Molecular genetic approaches to skin disease: keratins and keratinisation

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To date, the single largest group of genodermatoses for which specific genes have been identified are those caused by keratin mutations. The story of these discoveries is of interest for two reasons:

1. It attests to the importance of clinical investigation in answering fundamental biological questions.

2. These discoveries reflect a fertile interplay between two genetic strategies, namely positional cloning and candidate gene approaches (Box 1).

Box 1 Identifying disease causing genes

**Positional cloning** is the identification of a gene based upon the relation of its chromosomal position to a particular phenotype; knowledge of the corresponding protein or pathophysiology is not required — this is therefore the ultimate black box approach. In practice, multiple loci on the autosomes are examined for co-segregation of a particular disease with a Mendelian pattern of inheritance. The story of these discoveries is of interest for two reasons:

1. What disease would result from a mutated keratin gene ('gene looking for a disease')?
2. Keratin genes are highly expressed in skin, so would positional cloning of various skin diseases centre on the known clusters of keratin genes located on chromosomes 12 and 17 ('diseases looking for a gene')?

**Keratins and epidermolysis bullosa simplex**

Keratin genes, some of the first human genes to be identified, comprise over 30 related genes (K1, K2, etc), whose protein products show specific expression patterns in epithelial cells. Despite the many studies that tried to relate keratin gene expression to disease states, the exact function of keratins remained uncertain. Thinking genetically, two experimental approaches could be envisaged:

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**Positional cloning** is the identification of a gene based upon the relation of its chromosomal position to a particular phenotype; knowledge of the corresponding protein or pathophysiology is not required — this is therefore the ultimate black box approach. In practice, multiple loci on the autosomes are examined for co-segregation of a particular allele at a particular locus with a disease phenotype. This is a systematic approach rather than one led by hunch.

By contrast, in a **candidate gene approach** a particular gene is examined, either on the basis of known protein data or on insight into the pathophysiological basis of the disorder, to see whether gene mutations are found in individuals with the disease. The speed of progress in understanding keratins and disorders of keratinisation has occurred because there has been a rapid interplay between these two approaches.
Box 2 Epidermolysis bullosa

Epidermolysis bullosa simplex has three main clinical variants, the inheritance in most cases being autosomal dominant. The mildest form is Weber-Cockayne syndrome (OMIM #131800), characterised by blistering of the palms and soles. The Koebner (OMIM #131900) and Dowling-Meara (OMIM #131760) syndromes show a more severe phenotype, a herpetiform blistering pattern being evident in the latter (Fig 1). The mucosae may be affected. A reasonable genotype-phenotype correlation has emerged, with mutations in the rod domains associated with a more severe phenotype, whereas mutations elsewhere in the keratin genes are better tolerated and give rise to a milder phenotype.

As it turned out, the answers to both these experimental approaches were found within weeks of each other. Transgenic mice carrying a mutated K14 gene showed a phenotype strikingly similar to that seen in epidermolysis bullosa simplex (EBS) (Box 2). Almost simultaneously, positional cloning in kindreds mapped the cause of the same disorder on top of K14 – a coincidence too good to ignore (Fig 1). K14 combines with K5; so it was predicted, and subsequently shown, that the same phenotype could be produced from mutations in K5 (although K5 and K14 are located on different chromosomes). The answer to the fundamental question of what function keratins serve there fore relied on clinical science: blisters in response to traction are the morphological counterpart of cytoskeletal malfunction, which in turn is secondary to keratin mutation.

Keratins and other skin diseases: ‘phenotype walking’

Molecular geneticists use the term ‘chromosome walking’ to describe how, if they obtain sequence information on a particular chromosome, they are able gradually to move or ‘walk’ along that chromosome towards an area of particular interest: that is, to walk from the known to the unknown. An analogy can be made clinically: when it became known that defects in certain keratins caused a particular blistering disease, other inherited blistering diseases became candidates for mutations in other keratin genes and, in turn, any other phenotypic features of these disorders might overlap with yet other disorders, which themselves might result from further keratin gene mutations.

Most keratin genes are clustered on chromosomes 12 and 17, so it took little time and effort to deter-
mine whether other inherited blistering disorders might map to these loci. Bullous congenital ichthyosiform erythroderma of Brocq (BCIE) (OMIM #113800) is a rare disorder characterised by generalised blistering at birth, varying degrees of erythroderma, and the development in later life of a generalised hyperkeratosis most pronounced on the palms, soles and flexures of the large joints (Fig 2). Kindreds with this condition mapped to the keratin clusters, and were subsequently shown to have mutations of K1 and K10. BCIE has many similarities with ichthyosis bullosa Siemens (IBS), a less severe disorder without erythroderma, where the hyperkeratosis is mild and located on the flexural areas as well as the umbilical skin and shins (Fig 3). It was

Figure 2. Bullous ichthyosiform erythroderma of Brocq: (a) the characteristic hyperkeratosis (blisters and varying degrees of erythroderma); (b) histology showing hyperkeratosis and epidermolysis (incipient blister formation); (c) newborn child with erosions following blisters.

