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Mutations in the Genes for Epidermal Keratins in Epidermolysis Bullosa and Epidermolytic Hyperkeratosis

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**Background:** Clues from clinicopathologic studies of epidermolysis bullosa simplex (EBS) and epidermolytic hyperkeratosis (EH) have implicated abnormalities in keratin filaments as possibly underlying the pathogenesis of these diseases. Multiple avenues of study have now converged, which confirm this hypothesis.

**Observations:** The clinical spectrum of EBS and EH is reviewed together with classic histologic, electron microscopic, and immuno-electron microscopic studies. Linkage analyses have shown in EBS and EH that the disease traits are linked to the keratin gene clusters on chromosomes 12 and 17. Transgenic mice bearing mutations or deletions in genes coding for basal cell keratin K14 express the phenotype of EBS, and transgenic mice bearing abnormal K1/K10 genes resemble EH. Increasing numbers of point mutations in the human keratin genes have been found in both sporadic and familial cases of EBS in keratins 5/14 and EH in keratins K1/10 genes, respectively, particularly in highly conserved subdomains of the keratin proteins.

**Conclusions:** The recent and rapid progress in understanding the molecular biology of EBS and EH will also enhance knowledge about intermediate filament structure and function. Further studies of the effects of these mutations on the control of keratinocyte growth and differentiation are required. They will lead the way to rational pharmacologic or gene therapy.

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**LUCIDATING THE genetic basis of hereditary skin diseases has become possible with recent advances in research techniques, with increased accessibility of gene sequencing by the polymerase chain reaction. Target genes for genodermatoses have been identified in some cases from immunohistochemical and biochemical studies and, in other cases, by identifying genetic linkage in families. The studies characterizing keratin intermediate filament proteins, keratin genes, and their sequences, which have taken place in the last decade, have laid the scene for understanding changes in keratinocyte differentiation in hyperproliferative and malignant skin diseases. Multiple approaches in different laboratories have now identified keratin gene mutations underlying several diseases characterized by blistering: epidermolysis bullosa simplex (EBS) and pathologic features common to bullous congenital ichthyosiform erythroderma (BCIE) and ichthyosis bullosa of Siemens.**

**EPIDERMOLYSIS BULLOSA**

In hereditary and acquired epidermolysis bullosa (EB), the skin blisters following trauma. There are three groups of hereditary EB, separated according to the microscopic location of the rupture in the skin, which gives rise to a macroscopic blister. In EBS, the blister forms within the epidermis; in junctional epidermolysis bullosa (JEB), within the lamina lucida; and in dystrophic epidermolysis bullosa (DEB), below the lamina densa. The target gene for DEB is probably the major component of the anchoring fibril, type VII collagen, and for JEB, a further basement membrane protein, known variously as GB600, epiligrin, and kalinin, is implicated. Therefore, in EB, keratin gene mutations are confined to the simplex forms and we shall consider evidence from different studies giving rise to this conclusion, in the light of the clinicopathologic background of the disease.

Epidermolysis bullosa simplex was recognized as a separate category of hereditary
EB by 1898 and as a mendelian dominant trait by 1908. Koebner described cases with blisters on the hands and feet and occasionally elsewhere, but other families were described where localized blistering was confined to the hands and feet. Thus, EBS Weber Cockayne (EBS-WC) has been used to diagnose cases confined to the hands and feet, and EBS Koebner (EBS-K) was used for more generalized blistering. However, the disease spectrum probably crosses these boundaries with increasing disease severity.

**EPIDERMOLYSIS BULLOSA SIMPLEX-KOEKNER**

Generally, EBS-K begins within the first week of life and may be widespread on the trunk and limbs. Occasionally, mucosal sites are affected. Later in life, the blistering becomes localized to the hands and feet, but generalized blistering may still occur at sites of friction under clothing, triggered by physical stress, or by hormonal changes. The blistering is worse in summer, and the epidermis is fragile. The EBS-K is also associated with early-onset bruising. Epidermolysis bullosa simplex with mottled pigmentation is associated with generalized blistering, more like EBS-K, but has widespread mottled pigmentation and keratotic plugs on the palms.

