

Mouse Models for the Genetic Dissection of Atopy

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Key Words

Mouse models · Genetics · Linkage · Atopic phenotypes · Airways · Skin · IgE · Gene disruption · Transgenesis · ENU mutagenesis

Abstract

Atopic disorders are complex traits with a significant contribution of heritability. Several mouse models for atopic phenotypes such as allergen-induced and intrinsic asthma, atopic dermatitis and immediate cutaneous hypersensitivity have been used to systematically dissect the genetics of these diseases. Many of the chromosomal regions identified in genome-wide screens colocalize with linkage regions of autoimmune phenotypes suggesting common regulatory genes in combination (or addition) to disease-specific genes. Moreover, many of these regions correspond to regions found in the human system, thus strengthening the likelihood of a gene contributing to the phenotype. The role of individual candidate genes has been vigorously pursued in genetically modified mice by destruction or overexpression of the candidate gene. The complex interactions of multiple contributing genes may further be elucidated in randomly mutated mouse strains. Thus mouse models can contribute largely to the elucidation of disease genes.

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Introduction

Atopic diseases as many other immunological diseases are multifactorial complex traits with a significant impact of both genetic and environmental factors on the outcome of the disease. Inbred mouse strains have proven very valid for the dissection of such complicated phenotypes: (1) phenotypic variance between genetically identical individuals is caused solely by environmental and stochastic events; (2) segregation of genomic regions or candidate genes can be investigated in large offspring populations; (3) linked chromosomal segments can be further refined with congenic mouse strains, where parts of the candidate region are introgressed into an identical genetic background; (4) single and combined functions of candidate genes can be investigated by mutation through gene disruption, transgenesis and cross-breeding of such strains; (5) random mutations within the genome through chemicals can detect novel candidate genes, which are involved in the critical pathways and networks of these complex traits.

This review summarizes the genetics of atopic phenotype models of 'intrinsic' and allergen-induced asthma as well as immediate and chronic hyperreactivity of the skin and aberrant plasma IgE levels. The data results not only from linkage analysis of genome-wide microsatellite scans, but also from usage of gene expression profiling, which allows progression from a quantitative trait locus (QTL) to a gene.

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Table 1. Genetic linkage to atopy-related phenotypes of the respiratory system

Chromosome	Position cM	Syntenic human loci	Candidate genes	References
2	5	10p11-13 2q12-q14 9q22-q34	<i>Gata3</i> <i>Il1rn</i> <i>Il15ra</i>	Ewart et al. [17]
2	20	9q33-q34 2q14-q24	<i>Hc, Lcn2</i> <i>Ptgs 1</i>	Ewart et al. [17]
2	73	2q14	<i>Il1b</i>	De Sanctis et al. [15]
6	50	3p26-24	<i>Il5ra, Gnb, Ly36, Ly49</i>	De Sanctis et al. [14] Ewart et al. [23]
7	1.5	19q13	<i>Il11, Gaba receptors</i>	Ewart et al. [17]
7	20	19q13	<i>Klk</i>	De Sanctis et al. [14]
9	18	11q23	<i>Il10r, Il18</i>	Zhang et al. [1]
10	44	12q22-24	<i>Ifng</i>	Zhang et al. [1]
11	7	5q23-31	<i>Rel, Tpm1, Il4 cluster</i>	Zhang et al. [1]
11	52	17q12-q22	<i>Nos2, Scya11, CC chemokines</i>	Zhang et al. [1]
15	43	22q13	<i>Il2rb, Il3rb1, Il3rb2, Pdgfb</i>	De Sanctis et al. [15]
17	10	6q21	<i>Mhc, Tnfa, Mcpt6, Mcpt7</i>	Zhang et al. [1] De Sanctis et al. [14] De Sanctis et al. [15]

Genetic Linkage to Atopy-Related Phenotypes of the Respiratory System

Several studies have investigated the genetics of atopy-related phenotypes of the respiratory tract in inbred mouse strains. Airway hyperresponsiveness (AHR), airway eosinophilia (AE) and pulmonary inflammation (PI) after sensitization with ovalbumin (OVA) have been used as quantitative, allergen-induced asthma-like phenotypes. In addition, intrinsic asthma has been studied through the analysis of AHR after challenge with methacholine or acetylcholine without prior allergen sensitization.

