## **Online Supplement**

# Methods

## ALSPAC GWAS data generation and imputation methodology

In order to obtain the most robust GWAS data, individuals were excluded from further analysis on the basis of having: incorrect gender assignments; minimal or excessive heterozygosity (<0.320 and >0.345 for the Sanger data and <0.310 and >0.330 for the LabCorp data); disproportionate levels of individual missingness (>3%); evidence of cryptic relatedness (>10% Identity by Descent, IBD); and being of non-European ancestry (as detected by a multidimensional scaling analysis seeded with HapMap 2 individuals) in order to reduce the possibility of confounding by population substructure. EIGENSTRAT principal components analysis was used to generate the top 100 principal components after the removal of known regions of long linkage disequilibrium in the data<sup>1;2</sup>. This revealed no additional obvious population stratification and genome-wide analyses with other phenotypes indicate a low lambda. SNPs with a minor allele frequency of <1% and call rate of <95% were removed. Furthermore, only SNPs which passed an exact test of Hardy–Weinberg equilibrium (P>5×10-7) were considered for analysis.

Known autosomal variants were imputed with MACH 1.0.16 Markov Chain Haplotyping software<sup>3;4</sup>, using Centre d'etude du polymorphisme humain (CEPH) individuals from phase 2 of the HapMap<sup>5</sup> project (HG18) as a reference set (release 22). For each imputed SNP of interest, dosages were estimated based on probabilities calculated by the MACH algorithm (where 0 is the first homozygote, 1 is a perfect heterozygote, and 2 is the other homozygote); imputed genotypes were set to missing if the dosage was >0.3 either side of the integer dose.

#### PIAMA questionnaire data, genotyping and imputation methodology, and ethics

Questionnaires for parental completion, partly based on the International Study of Asthma and Allergies in Childhood core questionnaires, were sent to parents when the child was 8 years old. If parents confirmed that the child had been diagnosed with asthma by a doctor (ever), and had had asthma and/or one or more attacks of wheeze in the last 12 months, they were regarded as having current doctor-diagnosed asthma.

DNA was extracted from blood or buccal swabs at the age of 4 or 8 years. Children were genotyped on 3 different platforms. DNA of 1377 children was genotyped on the Illumina Omni Express Exome Chip and DNA of 288 children was genotyped with the Omni Express chip, both at the Genomics Facility of the University Medical Center Groningen. DNA of 404 children was genotyped at the Centre National de Genotypage (CNG, Evry, France) as part of the GABRIEL consortium<sup>6</sup>. SNPs were harmonized by base pair position annotated to genome build 37, name and annotation of strand for each platform. Discordant or duplicate SNPs or SNPs that showed large differences in allele frequencies (> 15 %) were removed. After quality control, a total of 1968 individuals remained and imputation was performed per platform using IMPUTE 2,0 against the reference data set of the CEU panel of the 1000 Genomes project (version March 2012). SNPs of high quality (info-score IMPUTE  $\geq 0.7$ ) were merged into one dataset using GTOOL and used for further analysis. Dosages of imputed SNPs were predicted based on the following assumptions; 0 is the first homozygote, 0.5 is a heterozygote and 1 is the other homozygote. The info scores of the imputed SNPs were all  $\geq 0.99$  which provided reliable dosages estimates. Analyses of SNPs most significantly associated with asthma in ALSPAC were performed using SNPtest v2.4.1 and IBM SPSS Statistics for Windows (Version 22.0, Armonk, NY).

The Medical Ethical Committees of the participating institutes approved the study, and all participants gave written informed consent.

#### Generation R questionnaire data, genotyping and imputation technology, and ethics

Information about wheezing and asthma was collected by a parental questionnaire at age 6 years<sup>7</sup>. Response rate for this questionnaire was 68%. Asthma was assessed with the question 'Was your child ever diagnosed with asthma by a doctor? [no; yes]'. Wheezing was assessed with the question 'Did your child ever suffer from wheezing in the last 12 months? [never, 1-3 times, >4 times]'. If parents confirmed that the child had been diagnosed with asthma by a doctor and had >=1 wheezing episode in the last 12 months, they were regarded as having current doctor-diagnosed asthma [no; yes].

