

## Human and computational models of atopic dermatitis

*A review and perspectives by an expert panel of the International Eczema Council*

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# Human and computational models of atopic dermatitis: A review and perspectives by an expert panel of the International Eczema Council



Kilian Eyerich, MD, PhD,<sup>a,b,\*</sup> Sara J. Brown, MD, FRCPE,<sup>c,d,\*</sup> Bethany E. Perez White, PhD,<sup>e,\*</sup> Reiko J. Tanaka, PhD,<sup>f,\*</sup> Robert Bissonette, MD,<sup>g</sup> Sandipan Dhar, MD,<sup>h</sup> Thomas Bieber, MD, PhD,<sup>i,j</sup> Dirk J. Hijnen, MD, PhD,<sup>k</sup> Emma Guttman-Yassky, MD, PhD,<sup>l</sup> Alan Irvine, MD, DSc,<sup>m</sup> Jacob P. Thyssen, MD, PhD, DMSci,<sup>n</sup> Christian Vestergaard, MD, PhD, DMSc,<sup>o</sup> Thomas Werfel, MD,<sup>p</sup> Andreas Wollenberg, MD,<sup>q</sup> Amy S. Paller, MD,<sup>r,‡</sup> and Nick J. Reynolds, BSc, MB BS, MD, FRCP<sup>s,†‡</sup>  
*Munich, Bonn, and Hannover, Germany; Chicago, Ill; Dundee, London, and Newcastle upon Tyne, United Kingdom; Montreal, Quebec, Canada; Kolkata, India; Davos, Switzerland; Rotterdam, The Netherlands; New York, NY; Dublin, Ireland; and Copenhagen and Aalborg, Denmark*

Atopic dermatitis (AD) is a prevalent disease worldwide and is associated with systemic comorbidities representing a significant burden on patients, their families, and society. Therapeutic options for AD remain limited, in part because of a lack of well-characterized animal models. There has been increasing interest in developing experimental approaches to study the

pathogenesis of human AD *in vivo*, *in vitro*, and *in silico* to better define pathophysiologic mechanisms and identify novel therapeutic targets and biomarkers that predict therapeutic response. This review critically appraises a range of models, including genetic mutations relevant to AD, experimental challenge of human skin *in vivo*, tissue culture models,

From <sup>a</sup>the Department of Dermatology and Allergy, Technical University of Munich; <sup>b</sup>the Center of Allergy and Environment (ZAUM), HMGU and Technical University of Munich; <sup>c</sup>the Skin Research Group, School of Medicine, University of Dundee and <sup>d</sup>the Department of Dermatology, Ninewells Hospital and Medical School, Dundee; <sup>e</sup>the Department of Dermatology and Skin Tissue Engineering Core, Feinberg School of Medicine, Northwestern University, Chicago; <sup>f</sup>the Department of Bioengineering, Imperial College London; <sup>g</sup>Innovaderm Research, Montreal; <sup>h</sup>the Department of Pediatric Dermatology, Institute of Child Health, Kolkata; <sup>i</sup>the Department of Dermatology and Allergy, University of Bonn; <sup>j</sup>the Christine Kühne-Center for Allergy Research and Education, Davos; <sup>k</sup>the Department of Dermatology, Erasmus University Medical Center (Erasmus MC), Rotterdam; <sup>l</sup>the Icahn School of Medicine at Mount Sinai Medical Center, New York; <sup>m</sup>Trinity College Dublin, National Children's Research Centre, Paediatric Dermatology Our Lady's Children's Hospital, Dublin; <sup>n</sup>the Department of Dermatology and Allergy, National Allergy Research Centre, Herlev and Gentofte Hospital, University of Copenhagen; <sup>o</sup>the Department of Dermatology, Aalborg University Hospital, Aalborg; <sup>p</sup>Medizinische Hochschule Hannover; <sup>q</sup>the Department of Dermatology and Allergy, Ludwig-Maximilians-Universität München; <sup>r</sup>the Departments of Dermatology and Pediatrics and the Skin Disease Research Center, Northwestern University Feinberg School of Medicine, Chicago; <sup>s</sup>Dermatological Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne; and <sup>t</sup>the Department of Dermatology, Royal Victoria Infirmary, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne.

\*These authors contributed equally to this work.

†These authors contributed equally to this work.

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Corresponding authors: Kilian Eyerich, MD, PhD, Biedersteiner Strasse 29, 80802 Munich, Germany. E-mail: [kilian.eyerich@tum.de](mailto:kilian.eyerich@tum.de). Or: Nick J. Reynolds, BSc, MB BS, MD, FRCP, Newcastle upon Tyne, NE2 4HH United Kingdom. E-mail: [nick.reynolds@ncl.ac.uk](mailto:nick.reynolds@ncl.ac.uk).