Linkage of Allergen-Induced Asthma-Related Phenotypes

The first genome-wide linkage analysis of allergen-induced AHR was carried out in a cross of Biozzi and BALB/c mice [1]. Biozzi mice develop PI, AE and AHR after immunization and subsequent challenge with OVA, whereas BALB/c mice show no response. Accordingly AHR and AE were chosen as principal phenotypes in this study. Four chromosomal regions on chromosome 9, 10, 11 and 17 were found for AHR, whereas only one region – on chromosome 11 distinct from that of AHR – showed linkage to AE (see table 1).

The region on chromosome 9 harbors the genes for interleukin-18 (*Il18*) and the IL-10 receptor (*Il10r*) in a distance of 3 cM. IL-18 has been shown to be increased in plasma of asthmatic patients [2]. Furthermore, IL-18 plasma concentrations might reflect the disease activity of recent asthmatic exacerbations [3]. IL-10 acts as a strong suppressor of Th1 responses and – like IL-18 – is elevated in serum of asthmatic patients [2]. Therefore, both genes represent reasonable candidate genes.

The region on chromosome 10 is syntenic to human chromosome 12q22–24, which has been shown to be linked to asthma and IgE levels in different ethnic populations [4–6]. The region of linkage is about 20 cM proximal to the location of the gene encoding the major Th1 cytokine interferon- γ (*Ifng*). IFN- γ is of great importance in suppressing allergic responses. However, the great distance of 20 cM between *Ifng* and the peak of linkage might suggest that other genes are responsible for the observed linkage effect.

The region linked to AE on chromosome 11 is syntenic to the human region 5q31 encoding the IL-4 cytokine cluster. Many population studies as well as meta-analyses of different human populations revealed linkage of this region to asthma, atopy, total IgE levels and AE [7–9]. Moreover, several associated polymorphisms within candidate genes have been identified. In the mouse system, one strong additional candidate gene of this region is

c-Rel, a transcription factor belonging to the NF κ B family. Specifically, c-Rel has been associated with AHR, PI, AE and total serum IgE levels [10].

The QTL for AHR on chromosome 11 includes two possible candidate genes: the genes for eotaxin (*Scya11*) and nitric oxide synthase 2 (*Nos2*). The syntenic region in humans, chromosome 17, has also been linked to asthma susceptibility. Eotaxin is responsible for the selective migration of eosinophils to the airways [11]. The effects are mediated via the chemokine receptor 3 (CCR3). Eotaxin can also bind to CCR5, a receptor shared with another chemokine, RANTES. RANTES is located in the syntenic human region and a functional promotor polymorphism has been shown to be associated with atopic dermatitis [12]. NOS2 has an important role in the regulation of the innate immunity and to a smaller extent in adaptive immunity. It has been suggested that it is involved in the development of asthma; however, NOS2 knockout mice show the same allergen-induced AHR as wild-type mice [13]. Thus it seems unlikely that *Nos2* has a critical role in the development of allergen-induced AHR in mice.

Finally, suggestive linkage of AHR was found to the MHC cluster (*Mhc*) on chromosome 17. The same region is linked to methacholine-induced AHR in different inbred mouse strains [14, 15]. The syntenic human region on chromosome 6p21 has been linked to various atopic phenotypes, and several polymorphisms in the *Mhc* and *Tnfa* genes are in association with asthma, specific sensitization and total IgE levels [16].

A second genome-wide linkage scan of OVA-induced AHR was performed in A/J $\times$ C3H backcross mice [17]. Out of 5 mouse strains tested, these 2 inbred mouse strains show the greatest differential expression of allergen-induced AHR, A/J mice expressing the high-responder phenotype and C3H the low-responder phenotype. Two distinct regions of linkage on chromosome 2 and one region with suggestive linkage on chromosome 7 were identified. Interestingly, none of these loci overlaps with the loci found in the genome-wide screen in Biozzi mice reflecting the impact of genetic heterogeneity even in the offspring of homozygous mouse strains.

The most promising candidate gene within one region on chromosome 2 is the gene for GATA3 (*Gata3*). GATA3 is a transcription factor, which has an important role for Th2 cytokine expression in mice. A dominant negative mutant of GATA3 inhibits allergen-induced inflammation in a murine model of asthma [18]. Other candidate genes of this region include the gene for the IL-1R antagonist (*Il1rn*), the mannose receptor and the IL-15R α subunit (*Il15ra*).

Part of the second locus on chromosome 2 is syntenic to human chromosome 9q, which was linked to human asthma susceptibility and elevated serum IgE levels [19]. It harbors the genes for complement factor 5 (*Hc*), lipocalin (*Lcn2*) and prostaglandin synthase 1 (*Ptgs1*).

