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## **Genome-wide association study of body mass index in 23,000 individuals with and without asthma**

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**Short title**

GWAS of BMI in asthmatic children and adults

**Key words:** Association; Asthma; BMI; Genetics; Genome-wide; Obesity

**Abstract**

**Background:** Both asthma and obesity are complex disorders that are influenced by environmental and genetic factors. Shared genetic factors between asthma and obesity have been proposed to partly explain epidemiological findings of co-morbidity between these conditions.

**Objective:** To identify genetic variants that are associated with body mass index (BMI) in asthmatic children and adults, and to evaluate if there are differences between the genetics of BMI in asthmatics and healthy individuals.

**Methods:** In total, 19 studies contributed with genome-wide analysis study (GWAS) data from more than 23,000 individuals with predominantly European descent, of whom 8,165 are asthmatics.

**Results:** We report associations between several *DENND1B* variants ( $p=2.2 \times 10^{-7}$  for rs4915551) on chromosome 1q31 and BMI from a meta-analysis of GWAS data using 2,691 asthmatic children (screening data). The top *DENND1B* SNPs were next evaluated in seven independent replication data sets comprising 2,014 asthmatics, and rs4915551 was nominally replicated ( $p<0.05$ ) in two of the seven studies and of borderline significance in one ( $p=0.059$ ). However, strong evidence of effect heterogeneity was observed and overall, the association between rs4915551 and BMI was not significant in the total replication data set,  $p=0.71$ . Using a random effects model, BMI was overall estimated to increase by  $0.30 \text{ kg/m}^2$  ( $p=0.01$  for combined screening and replication data sets,  $N=4,705$ ) per additional G allele of this *DENND1B* SNP.

*FTO* was confirmed as an important gene for adult and childhood BMI regardless of asthma status.

**Conclusions and Clinical Relevance:** *DENND1B* was recently identified as an asthma susceptibility gene in a GWAS on children, and here we find evidence that *DENND1B* variants may also be associated with BMI in asthmatic children. However, the association was overall not replicated in the independent data sets and the heterogeneous effect of *DENND1B* points to complex associations with the studied diseases that deserve further study.

## Introduction

During the past decades, the prevalence of asthma and obesity/overweight has markedly increased in children and adults, contributing substantially to morbidity and health costs worldwide.[1] Both asthma and obesity are complex disorders that are influenced by environmental and genetic factors. Twin studies have shown that genetic factors account for around 60-70% of the population variation in body mass index (BMI) and similar numbers have been presented for the heritability of asthma.[2-4] Numerous genetic variants have also been associated with each phenotype.[5-7]

Shared genetic factors between asthma and obesity have been proposed to partly explain epidemiological findings of co-morbidity between these conditions[8, 9], and longitudinal studies show that obesity seems to precede asthma.[1, 10, 11] Association analyses of known asthma and BMI genes show some evidence for a shared genetic predisposition to asthma and obesity, although other shared genetic determinants are likely to be identified.[12]

Recent genome-wide association studies (GWAS) have identified variants at several loci that are associated with BMI and/or obesity, with the strongest association to *FTO*, *TMEM18* and *MC4R*. [13-23] Of the BMI top GWAS hits published to date, the majority of loci were originally

identified in adult populations, although many of these loci have also shown associations with BMI or obesity in children.[23, 24]

While the genetics of BMI in the general adult population has been rather extensively studied, few candidate gene studies and no GWAS have been performed to evaluate the influence of genetics on BMI in asthmatics. Thus, it is currently not known if there are any differences between the genetics of BMI in healthy individuals and in an ascertained asthmatic population. Association analyses of genetic markers and BMI in asthmatics can give valuable insights in the complex interplay between genes, asthma and obesity.

The aim of this project was to identify common genetic variants associated with BMI in asthmatic children and adults, and to evaluate if there are differences between the genetics of BMI in healthy individuals and asthmatics.

## **Methods**

### **Ethics statement**

Written informed consent was obtained from all participants or their parents and all studies were approved by the local ethics committees.

### **Subjects**

All study subjects (totaling 23,562 individuals) were derived from existing studies (cohort, case-control and family based studies) on asthma and respiratory diseases from Australia, Canada, Central America, Europe, and the US (Table 1 and Supplement data). ALSPAC, BAMSE, Busselton Health Study, B58C (partly), CAPPS, ECHRS, EGEA, GABRIELA, MAGICS/ISAAC, MAS, PIAMA, SAGE, SAPALDIA and TOMSK are part of the GABRIEL consortium and described in detail elsewhere.[25] In the GABRIEL studies, doctor's diagnosis of asthma (ever) was used to define cases (subjects who reported to ever have had asthma were also

included as cases in ECRHS and SAPALDIA) and non-asthmatics were used as controls.

Established definitions for asthma were used in the other studies (CAMP, GACRS, GINI/LISA, Hartford/Puerto Rico and CAG) as described in the Supporting information.

Separate analyses were run in children (< 18 years) and adults, and some studies contributed samples to both age groups. BMI was measured at a clinical visit in each study and we used the measurement closest in time to the diagnosis of asthma. BMI values were not available for all individuals listed in Table 1, and somewhat lower figures are therefore presented in respective tables and figures with BMI results. Detailed information about each participating study is given in the Supporting information (Information S1).

### **Genotyping**

All genotyping in the GABRIEL studies were carried out at Centre National de Génotypage (Evry, France) using the Illumina Human610 Quad BeadChip (Illumina, Inc., San Diego, CA)[25], except for the MAGIC/ISAAC data set (Illumina HumanHap300 BeadChip, thus contributing with 300K SNPs in the analysis).[26] As reported previously, strict quality control (QC) procedures were applied to all genotyped data sets.[25, 26] CAMP probands were genotyped on Illumina's Human-Hap550 Genotyping BeadChip (Illumina, Inc., San Diego, CA) and used as cases in a case-control study design with 1,205 population controls obtained from Illumina's iControlDB public repository.[27] The SNP selected for replication in GACRS (rs4915551) was genotyped using the Sequenom MassArray platform with iPLEX chemistry. In CAG[28], GINI/LISA and Puerto Rican participants genotype information for the replication SNPs were extracted from existing imputed GWAS data. Detailed information about genotyping procedure in each participating study is given in the Supporting information (Information S1).

## Statistical analyses

The ancestry analysis was carried out in all studies with GWAS data using EIGENSTRAT 2.0[29], and outliers were flagged and eliminated from subsequent analyses.[25, 27] Separate association analyses were performed in each study and the GWAS results were then subject to meta-analysis (for asthmatic children; screening and replication data sets separately and finally combined). BMI was analyzed as a continuous trait, in asthmatics and non-asthmatics separately, with linear regression in PLINK[30], using an additive genetic model adjusted for age, gender and informative principal components (to adjust for potential population substructure). BMI standard deviation score (or equivalent such as z score) analyses were also performed in a subset of the child studies. The CAPPS, EGEA, and SAGE data sets comprised a mixture of unrelated and related cases and controls, and regression models adjusting for clustering of family genotypes was used in Stata Statistical Software (College Station, TX). In the family based studies CAMP and TOMSK, only probands were included in BMI analyses (siblings excluded). SNPs passing the following quality control criteria in each study were later included in the meta-analysis: 1) genotype missing rate < 3 % in both cases and controls; 2) minor allele frequency  $\geq$  1% in controls; 3) Hardy-Weinberg equilibrium p-value > 0.0001 in controls. Combined meta-analysis and tests for heterogeneity between studies were carried out in Metal, using a weighted z-score method (fixed effects) that accounts for the direction of association relative to a consistent reference allele (<http://www.sph.umich.edu/csg/abecasis/metal/index.html>).[21] A minimum of 1500 subjects were used as a cut off in each analysis. The weights are proportional to the square-root of sample size in each study and the squared weights sum to 1.0. Quantile-quantile plots (Q-Q plots), Manhattan plots and annotations were created in the WGAViewer software, which is based on the latest Ensembl Core, Variation, and GO databases.<sup>31</sup> Funnel plots

and random effects models were done in Stata Statistical Software (College Station, Tx). Plots of the regional association results were created using LocusZoom.[31] At individual study level, logistic regression analysis, adjusted for informative principal components (in PLINK or Stata), was used to estimate the association between specific *DENND1B* SNPs and asthma (followed by meta-analysis across studies using Metal). Power calculations based on reported effects of one of the major BMI genes, *FTO* [21] show that at least 2,500 individuals are required for robust association analyses (80% power based on Beta = 0.33, MAF 0.41 and significance level 0.05, one-sided p-value).

## Results

Table 1 shows the descriptive statistics of the child (screening and replication data sets) and adult studies and subjects included in this analysis after QC. The mean BMI values varied somewhat between studies, from 15.8 to 19.1 in children (age range 3.5-18 years) and from 24.3 to 28.4 in adults, but no large differences were seen between BMI in asthmatics and non-asthmatics. Figure 1 shows the QQ-plot based on 536,451 SNPs from the meta-analysis results on BMI in 2,691 asthmatic children using the screening data set (observed p-values on the y-axis to those expected on the x-axis for a null distribution). The tail marginally deviates from what is expected by chance without evidence of population stratification (genomic inflation factor 1.01), which suggests that true associations between some SNPs and BMI in asthmatic children exist in the data. We identified associations between several SNPs in *DENND1B* on chromosome 1q31 and BMI in asthmatic children (top SNP rs4915551, p-value= $2.2 \times 10^{-7}$ , Figure 2a and Table 2), and a locus on chromosome 7 containing *SLC12A9* was also indicated. A regional plot of association results for SNPs in the *DENND1B* loci on chromosome 1q31 is presented in Figure S1, where linkage disequilibrium values ( $r^2$  0.4-0.8 between rs4915551 and the other top SNPs) are also

indicated. The top 10 SNPs from the screening analysis, including *DENND1B* SNPs, were next analyzed in seven independent replication data sets comprising 2,014 asthmatic children from Europe, Central and North America (Table 1). One of the *DENND1B* SNPs was nominally significant also in the combined replication data sets (rs10737692,  $p=0.04$ ). The association for the top *DENND1B* SNP rs4915551 was nominally replicated ( $p<0.05$ ) in two of the studies (Figure 3), GACRS and CAPPS, and of borderline significance in GINI/LISA ( $p=0.059$ ). However, signs of heterogeneity were found for rs4915551, which indicate large inter-study variations and overall, the association was not significant in the replication data set,  $p=0.71$  (Table 3). Combined analyses of both screening and replication data ( $N=4,705$ ) confirmed highly significant tests for heterogeneity for all top *DENND1B* SNPs ( $p\text{-value} = 5.8 \times 10^{-3}$  to  $4.5 \times 10^{-5}$ , Table 3). The forest plot of rs4915551 in the combined analyses (Figure 3) also shows that BMI was estimated to change from -1.4 units in the Canadian study CAPPS ( $p=0.01$ ) to +1.7 units in the Russian study Tomsk ( $p=0.003$ ). Using a random effects model, BMI was overall estimated to increase by  $0.30 \text{ kg/m}^2$  ( $p=0.01$ ) per additional G allele of this *DENND1B* SNP. Minor allele frequencies for this SNP varied between 0.17 (Russia) and 0.37 (Puerto Rico), but showed no correlation with the direction of the effect on BMI ( $p>0.68$ ).

Because BMI changes with age in children, standard deviation (sds) BMI scores analyses (e.g. Z-scores, iso-BMI or specific cut-off such as above 85<sup>th</sup> or 95<sup>th</sup> percentile) are often suggested and we therefore compared our age and sex adjusted BMI results with sds BMI score results in studies with these data available (9 studies,  $N$  asthmatics = 3,696). Figure 4 shows that the sds score analyses are very consistent with the age and sex adjusted BMI results within each study and the pattern of clear heterogeneity is essentially unchanged ( $p<0.0001$  for heterogeneity,  $p\text{-value for association} = 0.008$  fixed effects and  $p=0.12$  random effects).

