

Molecular mechanisms in allergy and clinical immunology

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The genetics of atopic dermatitis

Nilesh Morar, FCDerm,^{a,b} Saffron A. G. Willis-Owen, DPhil,^b Miriam F. Moffatt, DPhil,^b and William O. C. M. Cookson, DPhil^b Oxford and London, United Kingdom

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Atopic dermatitis (AD) is a chronic itching (pruritic) skin disease. It results from a complex interplay between strong genetic and environmental factors. Genome screens of families with AD have implicated chromosomal regions that overlap with other skin diseases and with inflammatory and autoimmune diseases. These, together with candidate gene studies, provide novel insights into the pathogenesis of AD. The findings suggest a common theme of generalized epidermal dysfunction manifesting as a compromised skin barrier and failure to protect against, or aberrant responses to, microbial insults and antigens. Recent genetic advances with high-throughput methods for gene identification, such as DNA microarrays and whole-genome genotyping, will help further dissect this complex trait. This will aid disease-defining criteria and focused therapies for AD. (J Allergy Clin Immunol ■■■■;■■■;■■■-■■■.)

Key words: Atopic dermatitis, genetics, candidate gene, linkage, microarray

The term *atopic dermatitis* (AD) was first coined by Wise and Sulzberger¹ to describe “confusing types of localized and generalised lichenification, generalised neurodermatitis or a manifestation of atopy.” Today AD or atopic eczema is recognized as a strongly heritable,^{2,3} chronic, pruritic inflammatory skin condition that is most common in early childhood and that predominantly affects the skin flexures. Confusion about its cause, however, still persists.

The identification and characterization of the pathophysiologic mechanisms that underlie AD are of considerable biomedical importance. This is because of the

Abbreviations used

AD: Atopic dermatitis
LEKT1: Lympho-epithelial kazal-type related inhibitor
MMC1: Mast cell chymase 1
S100: S100 calcium-binding protein
SPINK5: Serine protease inhibitor, Kazal type 5

substantial and growing^{2,4} prevalence of the disease in industrialized countries (affecting some 10% to 20% of children and 1% to 3% of adults⁵) and because of the known relationship of AD with other atopic conditions, such as asthma and allergic rhinitis (the atopic triad). Around 60% of children given diagnoses of severe AD also have asthma,⁶ and 35% will have allergic rhinitis.⁷ Although there appears to be some genetic correlation between these conditions (as inferred from cross-phenotype familial clustering), at least a proportion of factors are likely to confer a liability that is specific to AD. This is supported by the observation that parental history of AD represents a more potent risk factor for AD in a child than either asthma or allergic rhinitis.^{8,9}

Current evidence indicates that AD is strongly genetic, with enhanced levels of phenotype concordance reported in monozygotic relative to dizygotic twin pairs (0.72-0.77 vs 0.15-0.23).^{2,3} The individual genetic factors or genes that contribute to the trait's cause are relative to other complex genetic diseases proving amenable to identification.

In this review we will present an overview of these data before turning to a brief discussion of recently emerging techniques founded on phenotype-related differences in gene expression. These new methods are likely to provide invaluable insights into the genetic architecture of AD and its underlying pathophysiology. Undoubtedly, they will result in better understanding of the disease and might ultimately lead to the identification of novel therapeutic targets.

BACKGROUND

Diagnostic criteria

Because the individual genetic factors that contribute to AD are thought to be of low effect size (with a genotype

From ^athe Wellcome Trust Centre for Human Genetics, University of Oxford, and ^bthe National Heart and Lung Institute, Imperial College, London.

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Reprint requests: Nilesh Morar, FCDerm, National Heart and Lung Institute, Airway Disease, Dovehouse St, London, SW36LY. E-mail: nilesh.morar@well.ox.ac.uk.

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relative risk of <2) and subject to complex environmental moderation, diagnostic accuracy is essential for the success of genetic studies. Variable or inaccurate diagnoses can obscure true genetic effects by increasing the signal-to-noise ratio. Unfortunately, over the years there has been a substantial variation in AD disease definition and diagnosis. This has been due to variable clinical presentations and the intermittent nature of the disease.

A minimum set of validated discriminators, which are based on the original consensus criteria by Hanifin and Rajka¹⁰ and further refined by a UK Working Party,¹¹ are now available and routinely used. The diagnosis requires evidence of a pruritic skin condition plus 3 or more of the following: history of involvement of the skin creases, history of asthma or hay fever, history of generally dry skin, onset at less than 2 years of age, and visible flexural dermatitis. These robust criteria might underpin the development of effective and reproducible genetic studies.