The relevance of the candidate genes within the three QTL regions was further investigated through pulmonary expression profiles with oligonucleotide microarrays [20, for a review see 21]. Only one locus was both differentially expressed in pulmonary tissue and a QTL in the genome screen: C5 was shown to be expressed to a large extent in low-responder mice and to a small extent in high-responder mice. The high-responder mice carry a 2-base pair deletion in the first exon of C5, which leads to a translation stop and to the absence of C5 protein. Thus C5 may have an important protective function in the development of allergen-induced AHR in mice.

Linkage of Intrinsic AHR

Genetic linkage of intrinsic AHR in mice was tested in three genome-wide linkage studies using various inbred mouse strains and slightly different phenotypes [for a review of the methods to determine AHR in mice, see 22]. The first study used a cross of A/J and C57BL/6J mice to test for linkage of methacholine-induced AHR [15]. The authors identified three potential regions of linkage on chromosome 2, 15 and 17. The region on chromosome 2 is syntenic to human chromosome 2q14 which has already been linked to human asthma in different studies. Possible candidate genes of this region are diverse IL-1-like molecules. The region on chromosome 15 also harbors different interleukin receptor genes like *Il2rb*, *Il3rb1* and *Il3rb2*. Finally, the region on chromosome 17 overlaps with the locus for allergen-induced AHR in Biozzi mice as described above [1].

In a second genome-wide linkage study AHR was induced by a single intravenous dose of the bronchoconstrictor acetylcholine in progeny derived from crosses between A/J and C3H/HeJ mice [23]. Only one region of significant linkage was identified, located on chromosome 6. By using multiple inheritance models the investigators concluded that the obtained data were best described with a mixed general model in which the inheritance pattern was determined by one major locus along with a polygenic component. The same region has been linked to immediate cutaneous hypersensitivity and in this context has been studied up to functional analysis of a candidate gene within this region [35]. The results are discussed in detail further on in this review article.

Table 2. Genetic linkage to atopy-related phenotypes of the skin

Chromosome	Position cM	Syntenic human loci	Candidate genes	Reference
1	80	1q22-31	<i>Il10, Tnfsf4, Tnfsf6, Fcer1g</i>	Daser et al., unpubl. data
6	46	3p24-26, 2p12-14	<i>Il5ra</i>	Daser et al. [35]
7	48	5q13, 11q13	<i>Il16</i>	Watanabe et al. [26]
9	34	11q22-23, 15q21-25	<i>Thy1, Cd3d, Cd3e, Il10r, Il18, Csk</i>	Kohara et al. [29]
11	44	17q11-p13	<i>Nos2, Scya11</i>	Daser et al., unpubl. data
13	51	5q13, 11q13	<i>Pik3r1</i>	Watanabe et al. [26]
14	32	8p21-23, 14q11.2	<i>Gata4, Tnfsf1</i>	Natori et al. [24]

Finally, a subsequent study used the same mouse strains (A/J-C3H/HeJ mice) to test for metacholine induced AHR [14]. Using different phenotypes, two QTL for AHR on chromosome 6 and 7 were reported. The QTL on chromosome 6 corresponds to the region found in the above-mentioned linkage study of acetylcholine-induced AHR and thus it is likely to represent the same locus. The region on chromosome 7 maps close to an interesting candidate gene, kallikrein (*Klk*). Kallikrein has been implicated in the pathobiology of human asthma. No significant interactions between the QTL on chromosome 6 and 7 could be identified by ANOVA analysis. Furthermore, suggestive linkage was found to a region on chromosome 17 already described in previous studies (see table 1).

Taken together the genome-wide linkage studies for atopy-related phenotypes of the respiratory system, there is only one common linked region for allergen-induced and intrinsic AHR – the *Mhc* cluster on chromosome 17.

Linkage of Atopy-Related Phenotypes of the Skin

The skin represents the second major compartment of the body in constant contact with the outer world and therefore with potential allergens. In fact, the first symptoms of allergy in humans are most often symptoms of the skin like atopic dermatitis or urticaria. Mouse models for this compartment of atopy have been rare to date and are summarized in the following section (see also table 2).

