Genetics for the Practicing Dermatologist

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In the era of robust genome sequencing, a working understanding of genetics has become important for the clinician. For the dermatologist, understanding the flow of genetic information from genotype to phenotype can aid in the delivery of effective patient care. In this article, we will review concepts in genetics and the human genome and how they contribute to clinical dermatology.
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The "Gene" Redefined

The concept of the "gene" has been an ever evolving notion since its conception by Mendel in 1866.[1] Initial work identifying the basic structure of the gene was synthesized by Crick and presented as the central dogma of molecular biology, outlining the linear flow of genetic information from DNA to RNA to protein.[2] With the recent completion of the Human Genome Project, the Encyclopedia of DNA Elements (EN-CODE), and the International HapMap Project,[3-9] the meaning of a genetic unit has undergone stark transformation and is now defined by the set of functional products produced by a genomic sequence.[10] This dictates that as clinicians we are forced to consider more than principles of classical genetics in the evaluation of our patients with complex medical diseases.

1. The Human Genome Project is an international scientific research project with a primary goal to determine the DNA sequence of all genes of the human genome. The Project formally began in 1990 and a complete draft of the human genome was published in 2003
2. The Encyclopedia of DNA Elements (ENCODE) represents a research consortium initiated by the US National Human Genome Research Institute in September 2003 with a goal to identify all functional elements in the human genome
3. The International HapMap Project began in 2002 and is aimed to develop a complete haplotype map (HapMap) of the human genome. It will focus on identifying common single nucleotide polymorphisms (SNPs)

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Phenotype Deconvolution and Genetic Skin Disease

The practice of medicine is focused upon linking a set of clinical phenotypes to specify and describe a single disease entity. In the past, blunt tools available for this task have made differentiating clinical disease difficult at times. In the era of advancing technology, molecular tools have become more precise, and thus the ability to link basic science and clinical medicine to define disease states has also sharpened. This trend is particularly applicable to dermatology, where subtle differences in cutaneous phenotypes can partially discriminate between highly similar disease states whereas robust tissue accessibility allows for frequent clinicopathologic correlation. Fine molecular correlation is often still unavailable. In a recent study that used the Online Mendelian inheritance in Man database, the authors attempted to systematically organize genetic skin disease by phenotype and genotype, in what has been termed "phenotype deconvolution."[11,12] From this, the current state of genodermatoses was defined to include 560 distinct disorders associated with mutations in 501 unique protein-encoding genes.[11] This comprehensive database and concept is only possible in part because of the knowledge gained during the past decade specifying molecular and genetic pathways. The goal of phenotype deconvolution is to resolve complex disease phenotypes into elemental features to discover hidden structure within the phenomic space and to mechanistically link entities that share overlapping features. The ras/mitogen activated protein kinase pathway (MAPK) syndromes exemplify this method. Noonan syndrome, LEOPARD syndrome, neurofibromatosis type 1 (NF1), cardio-facio-cutaneous (CFC) syndrome and Costello syndrome have several overlapping clinical features, including craniofacial dysmorphology, congenital heart disease, neurocognitive impairment and ectodermal features (Fig. 1). The RAS/MAPK pathway is known to play an important role in cell cycle regulation, differentiation and growth and in

Neurofibromatosis type 1	LEOPARD syndrome	Cardio-facio-cutaneous syndrome	Costello syndrome
Gene: *NF1*	**Gene:** *PTPN11*	**Gene:** *BRAF, MEK1, MEK2, KRAS*	**Gene:** *HRAS*
Cutaneous features: •Café au lait macules* •Plexiform neurofibroma •Intertriginous freckling* •Dermal neurofibromas	**Cutaneous features:** •Lentigenes •Café noir spots*	**Cutaneous features:** •Keratosis pilaris •Ulerythema ophryogenes •Melanocytic nevi* •Hemangiomas	**Cutaneous features:** •Loose skin on hands/feet •Hyperkeratosis of the palms and soles •Papillomata*
Extracutaneous features: •Optic glioma •Sphenoid wing dysplasia •Long-bone bowing •Learning disabilities •Lisch nodules	**Extracutaneous features:** •ECG conduction defects •Ocular hypertelorism •Pulmonic stenosis •Abnormal genitalia •Retardation of growth •Deafness	**Extracutaneous features:** •Failure to thrive •Congenital heart disease •Developmental delay •Pectus excavatum*	**Extracutaneous features:** •Risk for cancers (rhabdomyosarcoma, neuroblastoma, bladder)

Figure 1 Clinical features of the "Rasopathies."