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**integration of “omics” data sets, and development of predictive computational models. Although no one individual model recapitulates the complex AD pathophysiology, our review highlights insights gained into key elements of cutaneous biology, molecular pathways, and therapeutic target identification through each approach. Recent developments in computational analysis, including application of machine learning and a systems approach to data integration and predictive modeling, highlight the applicability of these methods to AD subclassification (endotyping), therapy development, and precision medicine. Such predictive modeling will highlight knowledge gaps, further inform refinement of biological models, and support new experimental and systems approaches to AD. (J Allergy Clin Immunol 2019;143:36-45.)**

**Key words:** Atopic dermatitis, atopic eczema, endotype, human models, machine learning, mechanistic models, precision medicine, tissue culture models, skin equivalents, systems biology

Atopic dermatitis (AD; synonym atopic eczema) has a complex etiology involving multiple genetic and environmental factors.<sup>1,2</sup> With its very high incidence in childhood, chronicity, devastating effect on quality of life for affected patients and their families, enormous socioeconomic costs, and limited therapeutic options to date, AD represents a major challenge. Furthermore, there is clear evidence that AD represents a systemic inflammatory disease with multiple comorbidities extending beyond the well-recognized atopic associations.<sup>3</sup> Consequently, a number of animal models have been developed and used by investigators and the pharmaceutical industry to better understand the disease and consider new pathways to target.<sup>4</sup> However, as recently reviewed, mouse models do not adequately reflect the transcriptomic and gene pathways activated in human AD skin,<sup>5</sup> and the intrinsic difference between mouse and human skin represents a barrier to direct translation of findings from animals into human disease. Consequently, there has been increasing interest in experimental studies in human subjects (in part facilitated by technological and “omics” developments), cell culture models using human tissue, and use of computational or mathematical models that are developed by integrating these data.

In this review article we have used the term *human AD model* to define representations of the disease state and interventions that enable scientific insight into disease pathogenesis, disease course, and response to therapy. We delineate and critically appraise these AD modeling approaches that range from experimental study of human skin *in vivo* (including challenge studies and detailed phenotyping and investigation of patients harboring specific genetic mutations) to generation of AD-relevant models by using immunologic, genetic, and molecular methods in 2-dimensional and 3-dimensional human tissue culture to development of *in silico* computational models using a systems biology approach. Although by definition a reductionist approach cannot recapitulate the full spectrum of AD, these models have greatly increased our understanding of the molecular drivers of AD and provide a powerful tool for preclinical drug development and target validation. However, just as the etiology, clinical expression, and severity of AD range broadly among patients, *in vitro* and *in silico* models of AD vary widely both in how the AD phenotype is induced and how the models are evaluated. Therefore we invited members of the International Eczema Council ([www.eczemacouncil.org](http://www.eczemacouncil.org)), a group of experts in AD,

#### Abbreviations used

ACD: Allergic contact dermatitis  
AD: Atopic dermatitis  
APT: Atopy patch test  
FLG: Gene encoding filaggrin  
LC: Langerhans cell

and associated authorities in the field to contribute to a scoping and development meeting and subsequently to evaluate and critically appraise the breadth of human AD and computational models to determine their strengths and weaknesses in how they recapitulate the pathophysiology of AD and enable therapeutics to be tested and validated.

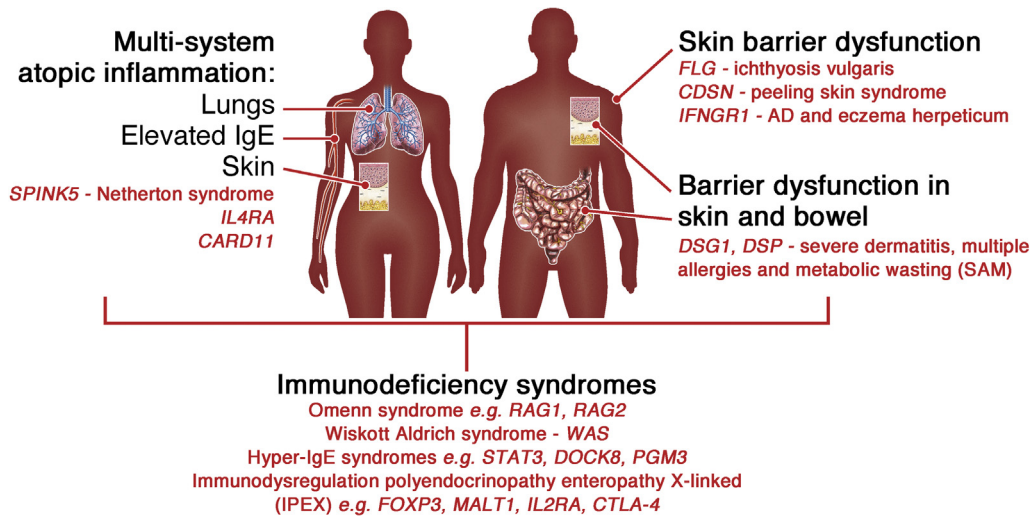
## IN VIVO MODELS OF AD

Two general approaches using human *in vivo* models have been followed to dissect the pathogenesis of AD: (1) the study of rare genetic variants with AD-like phenotypes and (2) the experimental challenge of patients with or without AD with allergens or irritants. Regarding the first approach, numerous studies have characterized genetic disorders that display skin barrier function abnormalities. Most often, these studies characterized ichthyosis vulgaris, a disease that allowed insight into the function of the epidermal differentiation gene filaggrin (*FLG*), in which mutations show the strongest association to AD development of all known genes (Fig 1).<sup>6</sup>

