

Epigenetic determinants of allergy and tolerance

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Summary

Not only genetics and environmental factors are responsible for the development of allergies. Epigenetic mechanisms – that are master regulators of gene transcription and thus of the accessibility and use of genome information – have been identified as a third power that is key in determining many features of allergy. So far, three epigenetic mechanisms have been identified in humans: histone modification, DNA methylation, and posttranscriptional modification by microRNA (miRNA) or small interfering

RNA (siRNA). In recent years, it became clear that epigenetic regulation of gene function does not only play a role in the development of allergy but may also play a key role in the induction of tolerance against allergens, holding the promise to truly heal or prevent atopic diseases in the future.

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Genetic predisposition and environmental factors contribute to the development of allergies at an early age

Genetic predisposition and environmental risk factors determine the development of allergic sensitization and atopic disease. Applying modern genetic technologies of genome wide mutation analyses, genetic variants in a number of genes have been associated with the susceptibility to become sensitized to allergens [1], to produce elevated levels of IgE [2], and to develop atopic diseases such as dermatitis [3], asthma [4], allergic rhinitis [5], and food allergies [6]. Intriguingly, very little of this genetic susceptibility is shared between these immune and disease entities that we like to consider related in clinical terms and summarize as atopic diathesis. Indeed, these findings contradict somewhat the idea that there is an atopic march where allergic sensitization and the development of atopic diseases is understood as a step by step process where one builds on the other, finally leading to a severe atopy phenotype. Rather, the genetic data suggests that there are distinct susceptibility patterns specific for certain disease phenotypes from the beginning and only very few genetically determined mechanisms are shared between more than one atopic disease.

For the role of the environment, it becomes increasingly clear that timing of risk factors is of

utmost importance for disease development. Many environmental factors conferring either risk (such as environmental tobacco smoke) or protection (such as growing up on farms) have their greatest impact on disease development when they are present very early [7, 8]. What “early” means in this context is a matter of debate: certainly early childhood, most likely first year of life and potentially already during fetal development, are viewed as distinct windows of vulnerability for disease development. It is important to note here that disease development does not necessarily (nor in the majority of cases) mean that there is an immediate clinical phenotype present. Rather, it is immunological changes and subclinical phenotypes that are observed early as precursors of atopic diseases. When atopic disease presents clinically for the first time in a patient, it is often difficult to assess and in many cases, first symptoms precede the diagnosis by months or even years.

Genetic susceptibility and environmental factors interact to determine allergies – but how?

Numerous studies have identified either genes or environmental factors that play a role in allergies. A gene by environment interaction was postulated suggesting that in individuals with a certain genetic

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predisposition, the exposure to a specific environment will lead to disease (when that exposure occurs at a vulnerable time) [9]. While this is an intriguing and unifying hypothesis able to combine individual findings, very little data exists where gene by environment interaction for allergic diseases was actually shown, let alone validated and replicated. One of the few examples for such an interaction is the observation that individuals who have a genetically determined deficiency in certain detoxification enzymes (glutathione S-transferases M1 and T1, GSTM1 and GSTT1, respectively) are more susceptible to develop asthma when exposed to environmental tobacco. This has been shown by our group for German children [10] and in parallel for a Southern Californian population of children [11].

On the other hand, gene environment effects have also been suggested for factors providing protection against the development of allergies: genetic variations in pathogen recognition receptors such as toll-like receptors (TLRs) and their co-receptors such as CD14 are associated with the development of asthma [12, 13] and allergies [1, 14]. For CD14, a gene environment interaction was reported: depending on a certain CD14 genotype in the promoter of the gene, exposure to bacterial lipopolysaccharides (LPS) had a dose-dependent effect on the development of asthma/allergy [15]. These findings are backed by numerous reports that genetic variants in different TLRs (most prominent in the TLR1/6/10 locus) and downstream signaling pathway proteins influence asthma and allergy [12]. In a systematic analysis of these pathogen recognition pathways, gene products of genes showing association with asthma and allergy were either located very upstream and involved in pathogen recognition or very downstream involved in effector mechanisms such as mitogen-activated protein kinases (MAPK) [16].

While these interactions could indeed and to some extent explain the development of allergies mechanistically, they have one caveat: the genetic setup is rather static and will change relatively slowly and only over generations. One could argue that here the driving force is the environment, changing so rapidly and massively that the allergy endemic within the last two generations could be explained. Another, more revolutionary theory is that DNA based mechanisms are not the only genetic code determining gene function and response to the environment. This so-called second code is called epigenetics.

Epigenetics – the missing link?