oncogenesis; more recently this pathway has been implicated in developmental disorders. Noonan syndrome, LEOPARD syndrome, NF1, CFC syndrome and Costello syndrome have been called the "RASopathies" because each have been found to be linked to genetic loci in the RAS/MAPK pathway.[13,14]

In some genetic syndromes, only 1 gene is known to cause a disorder, whereas in other conditions, multiple genes may be implicated. Genetic heterogeneity is attributed to situations in which identical clinical phenotypes are associated with mutations in several genes. An example of this is lamellar ichthyosis (a single disease entity with a single set of phenotypes), which can be produced by mutations in keratinocyte transglutaminase (*TGM1*), ATP-binding cassette member A12 (ABCA12), cytochrome P450 protein CYP4F22, or another unidentified gene

(several distinct genes causing an identical phenotype). Of the RASopathies, Costello syndrome has been shown to be caused by mutations in only 1 gene (*HRAS*) and CFC syndrome can be caused by mutations in 4 different genes (*BRAF, MEK1, MEK2* and, rarely, *KRAS*; Fig. 2A).[15-17] For the genetic skin conditions, mutations at single genetic loci are attributed in 84% of disorders whereas alterations at 2 or more genetic loci are identified in 15% of disorders.[11]

In contrast, clinical heterogeneity is defined by situations whereby mutations in single gene loci result in multiple different phenotypes and thus diseases. An example of this is mutations in connexin 26 or *GJB2* (a single gene) causing Vohwinkel syndrome, keratitis-ichthyosis-deafness (KID) syndrome, and Bart–Pumphrey syndrome (3 distinct dis-
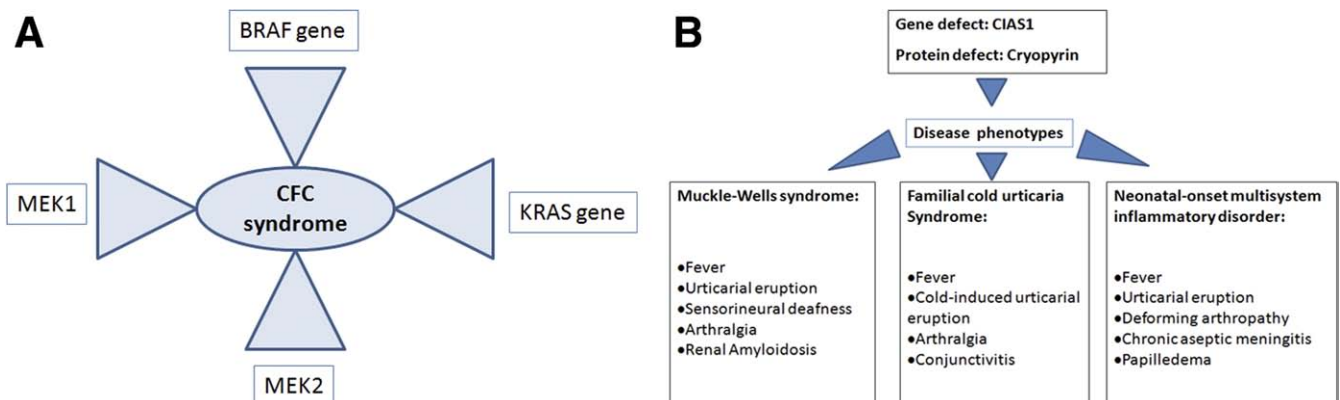

Figure 2 Genetic heterogeneity and clinical heterogeneity. (A) Genetic heterogeneity: CFC can be caused by mutations in 4 different genes. (B) Clinical heterogeneity: mutations in the same gene, CIAS1, can lead to different phenotypes as demonstrated in the auto-inflammatory diseases.

eases with different phenotypes). This concept can be further demonstrated with the cold-induced autoinflammatory syndrome 1 (*CIAS1*) gene which encodes the cryopyrin protein. Mutations in the *CIAS1* gene can lead to autoinflammatory diseases, including familial cold urticaria syndrome, Muckle-Wells Syndrome, and neonatal-onset multisystem inflammatory disorder.[18-20] The clinical features of these conditions are shown in Fig. 2B. Finally, mutations in 82% of the 501 unique protein-encoding genes associated with genetic skin disease cause a single unique disease, while only 3% were found to be causal in 4 or more unique diseases.

In the near future, we suspect that by using phenotype deconvolution in conjunction with the expected exponential increase of molecular knowledge that we will soon be able to redefine the meaning of both genetic and clinical heterogeneity in medical disease. This is likely to be accomplished by way of sharpening our ability to discern the relationship between molecular mechanisms and clinical phenotypes.