No association between *DENND1B* SNPs and asthma in children (case-control analysis, N=11,956) or adults (N=11,575) were seen (Table S1).

For BMI in non-asthmatic children (n=6,019 from data available in 8 studies), the top SNP on chromosome 9 (p-value =  $2.2 \times 10^{-7}$ ) is located in *DENND1A* (rs10818854), respectively (Figure 2b, Supporting Figure S2 and Table S2). A handful of additional SNPs in *DENND1A* were associated with BMI in non-asthmatic children (p-value  $\approx 10^{-4}$  to  $10^{-6}$ , Figure S3), but all SNPs had MAF around 0.03-0.04. *DENND1B* SNPs showed nominal p-values between 0.02-0.08 for BMI in non-asthmatic children, e.g. p=0.03 for rs4915551 (Figure 2b and Table S1), but the direction was generally in the opposite direction as in asthmatics. No association was found between rs4915551 and BMI in asthmatic or non-asthmatic adults (p=0.53 and 0.87). *DENND1A* rs10818854 was not associated with BMI in asthmatic children (p-value = 0.47, screening data set).

The meta-analysis of GWAS results on BMI in asthmatic adults (n=3,242) showed little evidence of strong genetic determinants (Figure 2c, Figure S4 and Table S3). The top SNP, rs643507 (p-value =  $8.0 \times 10^{-9}$ ), resides in a reasonable candidate gene for BMI, *LPIN2*, but has a minor allele frequency below 1% in 4 of the 7 adult studies. SNPs in *STARD13*, *PRKCQ* and *SLC24A2* with a p-value in the range of  $10^{-6}$  were also observed.

Only 3 SNPs were associated at p-values  $\leq 10^{-6}$  with BMI in non-asthmatic adults (n=7,679), all being intergenic SNPs (Figure 2d, Figure S5 and Table S4). The top SNP, rs7775861 on chromosome 6, was however close to genome wide significant at p= $8.6 \times 10^{-8}$ . Since *FTO* has been implicated as an important gene for BMI in several other GWAS, we tested the top *FTO* SNP reported in the literature (rs9939609 represented by rs8050136)[21] to evaluate if this SNP was associated with BMI in our data and found nominally significant associations (p-

values= $4.5 \times 10^{-4}$  and  $1.2 \times 10^{-3}$  in non-asthmatic and asthmatic adults, p-values= $6.1 \times 10^{-3}$  and  $2.6 \times 10^{-4}$  in non-asthmatic and asthmatic children, respectively).

## Discussion

Herein we report the results from unbiased GWAS analyses on BMI in a large collection of asthmatics and healthy individuals. Associations were found between several SNPs in *DENND1B* on chromosome 1q31 and BMI in 2,691 asthmatic children and also replicated in independent data sets; however, the effect estimates were very heterogeneous across studies from different geographical locations and overall not significant in the total replication data set. Only weak associations were found between *DENND1B* SNPs and BMI in non-asthmatic children and no associations were seen in asthmatic/non-asthmatic adults. A recent GWAS study identified *DENND1B* as a new susceptibility gene for asthma[32], and it is intriguing that we now find some evidence for association with another phenotype, BMI in asthmatic children. Given the overall lack of replication of the BMI results and the fact that the association did not reach genome-wide significance, we cannot completely rule out that other factors such as environmental exposures, other genetic variants or chance may have contributed to our findings. Additional replication of the association is necessary to validate our results.

Epidemiological studies have consistently found associations between asthma, particularly non-atopic asthma[33], and BMI/obesity, although temporal aspects of the asthma–obesity link seems to be rather complex in children.[34, 35] The shared genetic component between the two phenotypes is believed to be substantial but yet of moderate effect size; estimates from twin studies indicate that 8% of the genetic component of obesity is shared with asthma.[8] The literature is, however, sparse with examples of shared genetics between asthma and obesity. *LEP*, *PRKCA* and *TNF* genes have been evaluated for pleiotrophic effects in the

same population [36-38], as well as candidate genes from recent GWAS, including *GNPDA2*, *PTPRD* and *ROBO1*. [12] Our findings do not support the notion that *DENND1B* variants contribute to an increase in risk of either asthma or BMI in the general population, but rather that the identified variants affect BMI conditional on the child's asthma affection status. The association signal in asthmatics seemed to be restricted to *DENND1B* and not extend beyond the recombination hot spots up- and downstream of the gene.

Body mass index in children is known to change substantially with age. [39] Consequently, the definitions of overweight and obesity are age and sex specific. Cut-offs for these conditions have been established in certain reference populations, e.g. in the US [40], as well as Europe and Asia. [39] Similar to many other genetic studies in children [14, 37, 41], we used age and sex adjusted BMI as a continuous trait in the screening and replication data analyses. This maximized power by using a large number of cohorts and hence large overall sample sizes. However, the age and sex adjusted results were compared with z-score analyses (or equivalent sds BMI scores) for the top *DENND1B* SNP in 3,700 asthmatics, and the results were found to be very similar in respective cohort.

*DENND1B* (also known as DENN/MADD) is expressed in a wide range of tissues and cells, including adipocytes, but most predominantly in natural killer cells and dendritic cells. [32] *DENND1B* is predicted to exert its effects through the *TNF*-pathway via binding to the TNFR1, whereby MAP kinase activation and arachidonic acid release take place. [42] *DENND1B* has been proposed to have dual activities under normal and stress conditions such as hypoxia or macrophage invasion. [43] The *TNF*-pathway has been suggested to have a key role in both asthma and obesity, which makes the finding in our study biologically plausible. Children with asthma may be considered to suffer from chronic, inflammatory stress, and there is increasing

evidence that overweight/obesity is a proinflammatory state with chronic stress.[44] The link between DENND1B and the TNF-pathway has, however, been questioned and there is evidence that proteins in the DENN domain-containing family are involved in guanine-nucleotide exchange and endosomal-membrane trafficking.[45, 46] Further research is needed to determine potential interaction between DENND1B and TNF-related proteins. Recent meta-analyses of large GWAS implicate involvement of *DENND1B* also in the pathogenesis of Crohn's disease and primary biliary cirrhosis, which supports its role in inflammatory diseases.[47, 48]

In the original asthma GWAS, *DENND1B* variants were associated with decreased risk (e.g. the minor allele of the top hit, rs2786098) of asthma in children with European ancestry, while increased risk were seen in children with African ancestry.[32] Strong evidence of heterogeneity was found also in our study (especially for rs4915551) despite the fact that all cohort members in this study were predominantly of European descent, which is likely explained by the large geographical differences among the cohorts (Australia, Europe, Central and North America), as well as different exposure patterns (e.g. farm exposure as in GABRIELA[49]). A tendency towards a West-East gradient in the effect estimate of the top *DENND1B* SNP (rs4915551) was also seen ranging from significantly negative effect in a Canadian study to positive effect on BMI in the Russian study. In most of the included studies, a rather liberal asthma definition was used (doctor's diagnosis ever), similar to the GABRIEL study.[25] Other studies have used more stringent criteria, e.g. CAMP and the replication study GACRS (Supporting data for details), and different asthma criteria could also influence heterogeneity of effect. The top *DENND1B* SNP in our study (rs4915551), had  $p=3.12 \times 10^{-5}$  in the original asthma GWAS with OR = 0.73 (G vs A) in Europeans.[32] Similar to the results from the main

GABRIEL study[25] , the American EVE consortium[28] and an Australian study [50], we did not find any association between *DENND1B* and asthma per se.

Interestingly, *DENND1A* on chromosome 9q33 showed evidence of association in non-asthmatic children, albeit not at a genome-wide significant level and the top SNPs had a minor allele frequency of around 3-4%. Associations with polycystic ovary syndrome have also been reported for one of the SNPs, rs10818854 in Han Chinese.[51] *DENND1A* encodes for Connecdenn, which binds to Clathrin that mediates internalization of proteins and lipids through endocytosis.[46] Connecdenn shows 38% overall identity with the *DENND1B* encoded protein, which has also been named Connecdenn-2.

Analyses of genetic determinants for BMI in adults did not reveal any strong candidate genes. Two SNPs in *STARD13* (chromosome 13q12) were associated at  $p=3 \times 10^{-6}$  and  $1 \times 10^{-5}$  in asthmatics. *STARD13* has previously been associated with intracranial aneurysm[52], and it is unclear if this gene has any relevance also for BMI levels based solely on our data. Several SNPs on chromosome 6 showed associations to BMI in adult non-asthmatics, but these were intergenic and have not been associated with BMI or obesity before. SNPs in *FTO* did not reach genome wide significance in any of the groups, but were nominally associated with BMI in both adults and children regardless of asthma status. Although the data sets were rather large with adequate power to detect associations to common SNPs with the largest effect sizes on BMI, it is likely that an even larger study population would yield clearer results.

In summary, we find evidence that *DENND1B* variants identified using a GWAS approach may be associated with BMI in asthmatic children. Overall, the results were however not replicated in the independent data sets, and our results should therefore be interpreted with caution. The heterogeneous effect of *DENND1B* on BMI, as well as on BMI sds scores, in our

study and on asthma in a previous GWAS[32], point to complex associations with the studied diseases that deserve further study.

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### **Conflict of interest statement**

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### Legends to figures

Figure 1. Quantile-quantile (QQ) plot of SNPs after meta-analysis for association to BMI in the screening data set consisting of 2,691 *asthmatic children* (observed p-values on the y-axis to

those expected on the x-axis for a null distribution; i.e. no overall association between SNPs and BMI). Deviation of measures at the tail (upper right corner) may indicate true associations between some SNPs and BMI in asthmatic children.

Figure 2a. Manhattan plot showing the significance of association of all SNPs (n=536,451) across chromosomes 1-22 and X in the meta-analysis with BMI in *asthmatic children* (screening data set, n=2,691 individuals). SNPs are plotted on the x axis according to their position on each chromosome and association with BMI is indicated on the y axis (as  $-\log_{10}$  P value). The region containing *DENND1B* on chromosome 1q31 is indicated with an arrow.

Figure 2b. Manhattan plot showing the significance of association of all SNPs (n=527,642) across chromosomes 1-22 and X in the meta-analysis with BMI in *non-asthmatic children* (n=6,019 individuals).

Figure 2c. Manhattan plot showing the significance of association of all SNPs (n=532,694) across chromosomes 1-22 and X in the meta-analysis with BMI in *asthmatic adults* (n=3,242 individuals).

Figure 2d. Manhattan plot showing the significance of association of all SNPs (n=512,478) across chromosomes 1-22 and X in the meta-analysis with BMI in *non-asthmatic adults* (n=7,679 individuals).

Figure 3. Forest plot from the meta-analysis results of *DENND1B* rs4915551 G/A effects on BMI in asthmatic children (n children = 4,705 from both screening (S) and replication (R)

studies. The X-axis represents the beta values (ES) for association with BMI. Evidence of heterogeneity was found ( $p=0.001$ ) and random effects analysis was therefore used. The diamond represents the overall estimate (i.e. beta-coefficient, also represented by the dotted line) with 95% confidence intervals, which corresponds to the per-allele change in BMI (minor allele G as effect allele, additive genetic model). Sample size (n), beta-coefficient (ES) and p-values for the association to BMI, and study weight in the meta-analysis are given for each study. The grey box around each study's beta-coefficient is proportional to the weight (using the inverse variance method).

Figure 4. Forest plot from the meta-analysis results of *DENND1B* rs4915551 G/A effects on BMI standard deviation (sds) / z scores in asthmatic children (n children = 3,696) from studies with these data available. ALSPAC, CAPPs and SAGE had some additional cases available for these analyses (compared with age and sex adjusted analyses), and these studies are therefore labeled ALSPAC2, CAPPs2 and SAGE2, respectively. The X-axis represents the beta values (ES) for association with BMI. Evidence of heterogeneity was found ( $p<0.001$ ) and random effects analysis was therefore used. The diamond represents the overall estimate (i.e. beta-coefficient, also represented by the dotted line) with 95% confidence intervals, which corresponds to the per-allele change in BMI sds / z score (minor allele G as effect allele, additive genetic model). Sample size (n), beta-coefficient (ES) and p-values for the association, and study weight in the meta-analysis are given for each study. The grey box around each study's beta-coefficient is proportional to the weight (using the inverse variance method).