Epigenetics defines heritable mechanisms that influence gene function through gene expression not caused by changes in the nucleotide sequence of the

genetic code itself. Epigenetic mechanisms so far identified to be important in humans are post-transcriptional histone modification, small and other non-coding RNAs (siRNA and miRNA), and DNA methylation [17] (**Fig. 1**). Chromatin configuration, that is the organization of the genome, plays the central role as it controls accessibility of DNA (for transcription and thus use of DNA based information) by its confirmation. Open accessible chromatin (euchromatin) and closed non-accessible chromatin (heterochromatin) are controlled by modification proteins, so-called histones, that form the basic unit with DNA in which the genome is organized in the nucleus, the nucleosome. The nucleosome consists of an octamer of histone (H) proteins containing an H3-H4 tetramer flanked on either side with an H2A-H2B dimer around which exactly 146 base pairs of DNA are wrapped.

Histone proteins can be chemically modified by acetylation, methylation, phosphorylation, and ubiquitylation [18], changing their configuration and in turn the shape of the nucleosome from closed (e.g., acetylated) to open (e.g., deacetylated). Thus, the state of the nucleosome is seen as the master controller for translation as its accessibility is the first step necessary for gene transcription. If its configuration remains closed in a heterochromatin state in the area of a gene, this gene cannot be transcribed and translated into protein. The enzymes histone acetylases and histone deacetylases regulate histone modification by adding or retracting acetyl groups.

The second important epigenetic mechanism controlling gene expression by regulation of transcription accessibility is methylation of DNA [19]. It is the only genetically determined modification of DNA so far known in mammals. Methylation is the modification of the pyrimidine ring of the DNA nu-

Abbreviations

GSTM1	Glutathione S-transferase M1
GSTT1	Glutathione S-transferase T1
H	Histone
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
miRNA	MicroRNA
RNAi	RNA interference
siRNA	Small interfering RNA
SOTI	Specific oral tolerance induction
TLR	Toll-like receptor
Treg	T regulatory cell

cleotide cytosine by a methyl group. Cytosines are spread throughout the genome but they are found in a specific configuration as CpGs (which is a dinucleotide formed by cytosine and guanine) in regulatory regions of the genome such as gene promoters. Normally, the majority of CpGs are methylated acting as “stop signs” for transcription and thus, areas where they occur methylated, are closed for transcription. Induction of transcription is an active and targeted process that needs demethylation of a specific locus by enzymes. Only then transcription can start.

Once transcription from open unmethylated euchromatin DNA to messenger RNA (mRNA) has occurred, one further and last epigenetic mechanism controlling gene expression is RNA inter-

ference (RNAi). MicroRNAs (miRNAs), which are small single-stranded non-coding RNAs highly conserved across species, act as regulators of transcription efficiency by degrading and thus counteracting mRNA [20]. Sequences for miRNAs are localized in intergenic regions (between genes) and intronic regions (between exons of genes) and expressed in a tissue-specific manner.

All three mechanisms described above are capable of regulating biologically relevant and disease associated processes. Epigenetic control is especially important in immune regulation as it allows rapid adaption to danger signals and switching immune pathways on (and off) in a cell specific and delicately balanced manner. Epigenetics can potentially explain a great number of phenomena observed in complex diseases like allergies: population effects, changes in disease prevalence in a short period of time, inducibility of disease by the environment. But above all, epigenetics, other than genetics, holds one big promise: due to its reversibility it could be a key mechanism in inducing tolerance, reverting allergy, and true healing.

Epigenetic signatures are associated with allergic sensitization and allergic disease

The tools to study epigenetics in complex diseases have been developed just recently. High throughput assessment of epigenetic signatures on a genome-wide level is so far available for methylation analyses. Thus, there is the greatest progress and data pool available for association with human disease. On the other hand, immune mechanisms per se have been studied on a cellular level in great detail and so the role of epigenetic signatures has been established comprehensively and for all three levels of epigenetic regulation lined out above. Specifically, this has been shown in detail for T regulatory cells (Tregs), where methylation of the FOXP3 gene is the key event in the development of these cells from the T cell pool [21]. But also for Th1/Th2 development, epigenetic mechanisms, e.g., in the IL4/IL13 locus on chromosome 5q21, play a central role [22].

In humans, multiple methylation signatures across the genome are significantly associated with total IgE levels across populations as recently reported [23]. These differences in methylation marks are found in genes already previously linked to allergies and IgE levels such as IL4 and IL5 receptor but also in genes not related to these immune states so far such as LPCAT2 (involved in lysophospholipid metabolism), L2HGDH (a mitochondrial oxidoreductase), and in the transcription factor ZNF22. The top three association signals with methylation explain approximately 13 % in IgE variance in these populations, and thus far more than the top single