Genetic Modes of Inheritance and Genetic Skin Disease

When a patient presents to the dermatology clinic with signs of a genetic skin condition, it is helpful to be aware of the modes of transmission and inheritance of the disorder both to aid in the diagnosis and in family counseling. Mendelian inheritance patterns for single gene conditions include autosomal dominant (AD), autosomal recessive (AR), and X-linked. Additional mechanisms of inheritance include mitochondrial, imprinting, and complex or polygenic inheritance. Of the genetic skin conditions, AR inheritance is the most common (46%), followed by AD (37%), X-linked (6%), and mitochondrial (1%).[11]

AD genetic conditions occur when a mutation is present on at least 1 member of the gene pair (ie, allele) and the resultant abnormal clinical features are expressed. A genotype that has 1 normal and 1 mutated allele is often referred to as the heterozygous state. Tuberous sclerosis (TS) and NF1 are examples of the more common AD genetic skin conditions. Often an affected individual is the first member of the family to manifest the condition and this is the result of a de novo mutation in the affected individual. In TS, two-thirds of the cases are caused by de novo mutations, whereas only one third are inherited from a parent.

In AD conditions, allele penetrance is an important determinant of disease presence. In clinical genetics, penetrance refers to the proportion of individuals who both carry a disease-causing mutation and exhibit the clinical phenotype causing the disease state over a defined period. As such, reduced penetrance refers to individuals who carry the mutation but have mild or no features of the disease. Intrafamilial variability refers to the situation whereby different phenotypes are expressed among affected family members. This has been described in Birt–Hogg–Dube syndrome, which is caused by mutations in the folliculin (*FLCN*) gene in the mTOR pathway. Within a family, some members may have skin lesions (fibrofolliculomas) and renal cancer, whereas others may have pulmonary cysts and pneumothorax.[21] Variable expressivity may be explained in some cases by the Knudson "two hit" hypothesis, as in the case of tuberous sclerosis (TS). TS is caused by mutations in either of 2 tumor suppressor genes, hamartin (*TSC1*) and tuberin (*TSC2*). In this model, the first hit is the germline presence of a mutation in *TSC1* or *TSC2* and the second hit occurs randomly in somatic tissues and can result in the growth of various tumors, such as cardiac rhabdomyomas, renal angiomyolipomas and facial angiofibromas.[22]

In NF1, variable expressivity is demonstrated when a parent with numerous neurofibromas and hypertension has an affected child with a plexiform neurofibroma and an optic glioma but few neurofibromas. Of note, a subgroup of patients previously thought to exhibit a variant of NF1 with multiple café-au-lait macules and axillary freckling failed to reveal mutations in the *NF1* gene. With the new molecular tools described previously now available, a new syndrome known as Legius syndrome has been described and is attributable to mutations in the *SPRED1* gene.[23] Affected individuals present with multiple café-au-lait macules, axillary freckling, and macrocephaly. Legius syndrome differs from NF1 in that Lisch nodules, neurofibromas, and central nervous system tumors are absent. These subtle variations demonstrate how features (eg, café-au-lait macules vs freckling vs macrocephaly) may be more tractable currency for phenotypic comparison than disease complexes (eg, NF1 vs Legius syndrome), and thus the NF1 and Legius syndrome overlap represents an ideal case for phenotype deconvolution.

When a patient presents with a genetic skin disorder, such as TS or NF1, the individual's parents and siblings should be carefully examined to determine if they express mild features of the disorder. This is important for genetic counseling; if a parent is also found to have the AD condition, then the recurrence risk in future pregnancies is 50%. If the parents do not have any manifestations of the disease, then there is not a significant risk of recurrence in future offspring unless gonadal mosaicism exists. In rare cases, the mutation may be present in the germ line of 1 parent, but not in most other cells in the body; this is known as germ line or gonadal mosaicism, which carries a small risk of recurrence in future offspring.