**Table 1. Baseline Characteristics of Subjects in the Study Cohorts**

| Study name                                   | Study design     | Platform               | Country        | Asthma | Non-asthma | Age, years            | Gender, female | BMI, asthmatics       | BMI, non-asthmatics   |
|----------------------------------------------|------------------|------------------------|----------------|--------|------------|-----------------------|----------------|-----------------------|-----------------------|
| <i>Childhood onset - Screening data sets</i> |                  |                        |                |        |            | <i>Mean (min-max)</i> | <i>n, %</i>    | <i>Mean (min-max)</i> | <i>Mean (min-max)</i> |
| ALSPAC                                       | BC; Nested Ca-Co | Illumina 610-Quad      | United Kingdom | 571    | 573        | 7.5 (7.1-9.2)         | 510 (44.5)     | 16.3 (12.8-25.7)      | 16.2 (13.0-27.3)      |
| BAMSE                                        | BC;Ca-Co         | Illumina 610-Quad      | Sweden         | 239    | 246        | 8.3 (7.3-10.4)        | 216 (44.5)     | 17.3 (13.3-25.3)      | 17.0 (13.5-24.6)      |
| British 1958 Cohort                          |                  |                        |                |        |            |                       |                |                       |                       |
| - B58C GABRIEL                               | Nested Ca-Co     | Illumina 610-Quad      | United Kingdom | 197    | 723        | 7.3 (7.0-8.0)         | 390 (54.0%)    | 15.8 (11.5-23.9)      | 15.8 (10.7-25.2)      |
| - B58C WTCCC                                 | Pop-based cohort | Illumina 550K          | United Kingdom | 80     | 1204       | 7.3 (7.0-8.0)         | 589 (48.9%)    | 15.9 (9.5-21.9)       | 15.8 (9.3-23.6)       |
| - B58C T1DGC                                 | Pop-based cohort | Illumina 550K          | United Kingdom | 137    | 2092       | 7.3 (7.0-8.3)         | 994 (50.8%)    | 15.9 (12.5-23.9)      | 15.9 (10.5-25.7)      |
| CAMP*                                        | Fam.; Ca-Co      | Illumina 550K and 610K | USA            | 537    | -          | 8.8 (5.2-13.2)        | 205 (38.2)     | 17.9 (13.0-29.4)      | -                     |
| MAGICS / ISAAC                               | Ca-Co            | Illumina 330K          | Germany        | 507    | 346        | 10.3 (4-17)           | 357 (42)       | 18.6 (10.6-31.6)      | 17.8 (11.6-29.0)      |
| MAS                                          | BC; Ca-Co        | Illumina 610-Quad      | Germany        | 150    | -          | 9.6 (7-13)            | 65 (43)        | 17.5 (12.2-27.0)      | -                     |
| PIAMA                                        | BC;Ca-Co         | Illumina 610-Quad      | Holland        | 164    | 186        | 8.2 (7.4-9.7)         | 167 (47.7)     | 16.8 (13.0-24.6)      | 16.5 (13.0-23.2)      |

|                              |                  |                        |                   |          |       |                  |             |                  |                  |
|------------------------------|------------------|------------------------|-------------------|----------|-------|------------------|-------------|------------------|------------------|
| TOMSK Children               | Fam.             | Illumina 610-Quad      | Russia            | 109      | -     | 14.9 (3.5-51)    | 41 (37.2)   | 19.1 (10.0-69.3) | -                |
| <i>Replication data sets</i> |                  |                        |                   |          |       |                  |             |                  |                  |
| CAPPS <sup>†</sup>           | Trio / Cohort    | Illumina 610-Quad      | Canada            | 181 / 48 | 236   | 24.8 (6.2-49.7)  | 205 (49.2)  | 16.6 (13.4-25.3) | 16.6 (12.7-24.2) |
| GABRIELA                     | Ca-Co            | Illumina 610-Quad      | Germany           | 840      | 841   | 8.7 (5-14)       | 803 (47.8%) | 17.1 (10.0-37.3) | 17.0 (12.0-27.7) |
| GACRS                        | Fam.             | Sequenom               | Costa Rica        | 407      | -     | 8.7 (6.0-13.3)   | 171 (42.0)  | 16.8 (11.3-30.1) | -                |
| GINI/LISA                    | Pop-based BC     | Affymetrix 5.0 / 6.0   | Germany           | 72       | -     | 10.2 (9.9-11.1)  | 27 (37.5%)  | 17.2 (11.4-26.4) | -                |
| Hartford/ Puerto Rico        | Ca-Co            | Illumina Human Omni2.5 | Puerto Rico / USA | 514      | 421   | 10.0 (6-14)      | 451 (47.8)  | 21.1 (12.9-58.4) | 19.9 (11.8-39.6) |
| SAGE children <sup>†</sup>   | Trio / Cohort    | Illumina 610-Quad      | Canada            | 72       | 145   | 9.0 (7.7-10.2)   | 93 (42.9)   | 17.6 (13.6-29.0) | 17.5 (11.2-30.3) |
| CAG <sup>†</sup>             | Fam; Ca-Co       | Illumina 1Mv1          | USA               | 67       | -     | 28.2 (6-74)      | 21 (31.3)   | 24.4 (14.9-39.5) | -                |
| <i>Adult studies</i>         |                  |                        |                   |          |       |                  |             |                  |                  |
| British 1958 Cohort, B58C    |                  |                        |                   |          |       |                  |             |                  |                  |
| - B58C GABRIEL               | Nested Ca-Co     | Illumina 610-Quad      | United Kingdom    | 445      | 425   | 45.2 (44.3-46.0) | 466 (53.6%) | 27.6 (17.0-46.6) | 27.2 (17.0-54.7) |
| - B58C WTCCC                 | Pop-based cohort | Illumina 550K          | United Kingdom    | 180      | 1,249 | 44.9 (44.5-46.0) | 709 (49.6%) | 28.2 (18.4-56.3) | 27.3 (17.1-49.5) |

|                                   |                         |                      |                      |       |        |                       |                  |                      |                      |
|-----------------------------------|-------------------------|----------------------|----------------------|-------|--------|-----------------------|------------------|----------------------|----------------------|
| - B58C<br>T1DGC                   | Pop-<br>based<br>cohort | Illumina<br>550K     | United<br>Kingdom    | 339   | 2,248  | 45.3 (44.5-<br>46.0)  | 1,328<br>(51.3%) | 28.0 (17.3-<br>53.1) | 27.4 (17.4-<br>50.5) |
| Busselton<br>Health Study         | Pop.<br>based           | Illumina<br>610-Quad | Australia            | 509   | 703    | 53.4 (17.3-<br>91.4)  | 704<br>(58.1)    | 25.9 (18.7-<br>40.8) | 26.0 (15.8-<br>40.7) |
| ECHRS                             | Pop.<br>Based           | Illumina<br>610-Quad | Multiple<br>European | 678   | 1,527  | 33.9 (19.7-<br>48.1)  | 1181<br>(53.6)   | 24.8 (15.5-<br>44.8) | 24.8 (16.7-<br>54.1) |
| EGEA                              | Fam.;Ca<br>-Co          | Illumina<br>610-Quad | France               | 821   | 831    | 42.4 (15.1-<br>79.5)  | 675<br>(40.9)    | 24.3 (15.8-<br>44.0) | 24.3(16.7-<br>53.7)  |
| SAPALDIA                          | Pop.<br>Based           | Illumina<br>610-Quad | Switzer-<br>land     | 349   | 878    | 42.1 (18.3 –<br>61.8) | 572<br>(46.6)    | 24.4 (16.7-<br>39.3) | 23.9(16.9 –<br>45.5) |
| SAGE<br>Parents <sup>†</sup>      | Trio /<br>Cohort        | Illumina<br>610-Quad | Canada               | 77    | 192    | 44.6 (12.3-<br>68.1)  | 148<br>(54.6)    | 28.4 (20.3-<br>43.3) | 27.2 (19.8-<br>37.7) |
| TOMSK<br>adults                   | Fam.                    | Illumina<br>610-Quad | Russia               | 62    | 331    | 39.4 (19-78)          | 203<br>(61.3)    | 25.2 (16.9-<br>44.4) | 26.1 (13.6-<br>52.9) |
| Total sample size, child studies‡ |                         |                      |                      | 4,705 | 7,013  |                       |                  |                      |                      |
| Total sample size, adult studies  |                         |                      |                      | 3,460 | 8,384  |                       |                  |                      |                      |
| Total sample size, all            |                         |                      |                      | 8,165 | 15,397 |                       |                  |                      |                      |

\* In CAMP, 359 asthmatics included in GWAS analyses on asthma and 537 asthmatics included in GWAS analyses on BMI. 1205 Illumina controls did not contribute with BMI data. 205 were girls of the asthmatic children.

<sup>†</sup> In CAPPS, asthma in children and adults were analyzed together for power reasons. Out of 181 asthmatics, 48 were children. BMI was only available in CAPPS children. In SAGE, association to asthma in children and adults were analyzed together for power reasons. Association to BMI was analyzed separately in children and adults. In CAG, both children and adults were included in the BMI analyses (*DENNDIB* replication only).

<sup>‡</sup> Children with childhood onset of asthma were included in analyses of childhood asthma even if individuals were older (>18 years) at the time of enrollment, except for EGEA where individuals > 15 years were considered adults. Association to BMI was analyzed separately in children (<18 years) and adults. BMI values were not available for all individuals listed in this table, and somewhat lower are therefore presented in respective tables and figures with BMI results. BC = Birth cohort, Ca-Co = Case-control, Fam = Family based, Pop. based = Population based

**Table 2. Top 10 SNPs from meta-analysis of GWAS on BMI in asthmatic children, screening data set (n=2,691)**

| SNP        | Chr. | Position  | Min.<br>allele | MAF* | Nearby gene       | Distance<br>to gene | p-value <sup>†</sup><br>association | Test and Direction <sup>‡</sup> | p-value<br>heterogeneity |
|------------|------|-----------|----------------|------|-------------------|---------------------|-------------------------------------|---------------------------------|--------------------------|
| rs4915551  | 1    | 197508901 | G              | 0.24 | <i>DENND1B</i>    | 0                   | 2.2x10 <sup>-7</sup>                | A vs G -----                    | 0.07                     |
| rs7803865  | 7    | 100505792 | G              | 0.44 | <i>AC011895.1</i> | -1595               | 5.9x10 <sup>-7</sup>                | A vs G ---??---                 | 0.35                     |
| rs1775454  | 1    | 197727298 | T              | 0.22 | <i>DENND1B</i>    | 0                   | 8.6x10 <sup>-7</sup>                | T vs C ++++?--+                 | 0.09                     |
| rs314376   | 7    | 100457356 | G              | 0.45 | <i>SLC12A9</i>    | 0                   | 1.0x10 <sup>-6</sup>                | A vs G ---??---                 | 0.29                     |
| rs13232524 | 7    | 100512824 | T              | 0.47 | <i>AC011895.1</i> | -8627               | 1.0x10 <sup>-6</sup>                | T vs C ++++-----                | 0.03                     |
| rs1775456  | 1    | 197733055 | G              | 0.22 | <i>DENND1B</i>    | 0                   | 1.7x10 <sup>-6</sup>                | A vs G -----+-                  | 0.09                     |
| rs2111931  | 1    | 195795841 | C              | 0.23 | <i>DENND1B</i>    | 0                   | 1.8 x10 <sup>-6</sup>               | T vs C -----+-                  | 0.09                     |
| rs10737692 | 1    | 197548933 | A              | 0.23 | <i>DENND1B</i>    | 0                   | 1.8x10 <sup>-6</sup>                | A vs G ++++?--+                 | 0.08                     |
| rs1775444  | 1    | 196007313 | T              | 0.22 | <i>DENND1B</i>    | 0                   | 2.1 x10 <sup>-6</sup>               | T vs C +++++--+                 | 0.12                     |
| rs1924518  | 1    | 197738327 | A              | 0.22 | <i>DENND1B</i>    | 0                   | 2.4x10 <sup>-6</sup>                | A vs G ++++?--+                 | 0.11                     |

\* MAF = Minor allele frequency was estimated in the ALSPAC study (n=1,144 children; asthma cases and controls combined).