Other studies have focused on disorders characterized by systemic inflammation<sup>3</sup> and immunodeficiency with AD-like skin manifestations (Fig 1). One example is immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, which serves as a model to study how systemic imbalances in the regulatory T-cell population can drive cutaneous AD-like inflammation.<sup>7</sup> In addition, the link between type 2 immunity; transcription factors, such as Janus kinase or signal transducer and activator of transcription; and high levels of IgE was investigated in immunodeficiency syndromes, such as signal transducer and activator of transcription 3 and dedicator of cytokinesis 8 hyper-IgE syndromes or combined immunodeficiency disorders.<sup>8,9</sup>

Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) lists the main genetic conditions that have provided insight into AD pathogenesis to date. Although the study of rare variants offers the opportunity to delineate distinct molecular mechanisms and control pathways of a particular phenotype and thus can be regarded as “human models of AD,” a limitation of this approach is that not all observed phenomena are relevant in patients with AD, which is more complex and heterogeneous than monogenic disorders.

The second *in vivo* approach to study the pathogenesis of AD is standardized challenge with allergens or other environmental factors. The most commonly used model is the atopy patch test (APT), an epicutaneous challenge of specific allergens dissolved in vehicle,<sup>10</sup> which has provided insight into the temporal development of immune phenomena in patients with AD (see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).<sup>11</sup> Although developed in part to define clinically relevant reactions to aeroallergens, food allergens, and autoantigens,<sup>12-14</sup> its validity and predictive value depend on a variety of factors



**FIG 1.** Diagrammatic representation of “human knockout” monogenic models, providing insight into the pathomechanisms of AD. Specific genetic variants affecting the structural and/or immune functions of skin or other organs recapitulate features but not the entire phenotype of atopic inflammation and AD. *CARD11*, Caspase recruitment domain-containing protein 11; *CDSN*, corneodesmosin; *CTLA4*, cytotoxic T lymphocyte-associated protein 4; *DOCK8*, dedicator of cytokinesis 8; *DSG1*, desmoglein 1; *DSP*, desmoplakin; *FLG*, filaggrin; *FOXP3*, forkhead box protein 3; *IL2RA*, IL-2 receptor  $\alpha$ ; *IL4RA*, IL-4 receptor  $\alpha$ ; *IFNGR1*, IFN- $\gamma$  receptor 1; *MALT1*, mucosa-associated lymphoid tissue lymphoma translocation protein 1; *PGM3*, phosphoglucomutase 3; *RAG1*, Recombination-activating gene 1; *RAG2*, recombination-activating gene 2; *SPINK5*, serine protease inhibitor Kazal type 5; *STAT3*, signal transducer and activator of transcription 3.

in the protocol,<sup>15</sup> and the APT is not used routinely in clinical practice. Experimentally, the APT has provided insight into the temporal sequence of cutaneous cellular infiltrates. Acute skin lesions show a highly reproducible  $T_H2$ -dominant infiltrate,<sup>16</sup> although other cell types, including  $T_H17$  cells, are also present.<sup>17,18</sup> This  $T_H2$  dominance is in sharp contrast to other inflammatory skin diseases, such as psoriasis.<sup>19,20</sup> Time-course studies have shown that additional immune cell subsets, such as  $T_H1$  and  $T_H22$  cells, progressively infiltrate the skin during an ongoing APT reaction, mirroring the cellular composition of acute versus chronic human AD.<sup>17,21</sup> The APT has also been used to characterize dendritic cells within early lesional AD skin, such as inflammatory dendritic epidermal cells.<sup>18</sup> Furthermore, the APT has provided insight into the interaction of microbiota and our immune system, in particular the role of bacteria-derived superantigens acting as an amplifier of the allergen-specific cutaneous response in patients with AD.<sup>21-23</sup> In all these experimental APT studies, the population of patients with AD were well defined, with specific inclusion and exclusion criteria (although the precise definitions of AD varied); in most studies AD, together with specific IgE to the corresponding allergen used in the APT, was an inclusion criterion.