Cord blood samples including DNA were collected at birth. Samples were genotyped using Illumina Infinium II HumanHap610 Quad Arrays following standard manufacturer's protocols. Intensity files were analyzed using the Beadstudio Genotyping Module software v.3.2.32 and genotype calling based on default cluster files. Any sample displaying call rates below 97.5%, excess of autosomal heterozygosity (F<mean-4SD), and mismatch between called and phenotypic gender were excluded. In addition, individuals identified as genetic outliers by the IBS clustering analysis (> 3 standard deviations away from the HapMap CEU population mean) were excluded from the analysis. Genotypes were imputed for all polymorphic SNPs from phased haplotypes in autosomal chromosomes using the 1000 Genomes GIANTv3 panel. Analyses of single nucleotide polymorphisms (SNPs) most significantly associated with asthma in ALSPAC were performed using IBM SPSS Statistics for Windows (Version 21.0, Chicago, IL).

The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC 217.595/2002/20). Written informed consent was obtained from parents of all participants.

## References

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- (7) Jaddoe VWV, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HtA, Steegers EAP et al. The Generation R Study: design and cohort update 2010. *Eur J Epidemiol* 2010; 25:823-841.

**Key for study ID in Figures** (\_ch suffix denotes childhood onset asthma (ever), even if based on recall in adults; \_all suffix denotes whole cohort analysis (current doctor-diagnosed asthma) as per Results in Table 2):

GABAS: GABRIEL Advanced Surveys (Germany)

MAGMAS: MAGICS (Multicentre Asthma Genetics in Childhood Study) and MAS

(Multicentre Allergy Study) (Germany)

BAMSE: BAMSE cohort (Sweden)

EGEA: Genetics and Environment of Asthma (France)

TOMSK: Tomsk study (Russia); UFA: Ufa study (Russia)

ECRHS: European Community Respiratory Health Survey (Europe multicentre)

SAPAL: SAPALDIA (The Swiss study on Air Pollution and Lung Disease In Adults) (Switzerland)

KARELIA: Karelia Allergy Study (Finland)

KMSU: KMSU cohort (Russia)

MRCAE: MRCA and UKC (UK)

B58C: British 1958 Birth Cohort (UK)

BUSSEL: Busselton Health Study (Australia)

SLSJ: Saguenay-Lac-Saint-Jean Familial Collection (Quebec, Canada)

CAPSAG: Canadian Asthma Primary Prevention Study (CAPPS) and the Study of Asthma Genes and Environment (SAGE) (Canada)

 $C \land IN1/C \land IN2$ ; Canation of  $\land$  others International Network (C

GAIN1/GAIN2: Genetics of Asthma International Network (GAIN) (Multicentre)



Figure E1: Linkage disequilibrium between 31 child *TRPA1* SNPs in ALSPAC using the Haploview program. Values of  $r^2$  (x100) are shown.

# Figure E2: Forest plot showing meta-analysis of the per-allele association between *TRPA1* rs959974 and current asthma in ALSPAC and PIAMA, and asthma 'ever' across other GABRIEL studies

Study ID	ES (95% CI)	% Weig
Cohorts in this paper		
ALSPAC_all	1.30 (1.15, 1	.47) 17.35
PIAMA_all	1.35 (1.00, 1	.82) 2.94
Subtotal (I-squared = 0.0%, p = 0.818)	1.31 (1.17, 1	.46) 20.29
GABRIEL studies of children	<u>_</u>	
BAMSE_ch	0.98 (0.75, 1	.28) 3.72
GABAS_ch	0.79 (0.66, 0	.93) 8.69
MAGMAS_ch	1.05 (0.88, 1	.25) 8.69
Subtotal (I-squared = 64.5%, p = 0.060)	0.92 (0.82, 1	.03) 21.11
Other GABRIEL studies		
B58C_ch	0.96 (0.73, 1	.26) 3.46
BUSSEL_ch	0.96 (0.74, 1	.23) 4.20
CAPSAG_ch	1.05 (0.72, 1	.53) 1.87
ECRHS_ch	0.82 (0.67, 1	.01) 6.49
EGEA_ch	0.95 (0.80, 1	.13) 8.69
GAIN1_ch	0.97 (0.82, 1	.15) 9.10
GAIN2_ch	1.03 (0.70, 1	.50) 1.81
KARELIA_ch	1.39 (0.65, 3	.00) 0.45
KMSU_ch	1.04 (0.71, 1	.51) 1.83
MRCAE_ch	1.06 (0.80, 1	.40) 3.32
SAPAL_ch	1.20 (0.95, 1	.52) 4.86
SLSJ_ch	0.91 (0.75, 1	.11) 6.75
TOMSK_ch	1.15 (0.79, 1	.67) 1.87
UFA_ch	0.99 (0.76, 1	.28) 3.89
Subtotal (I-squared = 0.0%, p = 0.808)	0.98 (0.91, 1	.05) 58.60
Heterogeneity between groups: p = 0.000		
Overall (I-squared = 51.3%, p = 0.005)	1.02 (0.97, 1	.08) 100.0
	I	