† P-values for combined meta-analysis using a fixed effects model since the heterogeneity test was non-significant (except rs13232524, p=0.03)

‡ Direction reflects the effect (positive or negative on BMI-values) of the effect allele vs reference allele as indicated in the “Test and Direction” column.

“?” reflects missing data from that particular study. Study order corresponding to +/- position (alphabetical order) ALSPAC, BAMSE, B58C (sub-studies pooled), CAMP, MAGICS/ISAAC, MAS, PIAMA, and TOMSK. A total of 536,451 markers were analyzed (minimum number of children in the GWAS analysis, n=1500)

**Table 3. Top 10 SNPs from meta-analysis of GWAS on BMI in asthmatic children identified in the screening data set and association in replication data set and combined**

| SNP        | Nearby gene       | Replication studies |                         |                          | Screening and replication studies combined |                         |                          |
|------------|-------------------|---------------------|-------------------------|--------------------------|--------------------------------------------|-------------------------|--------------------------|
|            |                   | N                   | p-value*<br>association | p-value<br>heterogeneity | N                                          | p-value*<br>association | p-value<br>heterogeneity |
| rs4915551  | <i>DENND1B</i>    | 2014                | 0.71                    | 0.01                     | 4705                                       | $2.8 \times 10^{-5}$    | $3.6 \times 10^{-4}$     |
| rs7803865  | <i>AC011895.1</i> | 1460                | 0.52                    | 0.89                     | 3107                                       | $4.2 \times 10^{-5}$    | 0.10                     |
| rs1775454  | <i>DENND1B</i>    | 1460                | 0.12                    | 0.73                     | 3644                                       | $4.4 \times 10^{-3}$    | $6.6 \times 10^{-4}$     |
| rs314376   | <i>SLC12A9</i>    | 952                 | 0.62                    | 0.76                     | 2599                                       | $2.5 \times 10^{-5}$    | 0.11                     |
| rs13232524 | <i>AC011895.1</i> | 886                 | 0.70                    | 0.41                     | 3577                                       | $5.3 \times 10^{-5}$    | $5.2 \times 10^{-3}$     |
| rs1775456  | <i>DENND1B</i>    | 957                 | 0.20                    | 0.58                     | 3648                                       | $5.2 \times 10^{-4}$    | $3.8 \times 10^{-3}$     |
| rs2111931  | <i>DENND1B</i>    | 1368                | 0.63                    | 0.02                     | 4058                                       | $2.9 \times 10^{-4}$    | $5.5 \times 10^{-4}$     |
| rs10737692 | <i>DENND1B</i>    | 1461                | 0.04                    | 0.19                     | 3645                                       | $1.7 \times 10^{-2}$    | $4.5 \times 10^{-5}$     |
| rs1775444  | <i>DENND1B</i>    | 959                 | 0.21                    | 0.58                     | 3650                                       | $5.8 \times 10^{-4}$    | $5.8 \times 10^{-3}$     |
| rs1924518  | <i>DENND1B</i>    | 1355                | 0.87                    | 0.15                     | 3539                                       | $2.9 \times 10^{-4}$    | $5.6 \times 10^{-3}$     |

P-values for combined meta-analysis using a fixed effects model

Replication studies - CAPPS, SAGE, GACRS, GABRIELA, GINI/LISA, Hartford/Puerto Rico and Chicago. Combined: ALSPAC, BAMSE, B58C (sub-studies pooled), CAMP, MAGICS/ISAAC, MAS, PIAMA, TOMSK, CAPPS, SAGE, GACRS, GABRIELA, GINI/LISA, Hartford / Puerto Rico and CAG.