Hapten challenge to induce classical allergic contact dermatitis (ACD) in patients with AD has also broadened our understanding of AD pathogenesis (see Table E2). Whether patients with AD have an increased risk of ACD remains controversial and might depend on whether they harbor *FLG* mutations, which might have allowed increased allergen penetration. However, attenuated ACD reactions have been reported in patients with AD compared with control subjects in a severity-dependent manner.<sup>24,25</sup> This might be due to the fact that haptens induce distinct immune responses,<sup>26</sup> with fragrances mimicking the  $T_H2/T_H22$  dominance of AD, whereas nickel, 2,4-Dinitrochlorobenzene

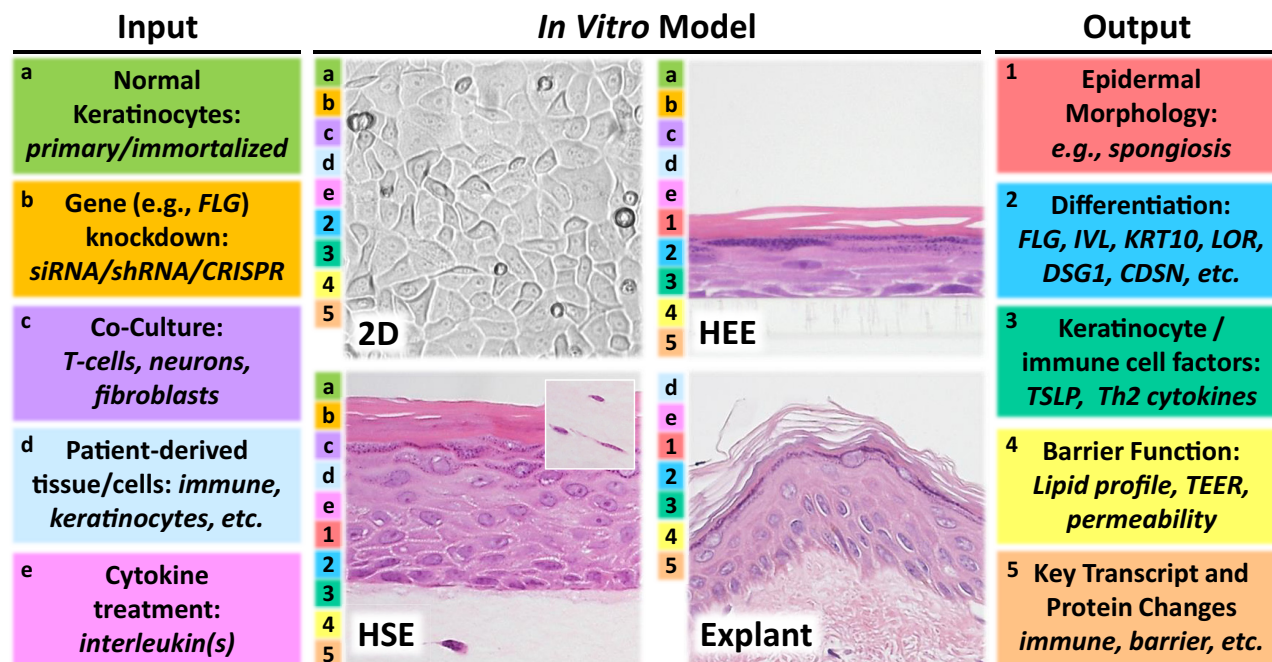
(DNCB), or imiquimod<sup>27</sup> induced  $T_H1/T_H17$ -skewed immune responses. Of note, patients with AD show a  $T_H2$ -skewed ACD reaction,<sup>28</sup> and this immune deviation might account for the diminished ACD prevalence in patients with AD. A  $T_H2$  immune reaction profile of patients with AD was also observed in an aero-challenge setup,<sup>29</sup> as well as when challenging patients with AD with physical factors, such as hard water.<sup>30,31</sup>

All current challenge models have some limitations (see Table E2) because they only represent acute reactions, and the small areas of application cannot reproduce the intense pruritus and sleep disturbances usually present in patients with AD. Furthermore, to date, they have not stratified for genetic differences/endotypes among patients with AD. For example, comparing APTs in patients with and without *FLG* mutations might be a useful line of future investigation. Moreover, in the future, molecular profiling of lesional skin from standardized challenge models adjusted according to AD endotype can be used in early clinical studies to evaluate the potential of new drugs to improve AD.<sup>32</sup>

## IN VITRO MODELS

As shown in Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org), there are several 2-dimensional cell-culture and 3-dimensional organotypic models for AD that complement each other in addressing specific experimental questions. Although 2-dimensional cell culture models (by definition) do not duplicate the architecture of skin, they are amenable to high-throughput techniques for drug discovery and target validation (2-dimensional model section, see Table E3). Accordingly, Otsuka et al<sup>33</sup> used 2-dimensional cultures to screen a chemical library for compounds that enhance *FLG* transcriptional activation and mRNA expression, suggesting a





**FIG 2.** Human *in vitro* models of AD. *In vitro* models can be designed to address specific experimental questions based on the input materials of the cultures. Assessment of the cultures or output depends on the type of culture. *CDSN*, Corneodesmosin; *DSG1*, desmoglein 1; *FLG*, filaggrin; *HEE*, human epidermal equivalent; *HSE*, human skin equivalent (inset, fibroblasts in collagen); *IVL*, involucrin; *KRT10*, keratin 10; *shRNA*, short hairpin RNA; *siRNA*, small interfering RNA; *TEER*, transepithelial electrical resistance; *TSLP*, thymic stromal lymphopoietin.

potential novel therapeutic agent for AD. On the other hand, 3-dimensional models replicate the stratified squamous epithelium of epidermis but require specific expertise and are time consuming. Epidermal equivalents consist of keratinocytes without a dermal compartment, whereas skin equivalents have a dermis, such as fibroblast-embedded collagen (3-dimensional model section, see Table E3). Both 2-dimensional and 3-dimensional models are amenable to treatment with disease-relevant cytokines, gene knockdown, use of patient-derived cells, and/or coculture (Fig 2 and see Table E3).