AD and atopy

AD, bronchial asthma, and allergic rhinitis are typically considered to represent a common syndrome of atopic diseases. The atopic state is characterized by either a positive skin prick test response to common environmental allergens, the presence of allergen-specific IgE in sera, an increase in total serum IgE levels, or a combination of these criteria. Around 80% of infants with AD exhibit increased total serum IgE levels (a phenotype that is approximately 47% heritable).¹² However, because this atopic trait marker is not present in all cases of AD, the disease has been subdefined into extrinsic and intrinsic AD. The extrinsic (allergic) form is associated with IgE-mediated sensitization, whereas the intrinsic (nonallergic) form of AD is characterized by a normal total serum IgE level and the absence of specific IgE responses to aeroallergens and food-derived allergens.^{13,14} These phenotype classifications, however, yield superficially indistinguishable clinical presentations in terms of lesion manifestation, distribution, CD4⁺ T-cell infiltration, and epidermal histology. Consequently, it has been hypothesized that IgE sensitization is not a necessary prerequisite for the development of the eczematous skin lesions that are characteristic of AD.¹⁵

Nevertheless, a number of defining primarily immunologic features can be identified, and these might relate to a partially independent underlying genetic cause. These include a reduced dermal infiltration of eosinophils and eosinophil granular proteins¹⁶ and a more moderate enhancement of lesional cytokine expression, including IL-13 and eotaxin,^{15,16} and reduced surface expression of the high-affinity receptor for IgE (FcεRI) on epidermal dendritic cells¹⁷ in intrinsic relative to extrinsic AD. In epidemiologic terms higher IgE antibody levels also appear to be associated with a more severe disease phenotype and worse long-term prognoses.¹⁸

Maternal transmission of AD

Parent-of-origin effects are thought to play an important role in the cause of AD (and other atopic conditions)

because infant disease risk is often,^{8,19,20} although not always,^{9,21} found to be more closely related to maternal than paternal disease status. This observation might be related to a number of underlying mechanisms, including genomic imprinting, mitochondrial transmission, and gene-environment interactions involving the *in utero* environment and/or exposure to the immunologic and nutritional properties of breast milk.

The first evidence of atopy-related maternal inheritance was obtained in the early 1990s when a linkage peak influencing IgE responsiveness was mapped to chromosome 11q through the maternal line.²² Although this observation has been implicated in a number of independent studies, as well as for AD,⁶ no regions exhibiting overtransmission of maternal alleles have been mapped *de novo* by using an AD phenotype.²³ Although this absence of line specificity might relate to a lack of maternally transmitted disease loci, it should also be noted that the majority of investigations do not explicitly test for parent-of-origin effects and thus are likely to have missed any small-effect, line-specific genetic determinants of AD. An exception to this is the gene encoding serine protease inhibitor, Kazal type 5 (SPINK5), in which maternally derived alleles have been associated with AD.²⁴

TRADITIONAL APPROACHES TO GENE IDENTIFICATION

Linkage studies

Linkage mapping is a method of genetic localization whereby disease-causing variants are mapped to discrete chromosomal regions by using patterns of inheritance of genetic variants (allele sharing by descent) in pedigrees. It does not depend on any hypotheses about disease cause. Individuals are genotyped at a series of consecutive molecular markers (eg, microsatellites or single nucleotide polymorphisms) spaced evenly across the genome, and regions are sought in which an excess of allele sharing is found to cosegregate with the disease phenotype. The resolution of this strategy depends on a number of factors related both to study design (eg, marker density and information content) and population history (eg, meiotic recombination frequency and patterns of linkage disequilibrium). Typically, the linkage regions identified in this way extend over large distances (>10 cM) that might contain hundreds of genes. This makes the ability to identify individual positional candidate genes a substantial task, except in gene-sparse regions and less complex model organisms.

This issue can be resolved through several convergent approaches, including fine mapping (genotyping additional markers for the saturation of implicated regions), linkage disequilibrium mapping, and the comparative use of data from alternative (but closely related) phenotypes or disciplines (eg, expression arrays).

To date, 4 genome screens for AD have been reported in the literature (Table I),^{23,25-27} providing genome-wide significant evidence of linkage on chromosomes 1q,²⁵ 3q,^{23,26}

TABLE I. Genome screens for AD

Study	Chromosomal location	Marker	Phenotype	Population and size of study
Lee et al, ²³ 2000	3q21	D3S3606*	AD	German/Scandinavian, 199 nuclear families (n = 839)
Cookson et al, ²⁵ 2001	1q21	D1S498*	AD	United Kingdom, 148 nuclear families (n = 383)
	17q25	D17S784*	AD	
	20p	D20S115*	AD & Asthma	
Bradley et al, ²⁶ 2002	3p24-22	D18S851*	AD	Swedish, 109 pedigrees (n = 470)
	3q14	D3S1768*	Severity score of AD	
	13q14	D3S2459*	Severity score of AD	
	15q14-15	D17S1290*	Severity score of AD	
	17q21	D15S118*	Severity score of AD	
Haagerup et al, ²⁷ 2004	18q21	D13S325*	AD with increased specific IgE	Danish, 100 nuclear families (n = 424)
	3p26-24	D3S3594-D3S3038†	AD with increased specific IgE	
	4p15-14	D4S2408†	AD with increased specific IgE	
	18q11-12	D18S877†	AD with increased specific IgE	

Only markers at the peak of linkage are shown.