In some cases a mosaic presentation in an adult may lead to generalized presentation in the next generation. An example of this can occur when the mosaic pattern in the adult is due to a postzygotic keratin 1 or keratin 10 mutation, so that only some cells in the body are affected, but these cells include the germline. Individuals with epidermal nevi of the epidermolytic hyperkeratosis type may have offspring with generalized epidermolytic hyperkeratosis. For that reason, skin biopsies of epidermal nevi are important to identify lesions with features of epidermolytic hyperkeratosis so that genetic counseling may be provided.[24]

AR conditions require that an individual carry both alleles with a gene mutation to express the clinical phenotype. If both parents are carriers, there is a 25% chance of transmission with each pregnancy. If 1 parent is affected by the dis-

ease and the other is a carrier, then the risk of transmission increases to 50% with each pregnancy because the affected parent is homozygous for the mutation. A special situation that can occur is when an affected patient carries 2 unique mutations at each allele loci. This is called compound heterozygosity and is presumable generated by each parent being a carrier as described previously, but for different disease associated allelic variants. Examples of AR genetic skin conditions include recessive dystrophic epidermolysis bullosa, Netherton syndrome, xeroderma pigmentosa, albinism, and many others. AR conditions present more commonly in offspring of consanguineous parents (when the mutant allele is inherited from a common ancestor) or when the mutant allele is common in the general population (eg, *CFTR* gene for cystic fibrosis). Carriers of gene mutations in AR conditions usually have no clinical manifestations (silent carrier or carrier state); however, occasionally carriers may show mild phenotypic features. For example, aquagenic wrinkling of the palms has been reported to be associated with the heterozygous carrier state for mutations in the *CFTR* gene for cystic fibrosis (Fig. 3).[25]

Epigenetics

Classical genetics maintains that differences in phenotypic expression are the result of structural alterations of the genome itself. Epigenetics is the principle that governs phenotype variation in the absence of underlying DNA change. Thus, an epigenetic trait is a stably transmissible phenotype resulting from changes in gene expression without alterations in the DNA sequence. While the intergenerational transmission of genetic mutations has been clearly linked to disease phenotypes, the inheritance of epigenetic marks across generations is less well-established. In 1 published example, an epimutation in the *MLH1* gene was transmitted from a mother to her son in a case of hereditary nonpolyposis colorectal cancer. The affected maternal allele was erased in the

Figure 3 Aquagenic wrinkling after brief water immersion demonstrated on the right hand of a 17-year-old female patient. A mild keratoderma on the mid palm of the left hand is the result of hyperhidrosis. Aquagenic wrinkling (keratoderma) can be a sign of the cystic fibrosis carrier status.

affected son's spermatozoa. This case demonstrates the rare inheritance of cancer susceptibility through transgenerational epigenetic transmission.[26] Various epigenetic mechanisms have been described and include histone modification, nonprotein coding RNA segments (ncRNA), and cytosine modification.[27-30] Data indicate that the pathogenesis of many human diseases, including cancer, heart disease, autoimmune disease, metabolic disease, and skin disease, are contributed to by epigenetic aberrancies of the genome.

DNA methylation acts to silence the transcription of many mammalian genes. DNA methylation occurs on cytosine residues of cytosine-guanine (CpG) dinucleotides, which are most often concentrated within the promoter region of genes. Epigenetic modulation through DNA methylation can regulate normal processes, including X chromosome inactivation, imprinting, and development. Aberrant gene silencing also exists in many pathologic disease states. The genesis of many conditions encountered in dermatology involves dysregulation of DNA methylation and includes genodermatoses (*NF1*, Von-Hippel–Lindau disease, hypomelanosis of Ito, dermatopathia pigmentosa reticularis), inflammatory dermatoses (atopic eczema, vitiligo, lupus erythematosus, psoriasis), developmental disorders with cutaneous features (Beckwith–Wiedemann syndrome, Silver–Russell syndrome, McCune–Albright syndrome (MAS), Albright's hereditary osteodystrophy, progressive osseous heteroplasia, Prader–Willi syndrome, Angelman syndrome), and tumorigenesis (melanoma, basal cell carcinoma, squamous cell carcinoma, cutaneous T-cell lymphoma).[31-35] Alterations in DNA methylation patterns vary for each aforementioned disease listed, and the specifics are beyond the scope of this review. In general, many diverse genes governing the aforementioned pathologic disease states have been reported to be regulated by CpG hypermethylation and encode for cell cycle regulators, tumor suppressors, DNA mismatch repair proteins, hormone receptors, and cell adhesion molecules. Studies have shown that in tumorigenesis, approximately 50% of the genes that are known to cause familial forms of cancer also undergo epigenetic silencing in various forms of sporadic cancer.[36,37] In epidermal cells of psoriasis patients, methylation of CpG islands in the promoter region of the *p16INK4a* gene was observed in 17 (30%) of 56 patients with psoriasis and correlated with higher Psoriasis Area and Severity Index (PASI) scores.[38] In cutaneous lesions of atopic dermatitis and lupus erythematosus, mRNA expression of DNA methyltransferases 1 and 3a, respectively, are altered because of differences in the methylation patterns of these gene promoters.[39-41] The contributions of DNA methylation to the pathogenesis of these and other dermatologic disease is currently under active research.

