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Joint influences of *Acidic-Mammalian-Chitinase* with *Interleukin-4* and *Toll-like receptor-10* with *Interleukin-13* in the genetics of asthma

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In the genetics of asthma, single genetic polymorphisms confer only a small individual risk factor. Haplotype-based association analyses, including joint analyses of several candidate genes, might therefore yield more convincing results than single-region statistics. We set out to test for joint influences of asthma genes previously identified in our study population that is acidic mammalian chitinase (*AMCase*), Toll-like receptor (*TLR*)-10, and the interleukins *IL-4*, *IL-13*, *IL-8*, and *IL-15*. In particular, we investigated whether haplotypes at two or three genes show stronger association with the trait than at a single gene alone. We genotyped 26 polymorphisms in 321 asthmatic children and 270 controls. Haplotype-based association analyses were performed by the program FAMHAP. Single-, two-, and three-gene analyses were conducted as well as conditional analyses for pairs of genes. In the two-region analyses, best evidence was found for a joint effect on asthma for *AMCase* and *IL-4* ($p_{raw} < 5 \times 10^{-7}$) as well as *AMCase* and *IL-13* ($p_{raw} = 5 \times 10^{-7}$). Besides, *IL-13* and *TLR-10* showed a stronger two-gene result ($p_{raw} = 0.001607$) than the respective single-gene analyses. Conditional analyses yielded similar results for these two-gene combinations and also revealed mutual additional effects for *IL-13* and *IL-4* ($p_{stratified} = 0.014831$ and 0.001525, respectively). The most significant results demonstrate a joint effect of *AMCase* with *IL-4* or *IL-13* on the trait. Furthermore, additional mutual effects were seen for *AMCase* and *IL-4* as well as for *TLR-10* and *IL-13*. The corresponding pathways might therefore be of particular importance in the genetics of asthma. Further studies are needed to elucidate the functional importance of these gene–gene effects and their precise role in asthma pathogenesis.

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Bronchial asthma is the most common chronic inflammatory disease of childhood, affecting 10% of all children in industrialized countries (1, 2). Studies in twins suggest that genetic and environmental factors have about the same impact on the development of asthma (3). Thus, the dissection of the genetic background of asthma is of great interest for at least two reasons: to develop new drugs for an effective and genotype-specific pharmacotherapy and to enable identification of predisposed individuals early in life to introduce preventive measures (4).

The genetics of asthma is highly complex. Since the report on the first genome-wide linkage analysis for asthma in 1996 (5), hundreds of linkage and association studies have been

conducted. Furthermore, the first genome-wide association study for asthma using microarray technology has been published recently (6). These numerous studies further underscored the complexity of asthma genetics as many asthma genes found in one study could not be confirmed in the next study. Recently, Vercelli reviewed all association studies performed so far in the genetics of asthma and summarized the genes that have been found in association with any atopic phenotype in at least five independent studies (7).

In our own study populations, we investigated all genes that have been found in association with asthma at least 10 times, except the human leucocyte antigen cluster. We were not able to confirm association of interleukin-10 (*IL-10*), monocyte differentiation antigen 14 (*CD14*), beta2 adrenergic receptor (*ADRB2*), IL-4-receptor (*IL-4R*), a disintegrin and metalloproteinase 33 (*ADAM33*), signal transducers and activators of a transcription 6 (*STAT6*), or high-affinity IgE receptor beta chain (*FCER1*) with asthma (8, 9 and unpublished data). Marginal association was detected with tumor necrosis factor alpha (*TNF-α*) and asthma.

However, association of *IL-13* (10) and *IL-4* (8) with asthma was confirmed. In addition, association of Toll-like receptor-10 [*TLR-10* (11)], acidic mammalian chitinase [*AMCase* (12)], *IL-8* (13), and *IL-15* (14) was detected with childhood asthma. Of all investigated candidate genes, polymorphisms within *AMCase* showed the strongest effect on the development of asthma in our population. Meanwhile, association of *AMCase* with asthma and heightened IgE levels has also been reported in the Indian population (15). Furthermore, chitinase-like proteins being involved in the same pathway have been demonstrated to be important in the development of asthma both functionally and genetically (16, 17).