* $P < .001$.

†Studies in which a maximum likelihood score of greater than 2 was present.

3p,²⁶ and 17q.²⁵ Additional loci have been mapped by using composite phenotypes, such as AD and asthma combined (20p),²⁵ AD with increased allergen-specific IgE levels (3p, 4p, and 18q),²⁷ and total serum IgE level (16q).²⁵ Suggestive evidence of linkage has also been documented on chromosomes 3q, 13q, 15q, and 17q on the basis of a semiquantitative AD severity score²⁶ and on chromosome 18q on the basis of a combined AD and increased allergen-specific IgE phenotype.²⁶ Some of these loci are of particular interest because they overlap with known psoriasis (1q, 3q, 17q, and 20p loci²⁵) or asthma (13q²⁸ and 20p²⁵) susceptibility regions.

Depending on the size of the genotyped population, the level of marker spacing, and the size of the underlying genetic effect, simulations have shown that the linkage peak location can vary some 20 to 30 cM from the disease locus.²⁹⁻³¹ On the basis of these observations and with the assumption that the phenotypes described above are comparable, replication has been established for chromosomes 3p26-22 (32-62 cM) and 3q14-21 (119-144 cM), with the chromosome 17 locus marginally exceeding this threshold (82-117 cM; based on Marshfield map coordinates: http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html). In a similar vein, other authors have attempted to replicate candidate loci (originally identified in analyses of AD or other atopic phenotypes) through selective region-specific linkage mapping in populations with AD. In this way additional evidence of linkage has been produced on chromosomes 5q,³² 13q,³² and 14q³³ on the basis of an AD phenotype and on chromosome 5q³³ on the basis of an AD severity score.

Candidate gene studies

In contrast to hypothesis-free linkage approaches, candidate gene studies are founded on pre-existing

knowledge of biologic function and disease pathways. Relative to linkage analyses, candidate gene approaches represent a temporally and economic alternative to disease gene identification but are fundamentally dependent on the accuracy and completeness of existing data. The approach is therefore limited in its potential for the discovery of novel targets and pathways. Candidate gene studies look for a statistical association between phenotype and genotype at one or more polymorphic loci within a gene of interest. In this section a selection of AD candidate genes is represented (Table II),^{6,24,34-62} with a detailed discussion of the evidence both for and against their involvement in AD pathology.

FcεRIβ (MS4A2)

FcεRIβ, the β subunit of FcεRI is encoded by a single gene, *MS4A2*, located on chromosome 11q12-13 within a region of confirmed linkage to atopy.^{63,64} Variants within this gene have previously demonstrated evidence of association with a range of atopy-related phenotypes (including bronchial hyperresponsiveness,^{65,66} grass and house dust mite allergy,^{65,66} and atopic asthma³⁴). Because of the known relationship between AD and atopy, the gene might therefore be of some relevance to the genetic dissection of AD.

The FcεRI receptor is expressed on a range of cells, including basophils, mast cells, monocytes, and Langerhans cells (with additional equivocal reports of gene expression on platelets, neutrophils, and eosinophils). The receptor binds to the constant region of the antigen-complexed IgE molecule and initiates the release of inflammatory mediators through an intracellular signaling cascade (degranulation). The full-length β subunit of the FcεRI receptor amplifies these cell activation signals⁶⁷ through an effect on the recruitment of Lyn kinase.⁶⁸ A

