significant genes increased (*IL13*, *TNF*, *IL4RA* and *ADAM33*), making in the overall no substantial differences in the genetic determinants between child-onset and adult-onset asthmas. The exception was for *LTC4S* and *GSTM1*, suggesting the former gene to be related with the development of asthma during childhood and the later one during adulthood. However, as *GSTM1* was significantly associated with childhood asthma in the comparison of childhood asthma vs adult control, it is difficult to conclude whether the polymorphism in this gene affected the susceptibility to new-onset adult asthma.

Our replication results were in agreement with several large-scale studies. A recent review of the literature revealed that five asthma candidate genes, *ADAM33*, *TNF*, *TBXA2R*, *CD14* and *LTC4S*, were the focus of several meta-analyses in which *ADAM33* and *TNF* had a modest association with asthma.[24] The first genome-wide replication study of 39 asthma candidate genes generated *IL4RA* results that were consistent with our observations.[25] In the most comprehensive replication study carried out to date, the reproducibility of 93 genes previously associated with asthma and/or asthma intermediate traits was tested.[26] *IL13* was associated with asthma, and *TBXA2R* was associated with atopy, as we also observed in this study. Our replication rate of 48% (11 genes out of 23; OR 1.15–1.62, if the outlier OR of 3.01 (95% confidence interval, 1.40–6.51) for *IL13* –1112C>T is excluded) was higher than that reported in previous large association studies (for OR see Table 4); the study of Daley *et al.*[26] (unrelated case–control sample of N=5565) and a genome-wide screen of 422 nuclear families using SNP arrays had low replication rates of 13% (12 out of 93 tested genes, OR <1.4) and 15.4% (6 out of 39 at SNP-level replication, OR 1.4–1.7), respectively. This better replication rate might be attributed to our sample size, as it is well documented that smaller studies have a tendency to have more favorable outcomes than larger ones.[27] Daley *et al.* concluded that many published associations for asthma and atopy may be false-positive results. Whereas Rogers *et al.*[25] suggested that the poor coverage of genome-wide association study genotyping platforms and lack of statistical power due to insufficient sample size were the main reasons for their low replication. We are more inclined to suspect the 'contextual' bias explaining our failure to replicate all candidate genes. By that we mean the confounding effect of the whole complex network of gene–gene and gene–environmental interactions. This can be seen from the controversy in the findings between this current study and our previous one. In this study, *CD14* –550C>T and *IL4RA* Ile50Val were not associated with total serum IgE level. Whereas, in our recent association study carried out on the same school children, these two gene variants had a modifying effect on the levels of total IgE later in life depending on the children's attendance of day care before 2 years of age.[15] This association could be detected because the day care attendance was taken in consideration as an environmental factor and the effect of a gene was investigated simultaneously with the effect of the other one.

In the gene–gene interaction analysis, we identified some statistical interactions that asserted the weak associations found in the individual gene assessment. Among them, significant interaction between *C3* and *IL4RA* and between *ADAM33* and *TBXA2R* were observed for both childhood and all asthma groups. Although straightforward functional evidences of such paired interactions are lacking, some plausibility can be inferred. C3 or complement component 3 is an important part of the innate immunity recognizing exogenous and endogenous molecular patterns. Some functions of its C3a subtype indicate a possible role for the complement system in asthma pathogenesis.[28] In allergen-sensitized mouse model of pulmonary allergy deficient in *C3* or in its receptor *C3aR*, Drouin *et al.*[29,30] have observed that in the mutant mice the characteristic manifestations of asthma were significantly attenuated compared with wild-type animals and that in the lung the number of interleukin 4 (IL4)-producing cells was decreased; whereas Kawamoto *et al.*[31] showed that the absence of *C3aR* in mice results in significantly increased level of Th2 cytokines (IL4, IL5 and IL10). In spite of the fact that the two groups' results are contradictory calling for further examination, the observed functional relationship clearly indicates a modulator role of C3 on IL4 cytokine expression. IL4 signal transduction is mediated through the α subunit of the IL4 receptor (IL4RA), which is IL4 specific. Thus, the *C3-IL4-IL4RA* axis might be one of the plausible models for the interaction between *C3* and *IL4RA*. With regard to *ADAM33* and *TBXA2R*, one common feature that could indicate their putative interaction is their involvement in angiogenesis, a process frequently underestimated in the pathophysiology of asthma.[32] Novel findings on *ADAM33* showed that its catalytic domain promoted endothelial cell (EC) proliferation *in vitro*, and formation of new vessels *ex vivo* and *in vivo*.[33] *TBXA2R* is also known to be implicated in neovascularization but in an opposed way: suppresses EC migration and angiogenesis by inhibiting the effector pathways of the vascular endothelial growth factor (VEGF), a key angiogenic and chemotactic regulator of EC.[34] Although the exact mechanism by which *ADAM33* exerts its proangiogenic effect is yet to be elucidated, the involvement of VEGF is likely to take place. In that case, the above findings will suggest interactive effect of *ADAM33* and *TBXAR* on VEGF regulation and consequently on angiogenesis and microvascular remodeling of conductive airways in asthma.