We were now interested whether the associated asthma genes and their polymorphisms are jointly associated with the disease in our population. More specifically, our aim was to investigate whether haplotypes at two or three genes exhibit stronger evidence of association than a single gene alone, thus reflecting a better explanation of the asthma affection status by a multi-region haplotype model.

Subjects and methods

Population of asthmatic children

Three hundred and twenty-two children with bronchial asthma (aged 5–16 yrs) were recruited from the southwestern part of Germany between July 2000 and January 2005. The probands were characterized at the Centre of Pediatrics and Adolescent Medicine, Freiburg, Germany, using a standardized clinical protocol as described previously (12). The diagnosis 'asthma' was based on a clear-cut history of asthmatic symptoms, the use of anti-asthmatic medication, and the presence of bronchial hyperreactivity. The anti-asthmatic drugs included typical betamimetics like salbutamol and standard corticosteroids used in asthma treatment like budesonide. Bronchial hyperreactivity was defined as a fall in FEV1 by at least 15% in histamine testing or exercise provocation.

Controls

Two hundred and sixty-nine randomly chosen probands were used as controls (aged 19–40 yrs). They originate from the same area in the southwestern part of Germany. No medical history was taken and no medical testing was performed on controls. Thus, some asthmatics might have been included in controls. However, this would increase the possibility of falsely negative results, whereas positive association still holds. Only Caucasians were included in the case and control populations.

Genes and single nucleotide polymorphisms (SNPs) under investigation

Six genes and 26 SNPs were included in our analysis. The numbers in brackets are the numbers that are used in the following in text and tables.

Acidic mammalian chitinase on chromosome 1q31: (1) rs12033184, (2) rs3818822, (3) K17R, (4) rs2275253, (5) rs2275254, (6) rs36011905 (F269S), and (7) rs2256721. Interleukin-8 on chromosome 4q12: (8) rs4073 and (9) rs2227306. Interleukin-15 on chromosome 4q31: (10) rs2857261, (11) rs7349640, and (12) rs10519613. Interleukin-13 on chromosome 5q31: (13) rs1881457, (14) rs1800925, and (15) rs20541. Interleukin-4 on chromosome 5q31: (16) rs2243250, (17) rs2070874, (18) rs2243274, and (19) rs2243290. Toll-like receptor-10 on chromosome 4p14: (20) rs4274855, (21) rs10856839, (22) rs11096957, (23) rs11096956, (24) rs11096955, (25) rs11466657, and (26) rs4129009.

Genotyping

Genotyping was performed by restriction fragment length polymorphisms as described previously (8, 10–14). Only individuals with known

genotypes for all investigated SNPs were included in subsequent analyses that is at minimum 267 cases and 221 controls (of 321 cases and 270 controls in total).

Statistical analyses

The distribution of genotypes for each of the 26 SNPs was tested for departure from Hardy–Weinberg equilibrium by a chi-squared-test with one degree of freedom using the program FINETTI (Thomas F. Wienker, http://finetti.meb.uni-bonn.de/). For the TLR-10 SNPs (24) rs11096955 and (25) rs11466657, the test yielded a p-value < 0.01 among the controls that may indicate genotyping problems. Therefore, these two SNPs were excluded from further analyses.