The immune system is a major driver of AD, and *in vitro* immune modulation with disease-relevant cytokines, such as interleukins, can lead to AD-like phenotypes in normal primary keratinocytes<sup>34</sup> and 3-dimensional models (3-dimensional cytokine model section, see Table E3).<sup>35-41</sup> Knockdown of filaggrin (*FLG*) in culture systems can provide insight into the molecular and proteomic changes associated with its loss in patients with AD,<sup>42</sup> and combining *FLG* knockdown with other perturbations, such as cytokine treatment, can be used to study the multifactorial drivers of AD. For example, Honzke et al<sup>43</sup> reported that *FLG* knockdown exacerbated epidermal responses to IL-4 and IL-13, including increased proliferation and keratinocyte-released cytokines in 3-dimensional skin equivalents. Patient-derived cells for 2-dimensional and 3-dimensional culture or tissue for explant culture are limited by access and availability but might be the most relevant in terms of modeling AD.<sup>44-47</sup>

Furthermore, patients' biopsy specimens can be a source of skin cells other than keratinocytes, allowing for coculture models. Given that multiple systems contribute to AD, coculture models that include immune cells, dermal fibroblasts, and neurons can

begin to address their interplay with keratinocytes. For example, Berroth et al<sup>47</sup> derived keratinocytes and fibroblasts from normal and AD skin and showed that AD-derived fibroblasts are sufficient to decrease *FLG* mRNA in normal-derived keratinocytes in 3-dimensional culture. Moreover, combining *FLG* knockdown with CD4<sup>+</sup> activated T cells uncovered direct cross-talk between keratinocytes and T cells that resulted in T-cell migration within the dermal compartment toward the epidermis.<sup>48</sup>

These studies highlight the levels of complexity that can be engineered into the 3-dimensional culture models. Three-dimensional culture systems have also been used to understand environmental influences on skin, including air pollution, UV radiation exposure, and bacterial infection.<sup>49-51</sup> Therefore these relevant environmental factors could be incorporated into *in vitro* models of AD. The 3-dimensional cultures and skin explants can also be used to assess the comparative efficacy and practical applicability of novel drug delivery systems.<sup>52,53</sup> Notably, despite the assorted methodologies applied in developing *in vitro* models of AD, there is overlap in the AD-like characteristics among the various models: most produce perturbed epidermal morphology, abnormal differentiation, and barrier dysfunction. Most often, disparities in reported phenotypes appear to stem, at least in part, from differences in the methodologies used in evaluating models (not necessarily because of the absence of the phenotype).

Although *in vitro* models might not mimic certain symptomatic and/or subjective aspects of the disease, such as pruritus and pain, they allow monitoring of changes in epidermal morphology and differentiation, gene and protein expression, lipid synthesis, and barrier function. Histologically, AD skin sections and most 3-dimensional models of AD show profound changes in the

epidermal compartment, including hypogranulosis, spongiosis, and increased cellularity because of hyperproliferation (3-dimensional model section, see Table E3). Changes in expression of genes (detected by using microarray, RNA-sequencing, or quantitative PCR) and protein (detected by using liquid chromatography–mass spectrometry, Western blotting, ELISA, or immunohistochemistry) can be used to evaluate disturbances in differentiation and immune response in 2-dimensional and 3-dimensional models. Lipid synthesis, which is required for optimal barrier function, can be monitored by expression of related enzymes or directly by using mass spectrometry. Epidermal barrier function can be monitored in 2-dimensional and 3-dimensional models, depending on the assay.

We recommend that the phenotype of any AD *in vitro* model should be extensively characterized and should include parallel analysis of epidermal morphology, differentiation status, loss or gain of key transcripts/proteins, analysis of immune components, and assessment of functional epidermal barrier parameters. Full characterization of any AD model can inform downstream evaluation of potential therapeutic agents with respect to reversing different aspects of the disease. Testing potential targets or drugs in several model types can add rigor and indicate whether a signaling pathway or protein is central to the diverse manifestations of AD.

## IN SILICO COMPUTATIONAL MODELS

A core element of a systems biology approach is development of *in silico* computational models (mechanistic models) by means of integration of different types of experimental and clinical data from multiple studies, including those associated with disease conditions. *In silico* experiments (ie, computer simulations or mathematical analysis of *in silico* models) can test model-specific hypotheses, predict disease prognosis or treatment outcomes, and identify knowledge gaps, guiding future experiments and clinical trials that produce further data. This iterative process refines *in silico* models, providing holistic systems-level mechanistic insight into how perturbations (treatments or risk factors) lead to whole-organism phenotypes.