TABLE II. Candidate gene studies of AD

Study	Gene symbol	Gene name	Chromosomal location	Population and study size
Palmer et al, ⁴⁶ 2006	FLG	Filaggrin	1q21*	Irish (n = 52), Scottish, (n = 279§), Danish (n = 142, 25†§)
Jones et al, ⁴⁸ 2006	CTLA4	Cytotoxic T lymphocyte-associated 4	2q33*†	Australian, 112 nuclear families
Moffatt et al, ⁴⁹ 2005	TLR9	Toll-like receptor 9	3p21.3*	British (n = 172)
Ahmad-Nejad et al, ⁵⁰ 2004	TLR2	Toll-like receptor 2	4q32*	German (n = 78)
Nishio et al, ⁵¹ 2001	IRF2	Interferon regulatory factor 2	4q35.1*†	Japanese, 49 pedigrees (n = 180)
Lange et al, ⁵² 2005	CD14	Monocyte differentiation antigen CD14	5q31.1*	German (n = 40)
Rafatpanah et al, ⁵³ 2003	GM-CSF	Granulocyte-macrophage colony-stimulating factor	5q31.3*	British (n = 113)
Tsunemi et al, ⁴⁵ 2002	IL13	IL-13	5q31-33*	Japanese (n = 185)
Liu et al, ⁴¹ 2000	IL13	IL-13	5q31-33†	German (n = 187)
Kawashima et al, ⁴⁰ 1998	IL4	IL-4	5q31-33*	Japanese, 88 nuclear families
Walley et al, ²⁴ 2001	SPINK5	Serine protease inhibitor, Kazal type 5	5q31-33*	British (n = 338)
Nishio et al, ⁴⁵ 2003	SPINK5	Serine protease inhibitor, Kazal type 5	5q31-33*	Japanese, 41 pedigrees (n = 177)
Kato et al, ⁴⁴ 2003	SPINK5	Serine protease inhibitor, Kazal type 5	5q31-33†	Japanese (n = 124)
Weidinger et al, ³⁶ 2005	CARD4	Caspase recruitment domain-containing protein 4	7p14-15*	German, 189 nuclear families (n = 454)
Cox H et al, ⁶ 1998	FcεRIβ	β Chain of the high-affinity receptor for IgE	11q12-13*	British, 60 nuclear families (n = 277)
Moffatt et al, ⁵⁴ 1992	FcεRIβ	β Chain of the high-affinity receptor for IgE	11q12-13†	British (n = 172)
Novak et al, ⁵⁵ 2005	IL18	IL-18	11q22*	German (n = 225)
Chae et al, ⁴³ 2003	TIM1	T-cell immunoglobulin- and mucin domain-containing molecule 1	12q12-13*	Korean (n = 112)
Jang et al, ³⁹ 2005	PHF11	PHD finger protein 11	13q14*	Australian, 111 nuclear families
Mao et al, ³⁴ 1996	MCC	Mast cell chymase	14q11.2*	Japanese (n = 100)
Mao et al, ³⁵ 1998	MCC	Mast cell chymase	14q11.2*	Japanese (n = 145)
Tanaka et al, ⁵⁶ 1999	MCC	Mast cell chymase	14q11.2†	Japanese (n = 169)
Iwanaga et al, ⁵⁷ 2004	MCC	Mast cell chymase	14q11.2*	British, 341 nuclear families
Weidinger et al, ³⁶ 2005	MCC	Mast cell chymase	14q11.2*	German (n = 24)
Oiso et al, ⁵⁸ 2000	IL4R	IL-4 receptor α chain	16p12-p11*	Japanese (n = 27)
Hosomi et al, ⁵⁹ 2004	IL4R	IL-4 receptor α chain	16p12-p11*†	Japanese (n = 101)
Kabesch et al, ⁴⁷ 2003	CARD15	Caspase recruitment domain-containing protein 15	16q12†	German (n = 330)
Nickel et al, ³⁷ 2000	RANTES	Regulated on activation, normally T cell expressed and secreted	17q11.2†	German (n = 268)
Tsunemi et al, ³⁸ 2002	EOTAXIN	Eotaxin	17q21.1-21.2†	Japanese (n = 140)
Arkwright et al, ⁶⁰ 2001	TGFβ1	TGF-β1	19q13.1*	British (n = 68)
Vasilopoulos et al, ⁶¹ 2004	SCCE	Stratum corneum chymotryptic enzyme	19q13.3*	British (n = 103)
Vavilin et al, ⁶² 2003	GSTT1	Glutathione S-transferase, Theta-1	22q11.2*†	Russian (n = 325)

Significance levels for the markers showing the strongest association are shown.

* $P \leq .01$.† $P > .01 \leq .05$.

‡Markers showing association when haplotype analysis was performed.

§Phenotype of AD and asthma combined.

truncated isoform (produced by alternative splicing) appears to downregulate FcεRI cell-surface expression, ultimately inhibiting FcεRI function. In the context of mast cells, the FcεRIβ monomer has also been shown to act as a target for peptides that inhibit the IgE-mediated secretory response.⁶⁹ These multifaceted antagonistic functions of the FcεRIβ chain indicate a central role in the modulation of the inflammatory response.

The involvement of FcεRIβ in the cause of AD has been investigated in 2 independent panels of families with AD. Consistent with previous linkage data, which have indicated a strong effect of maternally derived alleles at the chromosome 11 locus, an excess transmission of maternal FcεRIβ alleles has been observed in patients with AD relative to control subjects.⁶ These data indicate a potential role for FcεRIβ in the pathophysiologic processes that underlie AD, but because the variants examined were noncoding, their mechanism of action remains to be identified. Since this original investigation, several additional variants have been identified, which (because of their function) might represent useful targets for future analysis.⁷⁰

Mast cell chymase 1 (*CMA1*)

Mast cell chymase 1 (MCC1) is encoded by the gene *CMA1*, which maps to the long arm of chromosome 14q11, a region that has shown previous evidence of linkage with AD.³³ MCC1 is a glycosylated chymotryptic-like serine protease that is found at high levels in the secretory granules of mast cells and appears to operate in concert with histamine and tryptase to confer a range of pro-inflammatory effects on release from activated cells. Specifically, introduction of purified human MCC1 into the skin or peritoneum of guinea pigs yields an increased microvascular permeability⁷¹ and marked accumulation of inflammatory cells, including neutrophils, eosinophils, leukocytes, and macrophages.⁷² The microvascular permeability seen, although of lower amplitude, is of greater longevity than that of histamine.⁷¹