In human cells, DNA is wrapped around core proteins called histones and secondarily packaged into organized structural units called nucleosomes.[42] These serve as the building blocks of chromatin. Amazingly, the human genome when unwound reaches approximately 1.8 m in length, and when bound to histones stretches 90 mm, but as chromatin can condense to 120 μm in chromosomal form.[43] Epigenetic, posttranslational chemical modifications of his-

tones are responsible for regulating changes in gene expression. Specifically, histone proteins are subject to acetylation, methylation, phosphorylation, and ubiquitination.[44] In general, acetylation of lysine amino acid residues of histone complexes activates transcription; however, chemical modification of histones can either activate or inhibit gene expression. Aberrant histone modification has been shown to contribute to the pathogenesis of various dermatologic diseases, including melanoma and systemic lupus erythematosus.[34,41]

The fascinating potential for interplay between mechanisms of epigenetic gene regulation is beginning to be realized (Fig. 4). The completion of the human genome project has confirmed earlier notions that most DNA sequences are not protein coding regions. Importantly, these noncoding regions do not simply represent "junk DNA;" a role for noncoding RNA in regulating normal and pathologic states has emerged. Noncoding RNA (ncRNA) is defined as functional RNA molecules that are not translated into protein. Examples include transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar (snoRNA), microRNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), and the long ncRNAs (eg, Xist and HOT AIR). Within the human genome, the absolute number and true functional diversity of ncRNA is unknown; however, bioinformatics data suggest that there may be thousands of ncRNAs regulating up to half of all human genes.[44-49]

The most studied class of noncoding RNAs is the miRNAs, which comprise approximately 1% to 5% of the human genome. miRNAs are small endogenous RNA molecules 20 to 25 nucleotides in length that are generated by the processing of an RNA polymerase II transcript by 2 ribonuclease III proteins. miRNAs regulate gene expression by binding to specific target messenger RNAs (mRNAs) and either causing their degradation or directly inhibiting their protein translation. In dermatology, miRNAs have been shown to regulate many cellular processes, including development, stem cell differentiation, cancer biogenesis, signal transduction, metabolism, genomic stability, apoptosis, hair follicle development, wound healing, and inflammatory skin disease.[34,50-58] Within the epidermis, there is a discrete set of miRNAs expressed that contribute to the regulation of the aforementioned processes.[58] In fact, mice deficient of epidermal miRNA production display significant derangements in hair follicle morphogenesis and demonstrate hyperproliferation of the epidermis.[59,60] Dysregulation of miRNA expression has been hypothesized to contribute to chronic wound healing defects through control of cutaneous vascular endothelial growth factor and hypoxia-inducible factor-1).[55] In psoriasis, noncoding RNAs have been hypothesized to contribute to the unabated epidermal hyperproliferation and ultraviolet sensitivity via differential expression of miRNA-203, miRNA-146a, and miRNA-125 b.[61,62]

Finally, approximately 50% of genomic sequences encoding for miRNAs are physically located near fragile sites and regions of loss of heterozygosity, amplifications, and breakpoints in DNA that are commonly associated with carcinogenesis.[63] Human cultured melanoma cell lines reveal unique miRNA expression profiles and DNA copy number variation, which may contribute to tumorigenesis.[64,65] The future of clinical medicine will be inundated with disease- and eventually patient-specific noncoding RNA signatures providing tremendous potential for disease classification and treatment applications.
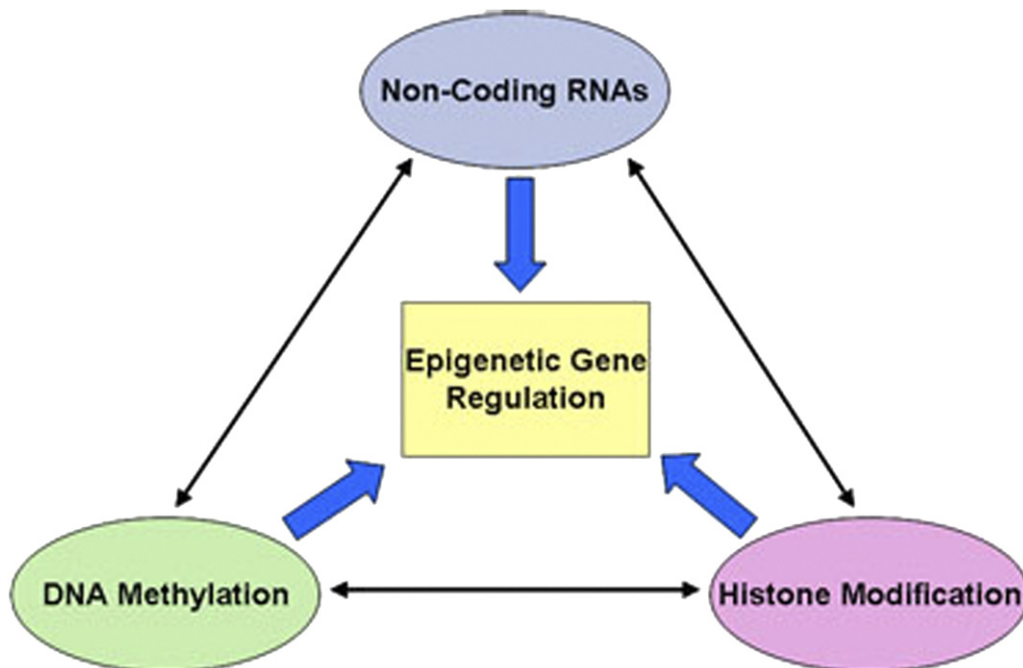

