Review paper

ADVANCES IN GENETICS OF INTEREST TO DERMATOLOGISTS
Part I

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ABSTRACT

The rapid expansion of recombinant DNA technology have made it possible to understand the essential molecular pathology in a number of diseases. For certain genodermatoses defective genes related to clinical phenotype are relatively good investigated: localization on chromosomes, as well as certain products of these genes. The following disorders are reviewed: epidermolysis bullosa hereditaria simplex, ichthyoses, epidermolysis bullosa hereditaria dystrophica, cutis hyperelastica, albinism, piebaldism, xeroderma pigmentosum, incontinentia pigmenti and neurofibromatosis.
Although the laboratory achievements in molecular biology do not at present offer therapeutical solutions for clinical problems, a practical application seems probable in the future.

KEY WORDS:
advances in genetics, review, disorders keratinization, hereditary epidermolyses, cutis hyperelastica, albinism, incontinentia pigmenti, xeroderma pigmentosum.

INTRODUCTION

There is a number of reasons why dermatologists should follow closely the new developments and trends in the broad field of genetics: 1. In certain dermatological disorders antenatal diagnosis and genetic counselling is possible; 2. Genetic mapping of a disorder may be helpful in establishing its cause and may lead to sequencing and cloning of the relevant gene; 3. Mapping and cloning of a gene provoking a disease promises real hope of gene-therapy through insertion of the missing gene into a a relevant cell line, which can then be reintroduced into the patient; 4. Careful evaluation of the available clinical information can contribute to the better understanding of certain diseases. An example may be the association of a known genetic disorder with unusual clinical symptoms, as may be observed in X-linked ichthyosis with hypogonadism.

In this essay we intend to discuss some aspects of gene mapping and the latest developments in identification of the genetic defect in a number of dermatologic disorders. The enormous breakthrough is reflected in the fact that over 1600 genes have been localized and 90 loci are relevant to the skin disorders (1). A number of rather sophisticated methods have to be introduced and painstaking efforts were made to discover the new facts.

KERATINIZATION

Many facts concerning the normal process of keratinization became known. Through two-dimensional gel-electrophoresis more than 30 different keratin molecules can be separated. They may be divided into larger ones, basic or type II, and smaller ones, acidic or type I keratins. The genes coding for type I keratins are located on human chromosome 17 and for type II on chromosome 12. Keratins form a major portion of the cytoskeleton of all epithelial cells. Because diameter of these structure is between 7 and 10 mm, they were called intermediate filaments (2). The type II (basic) keratins are labelled with numbers 1-9 and the type I (acidic) with numbers 10 - 20.

Abreviations::

cDNA:	complementary deoxyribose nucleic acid
STS:	steroid sulfatase
GPT:	glutamic-piruvic transaminase
RFLP:	restriction fragment langth polymorphism
PCR:	polymerase chain reaction
DOPA:	dihidroxyphenilanin
mRNA:	messenger ribonucleic acid
COL1A1:	collagen, type I, alpha 1
COL7A1:	collagen, type VII, alpha 1
OCA:	oculocutaneous albinism
XP:	xeroderma pigmentosum
IP:	incontinentia pigmenti
NF 1:	neurofibromatosis, type 1

In the epithelial tissue keratins are expressed in pairs: in the basal cells of all stratified epithelia keratins K14 and K5; K10 and K1 are found in the stratified layers of epidermis; K18 and K8 are found in early embryo and in some epithelial neoplasms; K17 and K7 in simple epithelia; K16 and K6 in stratified epithelia under proliferative conditions such as wound healing, psoriasis or squamous cell carcinomas. K13 and K4 are expressed in stratified nonkeratinizing tissues such as esophagus, while K12 and K3 are expressed in corneal epithelium (3). It is known that K1, K5, K10 as well as other keratins may be polymorphic, determined by multiple allelles. Although keratin genes have been mapped to the chromosomes 12 (type II keratins) amd 17 (type I keratins), the genes for single keratins responsible for development of hereditary palmoplantar keratodermas (HPK) were not unequivocally identified.