Additionally, MCC1 is also known to exhibit a number of properties that are consistent with a role in tissue remodeling. These include the ability to activate interstitial procollagenase,⁷³ to process procollagen into collagen (thereby promoting the formation of fibrils),⁷⁴ and to release (but not activation of) TGF-β1 from the extracellular matrix of epithelial and endothelial cells.⁷⁵ These features, combined with an abundant expression of the protein in the dermis, have rendered MCC1 (*CMA1*) an excellent candidate for genetic analyses of AD. Supportive of this observation is the increased number of CMA1-positive cells in chronic AD skin lesions⁷⁶ compared with that seen in both unaffected and psoriatic skin biopsy specimens.

Several investigations have now reported a significant association between genotypes at one or more polymorphic sites in *CMA1* and its promoter and AD status.³⁴ The most pronounced effects have been observed in individuals at the low end of the total serum IgE spectrum.³⁵ These data indicate that the genetic relationship between MCC1 and AD might be independent of IgE. Interestingly, a recent large-scale association study demonstrated

a specific effect of this locus on AD status, with no effect observed on serum IgE levels or any other atopic phenotype.³⁶ Although these data appear congruous, no clear conclusions can be drawn because a number of negative replications also exist within the literature.^{77,78} This might be due to variation in population ancestry and age parameters. However, these failures to substantiate the initial findings might relate to a lack of power to differentiate between IgE-dependent and IgE-independent mechanisms of AD pathogenesis.

CC chemokines: RANTES and eotaxin 1

Chemokines are a group of chemotactic cytokines that induce inflammatory cell mobilization through the presentation of a concentration gradient.⁷⁹ These molecules can be divided into 3 broad categories on the basis of their protein structure (and specifically the location of cysteine motifs conserved within the N-terminal domain). Both RANTES and eotaxin 1 are CC chemokines that have 2 juxtaposed N-terminal-conserved cysteine residues.

RANTES (which is encoded by *CCL5* on the long arm of chromosome 17 [17q11.2-q12]) exhibits several known functions, including the stimulation of histamine secretion from basophils, the activation of eosinophils, and the mobilization of monocytes, eosinophils, and memory T_H cells (with a preference for CD45RO⁺ and CD4⁺ subtypes). Although virtually all nucleated blood and tissue cells produce chemokines, the primary source of cutaneous RANTES appears to be dermal fibroblasts.

Eotaxin 1 is encoded by the gene *CCL11*, which is also located on the long arm of chromosome 17 (17q21.1-q21.2) adjacent to a linkage region previously identified as being linked to AD, AD severity, and psoriasis phenotypes. The eotaxin 1 chemokine is a selective chemoattractant and activator of both eosinophils⁸⁰ and T_H2 lymphocytes, and it might also operate as an indirect negative regulator of neutrophil recruitment.^{81,82}

Enhanced levels of both RANTES and eotaxin 1 have been identified in the sera of patients with AD relative to those of healthy control subjects,⁸³ with RANTES demonstrating a significant positive correlation with both total serum IgE levels and eosinophil numbers. Eotaxin 1 also demonstrates a significantly increased pattern of gene expression in lesional skin biopsy specimens taken from patients with AD compared with those from nonatopic control subjects.⁸⁴ Consistent with a role for these CC chemokines in the pathology of AD, tacrolimus (FK506) ointment, a clinically effective macrolide lactone AD treatment, has been shown to suppress the expression of both eotaxin 1 and RANTES in lesional AD skin.⁸⁵ UV-B irradiation, used in phototherapy, has also been shown to inhibit cytokine-stimulated RANTES expression in cultured epidermal keratinocytes.⁸⁶ Together these data indicate that CC chemokines, particularly RANTES and eotaxin 1, might represent useful future targets for the genetic dissection of AD.

A functional point mutation has recently been described in the proximal promoter region of the RANTES gene (*CCL5*). This variant results in the generation of a novel

consensus binding site for the GATA transcription factor family and has been associated with enhanced RANTES production in patients with AD.⁸⁷ The variant has now been shown to be associated with AD (but not asthma) in the children of the German Multi-centre Allergy Study,³⁷ although these results were not replicated in a second Hungarian cohort.⁸⁸

A number of polymorphisms have been identified in the gene that encodes eotaxin 1 (*CCL11*). Two of these variants (both located in the promoter of the gene) have been reported to be associated with total serum IgE levels in patients with AD.³⁸ However, no variants as yet have been shown to be related to AD susceptibility. This apparent specificity of effect might be related to the diminutive size of the genotyped sample of this single study,³⁸ the low magnitude of the underlying genetic effect, and the power advantage associated with the use of quantitative traits. Consequently, these data require replication, as well as further study in a more substantial cohort of subjects. An orally available antagonist of the eotaxin 1 receptor (*YM-344031*) does, however, lend some support to the genetic findings because it has recently been shown to inhibit both immediate and late-phase antigen-induced cutaneous inflammation in a mouse model of allergy.⁸⁹