Genome-Wide Screens for Atopic Dermatitis in Mouse Models

A genome-wide linkage analysis for susceptibility loci for atopic dermatitis in a mouse model was performed in NOA (Naruto Research Institute Otsuka Atrichia) mice [24]. The NOA mouse strain is regarded as a model for severe allergic dermatitis as the strain develops severe ulcerative skin lesions with accumulation of mast cells and significantly increased total serum IgE levels. By using backcross progeny of five different mouse strains a significant association between ulcerative skin lesions and markers in the middle of chromosome 14 could be identified. The candidate gene is encoded within an interval of about 17 cM distal from the T cell receptor alpha (TCR α) gene thus probably excluding the TCR α itself as a candidate gene for atopic dermatitis in mice. In humans the TCR α locus has been shown to be in association with specific sensitization [25], but not with atopic dermatitis.

The genetic background of the 5 parental strains had a significant impact on the incidence of skin lesions in the progeny: atopic dermatitis developed 3 times more often in progeny derived from Th2 responder mice than from Th1 responder mice. In a search for these modifier genes two chromosomal regions with candidate genes suppressing the development of atopic diseases were identified: in the middle part of chromosome 7 and in the telomeric region of chromosome 13 [26]. Both loci correspond to regions of synteny to human chromosomes where linkage to different atopic phenotypes has been described (chromosome 5q13 and 11q13, see also table 2). Furthermore, both regions contain candidate genes for atopic dermatitis.

The region on chromosome 7 encodes the gene for IL-16 (*Il16*). It has been shown that IL-16 mRNA is upregulated in acute atopic dermatitis and is associated with in-

creased numbers of CD4⁺ T cells at the site of inflammation [27]. For the region on chromosome 13 phosphoinositide-3-kinase regulatory subunit, polypeptide 1 (*Pik3r1*) represents a possible candidate gene. It belongs to a family of enzymes, which are involved in the generation of lipid mediators, which in turn activate a series of intracellular kinases [28].

A second genome-wide linkage screen for atopic dermatitis used NC/Nga mice as a model for atopic dermatitis [29]. Like NOA mice, NC mice show ulcerative dermatitis and elevated total serum IgE levels. The most significant linkage result was found on chromosome 9, termed Derm1. Several candidate genes are located near Derm1, for example thymus cell antigen 1, theta (*Thy1*), *Il10r*, *Il18* and cytoplasmic tyrosine kinase (*Csk*). Besides their above-mentioned regulatory role in asthma, IL-18 as well as the IL-10 receptor were recently shown to be important for the propagation of atopic dermatitis. Both are strongly downregulated in acute lesions of atopic dermatitis in humans and thus might present the best candidate genes of this region [30, 31]. But as the *Derm1* region lies about 16 cM proximal of the AHR locus (1), it is more likely that a different gene is responsible for the dermal phenotype.

To date, two genome-wide screens for atopic dermatitis in humans have been performed [32, 33], but no chromosomal region found in those screens corresponds to the regions found in the above-mentioned mouse models.

Genome-Wide Screen for Immediate Cutaneous Hypersensitivity in Mouse Models

Genetic variance of a candidate gene and proof of the functional relevance of these polymorphisms has been demonstrated in a study of immediate cutaneous hypersensitivity (ICHS) after sensitization with birch pollen extract [34]. The genome-wide linkage and gene analysis revealed the IL-5R α chain (*Il5ra*) on chromosome 6 as a strong candidate gene [35]. Allelic variation occurs on two levels: (1) a nucleotide exchange in the Kozak sequence of the 5' untranslated region, which leads to increased translation of a reporter construct, and (2) differential alternative splicing of the soluble isoforms in high- versus low-responder mice. Soluble receptors are thought to antagonize the effects of interleukins by inhibiting their binding to the receptors. Thus higher expression of soluble IL-5R would be expected to result in a reduction of signalling through the IL-5R and thereby fewer allergic symptoms. In conclusion, the functional impact of both variants clearly matches the association results.

Further support for the candidate gene status of the IL-5R α chain is provided in a knockout model. In this study

IL-5R α knockout mice were OVA sensitized and subsequently challenged [36]. Unlike the control wild-type mice the knockout mice did not develop AHR after repeated antigen inhalation. The number of eosinophils in BAL was reduced and airway eosinophilic inflammation was attenuated. Almost the same effects were seen in IL-5 knockout mice: after sensitization and challenge with OVA IL-5-deficient mice did not show AHR, AE or PI [37]. If IL-5 is overexpressed in the airway epithelium, mice show intrinsic AHR [38] which depends on the presence of CD4(+) cells and α_4 integrin signalling. Therefore, genetic variance in the IL-5/IL-5R α system proves relevant for various atopic phenotypes.