**EPIDERMOLYSIS BULLOSA SIMPLEX-WEBER COCKAYNE**

The onset of the blistering of hands and feet in EBS-WC usually occurs later than in EBS-K, and, as blistering is aggravated by walking, children tend not to be affected until they start to walk. Blisters tend to improve with cold weather and worsen in summer. They tend to restrict active sports, although can be helped by the placing of sports shoes in a refrigerator prior to important events.

**EPIDERMOLYSIS BULLOSA SIMPLEX-DOWLING MEARA**

An unusual variant of EBS was reported in 1954 by Dowling and Meara, when four children were found to have hereditary blistering together with palmoplantar hyperkeratosis, nail dystrophy, and some oral ulceration. A dominant mode of inheritance has been observed in families with multiple cases. Abnormalities are usually detected within the first 5 days of life, with blisters and erosions on the hands and feet, particularly with hemorrhagic and perungual lesions. Extensive skin involvement may be seen, and the disease can be fatal in the neonatal period, when affected infants are often diagnosed as having JEB or DEB. Later, grouped herpetiform blisters occurring after trauma are noted, although these also occur spontaneously on an erythematous base without trauma. Blisters tend to improve in later childhood and adult life, usually beginning around the age of 10 or 12 years. The palmoplantar hyperkeratosis usually starts in early childhood and becomes more marked with age, although within families there is variation in time of onset and severity. Nail involvement results in shedding of the finger or toenails or nail dystrophy with irregular thickening of the nail plates and ridging. The patients exhibit no seasonal variation and variable response to the presence of febrile illness.

On light microscopy, Dowling and Meara and Polani suggested that the blisters appeared subepidermally. More recently, on light microscopy of resin-embedded semithin sections, a fine layer of cellular debris can be seen lining the basal space (i.e., basal cell fragments at the dermal side of the blister cavity, suggesting that this has been intrapidermal, with darkly staining granules in the blister roof, blister base, and perilesional skin. The blister cavity contains an infiltrate of neutrophils, eosinophils, and red blood cells. Nowadays diagnosis is confirmed by electron microscopy, which reveals that intrapidermal blistering takes place with the cleavage often low within the basal cells, which creates difficulties in determining the plane of cleavage on light microscopy.

**CLUES FROM CLASSIC MORPHOLOGIC STUDIES**

In all forms of EBS, the intraepidermal blisters can be seen ultrastructurally to result from lysis of the basal keratinocytes. In EBS-Dowling Meara (EBS-DM), there is an additional very characteristic aggregation or clumping of tonofilaments, which gave an early clue that keratin filaments might be abnormal in EBS. However, it was not clear initially whether this filament aggregation occurred as a primary event, or whether it was a secondary effect of the cytolysis, but the extent of clumping suggested to Anton-Lamprecht and Schneider that this was a primary defect. A recent study of the distribution of tonofilament clumps in EBS-DM showed that the clumps were found in all lesions skin samples but also in perilesional and nonlesional skin. Keratin clumps were also observed in adnexal epithelia, including outer root sheath, sweat ducts, and sebaceous glands, and in cultured keratinocytes from affected individuals. Clearly these clumps occurred in the absence of cytolysis. Immuno-electron microscopy confirmed that these tonofilament clumps contained keratins K5 and K14, as they were strongly labeled with keratin monoclonal antibodies known to be monospecific for K5 and K14, whereas there was little reaction with antibodies to K1 and K10. These results, therefore, supported the suggestion that the tonofilament clumping was a primary event and, more particularly, that the keratin filament abnormalities involved the basal keratins, K5 and K14.

The degree of clumping, however, is very variable between individuals and, in some cases, the bundles themselves are thicker than normal, or form irregular aggregations of tonofilaments and, in other cases, they simply form homogeneous clumps. Similar abnormal tonofilament clumps were found in samples of a fetus affected with EBS-DM and in cultured keratinocytes from affected individuals. Electron microscopic findings in EBS-WC and EBS-K have shown no obvious abnormalities akin to EBS-DM. A case of EBS-R

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has been reported as showing abnormal tonofilament formation plus changes in keratin immunohistochemical staining in basal cells. Subtle alterations in amount or organization of tonofilaments in basal keratinocytes have been reported in EBS-WC, but this area obviously merits further investigation.