A mechanistic model describes causative interactions between the system's components involved in the phenomena of interest (eg, disease or treatment outcomes). Existing mechanistic models of AD vary widely, depending on the levels of interaction (tissue, cells, proteins, and genes) included in the model and mathematical methods used to describe the interactions.

Domínguez-Hüttinger et al<sup>54</sup> developed a multi-scale deterministic model that delineates interactions between the environment, skin barrier integrity, and immune activation using ordinary differential equations (Table I).<sup>54–61</sup> Two bistable “switches” are described: the first regulating the onset of AD flares and the second controlling progression to severe and persistent disease. The model predicts, for example, that genetic predisposition to barrier dysfunction (eg, *FLG* haploinsufficiency) predisposes to longer flares and more persistent disease and that prophylactic emollient use might be beneficial (Table I).

Application of optimal control theory to the hybrid mathematical model can inform the design of patient-specific optimal strategies for “proactive therapy” to prevent recurrent flares once the disease has been brought under initial control.<sup>55</sup>

For example, this computational model supports the need for a greater topical steroid treatment dose after disease worsening and the potential need for more frequent than 2 to 3 days per week application of topical steroid treatment to maintain remission<sup>62</sup> in patients with *FLG* haploinsufficiency (Table I), presenting a readily testable stratification treatment regimen based on genotype.

Polak et al<sup>56</sup> developed a stochastic Petri net model that delineates genetic regulatory mechanisms responsible for immune responses in Langerhans cells (LCs; Table I). The model describes reported interactions between interferon regulatory factors, interferon regulatory factor transcription partners, and DNA sequences in a logic-based diagram. *In vitro* experiments validated model predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that a phosphoinositide 3-kinase  $\gamma$  inhibitor reduces the ability of LCs to induce T<sub>H</sub>1 responses. These smaller-scale and focused mechanistic models can describe detailed interactions that are difficult to include and validate in multiscale models. Inclusion of the detailed interactions would make the multiscale models too complex to interpret and validate because of the current lack of quantitative dynamic data measuring the variables across different scales simultaneously.

Subramanian et al<sup>57</sup> used a pathway model that included manually curated skin-specific pathways and relevant genes (Table I). Pathway enrichment analysis using transcriptomic data sets of patients with AD provided mechanistic insights into drug actions of topical betamethasone and pimecrolimus. The pathway model would allow *in silico* experiments once the kinetics parameters for pathways are identified to provide quantitative and dynamic predictions of disease progression and treatment outcomes.

Population pharmacokinetic and pharmacodynamic models have also been developed to describe differences and variability in pharmacologic effects observed in large clinical studies for AD treatment.<sup>58,59</sup> The authors identified the model parameters that best fit the effects of nemolizumab and dupilumab measured in terms of AD severity score or pharmacokinetics (Table I).<sup>58,59</sup> Population pharmacokinetic and pharmacodynamic models could help achieve mechanistic understanding of pharmacologic effects if combined with mechanistic models.

One of the challenges in developing mechanistic models is identification of the components and pathways relevant to the model-specific hypothesis to be tested. This can be achieved by using unbiased multivariate analyses of a collection of large-scale data, for example by using machine learning data analysis. Application of machine learning methods to AD-related data is relatively limited at present, but some relevant works have been already published. Thijs et al<sup>60</sup> developed a piecewise linear mixed model to predict AD severity scores after different treatments, and Kiiski et al<sup>61</sup> developed a multivariate logistic regression model to predict a “good treatment response.” A sufficient level of cross-validation is crucial to reduce bias and ensure the general applicability of models with predictive power beyond mere data description.

All the models presented above were developed based on the published data derived from studies in which the inclusion and exclusion criteria for AD were specified. Although the majority of studies used the Hanifin and Rajka criteria and specified further clinical (including comorbidities) and demographic details, it is clear that patients with AD present with a wide spectrum of

**TABLE I.** *In silico* computational models of AD

Model type	Scientific merits	Clinical utility	Limitations	Key features	Key findings/ predictions	References
Multiscale mechanistic model	Mechanistic understanding of system-level effects of potential triggers and processes on disease state	Identification of therapeutic targets and their mechanisms for further clinical investigation  Prediction of dynamic effects of therapeutics, leading to patient stratification	Models developed based on hypothesized relationships that were previously described experimentally	A hybrid ordinary differential equation model of the dynamic interplay between skin barrier function, immune responses, and environmental stressors that determines AD pathogenesis	Preventive effects of emollients against AD progression (shown by clinical trials)  Synergistic effects of environmental (eg, microbiome) and genetic (eg, FLG) risk factors on AD progression (shown by mouse experiments with ovalbumin challenge or dose-dependent effects of FLG deficiency)	Dominguez-Huttinger et al <sup>54</sup>
				A hybrid model of treatment effects of corticosteroids and emollients on AD pathogenesis and exploration of optimal regimens for induction of remission and maintenance of remission	Poor adherence to the suggested optimal treatment schedule leads to higher treatment doses.  Application of corticosteroids for 2 consecutive days per week is optimal for the maintenance period.	Christodoulides et al <sup>55</sup>
Gene regulatory network model	Understanding of gene regulatory mechanisms behind disease processes	Identification of therapeutic targets and their mechanisms at the gene regulation level	Models developed based on published genetic interactions	Stochastic Petri net model of interferon regulatory factor gene regulatory network in response to <i>in vitro</i> treatment of LCs with TNF- $\alpha$ and TSLP	<i>In vitro</i> experiments validated predictions that the ability of LCs to present a peptide is altered by cytokine milieu and that PI3K $\gamma$ inhibitor reduces the ability of LCs to induce T <sub>H</sub> 1 responses.	Polak et al <sup>56</sup>
Pathway models	Understanding of disease mechanisms	Identification of therapeutic targets and their mechanisms	Models developed based on published pathways	A pathway model including 35 manually curated skin-specific pathways and >2600 genes	Pathway enrichment analysis using transcriptomic data sets of 10 patients with AD treated with betamethasone valerate and pimecrolimus predicted mechanism of action of both drugs on human skin.	Subramanian et al <sup>57</sup>

