Attempted replication of 50 reported asthma risk genes identifies SNPs in RAD50 and PTPRE as associated with childhood atopic asthma

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Rationale
Asthma is a common disease of childhood that is strongly influenced by genetic factors. To date, over 250 have been reported to be associated with an increased risk of asthma, although inconsistent replication of these findings is more often the norm than otherwise. We sought to replicate association with childhood atopic asthma in single nucleotide polymorphisms (SNPs) of the most likely candidate genes using subjects from the Perinatal Risk of Asthma in Infants of Asthmatic Mothers (PRAM) study.

Methods
Two data sources were combined to create a ranked list of candidate genes: 1) a systematic search of PubMed, which identified every gene reported to be associated with asthma, and 2) an exploratory genome-wide association study (GWAS) performed on a subset of the PRAM cohort (n=102; see Table 1). Each was assigned a “Gene ranking score” equal to the number of publications reporting its prior association (“Publication score”) plus the sum of the -log P-values of its significant SNPs in the GWAS, scaled to the SNP density of that gene (“GWAS score”). For the top 50 genes, the most commonly replicated SNP in the literature was chosen for genotyping in the full PRAM cohort (N=66; see Table 2). Genes with non-SNP polymorphisms, or those located in the HLA superlocus, were excluded in the ranked list.

SNPs were assayed on the TaqMan genotyping platform (Applied Biosystems). Assayed SNPs were excluded from the final analysis if cases or controls had a call rate < 95% or if controls were significantly out of Hardy-Weinberg equilibrium. Bonferroni correction was used for significance testing, with an alpha of 0.00094 (0.05 corrected for 53 potential comparisons). If a SNP was located in a gene that was selected due to prior evidence in the PRAM GWAS (i.e., had a non-zero GWAS score), then the association tests were also performed using only subjects that were not assayed in the GWAS. If the observed association was not replicated in the subset of non-GWAS samples, then that SNP was not considered in the final analysis.

Results
The literature search identified 251 asthma risk genes from 469 prior publications (Figure 1). From the eligible top 50 ranked genes, 53 SNPs were selected for genotyping. Of these, 43SNPs passed quality control measures.

Two SNPs were significantly associated with asthma using a strict Bonferroni-corrected level of significance: rs2706347 in RAD50, and rs10830196 in PTPRE (Figure 2). Two additional SNPs were associated with asthma at a nominal level of significance: rs1801275 in IL4R, and rs4523 in TBX42R. Seven SNPs with prior GWAS evidence demonstrated nominal association with asthma, including SNPs in Daq3, MEFFY, NOS1, IL16, DPP10, IL33, and PDGFRα. However, these associations were not replicated when restricted to non-GWAS samples, and so they were considered to be potentially spurious.

No evidence of association was seen for SNPs in asthma risk genes that have been frequently reported in the literature. Included in the genes TNF (31 prior replications), IL13 (15 prior replications), ADAM33 (13 prior replications), TGFβ1 (13 prior replications), and ADRB2 (2 prior replications). In the present study, we observed P-values of allelic association for these genes ranging from 0.10 to 0.93.

Conclusions
RAD50 and PTPRE may be promising candidate asthma risk genes. Lack of evidence of the most highly reported polymorphisms in the present study highlights the need for robust replication of candidate risk genes in asthma.