Hereditary epidermolysis bullosa simplex (EBS) arises from basal cell cytolysis. A minimal reduction and disorganization of tonofilaments has been observed in EBS earlier (4). Coulombe et al (5) demonstrated lately that a perturbation of basal cell karatin filament network was responsible for the clinical manifestations in EBS. In two patients with EBS of Dowling-Meara type a point mutation in a critical region of the K14 gene was observed: an arginin 125 cystin mutation disrupted keratin network formation in transfected keratinocytes (5). Using postembeding immunogold electron microscopy and antibodies to K5, K14 and K10, Yamamoto et al. (6) concluded that EBS Dowling-Meara was associated with an intrinsic abnormality of the keratin filament network involving keratins 5 and 14, In a family with EBS of Koebner type Bonifas and cow. (7) were able to map the defect to chromosome 17 by lod score calculation and also to identify the mutation in 3542 bp region. In a second family of EBS Weber-Cockayne type, the inheritance of the disease was linked to loci that map near the keratin 5 gene. There are also reports linking the EBS-Ogna type to glutamicpyruvic transaminase (GPT) gene, located on chromosome 8 (8).

Humphries et al (9) suggested a linkage of EBS (Koebner type) to the long arm of chromosome 1. The gene for human nidogen, which is a sulfated glycoprotein of 150 kd representing one of the major components of the basal membrane, was mapped to the chromosome 1 (locus 1q43) However, the restriction fragment length polymorphism investigations using four endonucleases support the exclusion of EBS (Koebner type) linkage to nidogen locus.

ICHTHYOSES

Ichthyoses are characterized by scales covering the entire skin surface. There is considerable variation in severity between patients; classification is arbitrary, the five main varieties are: autosomal dominant ichthyosis vulgaris, X-recessive ichthyosis vulgaris (XRIV), erythrodermia ichthyosiformis bullosa (epidermolytic hyperkeratosis), erythrodermia ichthyosiformis nonbullosa and ichthyosis lamellaris. Cutaneous symptoms similar to ichthyosis may be observed in certain syndromes: Refsum's, Netherton's, Rud's, and Sjögren-Larsen as well as in certain malignant diseases. All these conditions are separable on the basis of the mode of inheritance, and clinical features, light and electron microscopy; a few biochemical assays may also be useful in this respect. Molecular genetic studies are ongoing.

Of all the above mentioned disorders the best investigated is XRIV: In fibroblasts, leukocytes and keratinocytes there is a deficiency in the activity of the enzyme steroid sulfatase (STS), the consequence is an increased quantity of cholesterol sulfate in plasma and in stratum corneum (10). The enzymatic cleavage of cholesterol sulfate is an essential link in the process of normal keratinization and desquamation. While cholesterol sulfate seems to increase cohesion of corneocytes in the inner stratum corneum, free cholesterol is important for normal desquamation.

The STS gene has been assigned by deletion mapping, hybride studies and linkage analysis to the distal part of the short arm of the X-chromosome in Xp22.3 region (11,12,13).

Using anti-STS polyclonal antibodies, a cDNA probe for STS was isolated (14). This probe was used to investigate the molecular defect in patients affected either by XRIV or XRIV with hypogonadotropic hypogonadism (Kalmann syndrome). The results (14) showed that a deletion in the coding region of the STS gene was shared by both groups of patients. Therefore, the association of the two syndromes represents an example of the so-called contiguous gene syndromes (15), a group of diseases often due to small chromosomal deletions involving more than one gene. Combining the direct DNA analysis in STS deficient patients with the enzyme activity represents a powerful tool of diagnostic application.

The other disorders of the ichthyosis group were not studied to the same extent, but some results seem to be promising: in erythrodermia ichthyosiformis an increased content of n-alkanes in the horny layer was observed. In Refsum's syndrome phytanic acid is accumulated in stratum corneum due to deficiency of phytanic acid alpha-hydroxilase (16). There is a report on ichthyosiform scaling in two patients suffering from glycogen storage disease types 2a and 2b due to alpha-(1-4)-glucosidase deficiency (17).