Two other chromosomal regions showed suggestive linkage to ICHS [Daser et al., unpubl. data; (table 2)]. Linkage of ICHS to chromosome 11 is in close proximity to one locus already found in linkage to allergen-induced AHR in Biozzi mice [2]. As several immunological phenotypes show linkage with this region (fig. 1), this area could contain a common regulatory gene. Alternatively, it could contain a gene-dense cluster of disease-specific genes as further discussed below. Also the second suggestive linkage maps to a region where various phenotypes cluster: on chromosome 1 at 81 cM (fig. 1). Candidate genes of this locus are, for example, members of the tumor necrosis factor superfamily (*Tnfsf*), the genes coding for the γ chain of the high-affinity IgE receptor (*Fcer1g*) and IL-10 (*Il10*). The high-affinity IgE receptor represents one of the key molecules involved in allergic reactions and Fc ϵ RI γ is an essential part of this receptor as well as of other Fc receptors. As mentioned above, IL-10 is an important regulator of allergic responses and together with its receptor seems to be a strong candidate gene.

Genome-Wide ENU Mutagenesis

In addition to the strategies described in the previous sections another systematic approach is underway to detect responsible genes for polygenic traits. The chemical ethyl nitrosourea (ENU) introduces random point mutations into the germline of inbred mice [39, 40]. One advantage is that in contrast to homologous gene targeting and transgenesis, where candidate genes have to be defined, random gene mutagenesis does not require any knowledge of the genes and their products to be targeted. Moreover, through random mutation several point mutations are introduced into one animal, which may reflect the more complex situation of non-Mendelian traits and will include testing of interaction and epistasis of several