Figure 3. Ichthyosis bullosa of Siemens: (a) hyperkeratosis; (b) hyperkeratosis and epidermolysis, the latter high in the epidermis reflecting the expression pattern of keratin 2e.
Therefore not surprising to find that IBS was due to mutations of K2e. The expression of K2e is similar to that of K1 or K10 but more superficial, in keeping with the histological features.

A further example of how differences in expression of the various keratins can explain the particular pattern of disease is provided by a group of heterogeneous disorders, the palmoplantar keratodermas, all of which are characterised by abnormal keratinisation of the skin of the palms and soles. The standard classification, based primarily on morphology and associated features, is unsatisfactory but the recent identification of mutations underlying these disorders gives hope of an improved classification. The Verner type (OMIM #144200) shows diffuse thickening of the epidermis of palms and soles (Fig 4). K9 expression is largely restricted to the palms and soles, and has been shown to cause the Verner type of palmoplantar keratoderma. The position is less clear with some of the other clinical variants of palmoplantar keratoderma. For example, non-epidermolysis diffuse palmoplantar keratoderma (OMIM #600962) is associated with a mutation in K1 in one family, and no linkage to the keratin cluster has been found in others. Some palmoplantar keratodermas have been mapped to other chromosomal regions.

The process of phenotype walking can be taken even further. Pachyonychia congenita is characterised by dystrophic nail hypertrophy and hyperkeratosis of the nail beds, the soles and the palms (Fig 5). Two variants, the Jadassohn-Lewandowsky (OMIM #167200) and the Jackson-Lawler (OMIM #167210) types are recognised on the basis of associated syndromes. Jadassohn-Lewandowsky has been found to be caused by mutations in K6a and K16, and the Jackson-Lawler variety by K17 mutations. The clinical differences between the two forms of pachyonychia congenita can be explained on the basis of the different expression patterns.

There is thus clear overlap between pachyonychia congenita and the other keratin disorders. For instance, patients with pachyonychia may also show changes in the mouth akin to those of white sponge naevus syndrome, which is characterised by the presence of white thick plaques in the mouth and occasionally on the mucosa of the oesophagus, nose, genitals and rectum (Fig 6) (OMIM #193900). On the basis of this overlap, mutations of K4 or K13 were recently found to underlie white sponge naevus syndrome. However, patients with pachyonychia congenita also show cyst formation, abnormal

Figure 4. Palmoplantar hyperkeratosis (Verner type) secondary to a keratin 9 mutation.

Figure 5. Palmoplantar hyperkeratosis and subungual hyperkeratosis in pachyonychia congenita.
hair and mucosal abnormalities. Subsequent work has revealed that keratin mutations underlie other syndromes which also share some of these features, such as steatocystoma multiplex (OMIM #184500). Patients with pachyonychia also have mild abnormalities of the hair, particularly on their eyebrows. Recent mapping of the primary hair disorder monilethrix, characterised by beaded hairs that break easily and alopecia, has shown linkage to the keratin cluster (Fig 7). As this article goes to press, we and others have found mutations in a hair keratin as the cause of monilethrix.

Perhaps most unexpectedly, at least to the non-specialist, has been the identification of genetic abnormalities in naevus lesions (ie localised, as in a birthmark) similar to those found in some of the above disorders.

Figure 6. Characteristic changes of leukoplakia in white sponge naevus syndrome.
orders. These are therefore a result of genetic mosaicism — that is, due to a mutation in a keratin gene during early embryonic development, rather than in the germ line. It is a useful maxim that all genetic skin diseases will also exist in a localised mosaic (or naevoid) form.

Conclusion

Advances in the molecular genetics of skin disease have not been confined to diseases caused by keratins. Inherited diseases of the basement membrane zone, and the genes underlying familial melanoma and basal cell carcinoma have also fallen to the power of positional cloning and allied techniques. A cause of satisfaction is that more progress has been made in five years than in the previous 50 years — but it is open to question whether therapeutic innovation will follow close behind.

Further reading


Figure 7. Characteristic beaded appearance of the hair in monilethrix.