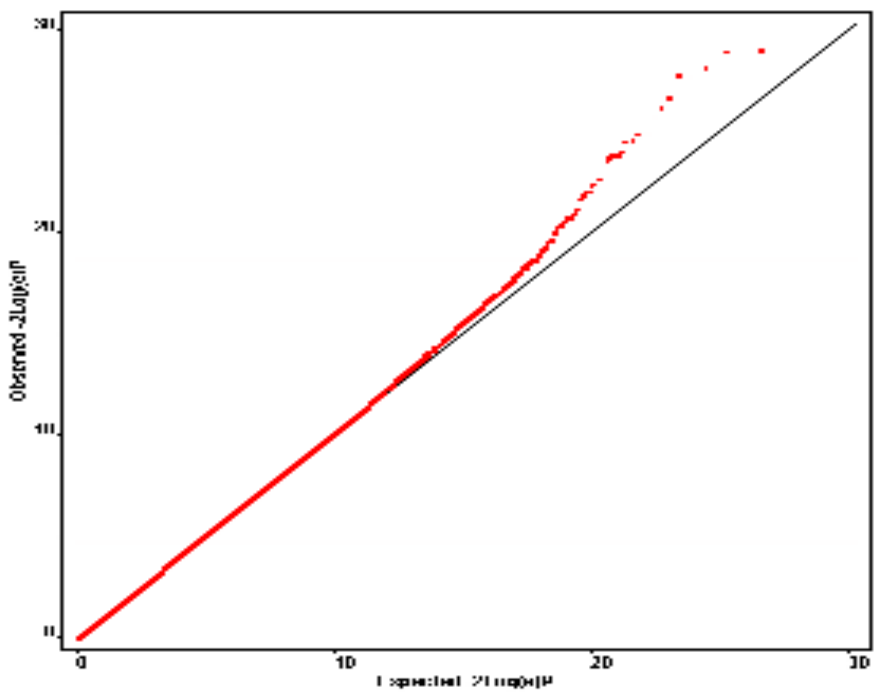


Figure 2a

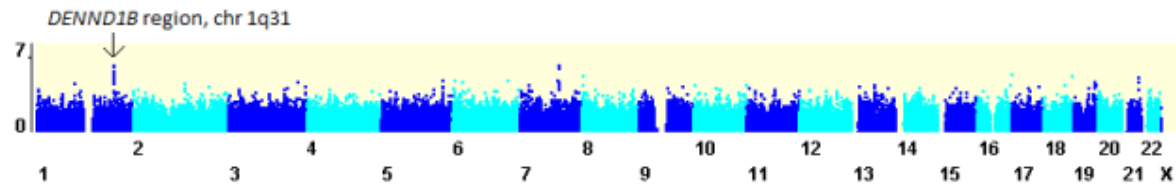


Figure 2b

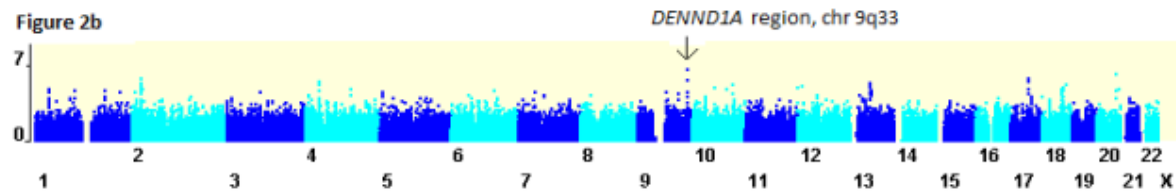


Figure 2c

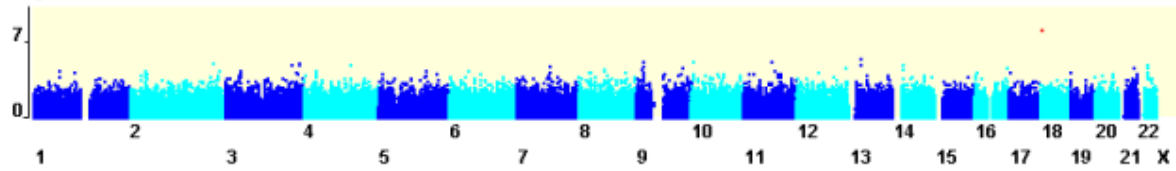
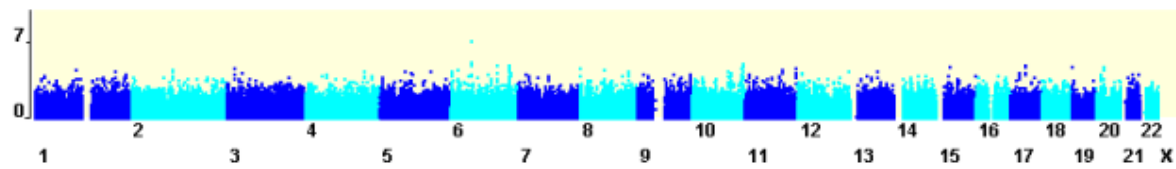
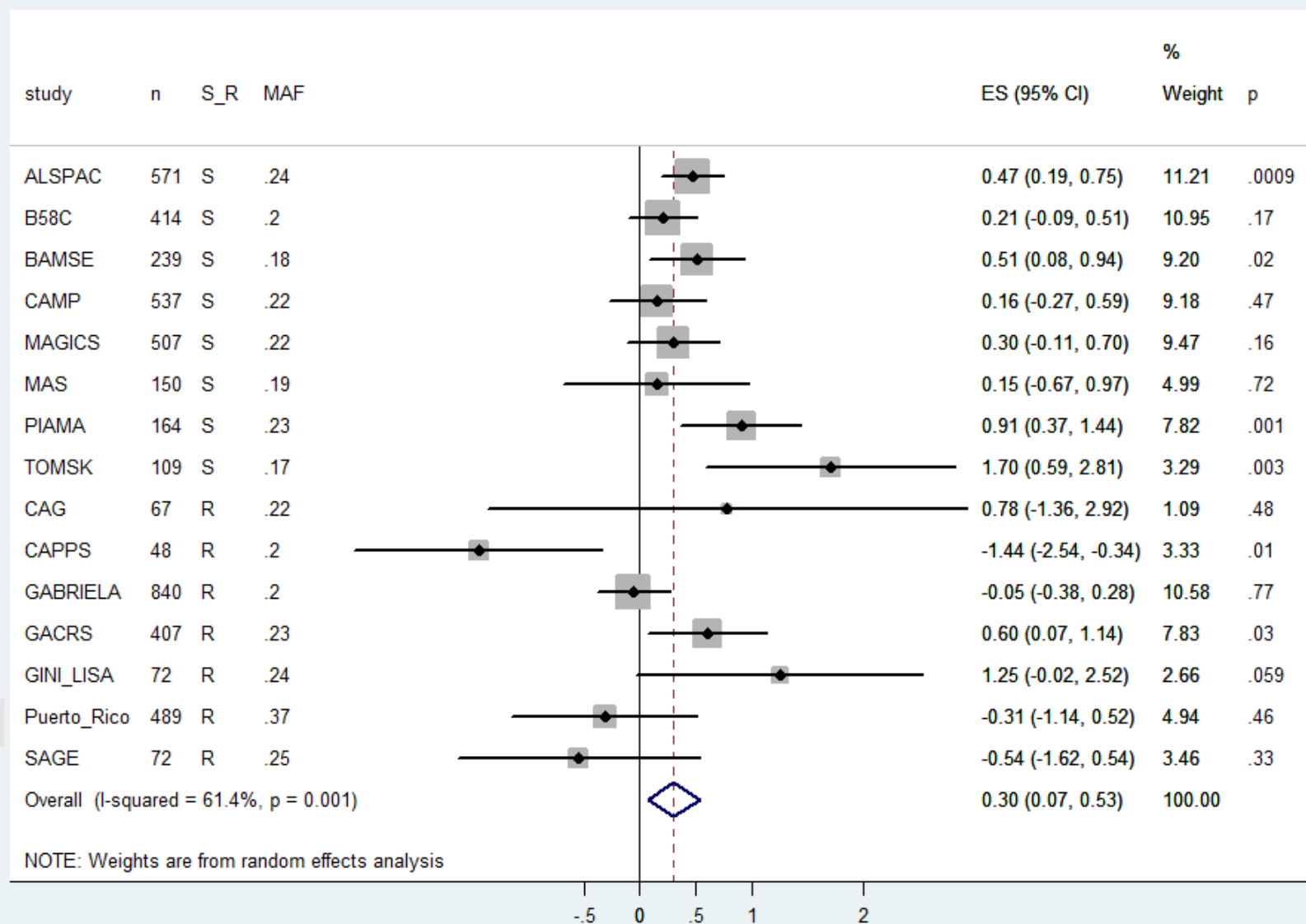
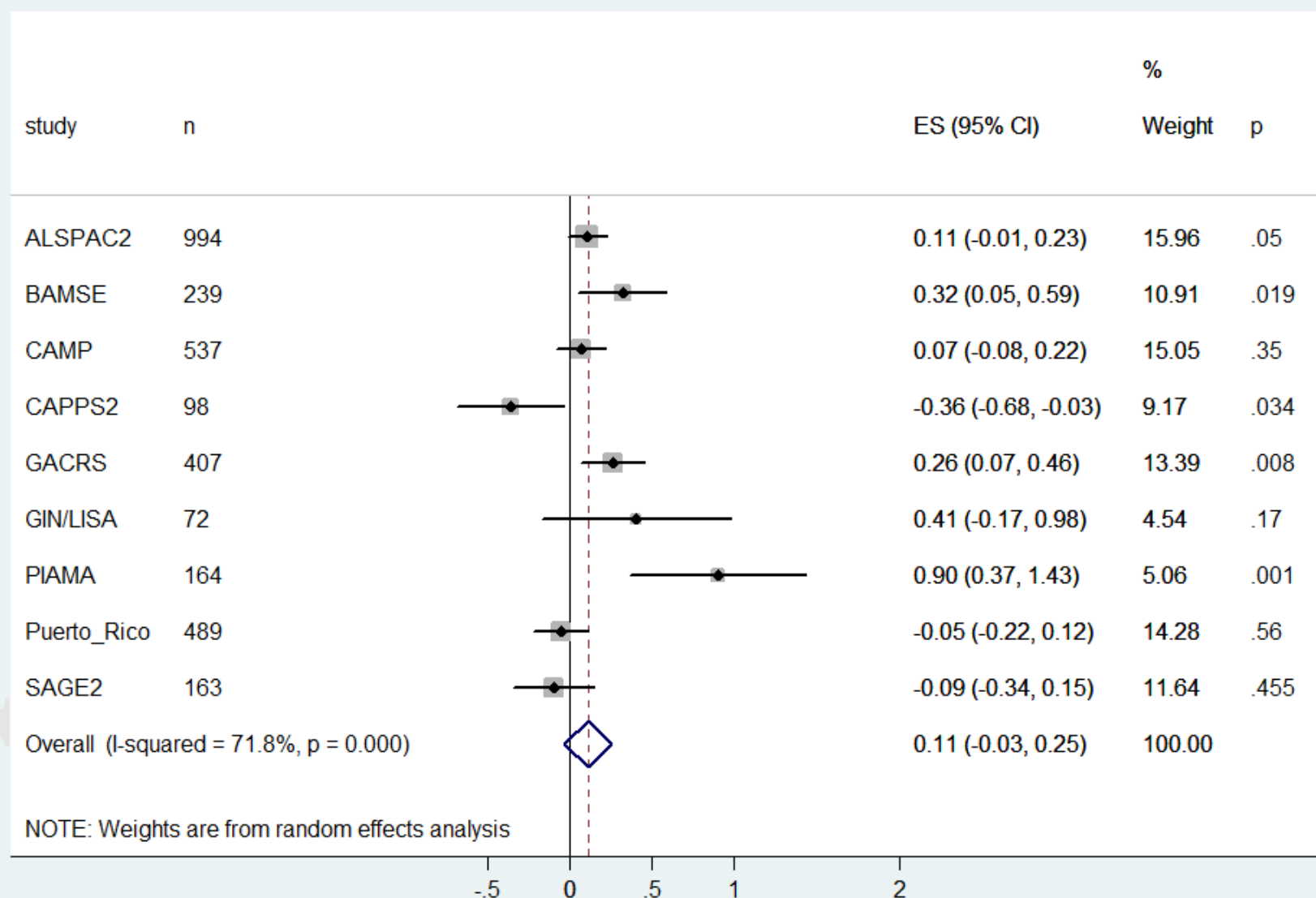


Figure 2d







## Supporting information

### Melén et al, Genome-wide association study of body mass index in 24,000 individuals with and without asthma

Figure S1. Regional plot of the *DENND1B* loci on chromosome 1q31 using LocusZoom.[1] Association results for SNPs from meta-analysis of BMI in asthmatic children ( $n = 2,691$  from screening data sets ALSPAC, BAMSE, B58C, CAMP, MAGIC/ISAAC, MAS, PIAMA and TOMSK) as a function of genomic distance (chromosomal position from National Center for Biotechnology Information build hg18). Each circle represents a SNP, with the color of the circle indicating the correlation (linkage disequilibrium represented by  $r^2$ ) between that SNP and the best SNP at the locus (meta-analysis  $p$ -value =  $2.2 \times 10^{-7}$  for rs4915551, purple circle). Light blue lines indicate estimated recombination hot spots in HapMap phase II CEU. Bottom panel shows genes at each locus as annotated in the UCSC Genome Browser Annotation Database.

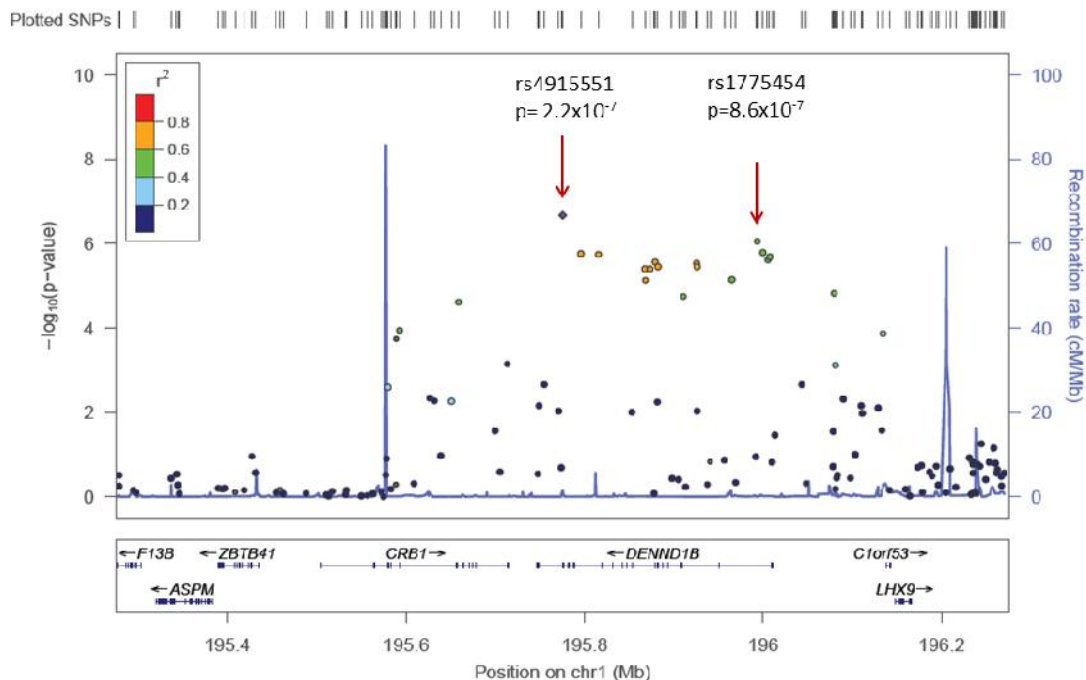


Figure S2. QQ- plot of SNPs after meta-analysis for association to BMI in *non-asthmatic children* (observed p-values to those expected for a null distribution). Deviation of measures at the tail (upper right corner) may indicate true associations between some SNPs and BMI. Genomic inflation factor ( $\lambda$ ) = 1.02.

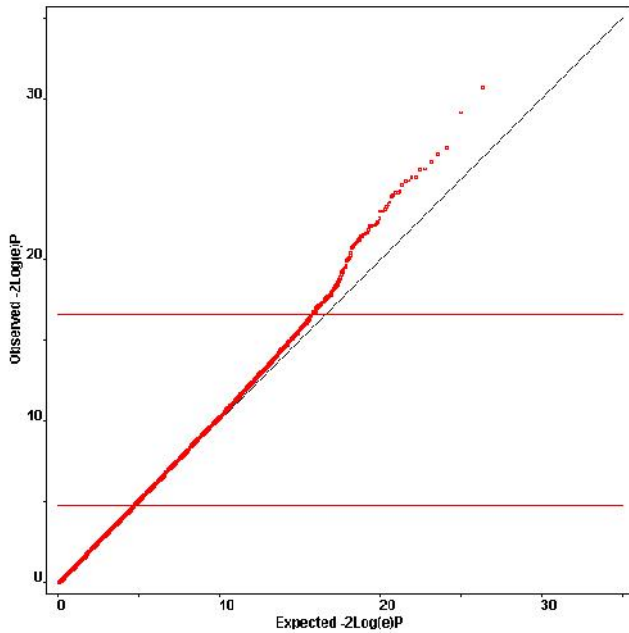


Figure S3.

Regional plots of the *DENND1A* loci on chr 9q33 using LocusZoom.[1] Association results for SNPs from meta-analysis of BMI in 6,019 non-asthmatic children in 11 studies ( $-\log_{10}$  P value) as a function of genomic distance (chromosomal position from National Center for Biotechnology Information build hg18). Each circle represents a SNP, with the color of the circle indicating the correlation between that SNP and the best SNP at the locus (rs10818854, purple circle). Light blue lines indicate estimated recombination hot spots in HapMap phase II CEU. Bottom panel shows genes at each locus as annotated in the UCSC Genome Browser Annotation Database.

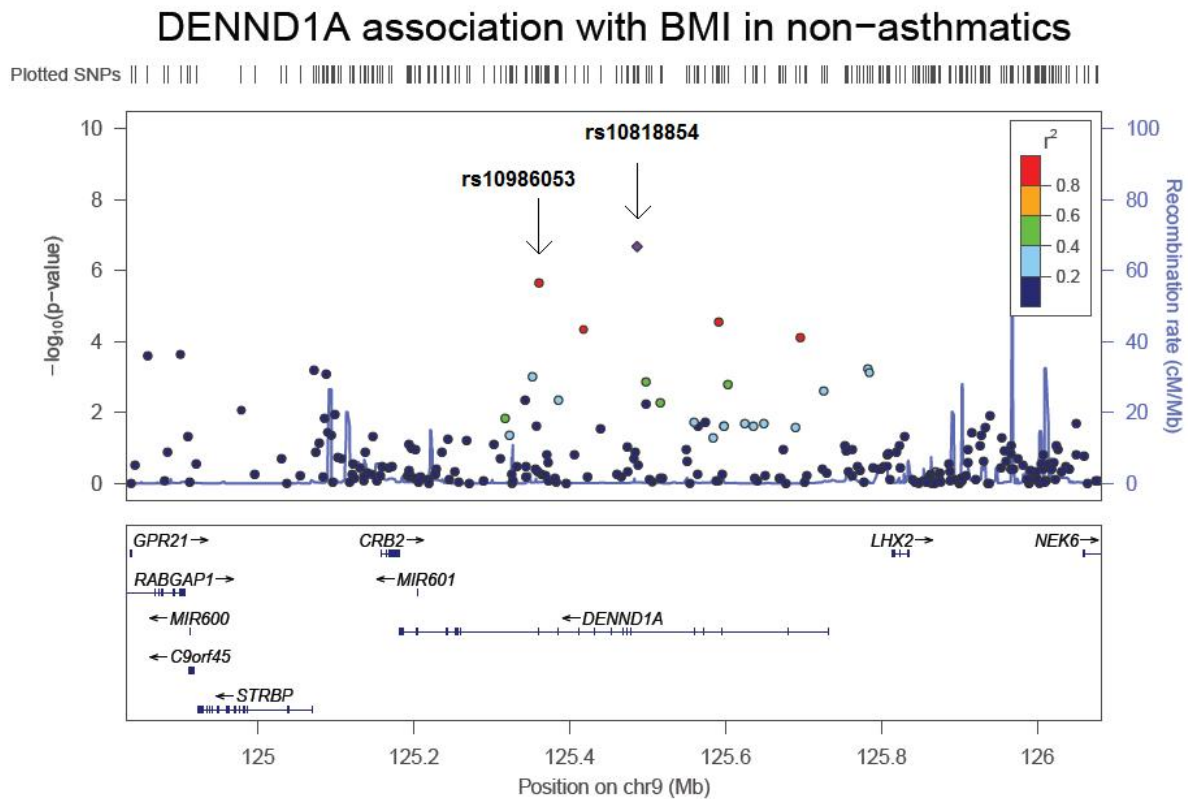


Figure S4. QQ- plot of SNPs after meta-analysis for association to BMI in *asthmatic adults* (observed p-values on the y-axis to those expected on the x-axis for a null distribution). Deviation of measures at the tail (upper right corner) may indicate true associations between some SNPs and BMI. Genomic inflation factor ( $\lambda$ ) = 1.01.