Figure E3: Forest plot showing meta-analysis of the per-allele association between *TRPA1* rs1384001 and current asthma in ALSPAC and PIAMA, and asthma 'ever' across other GABRIEL studies

Study ID	ES (95% CI)	% Weig
Cohorts in this paper		
ALSPAC_all	1.30 (1.15, 1.47)	17.34
PIAMA_all	1.35 (1.00, 1.82)	2.94
Subtotal (I-squared = 0.0%, p = 0.818)	1.31 (1.17, 1.46	20.28
GABRIEL studies of children	<u>_</u>	
BAMSE_ch	0.98 (0.75, 1.28)	3.72
GABAS_ch	0.78 (0.65, 0.93	8.69
MAGMAS_ch	1.05 (0.88, 1.25	8.69
Subtotal (I-squared = 66.5%, p = 0.051)	0.92 (0.82, 1.03	21.10
Other GABRIEL studies		
B58C_ch	0.96 (0.73, 1.26)	3.46
BUSSEL_ch	0.96 (0.74, 1.23)	4.20
CAPSAG_ch	1.05 (0.72, 1.53)	1.89
ECRHS_ch	<b>0.82</b> (0.67, 1.01)	6.49
EGEA_ch	0.95 (0.80, 1.13)	8.69
GAIN1_ch	0.97 (0.82, 1.15	9.09
GAIN2_ch	<b>1.03</b> (0.70, 1.50)	1.81
KARELIA_ch	1.39 (0.65, 3.00)	0.45
KMSU_ch	<b>1.06</b> (0.72, 1.54	1.85
MRCAE_ch	1.06 (0.80, 1.40	3.32
SAPAL_ch	1.20 (0.95, 1.52)	4.86
SLSJ_ch	0.91 (0.75, 1.11)	6.75
TOMSK_ch	1.12 (0.77, 1.63)	1.89
UFA_ch	0.99 (0.76, 1.28	3.89
Subtotal (I-squared = 0.0%, p = 0.812)	0.98 (0.91, 1.05	58.62
Heterogeneity between groups: p = 0.000		
Overall (I-squared = 52.0%, p = 0.005)	1.02 (0.97, 1.08	100.0

Figure E4: Forest plot showing meta-analysis of the per-allele association between *TRPA1* rs4738202 and current asthma in ALSPAC and PIAMA, and asthma 'ever' across other GABRIEL studies