Figure 4 Interplay between mechanisms of epigenetic gene regulation: noncoding RNAs, DNA methylation, and histone modification.

Genomic Imprinting and X-Inactivation

Genomic imprinting describes the situation in which a gene behaves differently depending on whether it is inherited from the mother or the father's genome. In other words, the clinical phenotype depends on the parent of origin of the genetic mutation.[32] MAS is an example of a condition affected by genomic imprinting. MAS is caused by an alteration in the guanine nucleotide-binding protein alpha-stimulating activity polypeptide 1 (*GNAS1* gene).[66] The clinical features include large, segmental café-au-lait patches, polyostotic fibrous dysplasia, and endocrine abnormalities; 2 of 3 of these features need to be present for a clinical diagnosis. Regulation of the *GNAS1* gene involves tissue-specific imprinting, whereby acromegaly in MAS is maternally imprinted and precocious puberty can be caused by maternal or paternal imprinting.[67]

Functional mosaicism occurs in females because of X chromosome inactivation. Very early in embryologic development (beginning as early as in the 4- or 8-cell embryo), each cell simultaneously and selectively turns-off or inactivates either the maternal or the paternal X chromosome. X-linked dominant conditions are presumed lethal in males and are expressed in affected females in a mosaic pattern as the result of X chromosome inactivation (X-inactivation results in the presence of some cells which have an active normal X chromosome). Female infants survive because many cells express the gene from the unaffected X chromosome because of random X-inactivation. Male infants with an XXY karyotype will survive because of the same mechanism. Male infants may also survive if the mutation was a postzygotic mutation resulting in somatic mosaicism; again because not all cells in the body would be affected. Incontinentia pigmenti (IP) is an X-linked dominant disorder caused by alterations in the NF-kB essential modulator (*NEMO*) gene. IP presents at birth with vesicles along the lines of Blaschko, which evolve into verrucous plaques during the first weeks to months. As the verrucous lesions resolve, hyperpigmented streaks and whorls along Blaschko's lines remain. In adulthood, the cutaneous findings consist of subtle residual hypopigmented and atrophic streaks with absent eccrine glands and hair follicles in the affected areas. Extracutaneous features can include peg-shaped teeth, dystrophic nails, and less commonly ocular and neurologic manifestations.[68] It is the random inactivation of X chromosomes and the migration patterns of resultant epidermal cells along the lines of Blaschko that results in the distinctive clinical cutaneous phenotype of IP.

The hyperpigmented streaks in IP are a classic example of the lines of Blaschko. In 1901, Blaschko noted that epidermal nevi follow distinct patterns on the skin and made the first diagrams mapping these nevi out in what are now known as the lines of Blaschko.[69] Blaschko observed and documented different patterns on the skin depending on the location on the body, specifically an S-figure on the sides of the torso and the V-shape on the back which has been referred to as the "fountain spray." The patterned lines on the skin seem to represent the migration pattern of the embryonic cells during development. The lines of Blaschko are highlighted in several genetic skin disorders, such as epidermal nevus syndrome, porokeratotic eccrine ostial and dermal duct nevus, IP, MAS, and Conradi-Hunerman-Happle syndrome (Fig. 5).[70,71]

Genome-Wide Association Studies and the Skin

A single-nucleotide polymorphism or SNP (pronounced *snip*) is a DNA sequence variation occurring at a single nucleotide (adenine, cytosine, guanine, or thymine) in the genome. The International HapMap initiative has identified more than 10 million human SNPs, thus providing a mech-