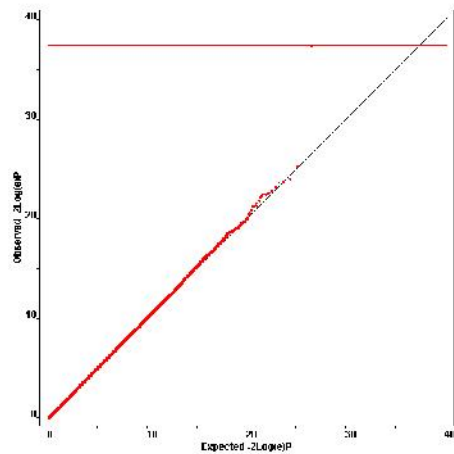


Figure S5. QQ- plot of SNPs after meta-analysis for association to BMI in *non-asthmatic adults* (observed p-values on the y-axis to those expected on the x-axis for a null distribution). Deviation of measures at the tail (upper right corner) may indicate true associations between some SNPs and BMI. Genomic inflation factor ( $\lambda$ ) = 1.02.

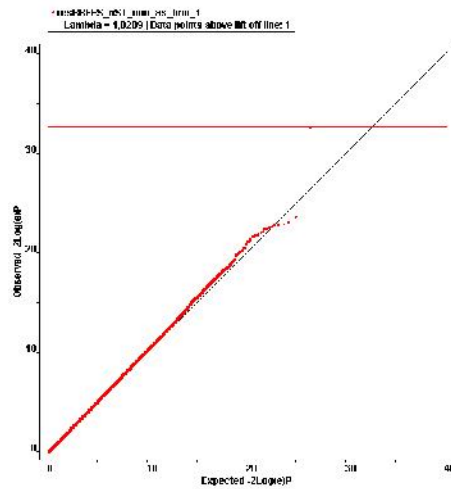


**Table S1. Top 5 *DENND1B* SNPs from meta-analysis of GWAS on BMI in asthmatic children (screening studies<sup>a</sup>) and association with BMI in non-asthmatics, and asthma in children and adults**

| SNP        | Chr | Position  | Gene           | Min.<br>allele | MAF <sup>a</sup> | Test   | p-value <sup>c</sup><br><i>BMI</i> in<br>non-<br>asthmatic<br>children,<br>n=6,437 | Direction,<br>non-<br>asthmatic<br>children |
|------------|-----|-----------|----------------|----------------|------------------|--------|------------------------------------------------------------------------------------|---------------------------------------------|
| rs4915551  | 1   | 197508901 | <i>DENND1B</i> | G              | 0.24             | A vs G | 0.03                                                                               | +++++--++                                   |
| rs1775454  | 1   | 197727298 | <i>DENND1B</i> | T              | 0.22             | T vs C | 0.03                                                                               | -----?++-                                   |
| rs1775456  | 1   | 197733055 | <i>DENND1B</i> | G              | 0.22             | A vs G | 0.03                                                                               | +++++---?                                   |
| rs2111931  | 1   | 195795841 | <i>DENND1B</i> | C              | 0.23             | T vs C | 0.09                                                                               | +++++---+                                   |
| rs10737692 | 1   | 197548933 | <i>DENND1B</i> | A              | 0.23             | A vs G | 0.08                                                                               | -----?++-                                   |

| SNP        | Chr | Position  | Gene           | Min.<br>allele | MAF <sup>a</sup> | p-value <sup>d</sup> ,<br><i>asthma</i> ,<br>children | p-value <sup>d</sup> ,<br><i>asthma</i> ,<br>adults |
|------------|-----|-----------|----------------|----------------|------------------|-------------------------------------------------------|-----------------------------------------------------|
| rs4915551  | 1   | 197508901 | <i>DENND1B</i> | G              | 0.24             | 0.99                                                  | 0.92                                                |
| rs1775454  | 1   | 197727298 | <i>DENND1B</i> | T              | 0.22             | 0.60                                                  | 0.49                                                |
| rs1775456  | 1   | 197612759 | <i>DENND1B</i> | G              | 0.22             | 0.17                                                  | 0.14                                                |
| rs2111931  | 1   | 195795841 | <i>DENND1B</i> | C              | 0.23             | 0.42                                                  | 0.83                                                |
| rs10737692 | 1   | 197548933 | <i>DENND1B</i> | A              | 0.23             | 0.53                                                  | 0.83                                                |

<sup>a</sup> Screening studies: ALSPAC, BAMSE, B58C (sub-studies pooled), CAMP, MAGIC/ISAAC, MAS, PIAMA and TOMSK.

<sup>b</sup> MAF = Minor allele frequency was estimated in the ALSPAC study (n=1,242 children).

“Direction” reflects the effect (positive or negative on BMI-values) of the effect allele vs reference allele as indicated in the “Test” column. “?” in the “Direction” column reflects missing data from that particular study.

<sup>c</sup> Based on all child studies with GWAS data; ALSPAC, BAMSE, B58C (sub-studies pooled), CAMP, CAPPS, GABRIELA, MAGIC/ISAAC, MAS, SAGE, PIAMA, and TOMSK plus Hartford / Puerto Rico in which data on *DENND1B* was available.

<sup>d</sup> Based on all studies with GWAS data; ALSPAC, BAMSE, B58C (sub-studies pooled), CAMP, CAPPS, GABRIELA, MAGIC/ISAAC, MAS, SAGE, PIAMA, TOMSK (n tot for children = 11,956) and Busselton, B58C (sub-studies pooled), SAPALDIA, ECHRS, EGEA, TOMSK (n tot for adults = 11,575)



**Table S2. Top 10 SNPs from meta-analysis of BMI in non-asthmatic children (n=6,019)**

| MarkerName | Chr | Minor allele | MAF  | Gene           | Distance to gene | p association | Test   | Direction | p heterogeneity |
|------------|-----|--------------|------|----------------|------------------|---------------|--------|-----------|-----------------|
| rs10818854 | 9   | A            | 0.04 | <i>DENND1A</i> | 0                | 2.2E-07       | A vs G | +++-----  | 0.70            |
| rs6097169  | 20  | A            | 0.10 | <i>NA</i>      | -31785           | 4.6E-07       | A vs G | +---+?+?  | 0.02            |
| rs8074700  | 17  | A            | 0.26 | <i>NA</i>      | 18052            | 1.2E-06       | A vs C | -+-----   | 0.56            |
| rs2033654  | 2   | A            | 0.42 | <i>ADCY3</i>   | 0                | 1.7E-06       | A vs C | +++--?++  | 0.53            |
| rs10986053 | 9   | T            | 0.03 | <i>DENND1A</i> | 0                | 2.1E-06       | T vs C | +++--?+?  | 0.5             |
| rs2326017  | 17  | T            | 0.28 | <i>NA</i>      | 0                | 2.3E-06       | T vs C | -+-+----  | 0.53            |
| rs1317830  | 4   | G            | 0.32 | <i>NA</i>      | 42799            | 2.8E-06       | A vs G | -----     | 0.94            |
| rs13407913 | 2   | G            | 0.43 | <i>ADCY3</i>   | 0                | 3.4E-06       | A vs G | ---+--?-- | 0.47            |
| rs1530016  | 2   | T            | 0.40 | <i>EFR3B</i>   | 0                | 3.4E-06       | T vs C | ---+--?+- | 0.65            |
| rs713587   | 2   | T            | 0.49 | <i>NA</i>      | 8224             | 3.7E-06       | T vs C | +++--?+?  | 0.19            |

“Direction” reflects the effect (positive or negative on BMI-values) of the effect allele vs reference allele as indicated in the “Test” column. “?” in the “Direction” column reflects missing data from that particular study. Study order corresponding to +/- position (alphabetical order): ALSPAC, BAMSE, B58C (sub-studies pooled), CAPPS, GABRIELA, MAGIC/ISAAC, PIAMA, SAGE.

**Table S3. Top 10 SNPs from meta-analysis of BMI in asthmatic adults (n=3,242)**

| MarkerName | Chr | Min Allele <sup>a</sup> | MAF <sup>a</sup> | Nearby gene                   | Distance to gene (bp) | P-value | Test   | Direction <sup>b</sup> | pvalue, heterogeneity |
|------------|-----|-------------------------|------------------|-------------------------------|-----------------------|---------|--------|------------------------|-----------------------|
| rs643507   | 18  | C                       | 0.01             | <i>LPIN2</i>                  | 0                     | 8.0E-09 | T vs C | ??++??                 | 0.01                  |
| rs2555603  | 13  | C                       | 0.25             | <i>STARD13 / RP11-81F11.3</i> | 0                     | 3.4E-06 | A vs C | +++++++                | 0.59                  |
| rs11259403 | 10  | C                       | 0.33             | <i>PRKCQ</i>                  | 0                     | 6.6E-06 | T vs C | +++++++                | 0.98                  |
| rs3780215  | 9   | A                       | 0.20             | <i>SLC24A2</i>                | 0                     | 7.4E-06 | A vs G | ++++-++                | 0.38                  |
| rs11603160 | 11  | T                       | 0.18             | <i>P2RY2</i>                  | -35071                | 8.0E-06 | T vs C | +++++++                | 0.68                  |
| rs12468557 | 2   | T                       | 0.38             | <i>CPS1</i>                   | 0                     | 9.4E-06 | T vs C | +++++++                | 0.25                  |
| rs1648703  | 3   | C                       | 0.20             | <i>RFC4</i>                   | 0                     | 1.1E-05 | A vs C | -----                  | 0.10                  |
| rs1656941  | 3   | C                       | 0.20             | <i>RFC4</i>                   | 0                     | 1.2E-05 | A vs C | -----                  | 0.10                  |
| rs2555595  | 13  | C                       | 0.21             | <i>STARD13 / RP11-81F11.3</i> | 0                     | 1.3E-05 | T vs C | +++++++                | 0.60                  |
| rs595817   | 22  | C                       | 0.19             | <i>SEZ6L</i>                  | 0                     | 1.4E-05 | T vs C | ++++-+-                | 0.82                  |

\* Minor allele and minor allele frequency (MAF) based on ECHRS data. *LPIN2* has MAF<0.01 in 4/7 adult studies and therefore not analyzed.

<sup>b</sup> “?” reflects missing data from that particular study. Study order corresponding to +/- position (alphabetical order): Busselton, B58C (substudies pooled), ECHRS, EGEA, SAGE, SAPALDIA, TOMSK.