EPIDERMOLYSIS BULLOSA HEREDITARIA DYSTROPHICA

Epidermolysis hereditaria dystrophica (EBHD) can be inherited either as a recessive or dominant pattern. The disease is characterized by subepidermal blisters which appear on pressure sites of the skin, mostly on hands, feet, knees, elbows, buttocks, but also on other parts of the skin, as well as on buccal and oesophagopharingeal .mucosa. It is usually expressed at birth. In the recessive type dystrophic scare develop following the blisters.

Previous ultrastructural studies have demonstrated morphological abnormalities and rarification of anchoring fibrils, which apparently provide stability for the attachment of the dermis to the basement membrane (18,19,20). Recent studies have demonstrated that type VII collagen is a major component of the anchoring fibrils (21). This Type of collagen has been shown to be absent in skin of patients with severe generalized recessive EBHD (22). In the RFLP study of 37 members of a Finish family with dominant EBHD using the PvuII restriction endonuclease and 1.9 kb type VII collagen clone, the RFLP cosegregated with the disease, thus strongly supporting genetic linkage in this family (23). The data indicated that EBHD mutation was closely linked to the COL7A1 gene on chromosome 3.

CUTIS HYPERELASTICA (EHLERS-DANLOS SYNDROME)

There are more than 10 recognized types of Ehlers-Danlos Syndrome (EDS). Type IV also known as "arterial" or "ecchymotic" is considered to be an autosomal dominant disease. It is the most severe type as the life expectancy is usually reduced due to occurence of spontaneous ruptures of large arteries and hollow organs. Structuraly abnormal and/ or reduced type III collagen was reported in tissues and cultured fibroblasts (24,25), Structural mutations of pro-alpha 1 (III) collagen have been shown to cause reduced secretion of type III molecules (25).

The fibroblasts from a severely affected type IV EDS patient were shown to synthesize normal-size and shortened type III procollagen chains. Two-dimensional PAGE of cyanogen cleavage products revealed that shortened chains lack two peptides, namely alpha 1 (III) CB 3 and CB 6. The triple helical domain sequences of these two peptides extend from amino-acid 1-95 and from 133-231 (26). Lee et al. (27) were able to prove the production of a mRNA that lacks the sequences corresponding to 16 exons of the triple helical domain of COL3A1. By sequencing 490 subclones produced by PCR, they documented a 1026 bp inframe deletion that resulted in exon 8 joining to exon 25 sequences. The shortened mRNA ultimately directs the synthesis of procollagen chains bearing a deletion of 342 amino-acids, from residue 46 to 387 (27). They were able also to identify a polymorphic DNA sequence in COL3A1 gene, which was mapped at chromosome 2q31 (27).

Extreme joint laxity, soft, but nonfragile skin displaying limited bruising and minimal hyperextensibility are the cardinal symptoms of the type VII EDS. This condition may be subdivided into EDS VIIA with structurally defective procolagenase alpha 1(I) and VIIB with deficient pro-colagenase alpha 2(I). In a child with EDS VII a structural defect in the amino-terminus of pro-alpha 1(I) collagen was shown. As a result type I procollagen molecules containing defective subunits are not converted to mature collagen. This protein structural defect is caused by a single base mutation (A for G) at the position 1 of the splice donor site of intron 6 of the pro-alpha 1(I) collagen gene (COL1A1). The affected allele produces transcripts lacking exon 6 sequences (28).

ALBINISM

Albinism (Oculocutaneous Albinism, OCA) is an autosomal recessive disease characterized by completely absent pigmentation in skin, hair, and eyes. Hypopigmentation of retina is associated with decreased visual acuity, photophobia and varying degrees of nystagmus. The basic anomaly is a deficiency in tyrosinase activity of the melanocytes. This copper containing enzyme is responsible for catalyzing the first two steps of the melanin formation: hydroxylation of tyrosine to dehydroxyphenilalanin (DOPA) and dehydrogenation of DOPA to DOPAQUINONE.