Plant homology domain finger protein 11 (*PHF11*)

PHF11 encodes the diffusely expressed, differentially spliced NY-REN-34 antigen⁹⁰ responsible for a serum IgE and asthma locus located on chromosome 13q14.²⁸ Variants within this gene have been successfully associated with both a severe bronchial asthma phenotype and serum IgE levels.²⁸ Consistent with evidence that both AD and asthma exhibit linkage to this locus, association has now been extended to a childhood AD phenotype, although this as yet remains to be replicated.³⁹ Although the function of the *PHF11* protein is largely unknown, the presence of a zinc finger motif, as well as distinctive splice variants in immune tissues,²⁸ suggests a role in the transcriptional regulation of immune-related products, such as IgE.

The cytokine gene cluster

The cytokine gene cluster is located on chromosome 5q31-33 and comprises a tightly linked group of functionally related genes that encode both intracellular and cell-surface mediators of the immune response. The genes include several ILs (*IL3*, *IL4*, *IL5*, *IL9*, *IL12*, and *IL13*), the GM-CSF (*GM-CSF*), the *CD14* antigen, T-cell immunoglobulin domain mucin domain protein 1 (*TIM-1*), and *SPINK5* (encoding the serine protease inhibitor LEKTI [lympho-epithelial kazal-type related inhibitor]). The cytokine gene cluster overlaps with a region of significant linkage originally identified for total IgE in a collection of Amish pedigrees.⁹¹ This linkage has since also been observed for a range of populations and atopic phenotypes, including both AD³² and AD severity,^{33,92} with weaker evidence of linkage identified for total IgE measurements in families selected for AD.²⁵

Consequently, several of the genes in the cytokine cluster have been analyzed as candidates for AD through analysis of association. Consistent with previous observations of linkage peak fractionation in complex traits,⁹³ significant evidence of association has been reported for variants in and around a number of the genes, including *IL4*,^{40,94} *IL13*,^{41,42,94} *TIM-1*,⁴³ and *SPINK5*.^{24,44,45} These effects might ultimately prove difficult to disentangle because of the increased level of linkage disequilibrium that exists across the region and the potential for gene-gene interactions through overlapping functional pathways.

Although some degree of nonreplication is reported in the literature,^{95,96} the causes of this inconsistency, such as type I error or other experimental confounders, are not yet clear. Certainly data derived from alternative sources (including expression, protein interaction, or both) indicate that at least a proportion of these genetic effects might be real. For example, *IL13* is found to show an enhanced level of gene expression in both subacute and lichenified AD skin lesions relative to healthy controls.⁹⁷ This effect is more pronounced in extrinsic forms of the disease.¹⁵ *SPINK5* is expressed in the uppermost epidermis and pilosebaceous units of the skin,⁹⁸ where its product, LEKTI, inhibits 2 serine proteases involved in desquamation and inflammation (stratum corneum tryptic enzyme and stratum corneum chymotryptic enzyme).⁹⁹

The epidermal differentiation complex

The AD linkage peak described on chromosome 1q21 overlies the epidermal differentiation complex. This complex contains a collection of genes that are known to be expressed during terminal differentiation of the epidermis. These genes can be divided into several functionally related clusters, with a small number of additional single-copy genes (trichohyalin, repetin, involucrin, filaggrin, and loricrin). The clusters include the S100 calcium-binding proteins (S100s), the small praline-rich proteins, and the late expressed cornified envelope proteins. The genes in this region represent good candidates for future genetic studies. Consistent with this suggestion, decreased levels of both filaggrin^{100,101} and loricrin¹⁰⁰ and increased levels of S100A7¹⁰⁰ and S100A8¹⁰⁰ expression have already been observed in skin biopsy specimens of patients with AD by using recently developed microarray technologies. Recently, loss-of-function variants in the gene encoding filaggrin (*FLG*) were found to be predisposing factors for AD and for asthma occurring in the context of AD in populations of European origin.⁴⁶ Mutations in this gene also cause ichthyosis vulgaris, a common inherited disorder of keratinization that is associated with the atopic diathesis.¹⁰² These preliminary results now need to be explored and confirmed. It also remains to be seen whether these functional variants play a significant role in AD pathogenesis worldwide and in non-European populations.

Although the candidate genes detailed in this review represent a bringing together of existing positional and biologic knowledge, nonreplication remains a significant issue that restricts the ability for clear interpretation of

findings. This problem is likely to be related, to a certain extent, to insufficient statistical power. This in turn limits the potential to detect small-effect disease loci, which are either in incomplete linkage disequilibrium with the genotyped marker locus or are characterized by a distinct distribution of allele frequencies.¹⁰³ This ultimately produces inconsistent patterns of replication across populations.