(Continued)



TABLE I. (Continued)

Model type	Scientific merits	Clinical utility	Limitations	Key features	Key findings/predictions	References
Population PK/PD models	Understanding of differences and variability in pharmacologic effects among a target population from clinical trial data	Prediction of optimal dose regimen Testing effects of weight, sex, etc	Requires large clinical data to have sufficient predictive power	PK/PD model for serum nemolizumab and pruritus VAS developed from 299 patients' time course data Two-compartment PK model for dupilumab developed from data of 197 healthy volunteers and patients with AD from 6 studies	An appropriate flat dose regimen that is independent of body weights is used. Production rate of IL-4Ra is similar for patients with AD and healthy volunteers and does not change over time.	Saito et al <sup>58</sup> Kovalenko et al <sup>59</sup>
Machine learning predictive models	Unbiased analyses of differences between disease and nondisease (including treated) tissue/patients and prediction of clinical outcomes (prognostic and therapeutic)	Identification of disease and therapeutic targets Findings can feed into mechanistic models	Causative mechanisms remain largely unknown Machine learning applications to atopic eczema relatively limited at present	Piecewise linear mixed models to predict EASI scores at 3 future time points from baseline biomarkers Developed from data of 150 serum biomarkers measured in 193 patients with AD Multivariate logistic regression model to identify predictors of long-term response to topical maintenance treatment in AD on 169 patients	Combination of TARC, IL-22, and sIL-2R provides a good predictor for future EASI score. Serum total IgE (rather than the initial severity) is the most important factor predicting a good long-term treatment outcome.	Thijs et al <sup>60</sup> Kiiski et al <sup>61</sup>

EASI, Eczema Area and Severity Index; IL-4Ra, IL-4 receptor antagonist; PI3K $\gamma$ , phosphoinositide 3-kinase  $\gamma$ ; PK/PD, pharmacokinetics/pharmacodynamics; sIL-2R, soluble IL-2 receptor; VAS, visual analog scale.

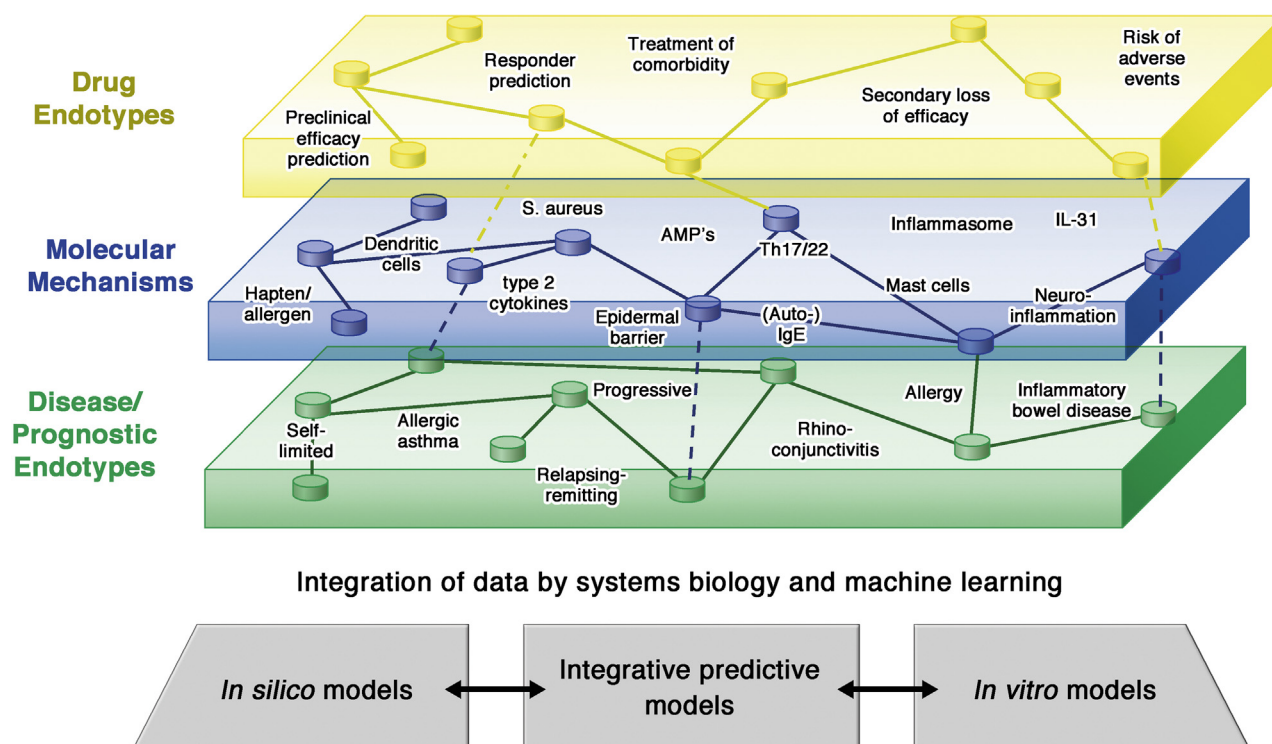
clinical and molecular features (including, for example, a greater heterogeneity in transcriptomic profile of lesional skin compared with psoriasis).<sup>63</sup>