**Table S4. Top 10 SNPs from meta-analysis of BMI in non-asthmatic adults (n=7,679)**

| MarkerName | Chr | Posititon | Min allele <sup>a</sup> | MAF <sup>a</sup> | Nearby gene          | Distance to gene (bp) | P-value | Test   | Direction <sup>b</sup> | pvalue, heterogeneity |
|------------|-----|-----------|-------------------------|------------------|----------------------|-----------------------|---------|--------|------------------------|-----------------------|
| rs7775861  | 6   | 51068717  | T                       | 0.38             | <i>AL139097.1</i>    | -87985                | 8.6E-08 | T vs C | +++++--                | 0.28                  |
| rs912714   | 6   | 50992143  | T                       | 0.35             | <i>AL139097.1</i>    | -11411                | 8.0E-06 | T vs C | +++++--                | 0.13                  |
| rs11816297 | 10  | 129604375 | G                       | 0.24             | <i>FOXI2</i>         | 64925                 | 1.0E-05 | A vs G | -----                  | 0.78                  |
| rs7093392  | 10  | 129614354 | G                       | 0.24             | <i>CLRN3</i>         | 61751                 | 1.1E-05 | A vs G | -----                  | 0.72                  |
| rs12526832 | 6   | 50992865  | T                       | 0.36             | <i>AL139097.1</i>    | -12133                | 1.1E-05 | T vs C | +++++--                | 0.16                  |
| rs1926177  | 10  | 129594161 | C                       | 0.31             | <i>FOXI2</i>         | 54711                 | 1.2E-05 | A vs C | -----                  | 0.65                  |
| rs10484664 | 6   | 51016523  | A                       | 0.35             | <i>AL139097.1</i>    | -35791                | 1.2E-05 | A vs G | +++++--                | 0.15                  |
| rs4792930  | 17  | 40805844  | T                       | 0.45             | <i>TUBG2</i>         | -5422                 | 1.3E-05 | T vs G | +++--++                | 0.44                  |
| rs6934225  | 6   | 116085882 | A                       | 0.26             | <i>RP11-721G11.1</i> | 12515                 | 1.4E-05 | A vs G | -----                  | 0.64                  |
| rs10457074 | 6   | 149359829 | G                       | 0.33             | <i>UST</i>           | 0                     | 1.4E-05 | A vs G | +++++--                | 0.61                  |

<sup>a</sup> Minor allele and minor allele (MAF) frequency based on ECHRS data.

<sup>b</sup> “?” reflects missing data from that particular study. Study order corresponding to +/- position (alphabetical order): Busselton, B58C, ECHRS, EGEA, SAGE, SAPALDIA, TOMSK.



## Information S1

### Study designs

#### ALSPAC

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based, birth cohort recruited during pregnancy. Pregnant women resident in Avon, United Kingdom with estimated dates of delivery between 1<sup>st</sup> April 1991 and 31<sup>st</sup> December 1992 were recruited through antenatal clinics. The study methodology has been published previously[2]. From 14,451 women recruited, there were 14,072 live births and 13,988 children were alive at age one year. Children were followed from birth using self-completion questionnaires sent to their mothers at approximately annual intervals and, from 7 years of age, in yearly dedicated research clinics.

Asthma was defined as a positive response to a question at 91 months (approximately 7½ years), “Has a doctor ever told you that your child had asthma?” A total of 3,030 children had DNA available for genotyping and data on asthma at 91 months as well as other asthma related phenotypes. Of these, 625 (20.6%) had asthma and an equal number of controls was selected at random from the remainder. Body mass index was measured in a research clinic at age 7-8 years. After ALSPAC-QC: 1) genotype missing rate <5% in both cases and controls; 2) minor allele frequency  $\geq 0.5$  in both cases and controls; 3) consistency with Hardy-Weinberg equilibrium in controls ( $P > 10^{-7}$ ); 4) exclusions due to ancestry, relatedness and sex mismatches, a total of 1,152 subjects (576 asthma cases and 576 controls) were retained in the GWAS analyses.

#### BAMSE

Between 1994 and 1996, 4,089 newborn infants were recruited in the BAMSE study, and questionnaire data on baseline study characteristics were obtained.[3] The catchment area included central and north-western parts of Stockholm. At approximately one, two, four, and eight years of age, parents completed questionnaires on their children’s symptoms related to asthma and other allergic diseases. The response rates were 96%, 94%, 92% and 84%, respectively. At eight years of age, all children of the BAMSE study were invited to clinical testing (including weight and height measurements), and blood samples were obtained from 2,480 children. DNA was extracted from 2,033 samples after exclusion of samples with too little blood, lack of questionnaire data, or if parental consent to genetic analysis of the sample was not obtained. From these samples, all children with a doctor’s diagnosis of asthma (ever) were selected as cases and children with no history of asthma or other allergic diseases were selected as controls. BMI values from the eight year follow up were used in this study. Age and sex specific BMI standard deviation scores in BAMSE were constructed using Swedish reference

values.[4] The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden. After QC a total of 485 subjects (239 asthma cases and 246 controls) were retained in the GWAS analyses.[5]

#### British 1958 birth cohort (B58C)

The British 1958 birth cohort (B58C) is an ongoing follow-up of persons born in Britain during one week in 1958 (<http://www.b58cgene.sgul.ac.uk/>). Asthma and wheezing illness were ascertained through parental interviews and measurements of height and weight were obtained at ages 7, 11 and 16. Cohort members themselves reported asthma and wheezing illness at ages 23, 33 and 42 years. At age 44-45, a full biomedical examination was performed [6] from which DNA samples were prepared for use as a nationally representative reference series for genetic case-control studies. Genotyping with Illumina 550K or 610K chips was performed on non-overlapping subsets of the cohort by the Wellcome Trust Case-Control Consortium [7], the Type 1 Diabetes Genetics Consortium [8], and the GABRIEL consortium [5]. Imputations to the HapMap 2 (release 21) panel were performed using MACH v 1.0.16.

#### BUSSELTON

Residents of the town of Busselton in the southwest of Western Australia have been involved in a series of health surveys since 1966. The population is predominantly of European origin. In 1994/95 there was a follow-up study involving a subset of those who had attended any of the previous surveys. Cases of asthma were defined as those who reported doctor-diagnosed asthma in the 1994 survey (answer 'Yes' to 'Has your doctor ever told you that you had asthma?').[9] Controls are those who have consistently answered 'No' to 'Has your doctor ever told you that you had asthma?' at all previous surveys that they have attended from 1966 to 1994. For the GWA study, a case control sample of unrelated individuals was selected. After QC a total of 1,212 subjects were retained in the GWAS analyses. To allow for meta-analysis across different marker sets, imputation of polymorphic HapMap European CEU SNPs was performed using MACH2.[10]

Website: <http://www.busseltonhealthstudy.com/>

#### CAMP

CAMP was a multicenter clinical trial in children with mild to moderate asthma.[11] All recruited children had asthma as defined by having 2 or more symptoms per week, using an inhaled bronchodilator at least twice weekly or asthma medication daily, and airway responsiveness to methacholine  $\leq 12.5$  mg/ml. Children with severe asthma or other clinically

significant conditions were excluded. Of the 1,041 children enrolled in the original clinical trial, 968 children and 1,518 of their parents contributed DNA samples. At randomization, baseline data, including measurements of BMI, were collected. For the BMI analysis included in this study, genotype data from additional probands obtained with the Illumina Infinium HD Human610-Quad BeadChip were available, and a merged HumanHap550/Human610-Quad dataset was created.[12] Written informed consent was obtained from parents of participating children. The CAMP study was approved by the Institutional Review Boards of Brigham and Women's Hospital and the other participating centers.

#### Canadian Childhood Cohorts: CAPPs & SAGE

CAPPs: Full details have been previously published [13] and are available at <http://www.genopha.ca/>. In brief CAPPs was initiated in 1995 to assess the effectiveness of a multifaceted intervention program on the primary prevention of asthma in high-risk infants. Infants at high risk for developing asthma and other allergic disorders were identified and their mothers were recruited during their third trimester of pregnancy. Infants at high risk were defined as those who had a parent with asthma or two first-degree relatives with allergies or atopic disorders. The study had two recruitment centres in Canada, (Vancouver and Winnipeg)[14-16]. In total, there were 545 families recruited into the study (549 infants, 4 sets of twins). Loss to follow-up was minimal, with 86% of the families completing the questionnaire at the 7-year time point. There have been numerous publications on this cohort evaluating the effectiveness of the intervention strategy[14-16]. For genetic analysis blood samples have been obtained from the children, and both parents. The Ethics Committees of the University of British Columbia and the University of Manitoba approved the study and parents provided written informed consent for participation.

SAGE is a population-based sample of 16,320 children, born in the province of Manitoba, Canada in the year of 1995. The SAGE study design has been previously described in detail [17]. In 2002, a one-page survey was mailed to families to enquire about their health and home environment exposure. Questions included several chronic conditions in childhood, including hay fever, food allergy and asthma. Parents were asked for permission to be contacted for further study and to link the survey data to their child's health care database records. Children were stratified according to the presence of asthma (n=392), to the presence of hayfever or food allergy (n=192) and neither (n=3,002). Children with neither condition were further grouped by rural and urban location; the latter were further stratified by low and high income neighbourhoods. All children in the asthma and allergy strata were invited to participate in the case-control study, as were a random sample (n=200) from the strata of children with neither condition. Following consent from parents to participate in the study, children were assessed for allergic phenotypes. After QC 899 subjects from the CAPPs and SAGE studies were retained in the analysis.

## Chicago Asthma Genetics Study: CAG

CAG included European American subjects collected at the University of Chicago from a) families ascertained through affected sib pairs, b) affected children and their parents, c) adults and children with severe persistent asthma, and d) nonasthmatic control subjects (>18 years of age). Samples were recruited in the adult and/or pediatric asthma clinics at University of Chicago Hospital. Asthma was defined based on the following criteria, 1) a physician's diagnosis (with no conflicting diagnosis), 2) the presence of at least 2 self-reported symptoms (cough, wheeze, shortness of breath), 3) current use of asthma medications, and 4) either bronchial hyperresponsiveness, defined as a  $\geq 20\%$  decrease in FEV1 after inhalation of  $\leq 25$  mg/mL methacholine, or reversibility to inhaled bronchodilator, defined as a  $\geq 15\%$  increase in baseline FEV1 after inhalation of a bronchodilator (albuterol) or after treatment. Subjects with a birthweight below 4.4 lb or with > 3 pack-years of cigarette smoking were excluded. The median age of affected subjects is 28 years with a range of 6-74 years.

Genotyping of the CAG study was performed using the Illumina 1Mv1 platform (Illumina, San Diego, CA), with individual genotypes called using clustering algorithms as implemented in the BeadStudio software by Illumina.[18, 19]

## ECRHS

Sixteen centres (eight countries) in the European Community Respiratory Health Survey (ECRHS) have contributed samples to GABRIEL. In each centre, a representative community-based sample of at least 3000 adults aged 20-44 years were invited to complete a brief postal questionnaire asking about respiratory symptoms (ECRHS I - Stage 1) between 1991-1993. A random sample of these (600 per centre) underwent intensive further investigation (ECRHS I - Stage 2 – random sample, including height and weight measurements). Participants who had symptoms highly suggestive of asthma but who had not been selected at random to take part in Stage 2, were also invited to undergo intensive investigations (ECRHS I - Stage 2- enriched sample). About ten years later all adults who had taken part in Stage 2 were recontacted (ECRHS II) and again asked about respiratory symptoms. Samples suitable for DNA extraction were collected. For the GABRIEL initiative all cases of asthma were identified (participants from the random or enriched sample who said yes to the question 'Have you ever had asthma?' at either ECRHS I or ECRHS II)[20, 21]. Controls were a random sample (of the random sample) who answered 'no' to the same question in both surveys.