ID	ES (95% CI)	% Weigł
Cohorts in this paper		
ALSPAC_all	<b>→</b> 1.22 (1.07, 1.39)	17.80
PIAMA_all	1.45 (1.01, 2.08)	2.36
Subtotal (I-squared = 0.0%, p = 0.377)	1.25 (1.10, 1.41)	20.17
GABRIEL studies of children	<u>i                                     </u>	
BAMSE_ch	1.12 (0.85, 1.48)	4.02
GABAS_ch	<b>•••</b> 0.82 (0.68, 0.99)	8.49
MAGMAS_ch	1.06 (0.87, 1.28)	8.32
Subtotal (I-squared = 58.6%, p = 0.089)	0.97 (0.86, 1.09)	20.84
Other GABRIEL studies		
B58C_ch	0.91 (0.68, 1.22)	3.65
BUSSEL_ch	0.94 (0.71, 1.24)	4.02
CAPSAG_ch	0.89 (0.58, 1.35)	1.75
ECRHS_ch	0.87 (0.69, 1.08)	6.04
EGEA_ch	<b></b> 1.04 (0.86, 1.26)	8.49
GAIN1_ch	<b>•••</b> 0.99 (0.82, 1.18)	9.44
GAIN2_ch	0.83 (0.55, 1.25)	1.87
KARELIA_ch	2.86 (0.96, 8.48)	0.26
KMSU_ch	1.07 (0.70, 1.62)	1.75
MRCAE_ch	1.00 (0.74, 1.35)	3.41
SAPAL_ch	1.22 (0.95, 1.57)	4.88
SLSJ_ch	<b>—</b> 0.95 (0.78, 1.16)	7.99
TOMSK_ch	1.21 (0.80, 1.81)	1.85
UFA_ch	0.93 (0.69, 1.24)	3.60
Subtotal (I-squared = 0.0%, p = 0.624)	0.99 (0.92, 1.06)	59.00
Heterogeneity between groups: p = 0.003 Overall (I-squared = 35.6%, p = 0.063)	1.03 (0.97, 1.09)	100.00

Figure E5: Forest plot showing meta-analysis of the per-allele association between *TRPA1* rs7010969 and current asthma in ALSPAC and PIAMA, and asthma 'ever' across other GABRIEL studies

ID	ES (95% CI)	% Weig
Cohorts in this paper		
ALSPAC_all	<b>→</b> 1.28 (1.13, 1.45)	17.80
PIAMA_all	1.23 (0.89, 1.70)	2.60
Subtotal (I-squared = 0.0%, p = 0.821)	1.27 (1.13, 1.43)	20.40
GABRIEL studies of children	li	
BAMSE_ch	1.04 (0.81, 1.35)	4.12
GABAS_ch	0.81 (0.68, 0.96)	8.72
MAGMAS_ch	1.07 (0.89, 1.27)	8.72
Subtotal (I-squared = 63.6%, p = 0.064)	0.95 (0.85, 1.06)	21.57
Other GABRIEL studies	li	
B58C_ch	1.04 (0.79, 1.37)	3.55
BUSSEL_ch	1.07 (0.83, 1.37)	4.31
CAPSAG_ch	0.89 (0.60, 1.32)	1.78
ECRHS_ch	0.79 (0.64, 0.97)	6.29
EGEA_ch	1.05 (0.88, 1.26)	8.53
GAIN1_ch	<b>—</b> 1.04 (0.87, 1.23)	8.92
GAIN2_ch	0.93 (0.64, 1.34)	1.96
KARELIA_ch	<b>2</b> .13 (0.78, 5.83)	0.27
KMSU_ch	1.09 (0.75, 1.59)	1.88
MRCAE_ch	1.19 (0.90, 1.58)	3.36
SAPAL_ch	1.28 (1.01, 1.62)	4.83
SLSJ_ch	0.98 (0.80, 1.19)	7.21
TOMSK_ch	1.10 (0.74, 1.65)	1.68
UFA_ch	0.91 (0.69, 1.20)	3.46
Subtotal (I-squared = 11.9%, p = 0.323)	1.02 (0.95, 1.09)	58.03
Heterogeneity between groups: p = 0.001		
Overall (I-squared = 48.5%, p = 0.010)	1.05 (1.00, 1.11)	100.0

Figure E6: Forest plot showing meta-analysis of the per-allele association between *TRPA1* rs3735945 and current asthma in ALSPAC and PIAMA, and asthma 'ever' across other GABRIEL studies