Table 1, Essentials on metabolic, morphological and genetic defects in dermatoses listed bellow.

Disease	Metabolic or morphological defect	genetic defect
EPIDERMOLYSIS BULLOSA HEREDITARIA SIMPLEX		
Dowling-Meara	Disrupted keratin network formation	Point mutation Arg-125/Cys of K14
Koebner	Disrupted keratin network formation	Substitution T for C in K14 gene on chr 17q21
ICHTHYOSIS VULGARIS RECESSIVA (X-LINKED)	Steroid sulfatase deficiency	Deletions in STS gene mapped to chr Xp22.3
EPIDERMOLYSIS BULLOSA HEREDITARIA DYSTROPHICA	Abnormal formation of anchoring fibrils, absent collagen VII	Mutation linked to COL7A1 gene on chr 3
CUTIS HYPERELASTICA		
type IV	Shortened type III procollagen chains	Deletion in COL3A1 gene on chr 2q31
type VII	Defect of procollagen A1 (I)	Substitution A to G, exon 6 of COL1A1 gene, chr 17q21
ALBINISM	Deficient tyrosinase activity	Mutation in tyrosinase gene on chr 11q14-21
PIEBALDISM	Lack of melanocytes and pigment	Mutations within the KIT protooncogen on chr 4q11-21
XERODERMA PIGMENTOSUM		Mapped to chr:
variant A	Deficient DNA excision repair	1q
variant B	Deficient DNA excision repair	2p
variant F	Deficient DNA excision repair	15
NEUROFIBROMATOSIS	Circumscribed cutaneous tumors	Translocation on chr 17q11.2
INCONTINENTIA PIGMENTI		
sporadic	Leaking of pigment from stratum basale into papillary layer	Xp11-21
familial	Leaking of pigment from stratum basale into papillary layer	Xq26-28

Legend: chr - chromosome, STS - steroid sulphatase, K14 - keratin 14, COL 7A1 - collagen VII alpha 1, COL3A1 - colagen III, alpha 1, Arg - arginin, Cys - cystein, A - Adenine, G - guanine, C - cytosine, T - thymine

In USA OCA occurs with a frequency of 1:17000 and more than 1% of the population are heterozygous for a gene producing OCA. The gene for tyrosinase has been mapped at the long arm of chromosome 11q14-21 (29). Several alleles for tyrosinase related OCA have been recognized in humans.

OCA type I results from deficient activity of melanocyte tyrosinase, In classic type IA tyrosinase (negative), tyrosinase activity and melanin biosynthesis are entirely absent. In type IB (yellow) tyrosinase activity and melanin production are greatly reduced. Specially interesting is a type I variant, characterized by depigmented hairs only in cold exposed ares (scalp, eyebrows) due to a cold sensitive tyrosinase. Type II OCA (tyrosinase positive) is associated with normal tyrosinase function, the biochemical or genetic mechanism has not been determined. Type III OCA are also known as minimal pigment cases.

The gene for tyrosinase is greater than 50 kb in lenght and contains five exons coding for 529 amino-acids. In two patients the amino-acid substitution occured in codon 47 (asparagin for leucin) and codon 371 (threonin for asparagin); in a third patient there was the same mutation in codon 47 and another in 81 (leucin for prolin) (29). Mutation on other codons were also described (30,31). In a patient with temperature sensitive tyrosinase deficiency a point mutation in codon 422 (arginin instead of glycin) was demonstrated (32).

PIEBALDISM

Piebaldism is an autosomal dominant genetic disorder

characterized by congenital patches of skin an hair that compeletely lack melanocytes and pigment: on the forehead, ventral side of the chest and abdomen as well as on extremities. A similar phenotype in the mouse "dominant white spotting" has recently been shown to result from deletions or point mutations within the KIT protooncogen (33), which encodes the tyrosine kinase transmembrane cellular receptor for mast/ stem cell growth factor. The human piebaldism locus was provisionally mapped to chromosome 4q12, on the basis of interstitial chromosomal deletion (34,35) coincident with location of the human KIT protooncogen in chromosome segment 4q11-12.