Figure 5 (A) Happle expanded on the classification of the lines of Blaschko with the use of the following descriptions; (1) type 1a: Narrow lines of Blaschko, (2) type 1b: Broad lines of Blaschko, (3) Checkerboard, (4) Phylloid (meaning leaf-like, which is seen most commonly with chromosomal anomalies), (4) type 5: Midline patch, (5) type 6: Lateralization. (B) Type 1a narrow lines of Blaschko demonstrated in a patient with an extensive epidermal nevus (Photo courtesy of Alfons Krol, MD). (C) Type 1b broad lines of Blaschko demonstrated in a patient with McCune–Albright syndrome (Photo courtesy of Alfons Krol, MD). (Used with permission from Itin PH, Burgdorf WHC, Happle R, et al: Genodermatoses, in Schachner LA and Hansen RC (eds): Pediatric Dermatology, (ed 3), Mosby, London, pp 263-384, 2003.)

anism to measure diversity of DNA at the level of the base pair. An individual can have any combination of those alleles (ie, 1 copy of 2 different alleles or 2 copies of a single allele). Variations between DNA sequences across human populations can be measured by observing variations in SNP allele frequency. Thus, a SNP allele that is common in 1 population subgroup may be much rarer in another. Data collected from recent human genome sequencing efforts and by the Hap-Map consortium suggest that the genome of 2 unrelated humans is approximately 99.5% identical.[48,72,73] Importantly, modern genomics has been able to make advances in dissecting the basis of complex and multigenic inheritance in disease using the aforementioned natural polymorphisms.

Many diseases in clinical dermatology are defined as complex and therefore exhibit non-Mendelian multigenic inheritance patterns. Diseases governed by complex traits include atopic dermatitis, psoriasis, rosacea, basal cell cancer, squamous cell cancer, melanoma, alopecia, autoimmune diseases, connective tissue diseases, hair color, and others.[74] Since the initial completion of the International HapMap project in 2005, there have been an abundance of studies searching for genomic sequence associations and changes governing the heredity of these complex diseases; so-called genome-wide association studies (GWAS).[75]

In genetics, a genetic linkage map is a format to display the relative position of DNA loci (genes) in the functional recombination frequency, rather than the specific physical distance. This is important because we understand that only a small (<1%) of the DNA primary sequence encodes for expressed proteins and that genes that segregate together or have linkage, are not always near to one another. For human DNA, it was Botstein and colleagues[76] who first reported the construction of a genetic linkage map using polymorphisms in 1980. Both linkage studies and GWAS are similar in that they provide an unbiased and systematic mechanism for discovering the molecular basis of disease and rely on basic principles of genetics. Early linkage analysis studies genotyped short sequences of common genetic variants across the genome that acted as proxies for a disease phenotype. Using inheritance patterns of SNPs as proxies for a disease phenotype is possible because of the principles of coinheritance dictating that disease loci and common polymorphisms that are in close genomic proximity are inherited together. Linkage analysis requires the synthesis of large pedigrees in which many family members exhibit the disease phenotype.[77] GWAS differ in that they use unrelated cases and controls in a population to determine if a common polymorphism variant segregates with a disease allele and phenotype (Fig. 6). Over the years, simple genetic linkage analysis has been used to successfully identify genetic determinants in well more than 2500 diseases.[78] For GWAS, technology has been rate-limiting; however, the recent development of rapid sequencing techniques has advanced genome-wide approaches into its current state. To date, over 150 risk loci in more than 60 common diseases and traits have been linked by assaying hundreds of thousands of SNPs in numerous genome wide association studies across medicine.[79,80]

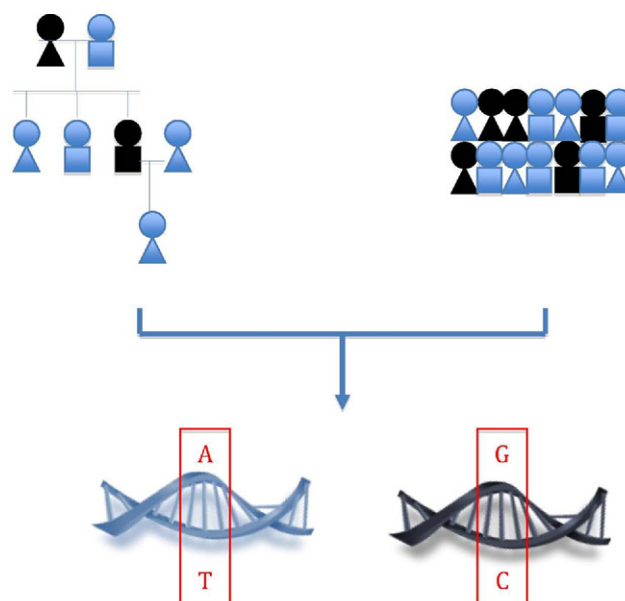In dermatology for example, recent GWAS have generated