Several approaches are required to combat these problems. First, genetic studies must be designed to maximize available statistical power by using large homogenous populations and adopting dense and informative marker sets. Second, phenotype definition must be closely matched to the underlying pathophysiology, thereby improving the signal-to-noise ratio. Finally, evidence should be sought from additional convergent sources, at least some of which focus on the identification of disease genes as opposed to causative allelic variants because these are not always coincident.¹⁰⁴

NOVEL APPROACHES TO GENE IDENTIFICATION

Microarray studies of AD

Gene expression arrays are a recently developed technology. They allow the simultaneous measurement of transcript abundance for several thousand genes through hybridization of cRNA to immobilized oligonucleotide probes. These data have multiple potential uses. These include the comparison of tissue- or cell-specific whole-genome expression profiles in samples of biologic or disease states, as well as the mapping of expression quantitative trait loci through the use of transcript abundance as a heritable quantitative phenotype. Expression quantitative trait loci are regions that contain modifiers of gene expression, and these are likely to play a fundamental role in disease pathogenesis.

Expression array technologies have only recently begun to be applied to the field of AD. Data are currently limited to the expression profiles of lesional skin biopsy specimens,^{100,105,106} purified epidermal cells,¹⁰⁷ total PBMCs¹⁰⁸ and peripheral blood T cells,^{109,110} eosinophils,¹¹¹ and monocytes.¹¹² Despite the typical low sample sizes, occasionally as few as 5 patients and 7 control subjects,¹⁰⁹ a number of potentially interesting observations have been made. For example, a downregulation of cornified envelope genes and concomitant increase in representation of the S100 keratinization pathway genes in AD lesional biopsy specimens compared with those from healthy control subjects has been reported.¹⁰⁰ Compromising these findings, however, is the reality that skin biopsy specimens contain a range of distinct cell types that might themselves vary both in their level of representation between samples, as well as in their individual expression profiles. Consequently, this might have a substantial effect and limit interpretation of disease-related changes in gene expression from biopsy studies.

Supportive of and highlighting the importance of single cell-type analyses, current expression data have demonstrated large disease-related differences in the gene

expression profiles of individual cell populations. Eosinophils exhibit an enhanced expression of several cytokine receptor subunits (including *GM-CSF α* and *GM-CSF β*), the *CD44* antigen, and the platelet-activating factor receptor in patients with AD relative to unaffected control subjects.¹¹¹ Monocytes show an upregulation of genes involved in MHC class I antigen presentation (the ATP-binding cassette transporter *TAP2*) and recognition of both bacterial pathogens and apoptotic cells, including the proteasome activator *PA28 β* ; the proteasome subunits *PSMB6*, *PSMB8*, and *PSMB10*; and β_2 -microglobulin (*β_2M*).¹¹² T cells have been shown to exhibit an increased expression of genes involved in chemotaxis, adhesion, and T_H2 polarization,¹⁰⁰ with genes involved in skin homing, proliferation, and apoptosis specifically upregulated in $CD4^+$ T cells.¹⁰⁹ Curiously, although the microglobulin *β_2M* appears to be upregulated in AD monocytes, the same gene product is reported to be downregulated in peripheral AD T cells.¹¹⁰ Although these discrepancies might relate to complex differences in the expression profile of individual cell types, the influence of type I error cannot be ruled out. The majority of current expression-array studies rely on small samples,¹⁰⁹ and many fail to make adequate provisions for patient-control subject matching, even just with regard to age¹¹¹ (considering that 70% of cases of eczema start in children <5 years of age¹¹³). These limitations significantly reduce the reliability of these data. Replication is required in large well-designed cohorts using whole-genome (hypothesis-free) technology to benefit from the full power of microarray technology.

AD AND INFLAMMATORY DISEASE

The predominance of atopic features in AD has fostered an expectation of heightened genetic overlap between AD and other allergy-related phenotypes. Contrary to this hypothesis, correlations have been described between AD and inflammatory traits, in particular those related to the skin (eg, psoriasis and leprosy) and gastric mucosa (inflammatory bowel disease). These observations suggest that inflammatory barrier-related mechanisms might play a central role in the pathophysiology of AD.

