB was established. Specialist units in the north and south of England provide expert clinical care for both children and adults with EB, as well as coordinating appropriate diagnostic tests (see *Useful addresses*). Although supported centrally by the NHS, several aspects of service development and provision have been initiated by the patient support group, Dystrophic Epidermolysis Bullosa Research Association (DebRA). The involvement of DebRA has been vital in translating several advances in patient care into service delivery.

## New treatments for EB

At present, there is no effective treatment for EB and the focus has been on preventing and supportively managing complications of the disease (Pai and Marinkovich, 2002). Research into treatments for EB is focused on three main areas: somatic gene therapy, improving wound healing, and prevention of cancer. Most gene therapy work is being targeted to the clinically severe recessive forms of EB and the strategies mainly involve ex-vivo gene replacement into stem-cell enriched cultured keratinocytes, skin fibroblasts or circulating bone marrow stem cells (Hengge, 2005). Protein replacement is also being assessed, although concerns about potential autoimmune responses to new antigens still need detailed consideration.

In most dominant forms of EB, mutations result in dominant–negative interference whereby the mutant gene product interferes with the function of the normal protein. In these circumstances gene therapy is predominantly being directed at silencing the mutant allele, and inducible mouse models have been established for therapeutic gene manipulation. At present, human gene therapy for EB has been limited to one ex-vivo trial of laminin 5-gene corrected keratinocytes in a patient with a form of autosomal recessive junctional EB. Restoration of functional laminin 5 protein was achieved but the skin graft, despite being autologous, was lost after a few months and the benefits did not persist. This trial used retroviral delivery of the transgene, but following safety and regulatory limitations, the next series of clinical trials for recessive dystrophic and junctional EB will be based on self-inactivating retroviral or lentiviral delivery systems or non-viral integrating plasmids. These trials of somatic gene therapy are expected to start before the end of 2007.

## Exploring other genodermatoses

Over the last decade, subsequent to the discovery of disease genes implicated in EB, the same techniques of electron microscopy and immunohistochemistry of skin samples, and genetic analyses using candidate gene approaches or genome-wide linkage, have been used to identify causative genes in other genodermatoses (Irvine and McLean, 2003). Mutations in structural components of other cell adhesion units, focal adhesions and desmosomes, have been identified, providing molecular explanations for conditions such as Kindler syndrome and skin fragility-ectodermal dysplasia syndrome (Ashton, 2004; South, 2004). These inherited blistering skin disorders show considerable clinical overlap with other forms of EB, and have also increased understanding about the roles of these structural components in maintaining normal epithelial integrity.

## Conclusions

Basic scientific research into the blistering skin disorder EB has provided a detailed insight into the intricate network of proteins that anchor the epidermis to the underlying dermis. Initially, these discoveries proved helpful in refining the classification of EB, making accurate diagnoses and establishing paradigms for genotype-phenotype correlation. However, the new molecular data have also led to better genetic counselling, the development of DNA-based prenatal diagnosis and a rationale for developing new forms of treatment, including somatic gene therapy. **BJHM**

*Conflict of interest: none.*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### Useful addresses

**The supraregionally funded epidermolysis bullosa service for England and Wales**

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### KEY POINTS

- Epidermolysis bullosa (EB) refers to a group of inherited diseases in which trauma to the skin and mucous membranes leads to blisters and erosions. About 10 000 people in the UK have EB.
- There are three main types of EB: simplex, junctional and dystrophic. Inheritance may be autosomal dominant (simplex, dystrophic) or autosomal recessive (junctional, dystrophic).
- EB is caused by mutations in 10 different genes encoding structural proteins at the junction between the epidermis and the dermis.
- Improved knowledge of the molecular pathology of EB, in combination with the development of a new national clinical and diagnostic service for individuals and families with EB, has led to more accurate diagnoses, better genetic counselling, and DNA-based prenatal diagnosis.
- Current translational research is being targeted to somatic gene therapy, improving wound healing, reducing the risk of skin malignancy, and expanding practical options for prenatal diagnosis.