Giebel and Spitz determined the DNA sequence of the normal human KIT gene, which is divided into 21 exons. They were also able to identify a large kindred of patients with the classical autosomal dominant piebaldism and to elaborate the pedigree. All the 21 KIT coding exons were amplified by PCR and sequenced. The analysis demonstrated only a single difference from the normal gene, GGA -AGT, glycine for arginin substitution within the codon 664, the last base of coding exon 13 (36). In the family assessed there was a perfect concordance between the heterozygosity for the codon 664 mutant allele and the piebald phenotype.

XERODERMA PIGMENTOSUM

Xeroderma pigmentosum (XP) is a rather rare autosomal recessive disease, which is characterized by sun sensitivity, cutaneous pigmentary anomalies and a high incidence of skin cancer. The incidence of XP may be as high as 1 in 40000, and it seems to be relatively frequent in certain countries e.g. Egypt (37,38). Clever reported in 1968 (39) that cultured fibroblasts from patients with XP were unable to perform DNA excision repair of UV-induced thymine dimers. Using various laboratory methods e.g. measurment of colony forming ability, autoradiographic detection of unscheduled DNA synthesis, complementation analysis and others, a number of XP types ca be differentiated. Cultured cells from patients can be assigned to complementation groups based on their ability to perform UV-induced unscheduled DNA synthesis (UDS) when fused to cells of different complementation groups,. By this method 9 types of different complementation groups designated with letters from A to I were established (40).

A further class of X patients with normal excision repair capacity but defective post-replication repair was described as XP variants. The wide variation in excision repair capacity in XP cells and the different clinical pictures seen in XP are quantitative reflections of genetic heterogeneity. As a matter of fact some of the XP variants are mapped to different chromosomes: e.g. variant A to 1q, variant B to 2p, and variant F to chromosome 15.

The tumors most frequently appearing in these patients are basal cell carcinomas (about 80%), which corresponds to the data in normal population. However, this is not the case in the XP groups C and D where the squamous cell carcinomas appear as frequently as the basal cell ones. In the XP-D group there is an outstanding preponderance of multiple lentigo maligna melanomas (41)

INCONTINENTIA PIGMENTI

Incontinentia pigmenti is a syndrome which may include additionaly to skin symptoms, defects of the eye, nervous and less often of the skeletal system. There are three types of skin lesions that usually appear in stages: intraepidermal vesicles, papular and warty lesions, and hyperpigmentation. According to Aubin the incidence of IP is 2,5 in 100000 (42). It is accepted that IP is due to a dominant gene linked to the X chromosome. The great majority of patients reported in literature are females of a familial appearance, the few cases in males seem to be sporadic. The familial cases of IP are mapped to Xq26-28 region (IP II gene) whereas the sporadic are linked to Xp11-21. Canizzaro and Hecht were able to show that the genetic defect in a girl was due to a translocation between chromosomes X and 10 (43). Similar other X/ autosome translocations were described in literature (44).

NEUROFIBROMATOSIS TYPE 1 (NF 1)

The condition was first described by von Recklinghausen in 1882. The clinical manifestations include cutaneous and subcutaneous neurofibromas, café au lait spots, Lisch nodules, learning disabilities, skeletal abnormalities and malignancies of the central and peripheral nervous system. With an incidence of about 1 in 3000 in all ethnic groups, it is one of the most common autosomal dominant disorder in man (45). The tumors are composed primarely of Schwann cells or fibroblasts with numerous perineural, endothelial and mast cells. The gene for NF 1 has been mapped to chromosome 17 (46). Subsequent collaborative multipoint mapping narrowed its genetic location to about 3 centi-Morgans of 17q11.2. The gene was supposed to be relatively large greater 300 kb (47). The large size may explain the high rate of sporadic occurence of this disease. Identification of two NF 1 patients and apparently balanced translocation with breakpoints 60 kb apart, represented a further step in elucidating the problem. Lately Wallace et al. succeed in identifying a large 13 kb transcript denoted NF1Lt, which most probably is the elusive NF 1 gene (48)

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