Inflammatory bowel disease (eg, Crohn's disease)

Crohn's disease is a chronic inflammatory bowel disease characterized by discontinuous, transmural inflammation of the gastrointestinal tract wall. The first linkage evidence for this disease was localized to chromosome 16,¹¹⁴ a region containing the *CARD15* gene. *CARD15* encodes the nucleotide-binding oligomerization domain protein NOD2. Variants within this gene^{47,115} and *CARD4/NOD1*,¹¹⁶ another member of the family that interestingly is also located within an IgE linkage peak,¹¹⁷ have shown association with AD, total serum IgE levels, or both.^{47,115,116} These observations suggest a novel barrier-based pathogenic mechanism underlying the

TABLE III. New therapies for AD are tabulated

Ceramide-dominant barrier creams decrease transepidermal water loss and are an important therapeutic adjunct in AD. ¹²³
Topical calcineurin inhibitors, such as tacrolimus/FK506, are effective in moderate-to-severe AD. They target several pathologic mechanisms: suppression of T-cell activation, inhibition of transcription of several cytokine genes and inhibition of cytokine release, decreased stimulatory activity of antigen-presenting cells, and inhibition of Fas-associated keratinocyte apoptosis.
Immunomodulation with CpG oligodeoxynucleotides commit T cells to the T _H 1 phenotype and diminished T _H 2 cell-mediated responses in an antigen-specific fashion in a murine AD model. ¹²⁴
Recombinant IFN- γ , a T _H 1 cytokine, has been effectively used to downregulate T _H 2 responses in severe AD. ¹²⁵
A novel CC chemokine receptor (CCR3) antagonist has been explored for its therapeutic efficacy in allergic diseases. ⁸⁹ CCR3 is the receptor for eotaxin 1.
The imbalance of serine proteases and their naturally occurring inhibitors has been targeted by protease inhibitors and are good potential therapeutic targets for AD. ¹²⁶⁻¹²⁸
The genes of the epidermal differentiation complex are also potentially important therapeutic targets for suppression of skin inflammation and maintenance of skin structural function.

Therapies focus on either reducing T-cell activation or on targeting molecules that have been shown to play a role in the pathogenesis of AD by means of immunogenetic studies.

development, maintenance, or both of AD. Both these genes encode cytosolic pathogen recognition receptors, with a preference toward recognition of muropeptides typically found in gram-negative bacterial peptidoglycans that have crossed the plasma membrane.

Leprosy

Leprosy is a chronic granulomatous disease predominantly affecting the skin and caused by the bacterial pathogen *Mycobacterium leprae*. Variation in host response to this pathogen carries a significant genetic component. Linkage to chromosome 20p has been reported,¹¹⁸ and the same region has previously been implicated in a composite AD and asthma phenotype,²⁵ psoriasis,²⁵ and systemic lupus erythematosus (a chronic multisystemic autoimmune disease).¹¹⁹ These data together suggest that defective barrier defenses and innate immunity are likely to play a significant role in the cause of AD. Consequently, a shift in research emphasis might be warranted, focusing to a lesser degree on atopic manifestations of the disease, with more weight placed on inflammation and host-pathogen interactions.

Psoriasis

Psoriasis is a chronic inflammatory disorder of the skin distinguished by thickened, pink epidermal plaques with characteristic silver scales. Strikingly, more than one third of AD susceptibility loci map to established psoriasis linkage peaks.¹²⁰ This suggests that at least a proportion of AD loci might confer an effect that is specific to the skin or barrier defenses. This hypothesis is supported by observations of an altered capacity for host microbial defense in both these conditions,^{107,121} with a reduced resistance in AD relative to psoriatic lesions.

FUTURE DIRECTIONS AND THERAPEUTIC IMPLICATIONS

Over the last decade, significant advances have been made in the genetic dissection of AD. A number of

replicable disease loci have been discovered, and a small number of positional, theory-based, or both candidate genes have been identified, with at least a proportion of these genes gaining empiric support in more than one population.

Perhaps the most striking product of this research is the lack of substantial overlap between the genetic architecture of AD and other atopic phenotypes (eg, asthma), with a few notable exceptions.^{28,39} Instead, a far greater degree of coincidence is observed between AD and psoriasis.²⁵ This challenges the historical view that AD is primarily mediated by IgE responses to common allergens, with a T-cell response skewed toward continued IgE production. It now seems possible that at least part of the predisposition toward AD might rest within the skin itself. A general barrier failure might help to explain why the majority of serum IgE is not directed against specific antigens, why intrinsic forms of AD exist, and why anti-IgE therapies are only effective in some patients with AD.¹²²

Traditional therapeutic strategies for AD have focused on moisturizers, topical and systemic steroids, and immunosuppressive agents. In contrast, there is a relative paucity of data on immunobiologic therapies, some of which are tabulated in Table III.^{89,123-128} New therapeutic approaches are likely to benefit from a shift in focus toward the salient pathologic features of AD, as identified through recent advances in research. T-cell immune dysregulation is currently considered to be the primary immunologic defect, although intrinsic immune keratinocyte immune deficiencies and abnormal stratum corneum barrier function might represent viable therapeutic targets for the future.

With the advent of new hypothesis-free technologies, such as gene expression microarrays and whole-genome association, we can hope to rapidly build on our current concepts of AD pathogenesis and identify both candidate genes and contributory disease pathways. Fundamental to the success of this approach will be an emphasis on study design and statistical power. Ultimately, an improved understanding of the genetic basis of AD is likely to assist with disease recognition and classification and culminate in the development of novel efficacious treatment options.

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