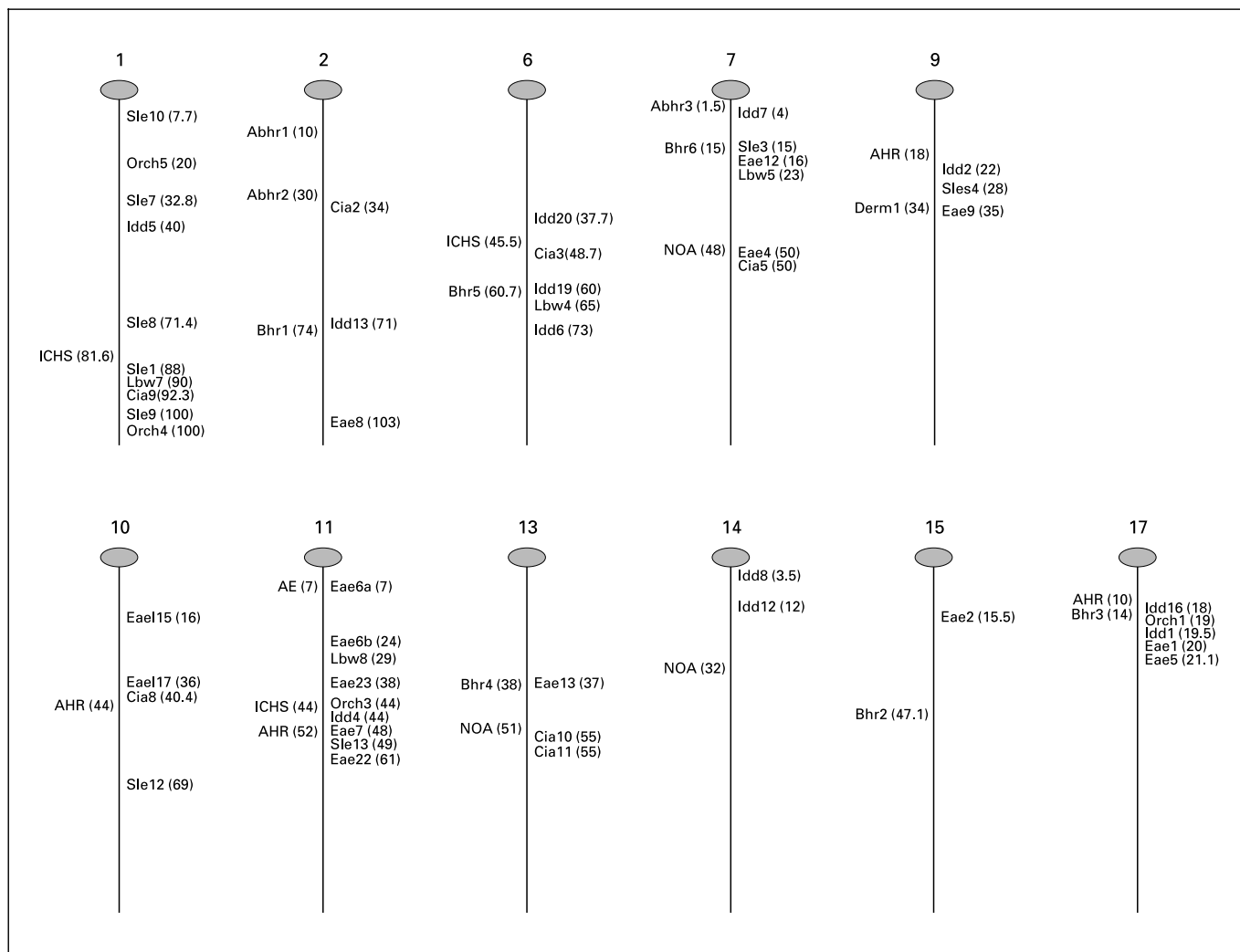


Fig. 1. Diagrams of mouse chromosomes with colocalizing QTLs of atopic and autoimmune phenotypes. Loci linked to the atopic phenotypes are depicted on the left side. Abbreviation of an atopic QTL not given in the text is NOA = atopic dermatitis in NOA mice. Loci of autoimmune phenotypes are given on the right side of the chromosomes. Abbreviations of QTLs are Sle = systemic lupus erythema-

tosis; Idd = insulin-dependent diabetes; Eae = experimental allergic encephalomyelitis; Cia = collagen-induced arthritis; Orch = autoimmune orchitis; Lbw = lupus NZB × NZW. Linkage positions in centi-Morgan (cM) are given in parentheses and are from the Mouse Genome Database (www.informatics.jax.org).

genes for the expression of a phenotype. Breeding of such mice makes possible the study of phenotypes of any interest in thousands of genetically different mouse lines [for review, see 41–43]. Such phenotype-driven screens are set up in several centers worldwide and first results show the high validity of the approach. Dominant mutations, which do show up already in the F1 generation, have been investigated in two programs in the UK [44] and Germany [45] and could also identify 39 mutations affecting the immune system [45].

One of those phenotypes is the occurrence of aberrant IgE levels in the plasma of untreated mice. So far 9 independent mutant mouse lines have been identified and the phenotypes have been confirmed as genetically inherited through backcrossing to a wild-type strain. They subdivide into abnormally high and abnormally low IgE plasma concentrations as compared to plasma concentrations of the wild-type strain [46]. Breeding of the mutant lines will allow investigating both the impact of the abnormal IgE levels on allergen-induced phenotypes and the investiga-

tion of the mutated gene(s) responsible for the phenotype.

To test for recessive traits, a breeding scheme has been set up that obtains homozygosity in three generations and if bred as a two-founder strategy will receive homozygosity of about 100 mutations in one G3 offspring animal [43]. In a first, small screen of 185 founder mice more than 20 recessive mouse lines with immunological phenotypes have been identified [43] and there are more to come, probably including atopic phenotypes, as this ENU mutagenesis screen is run on a very large scale.

Common and Disease-Specific Loci

Atopic disorders share certain features with most other immunological disorders: (1) they are multifactorial diseases with a significant component of heritability, which is inherited in a non-Mendelian fashion and (2) they use identical immunological pathways terminating in the specific diseases. Major distinctions between these complex diseases are the sites of immune attacks and specific environmental conditions.

The MHC complex contributes critically to most of them, but is not sufficient [47]. Several other susceptibility or protective alleles add to the expression of the phenotype and do so in varying combinations. The comparison of the chromosomal regions or genes linked to various immunological diseases in mice and men led to the hypothesis that autoimmune and inflammatory diseases are caused by the combinatorial interaction of (1) disease-specific genes, (2) nonspecific immunoregulatory genes and (3) specific environmental conditions [48–50]. An example from human studies is the colocalization of a locus at 7p15 with asthma and multiple sclerosis, Crohn's disease, systemic lupus erythematosus (SLE) and celiac disease. In mice extensive clustering of autoimmune phenotypes with certain chromosomal regions has been found [51, 52], and to a lesser extent also between autoimmune and atopic phenotypes (fig. 1).