ID	ES (95% CI)	% Weigl
Cohorts in this paper		
ALSPAC_all	1.30 (1.09, 1.55)	20.23
PIAMA_all	1.01 (0.61, 1.67)	2.48
Subtotal (I-squared = 0.0%, p = 0.355)	1.26 (1.07, 1.49)	22.71
GABRIEL studies of children		
BAMSE_ch	1.49 (0.98, 2.26)	3.61
GABAS_ch	0.83 (0.62, 1.12)	7.09
MAGMAS_ch	0.83 (0.63, 1.10)	7.90
Subtotal (I-squared = 66.9%, p = 0.049)	0.93 (0.78, 1.12)	18.61
Other GABRIEL studies		
B58C_ch	0.98 (0.60, 1.60)	2.58
BUSSEL_ch	1.33 (0.88, 1.99)	3.79
CAPSAG_ch -	0.84 (0.47, 1.48)	1.94
ECRHS_ch	0.98 (0.71, 1.34)	6.24
EGEA_ch	0.99 (0.76, 1.28)	9.55
GAIN1_ch	1.18 (0.91, 1.54)	8.99
GAIN2_ch	1.13 (0.65, 1.98)	2.02
KARELIA_ch	0.71 (0.21, 2.37)	0.43
KMSU_ch	1.13 (0.62, 2.07)	1.72
MRCAE_ch	0.95 (0.60, 1.51)	2.94
SAPAL_ch	1.20 (0.82, 1.75)	4.40
SLSJ_ch	1.05 (0.78, 1.41)	7.09
TOMSK_ch	1.48 (0.92, 2.39)	2.78
UFA_ch	1.11 (0.75, 1.63)	4.22
Subtotal (I-squared = 0.0%, p = 0.944)	1.08 (0.98, 1.20)	58.68
Heterogeneity between groups: p = 0.054	1	
Overall (I-squared = 4.1%, p = 0.406)	1.09 (1.01, 1.18)	100.0

SNP	Position	Allele*	MAF	SNP genotyped	N missing replaced with imputed	SNP imputed	N
rs12540984	72927920	A/G	0.1425	-		5,034 A/A 1,683 A/G 143 G/G	6,860
rs4738201	72930711	A/G	0.4859	1,621 A/A 3,419 A/G 1,860 G/G	41	-	6,900
rs6996723	72933632	C/T	0.1769	4,664 C/C 2,024 T/C 213 T/T	0	-	6,901
rs7827617	72934032	A/G	0.1711	4,758 A/A 1,937 A/G 206 G/G	25	-	6,901
rs959974	72935839	G/T	0.4708	1,920 G/G 3,476 T/G 1,505 T/T	1	-	6,901
rs959976	72936145	T/C	0.1717	4,751 T/T 1,942 C/T 208 C/C	4	-	6,901
rs1384001	72936237	C/A	0.4708	1,919 C/C 3,477 A/C 1,505 A/A	1	-	6,901
rs13279503	72939626	G/C	0.3545	-	-	2,854 G/G 3,168 C/G 848 C/C	6,870
rs4738202	72940861	A/G	0.3105	662 A/A 2,985 A/G 3,254 G/G	16	-	6,901
rs13280644	72948588	C/T	0.0982	5,608 C/C 1,227 T/C 66 T/T	0	-	6,901
rs13249568	72949209	T/C	0.2495	3,866 T/T 2,620 C/T 415 C/C	8	-	6,901
rs10504523	72951490	G/A	0.2493	3,868 G/G 2,619 A/G 414 A/A	3	-	6,901
rs1025926	72953158	C/T	0.251	3,856 C/C 2,611 T/C 434 T/T	51	-	6,901
rs10504524	72955891	G/T	0.2493	-	-	3,868 G/G 2,618 G/T 415 T/T	6,901
rs13255063	72959535	T/A	0.2493	-	-	3,867 T/T 2,618 A/T 415 A/A	6,900
rs1025927	72963135	A/G	0.0984	-	-	5,601 A/A 1,231 A/G 66 G/G	6,898

Table E1: Summary of *TRPA1* genotype data, including SNP position, minor allele frequency (MAF) and whether SNP was genotyped or imputed in white ALSPAC children