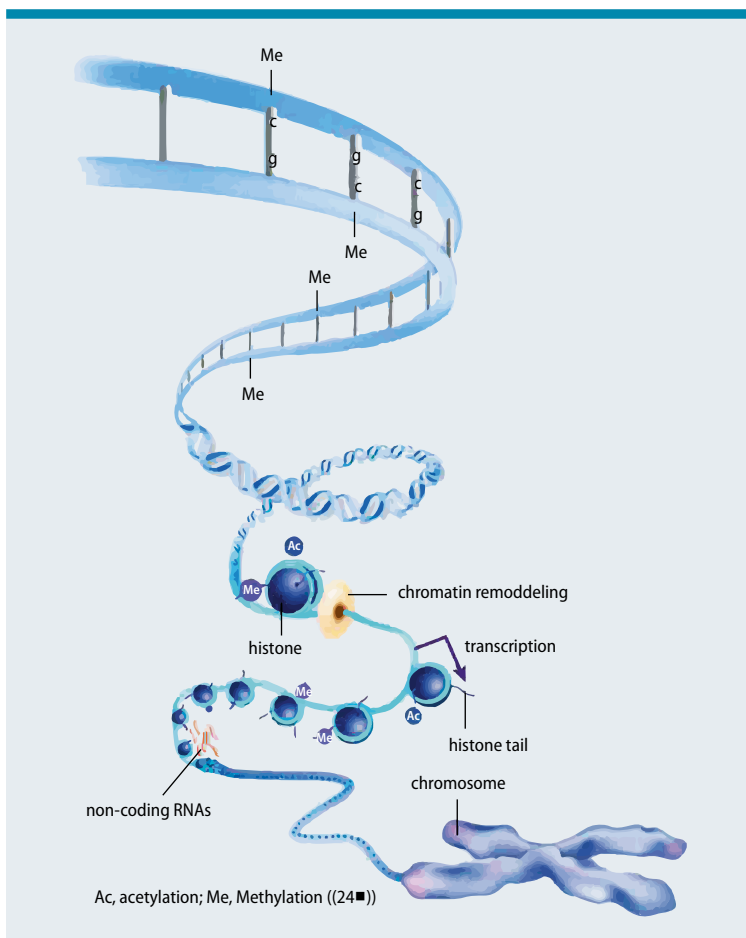


Fig. 1: Major epigenetic mechanisms. Genetic information of the coding nucleotide sequence (genome) is organized in a tertiary structure as chromatin interacting with supporting proteins (histones) and forming nucleosomes. Methylation of cytosine defines the accessibility of promoter regions for transcription, histone modification influences the state of nucleosomes and the opening and closing of chromatin. Non-coding RNAs (miRNA, siRNA) is a posttranscriptional mechanism to tightly control levels of protein translation.

nucleotide polymorphisms linked to IgE previously. In this study, patients with established allergies and asthma were included in an analysis of methylation and IgE levels at only one time, and therefore IgE levels as well as methylation may be driven in part by disease state.

In atopic dermatitis, a pilot study [24] investigated differences in DNA methylation in patients with atopic dermatitis and healthy controls in a number of tissues (affected and unaffected skin) and different subsets of blood cells (whole blood, T cells, B cells) using a first generation methylation chip (Infinium Human Methylation 27 Bead Chip, Illumina, Inc.) and RNA expression chip (Human-HT-12 v3, Illumina, Inc.). In this very well designed study, major differences in methylation and gene expression were mainly found in genes involved in the epidermal differentiation complex when lesional to non-lesional epidermal samples were compared, whereas blood cells were a less optimal surrogate for epigenetic and transcriptional mechanisms in atopic dermatitis. While with larger sample sizes also significant differences in blood may have been found between individuals with and without atopic dermatitis, local changes on the site of inflammation in atopic dermatitis may be missed if blood only is used in such analyses.

It is not clear from these studies whether methylation of certain loci are the consequence of the disease they are associated with (biomarker) or whether methylation changes precede the occurrence of the disease (predictor). If methylation changes precede disease, this would be an indication that they are functionally involved in the development of the disease. This can only be assessed in time series of individuals that are studied before and after the onset of the disease. Such an analysis has been performed in a small study of children developing food allergy [25]. Genome-wide DNA methylation profiles from CD4+ T cells at birth and at the age of one year were analyzed in 12 children with and 12 children without IgE mediated food allergy diagnosed at the age of one year. Differences in CD4+ methylation signatures were found in 179 loci at the age of one year and in 136 loci at birth. Approximately 30% of these different methylation signatures coincided with previously annotated gene polymorphisms, and thus are potentially driven by genetic predisposition. However, almost 100 methylation differences were found at birth when individuals were initially disease-free, potentially implicating these loci play a causal role in the development of food allergy. Pathway analysis of differentially methylated genes identified several MAPK signaling molecules. Therefore, the authors conclude that dysregulation of DNA methylation at MAPK

signaling-associated genes during early CD4+ T cell development may contribute to the development of food allergy.