**EPIDERMOLYTIC HYPERKERATOSIS**

Epidermolytic hyperkeratosis is seen in a variety of hereditary skin diseases, including BCIE, ichthyosis hystrix of Curt-Macklin, and ichthyosis bullosa of Siemens. It may also be found confined to the palms and soles in palmoplantar keratoderma of Verner. The pathologic characteristic of EH refers to epidermal acanthosis, cytology of the keratinocytes with tonofilament clumping, and hyperkeratosis. In recent articles, EH has been used to refer specifically to BCIE. Bullous congenital ichthyosiform erythroderma was described in 1902 by Brocq. The children present soon after birth with erythroderma and severe blistering, although this usually improves within the first year of life. Progressive hyperkeratosis develops, often affecting flexures, with a hyperkeratotic, sometimes spiny, appearance. Blistering is uncommon in adult life, and the hands and feet are usually spared, although not invariably. Although BCIE follows an autosomal dominant inheritance in a number of cases, many cases are sporadic. Several reports document parental involvement confined to a hyperkeratotic nevus with the pathologic features of EH and the children developing a full-blown BCIE phenotype. This can be interpreted as a somatic mutation arising early in the development in the parent affecting the germ line to result in gonadal mosaicism.

Histologic features of BCIE include marked hyperkeratosis, a broad degenerate granular layer with large keratohyalin granules, and intracellular edema of the stratum corneum keratinocytes, with basal cells appearing normal. There is usually minimal infiltrate, and blister formation usually occurs around the level of the granular cell layer. Investigation of the kinetics of EH by Frost and Van Scott suggested that EH was hyperproliferative in nature as the number of mitoses per centimeter of basal cell line/surface cell line greatly exceeded normal skin and approached that of psoriatic erythroderma.

Ichthyosis hystrix of Curt-Macklin shows features of very severe skin changes invariably associated with palmoplantar keratoderma. The skin shows erythroderma and severe hyperkeratosis with hyperkeratotic spines. It is not clear whether this should not be regarded as an extension of BCIE.

Ichthyosis bullosa of Siemens was reported in a Dutch family in 1937. The patients had minor blistering following trauma, but without any erythroderma. The patients again blister from blistering from birth, with hyperkeratosis developing in flexural sites. Variation in phenotype may be seen within the families. An additional characteristic feature is suprabasal vesiculation and denuding, which is described as the Mauersberg phenomenon. The histologic features show much less marked epidermolytic hyperkeratosis, which is within the stratum granulosum and stratum corneum, but not the stratum spinosum. A case report of a patient showing Mauersberg phenomena, but with infantile erythroderma, suggested some overlap of features with BCIE.

**ULTRASTRUCTURE OF EPIDERMOLYTIC HYPERKERATOSIS**

Morphologic changes in keratin filaments in EH were suggested by early ultrastructural reports to precede the other ultrastructural changes of vacuolation of the spinous and granular cell layers. Selective involvement of keratins K1 and K10 in this cytoskeletal abnormality of EH was illustrated by a recent study using both electron microscopy and immuno-electron microscopy, where the abnormal tonofilaments were found restricted to cutaneous epithelia. The aggregated tonofilaments expressed keratins K1 and K10 rather than the basal cell keratins K5 and K14, which suggested again that BCIE/EH might be primarily disorders of these keratins. Fetuses affected with BCIE have also been shown to develop suprabasal tonofilament clumps at a stage of skin development where keratohyalin granules are not yet expressed.

The electron microscopy of ichthyosis hystrix of Curt-Macklin shows features similar to BCIE, but the tonofilaments were reported to aggregate into concentric perinuclear shells, and the peripheral tonofilament may undergo further keratinization. In ichthyosis bullosa of Siemens changes are very restricted in distribution around the stratum granulosum.

**KERATIN EXPRESSION PATTERNS**

Implications of keratin involvement in genodermatoses still leaves a large number of genes that may be involved, since there are over 30 different keratin genes known in human tissues. Keratin proteins are classified by number according to their migration on two-dimensional gel electrophoresis, i.e., on the basis of charge and molecular weight. Keratins will only form filaments if both type I and type II keratins are present. In the absence of any complementary partner, single keratins will be rapidly degraded. In vitro heterodimers can form from many different type I and type II keratins, but in vivo type I and type II keratins are coexpressed in particular pairs that are characteristic of the epithelium and of the state of differentiation. Primary keratins K5 and K14 are characteristic of the keratinocyte phenotype, and keratins K8 and K18 are the primary keratins of simple epithelia; whereas secondary keratins (differentiation specific) vary between different patterns of epithelia. Keratinocytes synthesize keratins K5 and K14, while they are in contact with the basal lamina, but this is superseded by synthesis of the differentiation-specific keratins K1 and K10 as keratinocytes move up into suprabasal positions. Normal interfollicular suprabasal epidermis, therefore, expresses only the keratin pair, K1 and K10, although
keratin expression in hair follicles and skin appendages is more complex. Hyperproliferative epidermis, such as in psoriasis and during wound healing, can express the keratin pair K6 and K16, which is normally expressed suprabasally oral and genital mucosal stratified squamous epithelia.