Website: <http://www.ecrhs.org/>

## EGEA

Is a 12-year longitudinal survey which combines a case-control study and a family study. The first survey (EGEA1) took place between 1992 and 1995.[22] The study population included 388

asthmatics recruited in chest clinics and their 1,244 family members plus 415 population-based controls (total of 2,047 subjects). The probands (asthmatics and controls) were between 7 and 70 years old at time of study. All probands and their two parents were of European ancestry and were born in France. The second survey (EGEA2) was conducted between 2003 and 2007 and included follow-up data in 1,543 subjects from the initial cohort and 58 new family members.[23] Data collected through face-to-face interviews and examination included extensive phenotypic characterization (detailed clinical data based on standardized questionnaire, skin prick tests, lung function tests, bronchial responsiveness, blood samples, white blood cell counts, total IgE), data on risk factors (environmental exposures, diet, physical activity, hormone-related events) and drug consumption. Detailed description of the study can be found in Kauffmann *et al* [22] and Siroux *et al* [23] and at <http://cesp.vjf.inserm.fr/~egeanet>. Written informed consent was obtained from all subjects (or their parents) participating in the study under an Institutional Review Board-approved protocol. After QC, a total of 1,652 subjects with BMI measured in adulthood (at EGEA2 if available or at EGEA1) were retained in the present GWAS analyses. Asthma was defined with a positive answer to “Have you ever had attacks of breathlessness at rest with wheezing?” or “Have you ever had asthma attacks?” or being recruited as an asthma case in chest clinics. Individuals > 15 years were considered adults.

## GABRIELA

The GABRIELA study was carried out as a population survey in five rural regions of Europe: Baden-Württemberg and Bavaria in Germany, North/Central Switzerland, Tyrol in Austria, and Lower Silesia in Poland[24, 25]. The Polish center was not included in the GWAS analysis. Children aged 5–13 years were recruited through primary schools. A recruitment questionnaire was sent out to 132,366 children, of whom 79,888 (60.4%) returned their completed questionnaires (phase 1). Of these children, 34,491 were eligible for phase 2 as defined by a documented parental consent to dust sampling, blood sampling, and genetic analyses and nationality of their study country. To enrich informative observations a stratified random selection process was applied in two steps. In a first step, 9,668 children were selected for phase 2 within the three exposure strata per center. In the second step, 1708 children were selected for genotyping within exposure and outcome strata per center. Of those, 1681 had non-missing information for gender, age, BMI and asthma.

## GACRS

The GACRS is a family-based study of children with asthma in Costa Rica. Detailed information about this sample can be found elsewhere.[26, 27] Briefly, screening questionnaires were sent to the parents of 13,125 children ages 6 to 14 years enrolled in 113 schools in the Central Valley of Costa Rica from February of 2001 to December of 2006. Of the 7,282 children whose parents

returned screening questionnaires, 2,714 had asthma (defined as physician-diagnosed asthma and either at least two respiratory symptoms or asthma attacks in the previous year). Of these 2,714 children, 616 (22.7%) unrelated children had high probability of having at least 6 great-grandparents born in the Central Valley of Costa Rica and were willing to participate in our study. To enhance comparability with the asthma definition used in CAMP [28], we only considered 463 children who, in addition to asthma (as above), had either increased airway responsiveness ( $PD_{20} \leq 16.81 \mu\text{mol}$  of methacholine) or (for children unable to undergo methacholine challenge testing) increased bronchodilator response (improvement in baseline  $FEV_1$  of 12% or greater (at least 200 ml) after albuterol administration) for this work. Of these 463 children, 407 (88%) had BMI data and these were genotyped (for rs4915551) using the Sequenom MassArray platform with iPlex chemistry; all HWE-pvalue  $>0.05$ ). Written parental consent was obtained for participating children, for whom written assent was also obtained. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San José, Costa Rica) and Brigham and Women's Hospital (Boston, Massachusetts).

## LISA/GINI

The influence of Life-style factors on the development of the Immune System and Allergies in East and West Germany PLUS the influence of traffic emissions and genetics (LISApplus) Study is a population based birth cohort study. A total of 3097 healthy, fullterm neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases.[29]

A total of 5991 mothers and their newborns were recruited into the German Infant study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life.[30] All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the non-interventional arm. Detailed descriptions of the LISApplus and GINIplus studies have been published elsewhere. [29, 30]

Information on ever having physician-diagnosed asthma was collected using self-administered questionnaires completed by the parents. The questionnaires were completed at 6, 12, 18 and 24 months and 4, 5, 6 and 10 years of age in the LISApplus study and 1, 2, 3, 4, 6 and 10 years in the GINIplus study asking for each year of age since the previous follow-up. Based on these questions for asthma for each year of age up to 10 years, a binary outcome variable for ever having a diagnosis was defined. Weight and height were measured at the 10 year follow-up physical examination and BMI is defined as weight in kilogram divided by squared height in meter.. BMI was transformed to age- and sex-specific standard deviation scores (z-scores) according to the World Health Organisation (WHO) growth standards (<http://www.who.int/growthref/en/>).

DNA was collected at the age 6 and 10 years. For both studies, approval by the local Ethics Committees and written consent from participant's families were obtained.

72 children from Munich with ever having asthma from both studies were included (45 (62.5%) children from the GINIplus study and 27 (37.5%) children from the LISAplus study).

Statistical analysis was performed using the statistical software R, version 2.13.1 (<http://www.R-project.org>)[31], regressing expected allelic dosage on BMI adjusted for age at BMI assessment and sex.

Genotype information for the SNP rs4915551 was extracted from GWAS data. 68 individuals (45 from the GINIplus study and 23 from the LISA study) were analysed using the Affymetrix Human SNP Array 5.0 and 4 individuals from the LISAplus study were analysed using Affymetrix Human SNP Array 6.0. Genotypes were called using BRLMM-P algorithm (5.0), respectively BIRDSEED V2 algorithm (6.0), and imputed in IMPUTE2.[32]

## MAGICS/ISAAC

As previously described [33], 1255 children were recruited for the Multicenter Asthma Genetic in Childhood (MAGIC) study in seven German and Austrian clinical asthma centers located in Bochum, Cologne, Feldkirch, Freiburg, Munich/ Rosenheim, Vienna and Wesel from 2001 to 2007. Asthma was diagnosed by a pediatric pulmonologist according to clinical guidelines and objective measures such as lung function tests, clinical examination, history and allergy testing. 800 German children (non-asthmatics) were randomly drawn from the cross sectional International Study of Asthma and Allergies in Childhood phase II (ISAAC II) as a reference. The ISAAC study assessed the prevalence of asthma and allergies in schoolchildren and the asthma status was based on the parent's report of a physician's diagnosis of asthma at least once, or of spastic or asthmatic bronchitis more than once in self-administered questionnaires. BMI was assessed when children visited the study clinics for lung function measurements. Written informed consent was obtained from all parents of children included in these studies and study methods were approved by the respective ethics committees. All children were of German origin to control for population admixture. After all quality controls 1303 children at an average age of ten years remained for further analysis. Due to continuous quality checks, drop outs and update of consent in a follow up study, the number of children contributing to this analysis is slightly smaller than that in previous studies[33]. 581 (all asthmatic) children were analyzed from the MAGIC and 722 (70 asthmatics) from the ISAAC study. However, only 507 of the asthmatics had BMI data available for analyses.

## MAS

Consists of 1,314 healthy mature children born in 5 German cities in the year 1990. All children were followed at the age of 1, 3, 6, 12, 18, and 24 months and at yearly intervals thereafter until

age 15 years.[34, 35] Clinical and environmental assessment included standardized interviews, questionnaires, physical examinations, and environmental studies. Total and specific IgE antibodies to hen's egg, cow's milk, wheat, and soy were determined at the age of 1, 2, 3, 5, 7, and 10 years. DNAs from all children with a doctor diagnosis of asthma were provided for the GWA study. After QC a total of 171 subjects were retained in the GWAS analyses.

## PIAMA

The PIAMA study is a birth cohort study of children born between 1996-1997. Details of the study design have been published previously.[36] 10,232 pregnant women completed a validated screening questionnaire at their prenatal health care clinic (n=52). Based on this screening, 7,862 women were invited to participate, of whom 4,146 women agreed and gave informed consent. Mothers reporting a history of asthma, current hay fever or allergy to pets or house dust mite were defined as allergic. Children were recruited during the first trimester of pregnancy. Follow-up of the children took place at 3 months of age and yearly from 1 to 8 years of age. At eight years of age, all children were invited to clinical testing including weight and height measurements and collection of blood samples for DNA extraction. BMI-SDS were calculated using reference data from the 1997 Dutch Growth Study.[37] The Medical Ethical Committees of the participating institutes approved the study, and all participants gave written informed consent. The response rates to the annual questionnaires ranged from 3030 (92%) at age 1 to 2732 (83%) at age 8 years. For the GWA study, DNA from childhood asthma cases and a set of matched non-asthmatic controls were provided. After QC a total of 350 subjects were retained in the GWAS analyses.

## HARTFORD/PUERTO RICO

Full details on subject recruitment and study design were recently published.[38] In brief, children ages 6 to 14 years were recruited in Hartford, CT (n=449) and San Juan, PR (n=677). At both sites, the main recruitment tool was a screening questionnaire given to parents of children ages 6 to 14 years. All participants had to have four Puerto Rican grandparents. Children with asthma (cases) were selected on the basis of physician-diagnosed asthma and wheeze in the prior year. Controls had no physician-diagnosed asthma and no wheeze in the prior year. Height and weight were measured before spirometry, and BMI z scores were calculated from CDC growth charts.[39] After subject-level QC, there were a total of 935 subjects with both genotypic and BMI data available for analysis. Participants were genotyped using the Illumina Human Omni 2.5 BeadChip. After marker cleaning following Illumina guidelines, there were ~1.7 million SNPs available for analysis.

## SAPALDIA

Contributed all self-reported asthma cases as well as a random sample of controls. These subjects were obtained from among 6,055 SAPALDIA cohort subjects that participated in both, the baseline (1991) and follow-up (2002) examinations and agreed to providing blood for genetic analysis. SAPALDIA is a population-based cohort that originally recruited subjects aged 18 to 60 from population registries in eight Swiss communities representing the three largest language groups (German, French, Italian) as well as different levels of air pollution, altitude and degrees of urbanization.[40-42] At both baseline and follow-up examination subjects underwent spirometry, height and weight measurements as well as a detailed interview on respiratory health, smoking history, lifestyle factors and anthropometry. At follow-up, 8,047 of 9,651 baseline subjects re-participated in at least one part of the study and a formal biobank was established. SAPALDIA questions about smoking and asthma status were equivalent to those used by the ECRHS. Asthma status was defined by an affirmative answer to the question “Have you ever had asthma” at baseline and/or follow-up interview. After QC a total of 1,521 subjects were retained in the GWAS analyses.

## TOMSK

Is a population-based family study conducted by the Research Institute of Medical Genetics and Siberian State Medical University (Tomsk, Russia) from 1998 onwards.[43, 44] Both nuclear families and extended pedigrees were recruited through atopic bronchial asthmatic probands. All participants were Russians or of a mixed ethnic origin due to marriages between Russians and major East Slavonic populations (Ukrainians, Byelorussians). Altogether, 196 families were studied, out of which 150 families were recruited in Tomsk Region Children Hospital and Tomsk Region Hospital (Tomsk, Russia), and 46 families were recruited in the city of Irkutsk hospitals by the staff of the Irkutsk State Institute of Doctor’s Advanced Training (Irkutsk, Russia). Both probands and their relatives were clinically examined to establish diagnosis of asthma and atopy by the GINA criteria (Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. <http://www.ginasthma.org>). Besides the clinical examination, laboratory and functional testing were conducted to assess common IgE levels (solid-phase immune-enzyme assay), specific sensitization (skin-prick tests), lung volumes (spirometry), and airway responsiveness (bronchoprovocative tests with methacholine). For BMI analyses, only probands were included (siblings excluded).

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## SAPALDIA

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