FAMHAP version 17 (18) was used to investigate the association of the remaining 24 SNPs with the dichotomous asthma phenotype (i) by looking at haplotypes for SNPs of each particular gene separately and (ii) by simultaneously taking two and three genes into account. In particular, the null hypothesis is tested that no haplotypes or SNPs in neither region are associated with the trait. For a given marker combination, with the analysis option 'hapcc', FAMHAP tests whether the haplotype distribution, determined by an expectation–maximization-based algorithm, differs significantly between cases and controls. This is carried out by a chi-square statistic for ($2 \times$ k) tables with counts of k different haplotypes for cases and controls. Because it is not known prior to the analysis which markers are involved in an association with the trait, the option 'allcombi' of FAMHAP was used for each choice of genetic region(s) to perform separate tests for all possible marker combinations. This includes the association test for every single SNP (i.e., combinations that consist of only one marker). For the analyses jointly taking two or three regions (i.e., genes) into account [analysis option 'region' (19)], haplotype reconstruction is performed separately for SNPs within a genetic region, while the analysis is still performed for all possible marker combinations. Such an analysis with two or three genetic regions can be able to detect association even if interaction effects between haplotypes at different genes are present and no marginal single-region effects can be seen (19). Empirical p-values were determined by simulations, with at least one million replicates, in which case and control status is randomly permuted. Besides the nominal p-values for all marker combinations (p_{raw}), FAMHAP reports the corrected p-value for the marker combination with the strongest association result (p_{global}), which accounts for the multiple tests, that is all possible marker combinations (20). It should be noted that this procedure leads to a more stringent correction for regions or combinations of regions with a larger number of SNPs, and hence more marker combinations, than for analysis scenarios with less SNPs. Therefore, to compare association results for different regions, we have focused on the nominal p-values that are also based on at least one million simulated replicates but do not depend on the haplotype length and number of tested marker combinations.

In addition, a conditional two-region association analysis was performed in which the additional effect of the haplotypes at a SNP combination of the second region is tested, on top of the background effect of the first region, that is the effect that is explained by the SNP combination at the first region alone (analysis option 'strat'). In this context, the analysis is stratified by the different haplotypes at the SNPs of the first region. The null hypothesis corresponds to no association of the haplotypes at SNPs of the second region, given the association of the haplotypes at SNPs of the first region. We tested the SNP combinations that led to the strongest association results obtained by the single-region analyses.

A substantial additional effect on the disease of each of two genes, given the effect of the other gene, can be declared when both mutual stratification tests lead to a statistically significant result.

Results

Single-region analyses

The results of the haplotype-based association analyses separately for each gene, together with the combinations of SNPs with the highest association to asthma, are shown in Table 1. As expected by the results of our previous studies, *AMCase* showed the highest association with $p_{raw} = 2 \times 10^{-6}$, combining the five SNPs 2, 3, 4, 5, and 7.

AMCase is followed by *IL-4* with $p_{raw} = 0.001203$ and *TLR-10* with $p_{raw} = 0.003947$. Haplotypes of *IL-13* showed weak association ($p_{raw} = 0.010138$). Regarding *IL-8*, the best association was seen with one single polymorphism rather than with haplotypes of both SNPs ($p_{raw} = 0.033669$, SNP 9).

Table 1. Single-region analyses

Gene	Investigated SNPs	Best combination	p_{raw}	p_{global}
AMCase	1 2 3 4 5 6 7	2 3 4 5 7	0.000002	0.000088
Interleukin-13	13 14 15	13 14	0.010138	0.037323
Interleukin-8	8 9	9	0.033669	0.060908
Interleukin-15	10 11 12	11	0.615314	0.9161
Interleukin-4	16 17 18 19	17 18	0.001203	0.00842
Toll-like receptor-10	20 21 22 23 26	20 21 22 26	0.003947	0.029759

Results of the haplotype analyses for each gene separately. Shown is the combination of single nucleotide polymorphisms (SNPs) with the highest association to bronchial asthma for each gene. The rs numbers of the SNPs are given in the methods section. p_{raw}: simulated nominal p-value, p_{global}: corrected p-value, for the marker combination with the strongest association result, which accounts for testing all possible marker combinations within a gene.

Finally, *IL-15* did not show association with asthma in this analysis ($p_{raw} = 0.615314$, SNP 11).

Two-region analyses

In the next step, we tested all possible combinations between two genes by performing two-region analyses. In Table 2, the respective pairs of genes and the best combinations of SNPs within each group are listed. The best test statistic was found for *AMCase* and *IL-4* ($p_{raw} < 5 \times 10^{-7}$, meaning that of two millions of replicates none showed a higher test statistic than the real data sample, see also Table 2) and *AMCase* and *IL-13* ($p_{raw} = 5 \times 10^{-7}$).