DIFFERENT ANALYTIC PATHWAYS LEAD TO THE SAME CONCLUSIONS

Linkage Analysis

Linkage analysis is the technique of looking for chromosomal location of an unknown gene by comparing its inheritance pattern with that of a marker of known chromosomal location. Genomic DNA is analyzed for evidence that an identifiable polymorphic marker sequence is consistently inherited by individuals carrying an observable disease trait, and not by the unaffected family members, in different generations. The more individuals in whom association with a given marker is seen, the greater becomes the statistical probability of the unknown disease gene being located close to the marker. This is because the likelihood of the two genes being separated by random recombination events decreases with decreasing chromosomal distance between them.

Keratin proteins are the product of genes belonging to the intermediate filament gene family. Type I keratin genes code for the keratins 9 through 20 and trichocyte keratins Ha 1 through 4 and Ha X, whereas the type II keratin genes code for keratins 1 through 8 and trichocyte keratins Hb 1 through 4 and Hb X. Each keratin is the product of an individual gene. The chromosomal location of many keratin genes has now been identified, and they show significant clustering. In man, clusters of type I keratins occur on the long arm (17q 12-21) and short arm (17p 11-12) of chromosome 17 and there is a cluster of type II keratins on chromosome 12 at 12q 11-12. Studies of mouse/human somatic hybrids have revealed that both keratins 8 and 18 genes are located on chromosome 12, which suggests that these primary embryonic or simple epithelial keratins evolved before stratified squamous epithelia.

Families that have multiple affected members with the disease suspected to result from keratin mutations can, therefore, be analyzed by analysis of linkage of the clinical trait and the inheritance of genetic tracking markers known to reside close to the regions encoding keratin genes. This has shown good evidence of positive linkage to keratin in both EBS and EH. Several Koeber and Weber Cockayne families have been mapped to human chromosomes 17 or 12. Three families with BCIE have also been linked to the region of the type II keratin gene cluster of chromosome 12q, with no recombinants with a combined Lod score of 3.82. Genes encoding with retinoic acid receptor also mapped to this region of 12q. A further large family with epidermolytic hyperkeratosis has been mapped to chromosome 12q.

Manipulation of Keratin Structure

All intermediate filaments share the same pattern of molecular structure, even though the details of their protein and DNA sequences vary. A central a-helical rod domain comprises four subdomains, 1A, 1B, 2A, and 2B, separated by three stretches of nonhelical linker regions, L1, L12, and L2. There is a skip residue or “stutter” in the middle of helix 2B. Single polypeptides rapidly form coiled heterodimers of type I and type II keratins due to interaction along a strip of hydrophobic residues along the a-helical cylinder, and then from this the keratin filament is built up by forming tetramers and then protofilaments of 2 to 3 nm until intermediate filaments of approximately 10-nm diameter are formed. At certain points, the amino acid conservation of the helix is particularly high, for instance at the ends of the helical subdomains, and especially at the consensus helix termination peptide, at the carboxy terminal end of helix 2B. Head and tail regions provide the areas of greatest diversity between individual keratins and probably encode the tissue-specific fine-tuning of keratin function. Peptide sequences within the tail domains have been used to make monoclonal and polyclonal antibodies to individual keratins.

Useful information about keratin filament assembly has come from the expression of mutant keratin genes into tissue culture cells. Expressing keratin pairs with deletions in head or tail domains has shown that tail domains are necessary for production and maintenance of a filament network. Mutant keratins with deletions or mutations of the conserved helix termination peptide can produce apparent negative phenotypes when expressed in epithelial cells, causing filament network collapse. Internal deletions from the rod domain were also incompatible with normal filament structure, but helical pairing (with no end domain interactions) did protect from proteolysis. Vassar and colleagues subsequently introduced truncated keratin 14 genes into the germ line of transgenic mice, again producing a dominant negative effect (autosomal dominant fashion) and causing dramatic blistering and pathologic features reminiscent of the EBS-DM phenotype with clumping of the tonofilaments. Less damaging mutations in keratin 14 filaments produced a milder phenotype of blistering closer to the clinical features of EBS-WC. Transgenic mice expressing truncated keratin 10 gene gave rise to a different phenotype with blistering and epidermal thickening bearing some resemblance to Eh.