When we analyzed children growing up on farms for epigenetic signatures across the genome in a pilot project, we found that methylation marks differed strongest between farm children and non-farm children at birth (measured in cord blood), while differences between children with and without asthma were more pronounced at age 4.5 years compared to birth. However, the most significant differences were observed when changes in methylation over time from birth to age 4.5 years were compared between children that developed asthma and those that did not (own unpublished data). When pathway analyses were performed, methylation differences clustered in signaling cascades of the innate immune system and around MAPK signaling genes, surprisingly similar to methylation findings on food allergy development.

Epigenetic mechanisms play a role in tolerance inductions

Epigenetic mechanisms may not only be involved in the development and maintenance of allergic diseases but also in the induction of tolerance against allergens. This can be studied in individuals undergoing immune therapy for allergies. Indeed, one study has evaluated the effects of specific oral tolerance induction (SOTI) to peanuts investigating immunological and epigenetic changes due to therapy [26]. Three groups of individuals allergic to peanuts were analyzed: individuals with peanut allergy that did (group 1, n = 7) and did not (group 2, n = 13) respond with tolerance induction to SOTI and those that did not receive therapy but avoided peanuts (group 3, n = 20). While neither changes in IgE nor IgG levels before and after treatment were significantly associated with successful immune therapy and tolerance induction, the level of antigen induced T regulatory cells was significantly increased in individuals with positive reaction to SOTI. These Tregs showed increased migratory activity correlating with changes in FOXP3 methylation, a single feature differentiating individuals responsive to SOTI to those that were not.

Is epigenetic the key to manipulate fate (at least in allergy)?

The data recently evolving in epigenetics suggests that epigenetic mechanisms, and here we have only discussed methylation in detail, are involved and relevant in all aspects of allergy (Fig. 2). Epigenetic signatures

- a. identify children susceptible for the development of allergy (allergic sensitization and subclinical

disease development) before clinical disease is present,

- are associated with the presence of allergic diseases, and
- are associated with the successful induction of tolerance in patients with allergic diseases.

Epigenetic marks change over time and can be manipulated. Therefore, it is obvious that modification of epigenetic signatures is a potential therapeutic concept. In fact, epigenetic modulation is already a key issue in allergy therapy: corticosteroids, the most potent medication for allergies is an extremely strong epigenetic modifier. However, corticosteroids are untargeted epigenetic modifiers and what one would like to achieve is targeted manipulation of epigenetics as nature is capable of doing in a cell specific and timely manner. Observation and better understanding of the natural mechanisms and course of methylation in allergy will be the first steps necessary towards targeted epigenetic therapy and/or prevention. Many obstacles will have to be overcome but the promise is that epigenetic modification can lead to true healing of a disease that is affecting millions of people worldwide for all their lives. Pharmaceutical industry has already invested heavily in drug development [27] for the use in cancer and there is

hope that – learning from this field – epigenetic therapy will also reach allergies.

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Conflict of interest

The author declares that there are no conflicts of interest.

Annotation

This review article is based on a presentation at the German Allergy Congress 2015.

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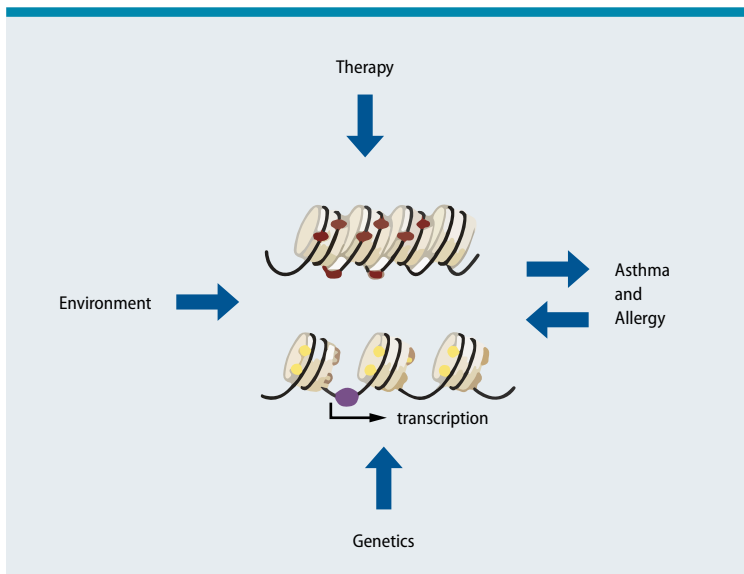


Fig. 2: Chromatin state (open euchromatin and closed heterochromatin) and accessibility of transcription is determined by environmental exposure and genetic predisposition (as mutations and variants can influence methylation sites). Epigenetic signatures are important for disease development. On the other hand, disease itself can modify epigenetic signatures (e.g., through chronic inflammation and transcription factor activation). Epigenetically active therapy can reverse epigenetic signatures.

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