Table 2. Two-region analyses

Gene 1	Gene 2	Best combination(s) of SNPs	p_{raw}	p_{global}
AMCase	*IL-8*	2 3 4 5 7 *9*	0.000004	0.000297
AMCase	*IL-13*	3 4 5 7 *13 14*	0.0000005	0.000114
		2 3 4 5 7 *15*		
		2 3 4 5 7 *13 14*		
		1 2 3 4 6 7 *14 15*		
AMCase	*IL-4*	2 3 4 5 7 *17 18*	<0.0000005	0.0001045
AMCase	*TLR-10*	4 5 7 *20 21 22 26*	0.000012	0.001783
IL-8	*IL-13*	8 9 *13 14*	0.003606	0.02854
IL-8	*IL-4*	9 *17 18*	0.000762	0.010178
IL-13	*IL-4*	13 14 *17 18*	0.003947	0.050383
IL-13	*IL-15*	13 14 15 *10*	0.009142	0.08734
IL-13	*TLR-10*	13 *20 21 22 26*	0.001607	0.033877
IL-4	*TLR-10*	17 18 *20 21 22 26*	0.000333	0.012071

This table lists the results of the two-region analyses. To facilitate reading of these data, the second gene and the corresponding single nucleotide polymorphisms (SNPs) are written in italics. The rs numbers of the SNPs are given in the methods section. Two-region results that yield a best combination of SNPs within one single gene are not shown. p_{raw}: simulated nominal p-value, p_{global}: corrected p-value, for the marker combination with the strongest association result, which accounts for testing all possible marker combinations for a particular pair of genes.

Besides gene combinations involving *AMCase*, there were also convincing results for *IL-4* with *TLR-10* ($p_{raw} = 0.000333$), *IL-13* ($p_{raw} = 0.00013$), and *IL-8* ($p_{raw} = 0.000762$), between *TLR-10* and IL-13 ($p_{raw} = 0.001607$) and, noteworthy enough, between *IL-15* and *IL-13* ($p_{raw} = 0.009142$), the former gene being otherwise completely without any effect throughout the whole study.

Stratification of the two-region analyses

The association result of the single gene *AMCase* with asthma is already so strong that the additional effect of any other gene can hardly be quantified by looking at the result of the respective two-region analysis, that is the degree to which the p-value decreases when jointly taking into account two regions compared to a single-region analysis. Thus, it is difficult to decide whether a significant additional effect is brought about by a second gene on top of the first, for example *AMCase*.

To formally test for a significant additional effect of a second gene, conditional on the effect of a first gene, mutual stratified tests of all combinations of two genetic regions from the two-region analyses were performed. The results of the tests can be found in Table 3. The results

Table 3. Stratification of two-region analyses

Gene pair and SNPs	$p_{raw\text{-}single}$	$p_{raw\text{-}two\text{-}regions}$	$p_{stratified}$
AMCase (2, 3, 4, 5, 7)	0.000002	0.000004	0.000038
IL-8 (9)	0.033669		0.157146
AMCase (2, 3, 4, 5, 7)	0.000002	0.0000005	0.000101
IL-13 (13, 14)	0.010138		0.746825
AMCase (2, 3, 4, 5, 7)	0.000002	<0.0000005	0.001740
IL-4 (17, 18)	0.001203		0.032851
AMCase (2, 3, 4, 5, 7)	0.000002	0.000017	0.002092
TLR-10 (20, 21, 22, 26)	0.003947		1
IL-8 (9)	0.033669	0.006001	0.072493
IL-13 (13, 14)	0.010138		0.024524
IL-8 (9)	0.033669	0.000762	0.167279
IL-4 (17, 18)	0.001203		0.003627
IL-13 (13, 14)	0.010138	0.00013	0.014831
IL-4 (17, 18)	0.001203		0.001525
IL-13 (13,14)	0.010138	0.047467	0.024100
IL-15 (11)	0.615314		0.776055
IL-13 (13, 14)	0.010138	0.002998	0.003308
TLR-10 (20, 21, 22, 26)	0.003947		0.012908
IL-4 (17, 18)	0.001203	0.000333	0.124716
TLR-10 (20, 21, 22, 26)	0.003947		0.101508