Colocalization of a disease-linked genomic region does not prove the identity of the genes involved, but could well be attributed to the clustering of various immune genes in one region. For example, the autoimmune locus *Sle1* on chromosome 1 (fig. 1) did split up into 4 distinct loci with unique contributions to the overall phenotype SLE upon fine mapping with congenic mice [53]. Similar findings have been made for 5 loci of *Idd* [for a review, see 54]. Moreover, many important immunological genes like the MHC complex, cytokine and T and B cell receptor loci

are clustered in a few regions of the genome. Therefore, the close colocalization of linkage with other immunological phenotypes including ICHS could be attributed to the clustering of several genes in close vicinity. In this respect linkage of the IL-4 gene cluster with various atopic phenotypes does not necessarily mean involvement of identical genes, but could well be a linkage to disease-specific genes just next to each other.

Common Candidate Genes in Mice and Humans

Until now the most convincing candidate genes for asthma and atopy are probably those which have been found in association and/or functional studies in humans and at the same time have been involved in the development of asthma or atopy in knockout and/or transgenic mice. Table 3 gives an overview of these genes. With only one exception – the gene for nitric oxide synthase 1 (*Nos1*) – these 'common' genes are restricted to interleukins, their receptors and transcription factors.

The most intensively studied field in this context is the IL-4/IL-13 signalling pathway. IL-4R α knockout mice have been shown to lack eosinophils in BAL and mucus production in the lungs during asthmatic attacks [55]. In humans the gene for the IL-4R α chain is highly polymorphic. Association of several amino acid variants with atopic phenotypes was found in different ethnic groups and further analysis has shown a functional impact for at least three of these variants [see 56, for a review]. The IL-4R α chain is part of the receptor complex for both cytokines, IL-4 and IL-13. Therefore, the effects seen in IL-4R knockout mice might be explained by inhibiting signalling of both cytokines and the contribution of the single cytokines can hardly be distinguished. A better insight is gained with mouse models in which the cytokines are altered separately. For example, IL-4 knockout mice lack eosinophils in BAL and the pulmonary inflammation is significantly decreased [57]. Accordingly overexpression of IL-4 in transgenic mice leads to accumulation of eosinophils, neutrophils and macrophages [58]. On the other hand, overexpression of IL-13 in the lung of mice causes overproduction of mucus and subepithelial fibrosis [59], whereas IL-13 knockout mice show a decreased production of Th2 cytokines and decreased serum total IgE levels [60]. However, the actions of IL-4 and IL-13 can hardly be distinguished from each other and more studies in this field are clearly needed.

Both cytokines have been demonstrated to be in association with human atopic phenotypes as well. A func-

Table 3. Genes found to be involved in the induction of atopic phenotypes in mice and humans

Gene	Knockout mice	Transgenic mice	Association in humans
<i>Il4ra</i>	Absence of eosinophils in BAL and mucus production [55]	–	Functional amino acid variants in association with asthma, atopic dermatitis and atopy [56]
<i>Il4</i>	Decreased PI and decreased eosinophils in BAL [57]	Epithelial cell hypertrophy, accumulation of macrophages, lymphocytes, eosinophils, and neutrophils [58]	Functional promotor polymorphism in association with asthma, atopic dermatitis and atopy [61]
<i>Il13</i>	Decreased Th2 cytokines and decreased total IgE levels [60]	Increased PI, mucus hypersecretion, subepithelial fibrosis and increased eotaxin production [59]	Association of an amino acid variant with asthma, total IgE levels and atopic dermatitis [62]
<i>Il10</i>	Attenuation of allergen-induced AHR [72]	–	Association of a promotor polymorphism with asthma and total IgE levels [73]
<i>Stat6</i>	Protection of AHR and mucus production, no specific IgE, no eosinophils in BAL, decreased PI [63, 64]	–	Association of a noncoding polymorphism with asthma [65]
<i>Bcl6</i>	Spontaneous airway inflammation, heightened total IgE levels [67]	–	Association of a promotor polymorphism with atopy [66]
<i>Nos1</i>	Significantly less AHR [13]	–	Association of intragenetic variants with asthma [74]

tional promotor polymorphism in the gene for IL-4 is associated with human asthma and atopy [61] and a coding variant in IL-13 was shown to be in association with asthma, atopic dermatitis and elevated total serum IgE levels [62].