Identification of Mutations Using Immunohistochemistry

Many monoclonal antibodies have been raised to keratin polypeptides. The high homology within acidic and basic families mean that many antibodies to crude antigens cross-react with multiple keratins. However, an increasing number of monospecific antibodies are available (for review see Lane and Alexander, 1990). The use of monoclonal an
A point mutation in helix 1A of K1 was reported in a family with BCIE. Although all members of this family had the same mutation, two had a mild and two had a severe phenotype, which did not correlate with insertional polymorphisms of the V2 domains of K1 and K10. Additional mutations in K10 1A rod domain have also been found.

In conclusion, this gives a total of at least four regions to date in which mutations have been identified. These are as follows: (1) the type-specific helix initiation peptide at the start of helix 1A, (2) the intermediate filament consensus helix termination peptide at the end of helix 2B, (3) the prehelix motif in the II head subdomain found in type II keratins, and (4) an area near to the stuffer in helix 2B.

The subdomains at the start and end of the α-helical rod domain were predicted to be critical for filament assembly on the basis of various experiments with deletion mutants, and on the basis of their high-sequence conservation. The helix termination peptide is also thought to be a critical alignment site for elongating the polymer during filament assembly, based on data from paracrystal alignment. The prehelix motif of type II keratins and the stuffer in helix 2B are both known to be well conserved, but we do not yet understand why, as their role in filament function has not been demonstrated. However, since we can see that mutations at these residues clearly lead to an ineffective keratin cytoskeleton, and severely compromised epithelial resilience, we can be sure that these regions of the molecules are, indeed, important.

While we must await further publications before firm conclusions can be drawn about the identification of mutational clusters and their functional significance, these naturally occurring pathologic mutations are likely to tell us more about the way in which keratins are put together in the cell and, consequently, about the way in which they function, and why they need to be so diverse in some regions and so conserved in others.

**BACK TO THE PATIENTS**

There remain a number of important clinical conundrums to solve even with the current information. What is the mechanism of clinical improvement seen with age in EBS-K, EBS-DM, and BCIE? How does the variation in phenotype within families of BCIE develop? Could this be due to polymorphisms and changes in the partner keratin alleles? Will disorders of keratinization require reclassification once the genetic analyses are more extensive to clarify the relationship of IC-CM, lamellar ichthyosis, and non-bullous ichthyosiform erythroderma to BCIE? Are there other skin diseases that could be caused by keratin mutations? Epidermolytic hyperkeratosis has been reported in several forms of palmoplantar keratoderma, and palmoplantar keratoderma is a feature of both BCIE and EBS-DM, so this would appear to be an important subject for study. Linkage analysis has now established that in two families palmoplantar EH is linked to the type I keratin cluster on chromosome 17, suggesting possible abnormalities in...
the palm-sole-specific keratin 9. Gross abnormalities of keratin clumping without cytolysis have been found in a family with tylosis and esophageal cancer.9,90

Does it help the patients to know the biological basis of their disease? The prenatal diagnosis of EB5/BCIE currently requires a skin biopsy at 16 to 18 weeks' gestation. Chorionic villus sampling can now clearly be performed earlier in a pregnancy, once the family mutation has been diagnosed, and this is easier when a new restriction enzyme site has been created. The patients must receive adequate genetic counseling and support on genetic diagnosis to discuss these issues. In personal experience, many milder cases will be opposed to prenatal intervention. Therapeutic options currently depend on the use of retinoids, which are very helpful to some families with BCIE, but pharmacologic manipulation of patient-derived cultured keratinocytes or derived lines may give rise to new drugs or approaches to treatment. Gene therapy in the established patient is unlikely to be beneficial in the near future. Keratinocytes could be grown from affected individuals and genetically manipulated, but the problems of grafting the entire integument and mucous appear insurmountable at present and will require systemic gene therapy delivery systems.

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