Results of stratification tests for the best single nucleotide polymorphism (SNP) combinations of the single-region analyses. The rs numbers of the SNPs are given in the methods section. Regarding the fourth column ($p_{stratified}$), the first and second line give the result of the conditional analysis testing for an additional effect of the gene mentioned on the same line in the first column, using the other gene as baseline.

of the stratified tests further support a strong impact on asthma by *AMCase* polymorphisms alone, as additional information from a second region (e.g. *IL-8*, *IL-15*, *IL-13*) contributes little to the already underlying effect of *AMCase*. Notwithstanding, a measurable additional effect in both directions was detected for *AMCase* with *IL-4* ($p_{stratified}$ = 0.001740 and 0.032851). Remarkably, *IL-13* and *IL-4* show mutual additional effects ($p_{stratified}$ = 0.014831 and 0.001525) as well as *IL-13* and *TLR-10* ($p_{stratified}$ = 0.003308 and 0.012908). *IL-4* and *TLR-10* did not show significant results in the stratified tests, indicating that the disease status can be largely explained by the haplotypes at a single gene alone. Furthermore, borderline mutual additional effects of *IL-8* with *IL-13* ($p_{stratified}$ = 0.072493 and 0.024524) could be found. Regarding *IL-13* and *IL-15*, the test for an additional effect of *IL-13* when conditioning on the haplotypes of *IL-15* was significant; while the reciprocal stratified test for *IL-15*, that is the gene showing the weaker effect in the single-region analyses, was not significant when conditioning on *IL-13*.

For illustrative purposes, a Venn diagram (Fig. 1) summarizes our findings of the association tests. It shows how the phenotypes of the whole data sample can be explained by the haplotypes at different genes. Even in case of a strong joint two-region association signal can the conditional analysis yield a non-significant result in one of the two directions. This happens if the second gene does not cover a substantial area that is not already covered by the first gene that serves as baseline.

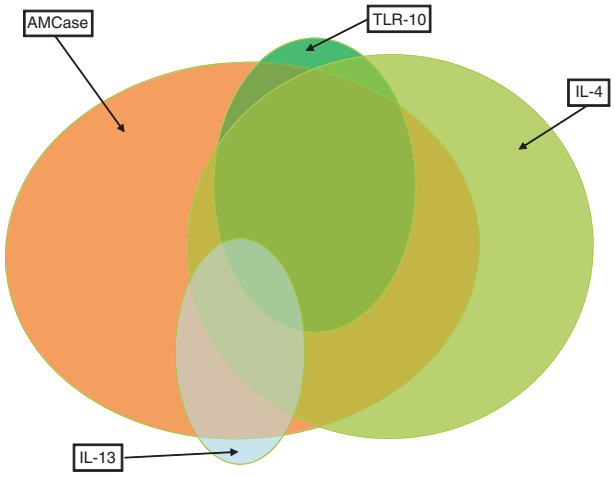


Fig. 1. Schematic illustration of explanation of the phenotype by haplotypes for different genes. The entirety of the rectangle represents all cases and controls. Each coloured ellipse corresponds to a particular gene; the area roughly depicts the proportion of individuals whose phenotype (i.e., case-control status) can be explained or discriminated by haplotypes at that gene. For persons outside the ellipse, haplotypes at the particular gene are not of discriminative value regarding their asthma phenotype. Haplotypes at two genes can explain the asthma phenotype for a larger portion of individuals, represented by the union of the areas of the corresponding ellipses, than a single gene alone. This union of the areas for two genes corresponds to the strength of the joint two-region association signal. The phenotype of individuals corresponding to the overlap of the areas for two genes (i.e., the intersection) can be explained by either of the two genes. The conditional analysis (stratified test) picks up that part of the area for the additionally tested second gene that does not overlap with the area for the first gene. Even in case of a strong joint two-region association signal can the conditional analysis yield a non-significant result in one of the two directions. This happens if the second gene does not cover a substantial area that is not already covered by the first gene that serves as baseline.