rs1025928	72963258	T/C	0.4156	1,188 T/T 3,362 T/C 2,351 C/C	0	-	6,901
rs10504525	72965123	C/T	0.1508	4,966 C/C 1,788 T/C 147 T/T	14	-	6,901
rs3735942	72965973	G/A	0.335	-	-	3,066 G/G 3,058 A/G 777 A/A	6,901
rs3735943	72966002	G/A	0.4858	1,838 G/G 3,431 A/G 1,632 A/A	2	-	6,901
rs10504526	72966552	A/G	0.4858	1,837 A/A 3,432 G/A 1,632 G/G	3	-	6,901
rs12548486	72971527	C/T	0.3351	-	-	3,061 C/C 3,059 C/T 777 T/T	6,897
rs10109581	72974329	G/T	0.2599	3,767 G/G 2,669 T/G 465 T/T	1	-	6,901
rs3735945	72974806	C/T	0.1091	5,478 C/C 1,332 T/C 91 T/T	1	-	6,901
rs920829	72977703	C/T	0.1091	-	-	5,477 C/C 1,328 C/T 91 T/T	6,896
rs1443952	72980652	C/T	0.3351	3,064 C/C 3,060 T/C 777 T/T	8	-	6,901
rs7010969	72982365	A/C	0.405	1,139 A/A 3,307 A/C 2,455 C/C	36	-	6,901
rs7011431	72982398	G/A	0.26	3,769 G/G 2,663 A/G 469 A/A	0	-	6,901
rs4738206	72986348	T/G	0.3343	3,076 T/T 3,054 G/T 771 G/G	1	-	6,901
rs2278655	72987277	C/T	0.071	-	-	5,889 C/C 836 C/T 32 T/T	6,757
rs13268757	72987638	G/A	0.1492	-	-	4,962 G/G 1,742 A/G 136 A/A	6,840

\*Allele order as per Ensembl database (<u>http://www.ensembl.org/index.html</u>)

Table E2: Per-allele associations between child *TRPA1* SNPs and total IgE (log transformed) at 7.5 years in ALSPAC

SNP	Position	Ln-IgE levels				
		Ν	OR (95% CI)	P value		
rs12540984	72927920	3808	0.93 (0.84-1.04)	0.210		
rs4738201	72930711	3834	1.05 (0.97-1.13)	0.203		
rs6996723	72933632	3834	1.06 (0.96-1.17)	0.262		
rs7827617	72934032	3834	1.03 (0.93-1.14)	0.574		
rs959974	72935839	3834	1.08 (1.00-1.7)	0.043*		
rs959976	72936145	3834	1.03 (0.94-1.14)	0.533		
rs1384001	72936237	3834	1.08 (1.00-1.17)	0.042*		
rs13279503	72939626	3822	1.06 (0.98-1.15)	0.119		
rs4738202	72940861	3834	1.09 (1.01-1.19)	0.031*		
rs13280644	72948588	3834	1.06 (0.93-1.20)	0.391		
rs13249568	72949209	3834	1.07 (0.98-1.17)	0.140		
rs10504523	72951490	3834	1.07 (0.98-1.17)	0.139		
rs1025926	72953158	3834	1.04 (0.95-1.14)	0.361		
rs10504524	72955891	3834	1.07 (0.98-1.17)	0.139		
rs13255063	72959535	3834	1.07 (0.98-1.17)	0.139		
rs1025927	72963135	3832	1.06 (0.93-1.20)	0.373		
rs1025928	72963258	3834	0.94 (0.87-1.02)	0.115		
rs10504525	72965123	3834	1.05 (0.95-1.17)	0.317		
rs3735942	72965973	3834	1.01 (0.93-1.10)	0.767		
rs3735943	72966002	3834	0.96 (0.89-1.04)	0.319		
rs10504526	72966552	3834	1.04 (0.96-1.12)	0.308		
rs12548486	72971527	3832	1.01 (0.93-1.10)	0.768		
rs10109581	72974329	3834	1.05 (0.97-1.15)	0.218		
rs3735945	72974806	3834	1.04 (0.92-1.17)	0.576		
rs920829	72977703	3831	1.04 (0.92-1.17)	0.530		
rs1443952	72980652	3834	1.02 (0.94-1.10)	0.694		
rs7010969	72982365	3834	1.06 (0.98-1.14)	0.166		
rs7011431	72982398	3834	1.05 (0.97-1.14)	0.247		
rs4738206	72986348	3834	1.01 (0.93-1.09)	0.795		
rs2278655	72987277	3751	0.91 (0.78-1.06)	0.205		
rs13268757	72987638	3803	1.06 (0.95-1.18)	0.288		

\*p-value<0.05