Binding of IL-4 or IL-13 to the IL-4R α chain is followed by activation of the transcription factor STAT6 (signal transducer and activator of transcription). Thus signalling of both cytokines can be reduced by blocking STAT6. Accordingly, STAT6-deficient mice are protected from the development of AHR, show less mucus production and neither eosinophils in BAL nor specific IgE [63, 64]. Again, a polymorphism within the gene is associated with human asthma, underscoring the results gained in mice [65].

Finally the transcription factor BCL 6 (B cell lymphoma) is associated with human atopy [66] and also of importance in mouse asthma models. Its gene product functions as a transcriptional repressor by binding to DNA motifs recognized by the STAT family. One such motif is the promotor of the immunoglobulin heavy chain to which both BCL6 and STAT6 can bind. BCL6 knockout mice develop high levels of IgE and IgG1 with tissue inflammation and infiltrations of Th2 cells, eosinophils and monocytes in the lung [67].

Additional Candidate Genes in Mouse Models

In addition to the above-described genes located in regions of linkage to atopic phenotypes, a few more genes – not located in linked regions – have also been found to be involved in the development of atopic diseases in mouse models (see table 4).

Only recently, CD81 $^{-/-}$ mice have been demonstrated to develop an impaired Th2 response. Furthermore, CD81 $^{-/-}$ BALB/c mice show no allergen-induced AHR after OVA sensitization and challenge and reduced PI in comparison to CD81 $^{+/+}$ littermates [68]. Interestingly, OVA-specific IgE levels are similar in CD81 $^{-/-}$ and CD81 $^{+/+}$ mice, whereas the production of Th2 cytokines is dramatically reduced in CD81-deficient mice.

The function of B7-1 (CD80) and B7-2 (CD86) in the development of allergen-induced AHR was investigated by using mice with germline deletions of the B7-1 and/or B7-2 molecules [69]. Mice lacking both molecules did neither show AHR, nor AE nor elevated IgE levels. Mice lacking either B7-1 or B7-2 showed a reduction of the same disease parameters. In addition, the production of Th1 cytokines was increased in B7-1/B7-2-deficient mice.

Table 4. Genes investigated in knockout mice with effects on atopic phenotypes but no association data in humans

Targeted gene	Phenotype	Effect	Reference
<i>Il5</i> -/-	AHR BAL eosinophils Specific IgE	Absence Absence As wild type	Foster et al. [37]
<i>Il5ra</i> -/-	AHR BAL eosinophils AE IL-5 Specific IgE	Protection Decrease Decrease As wild type As wild type	Tanaka et al. [36]
<i>Cd80</i> -/- <i>Cd86</i> -/- <i>Cd80, Cd86</i> -/-	AHR AE PI Total IgE Th2 cytokines Th1 cytokines	Absence Absence Absence Decrease Absence Increase	Mark et al. [69]
<i>Gata3</i> -/-	AE Mucus Specific IgE	Decrease Decrease Decrease	Zhang et al. [18]
<i>Cd81</i> -/-	AHR Th2 cytokines AI	Protection Decrease As wild type	Deng et al. [68]
<i>Dp</i> -/-	PI Th2 cytokines	Decrease Decrease	Matsuoka et al. [70]
<i>Rel</i> -/-	AHR AI BAL Total IgE	Protection Absence Absence Absence	Donovan et al. [10]

Finally, the role of prostaglandins in the induction of allergen-induced AHR was investigated by the generation of mice deficient in the receptor for prostaglandin (*Dp*) [70]. Prostaglandin D2 is expressed in mast cells and released upon activation by IgE-mediated allergic responses. After OVA sensitization and challenge the homozygous mutant (*DP*-/-) mice showed an increase of IgE levels similar to wild-type mice. However, the accumulation of lymphocytes and eosinophils in the lung and the expression of Th2 cytokines were significantly reduced in the *DP*-deficient mice. Accordingly these mice did not develop AHR.

Further Use of Mouse Genomics

Finally, the insights we gain from mouse genetics to help in the dissection of human genetics are not restricted to the mere comparison of regions found in linkage with a

disease in both species or to transfer candidate genes from one species to the next. In addition, the direct comparison of the sequences of both genomes facilitates the location of regulatory sites [71]. This technique has become especially important with the completion of the human draft sequence. Under the assumption that regulatory sites are more conserved between human and mice than noncoding random sequences, it helps to narrow down the region of possible functional importance and thus reduce the amount of time and money to find the region of interest. However, because mice and humans have extensive similarities in their genomes, it is highly likely that even non-regulatory 'nonsense' sequences are sometimes conserved between both species leading to an overestimation of regulatory sequences.

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