Three-region analyses

To further investigate the proposed relationships between the associated markers found by the two-region approach and the subsequent stratification tests, we conducted three-region analyses with all markers under study, allowing again for all possible combinations of markers of three different genetic regions. By these computationally intensive analyses, the joint genetic effect of all three regions is taken into account. We only report the most prominent results for combinations involving at least two regions that have shown evidence of association in the stratified tests. Such findings of joint association of three regions were obtained for *IL-4*, *IL-13* and *TLR-10* (p_{raw} = 0.000127), for *IL-4*, *IL-13* and *IL-8* ($p_{raw} = 6.3 \times 10^{-5}$) and for *IL-8*, *IL-13,* and *AMCase* ($p_{raw} = 10^{-6}$). Like these findings, other results are also in line with the results obtained by the single-region, two-region, and stratified tests; they are omitted here for reasons of conciseness.

Discussion

We set out to identify joint associations of candidate genes in the genetic predisposition to pediatric asthma in the German population. We included all genes that we have previously found in association with bronchial asthma in our paediatric population that is *TLR-10*, *AMCase*, and the different interleukins *IL-13*, *IL-4*, *IL-8*, and *IL-15*. Of these genes, *IL-13* and *IL-4* have already been investigated for possible interaction with additional genes: Gene–gene interaction has been repetitively reported between *IL-13* and the alpha chain for the IL-4-receptor (*IL-4Ra*) (21–23), as well as the alpha chain of the *IL-13 receptor 1* (*IL-13Ra1*) (24). Furthermore, *IL-4* interacts

with polymorphisms of *IL-4Ra* in Korean asthmatic children (25). In our study population, neither *IL-4Ra* nor *IL-13Ra1* is in association with asthma (Heinzmann A, Deichmann K).

Initially, single-region analyses were performed to identify for each gene separately the combination of SNPs in highest association with asthma (Table 1), followed by two-region analyses (Table 2). If a two-region combination leads to a more pronounced association result than either of the two genetic regions analyzed alone, as expressed by a markedly smaller p-value (p_{raw}), this can be regarded as a hint to both genes being jointly associated with asthma. However, to finally conclude whether each of the two genes explains a substantial additional effect when compared to the other gene alone, a formal statistical test such as the conditional test implemented in FAMHAP by the analysis option 'strat' needs to be performed.

As expected from our previous results, *AMCase* showed the strongest association with asthma among the single-region analyses ($p_{raw} = 2 \times 10^{-6}$). *IL-15* did not show association with asthma ($p_{raw} = 0.615314$). This is in contrast to our previous studies, in which we found association of *IL-15* with pediatric asthma in our own study population as well as in the cohort of the multi-centre allergy study (14, 26). The conflicting results are most likely because of the selection of SNPs. In the original study in 231 children, we genotyped five SNPs. In contrast, here we only genotyped the three most informative SNPs in the extended study population. Thereby we may have missed the disease-causing haplotypes. Indeed, neither convincing joint association nor haplotype interaction was seen for *IL-15* and any of the other genes. The only exception was a two-region p-value ($p_{raw} = 0.009142$), for *IL-15* and *IL-13*, which was marginally smaller than the single-region p-value for *IL-13* alone ($p_{raw} = 0.010138$). This might lead to the conclusion that the two-region SNP combination reflects an interaction between haplotypes at both genes. However, the stratified test for an additional effect of *IL-15* with *IL-13* as baseline was not significant.

In the two-region analyses, we found the best test statistic for *AMCase* and *IL-4* ($p_{raw} < 5 \times 10^{-7}$). As *AMCase* per se is already strongly associated with asthma, any additional effect by another gene is hard to quantify. Thus, mutual stratification tests were performed in addition to the two-region analyses, yielding $p_{stratified} = 0.032851$ for the additional effect of SNPs of *IL-4* to the development of asthma, given *AMCase* as baseline, and $p_{stratified} = 0.001740$ for the additional effect of *AMCase*, given *IL-4* as baseline. Thus, each of the two genes has a substantial additional effect on the asthma phenotype when compared to the effect of the other gene alone. This corresponds to a substantial portion of individuals whose phenotype can be explained when additionally considering haplotypes at the second gene, in comparison with taking into account only the first gene (see schematic illustration of the areas representing *AMCase* and *IL-4* in Fig. 1). None of the other genes showed significant results in the stratification tests with *AMCase* as baseline.