## FUTURE DEVELOPMENTS

The development of more sophisticated human and computational models of AD that integrate large-scale clinical and “omics” data offer the potential for a deeper understanding of disease endotypes, molecular mechanisms underlying key pathogenic events and clinical hallmarks of AD, as well as prediction of therapeutic outcomes, including comorbidity at the level of an individual patient. Accepting that, by definition, these models are based on a reductionist approach, they need to reflect the complexity of AD pathogenesis, including epidermal barrier dysfunction, altered penetration of chemicals and allergens, host/environment interaction, type 2 immunity, and tissue remodeling. We have illustrated in this review that the main

approaches available today are *in vitro* models, identification and characterization of human inherited syndromes resembling AD, *in vivo* challenges of patients with AD, and *in silico* models. Here we speculate how the future of AD research will likely inform the development of more refined models of AD.

Refinement is likely to depend, at least in part, on methodological advances in the field and the additional information generated by novel approaches. For example, single-cell sequencing has recently identified novel rare but important immunologic subsets,<sup>64</sup> and intravital photon microscopy has enabled visualization of cell-cell communication during inflammation.<sup>65,66</sup> Application of this technology to AD is likely to inform the inclusion of distinct epithelial and immune cell types<sup>64</sup> and/or genetically modified primary human cells.<sup>67</sup> Furthermore, small-scale spheroid organoids can enhance high-throughput approaches in the field.<sup>68</sup> Finally, we expect that a technological breakthrough in the development of 3-dimensional skin models will be facilitated by cell printers.<sup>69,70</sup>



**FIG 3.** Interconnected multilayer networks: the future of human AD modeling. A combination of innovative *in vitro* and *in silico* models obtained by a systems biology approach and machine learning algorithms will be needed to answer clinically relevant questions, such as identification of distinct disease endotypes, elucidation of molecular pathomechanisms, or prediction of therapeutic response.

Deep neural networks are being applied as artificial intelligence tools to facilitate physician interpretation in the field of melanoma diagnostics<sup>71</sup> and increasingly as methods to enable large data set integration. The first examples of disease classifiers<sup>72</sup> and prediction of disease severity from biomarker sets<sup>61,73,74</sup> have recently been published, and we expect this line of development to continue while ensuring a sufficient level of validation. We anticipate that refinement of these methods, in combination with *in silico* models, can lead to computational approaches and predictive models applied to diagnostics and therapeutic stratification.

The descriptive disease ontology of inflammatory skin diseases will need to be revised by shifting to pathogenesis-oriented structure<sup>75</sup> and, in the future, by better definition of disease endotypes based on integration of multiomics data, clinical features, and clinical response to therapy in light of *in silico* models as assessed in large-scale and longitudinal cohorts.<sup>76</sup> These advances are likely to inform the development of many of the current models.

However, to achieve a substantial breakthrough, we expect that different approaches will need to be combined, integrated, standardized, and performed at larger scale (Fig 3). For example, observations made in rare human disease variants or through specific challenge models in patients with AD can be validated *in vitro* and mapped to disease signatures *in silico*. Validation of functional hypotheses will increasingly depend on cross-referencing of data derived from clinical samples with outputs from *in vitro* models. Integration of clinical, biomarker, pharmacokinetic and pharmacodynamic (topical and/or systemic), and clinical outcome data will inform therapy

development and precision medicine. Notably, all of our models depend on how precisely a particular question is asked and the quality of the clinical input, including the clinical metadata and integration with “omics” data derived from clinical samples. Finally, advanced statistical and machine learning analysis combined with *in silico* predictive modeling will be required to integrate information throughout all described layers and data sets to elucidate underlying mechanisms (and endotypes), further highlighting the importance of data standardization and scientific networking.

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