Figure 6 Linkage studies and Genome-wide association studies. In linkage studies, differences in small sequences of DNA are compared between family members with the disease (black) to those without the disease (blue) through analysis of large pedigrees from families in which multiple members express the disease phenotype (left). In contrast, genome-wide association studies compare polymorphisms between unrelated persons with the disease (black) and controls without the disease (blue) in the population at large (right). In this example, the disease state is associated with the single-nucleotide polymorphism, GC, whereas the absence of disease is associated with an AT base pairing at the same nucleotide position. In both cases, segments of the disease-associated chromosomes are mapped through the use of SNPs.

novel insights into the immunopathogenesis of psoriasis, atopic dermatitis, alopecia areata, and systemic lupus erythematosus.[81-84] In the first psoriasis GWAS series published in 2007 and 2008, it was confirmed that HLA-C (HLA-Cw6), IL12B, and IL23R are highly significant genomic factors associated with the disease state and that IL-23A, IL-4/IL-13, TNFAIP3, and TNIP1 are novel loci also associated with psoriasis.[85-87] Subsequent GWAS have confirmed these loci and expanded the list of associated psoriasis genes to include more HLA types and the late cornified envelope gene cluster.[82]

New insights into the pathogenesis of atopic dermatitis have been gained with the identification of filaggrin mutations, enhanced protease activity, decreased synthesis of the lipid lamellae, and increased inflammatory gene expression and consequent barrier defects.[88-90] It has also become evident that there is significant interplay between the environment and genetic determinants governing the extent of disease. In a search to identify additional genomic aberrancy, a GWAS was performed involving approximately 1000 patients with atopic dermatitis and 1000 controls. A highly significant association with the homozygous state in Europeans of 1 SNP allele (rs7927894) located on chromosome 11q13.5 and the development of atopic dermatitis was shown.[83] Interestingly, this very SNP was previously identi-

fied as a susceptibility factor for Crohn's disease.[91] The molecular mechanism by which the nearby genes to this single-nucleotide polymorphism contribute to an increased relative risk of developing atopic dermatitis alone or in the setting of Crohn's disease is unknown and under research.[92]

It is well-understood that the pathogenesis and course of complex disease is governed by both genetic and environmental factors. Novel observations identifying associations between the primary genomic sequence and environmental influence on disease states have been elucidated by use of the HapMap, GWAS, and the age of efficient genomic sequencing. Recently, researchers have used SNPs to partially dissect the interactions between environmental signals, metabolic potential, the likelihood of adverse reactions to medications, and immune response to infection and vaccines. One direct application of this knowledge is in the development of tests to predict which drugs or vaccines would be most effective in individuals based upon their particular genotype. Early applications of this includes genotyping of individuals to determine their potential for warfarin metabolism, chance of abacavir hypersensitivity with the presence of HLA-B*5701, and possible induction of Stevens–Johnson syndrome in patients with haplotype HLA-B*1502 when exposed to carbamazapine.[93-95]

Practical Applications of Genetic Advancements

Significant advancements in the ability to provide preimplantation genetic counseling and diagnosis are becoming available because of the identification of the mutations underlying many genetic skin diseases. In preimplantation diagnosis, an embryo is conceived using in vitro fertilization and 1 cell from the embryo is tested for the genetic mutation and only embryos that do not carry the mutation are used for implantation. There are many associated ethical considerations for these techniques; nonetheless, we hope that improved treatments and even cures for genetic skin diseases may soon be offered using gene therapy, protein replacement therapy and bone-marrow-derived stem cell transplant. These technologies are still in their infancy, but hold great promise for the future.

Conclusions

The integration of basic and novel principles of genetics in the context of a rapidly evolving technology and scientific understanding of the mechanics and function of the human genome is particularly important for effective clinical care of our patients with genetic skin diseases, and for those with complex disorders, including autoimmune conditions and cancer. This review article has provided an overview of phenotype deconvolution, modes of inheritance, epigenetics, genomic imprinting, and GWAS in an effort to provide clinicians with a basic working knowledge of these complex concepts. Hopefully, this will translate into improved understanding of the literature and enhanced interactions with affected patients and families.

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