Significant joint association and mutual additional effects were obtained for *IL-13* and *IL-4* ($p_{stratified} = 0.014831$ and 0.001525 for the additional effects of *IL-13* and *IL-4*, respectively). A joint association could be detected for *IL-8* with *IL-13* (two-region $p_{raw} = 0.003606$), while the stratified test for *IL-8* conditioning on *IL-13* was at best borderline significant ($p_{stratified} = 0.072493$).

However, the most interesting and significant result besides the one for *AMCase* and *IL-4* is the joint association of *TLR-10* and *IL-13* ($p_{stratified} = 0.012908$ and 0.003308 for testing an additional effect of *TLR-10* and *IL-13*, respectively, taking the other gene as baseline). This is reflected by the small overlap of the corresponding areas in Fig. 1. All other possible combinations of regions, as presented in Table 3, showed no evidence for an additional effect, at least not in both directions. This is despite the fact that the two-region p-value is somewhat smaller than the corresponding single-region p-values. In this context, it should be noted that the raw two-region p-value is by definition at least as small as each of the two single-region p-values, because the combinations involving only markers of one region are included in the two-region analyses as well.

Taken together, we were able to consistently find *AMCase* to be strongly associated with asthma and could even demonstrate a significant additional effect of *IL-4*. The other most prominent genes in association with the disease, that is *TLR-10* and *IL-13*, were also shown to have significant mutual additional effects on asthma. Interestingly, the genes *IL-13* and *IL-4*, whose gene products are thought to be comparable and to act in a similar way, were as well jointly associated. These combined genetic effects might therefore be of particular importance in the genetics of asthma. If the areas corresponding to a pair of genes in Fig. 1 have large non-overlapping parts, this can be interpreted as

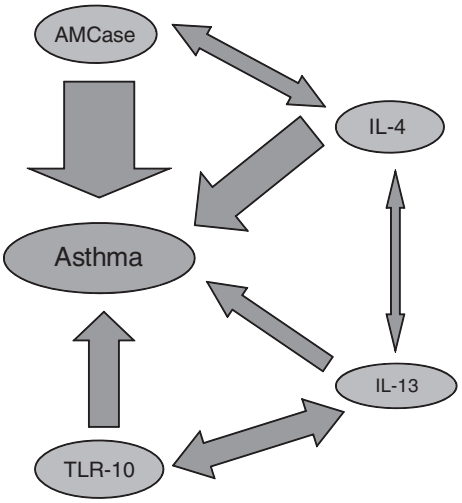


Fig. 2. Schematic illustration of the single and joint genetic effects on the asthma phenotype. Association signals of single genes are depicted by one-sided arrows, whereas two-sided arrows represent substantial additional effects for each of the two genes on asthma, when taking the effect of the other gene as baseline (stratified test). Thickness of arrows and letters represents the strength of signal.

predisposition to asthma in different individuals because of disease-causing haplotypes at different genes. Proceeding from Fig. 1, we further hypothesized a model of gene action shown in Fig. 2. It depicts the genes being in strongest association with asthma according to the single-region analyses and furthermore illustrates the findings of the conditional analyses, that is substantial mutual effects of two genes on the asthmatic phenotype. To date, there is no direct evidence in the literature that *TLR-10* and *IL-4*/*IL-13* directly interact with each other in a common pathway. However, as all these proteins are involved in innate immunity, an interaction seems likely. Nevertheless, it should be noted that interpreting the model obtained by a statistical analysis in a biological sense is generally not permissible (27). This is because of the fact that several causal underlying biological mechanisms can lead to the same statistical model. Hence, future molecular studies are needed to postulate a biological model that is consistent with our current findings, such that the functional importance of these joint associations, especially of *AMCase* and *IL-4* as well as *TLR-10* and *IL-13*, and their precise mode of action during asthma pathogenesis can be revealed.

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