Nevertheless, the significant results of our replication study as well as of the gene–gene interactions investigation should be interpreted with caution for inflation of type 1 errors. We have presented our findings based on the nominal α threshold of <0.05 without taking into account multiple testing. Relative to the replication study, this study is not an exploratory study aimed to find a 'significant' gene from multiple candidates but rather to test for confirmation of previously well-established hypotheses. Indeed, the genes from Group 1 and Group 2 are the top asthma and allergy related genes, each replicated in at least six or more independent populations, meaning they all have a high previous probability to show true associations even in the case of a relaxed threshold value for significance. However, if we adjusted for multiple comparisons by the Bonferroni method, none of our significant findings would survive this stringent level of correction. It is obvious that the power is enough to detect genetic effect with OR of around 1.4 with the current sample size, but not if we consider multiple testing. The same is for the results obtained from the screening of the interactions between two polymorphisms. If we strictly applied Bonferroni correction, the significant P-values would need to be in the order of 9.46×10^{-5} (0.05/528) because we carried out $33C^2$=528 tests for each phenotype; no P-value reached this value. Thus, our findings for the potential gene–gene interactions must be evaluated physiologically or by analyses of other sets of samples to validate these observations.

There are other limitations to this study. We focused on the effect of genetic polymorphisms on dichotomous phenotypes and ignored clinical severity and environmental factors. There was also a delay between the recruitment of child asthma cases and child control samples, which could be a source of bias due to differences in DNA processing as well as in environmental exposure. Although population stratification was not controlled in this study, we consider the confounding effect of this factor to be of a lesser extent in comparison to studies conducted on North American[35,36] or Western European[37,38] populations. From the genetic point of view, this

Table 4 Odds ratio and 95% CI of significant polymorphisms found in the basic association studies

		OR (95%CI)		
Gene	*Polymorphism*	*Allele*	*Dom.*	*Rec.*
Childhood asthma vs child control				
Group 1				
IL13	Arg110Gln	×	×	×
	−1112C>T	1.40 (1.07–1.84)	×	3.01 (1.40–6.51)
ADAM33	Met764Thr	×	×	×
	3236C>T	×	×	×
Group 2				
LTC4S	−444A>C	1.40 (1.05–1.88)	1.47 (1.05–2.06)	×
CCL5	−403A>G	×	×	×
	−28C>G	×	1.43 (1.00–2.05)	×
Group 3				
IL12B	−6415CTCTAA>GC	1.33 (1.07–1.66)	×	1.78 (1.25–2.55)
	1146 C> A	1.30 (1.04–1.61)	×	1.73 (1.21–2.48)
C3	Block 2 (haplotype 6)	1.92 (1.12–3.31)	×	×
	Block 4 (haplotype 1)	×	×	×
Adult asthma vs adult control				
Group 1				
TNF	−1037C>T	1.53 (1.20–1.96)	1.62 (1.22–2.16)	×
ADAM33	Met764Thr	1.47 (1.10–1.96)	1.57 (1.14–2.15)	×
	13236C>T	×	×	×
Group 2				
NOS1	GT repeat intron 2 (187allele)	1.42 (1.20–1.71)	1.55 (1.19–2.03)	1.56 (1.10–2.19)
	GT repeat intron 2 (183allele)	1.29 (1.07–1.55)	1.48 (1.11–1.97)	×
Group 3				
C3	Block 2 (haplotype 6)	×	×	×
	Block 4 (haplotype 1)	1.34 (1.14–1.64)	1.38 (1.04–1.84)	1.60 (1.19–2.14)
SOCS1	−1478CA>del	1.73 (1.27–2.36)	1.69 (1.20–2.37)	3.93 (1.20–12.86)
All asthma vs all controls				
Group 1				
TNF	−1037C>T	1.32 (1.10–1.59)	1.36 (1.10–1.68)	×
ADAM33	Met764Thr	1.26 (1.02–1.56)	1.28 (1.02–1.61)	×
	13236C>T	1.19 (1.01–1.40)	×	×
Group 2				
NOS1	GT repeat intron 2 (187allele)	1.17 (1.02–1.35)	×	×
	GT repeat intron 2 (183allele)	1.15 (1.00–1.33)	×	×
Group 3				
IL12B	−6415CTCTAA>GC	1.20 (1.04–1.37)	×	1.44 (1.14–1.81)
	1146 C> A	1.24 (1.08–1.42)	×	1.49 (1.18–1.88)
C3	Block 2 (haplotype 6)	1.53 (1.08–2.15)	1.58 (1.11–2.25)	×
	Block 4 (haplotype 1)[a]	1.27 (1.10–1.45)	1.39 (1.21–1.73)	1.31 (1.05–1.63)
SOCS1	−1478CA>del	1.47 (1.16–1.96)	1.43 (1.11–1.86)	3.09 (1.17–8.16)

Abbreviations: Allele, χ^2-test of allele frequency; CI, confidence interval; dom., 2×2 dominant model genotype χ^2-test; OR, odds ratio; rec., 2×2 recessive model genotype χ^2-test.
[a]For haplotype description please refer Inoue *et al.*[11]
Polymorphisms with a *P*-value ⩾0.05 in all association tests of the four genetic models are not shown.
×$P \geqslant 0.05$.

assumption is based on the fact that our control subjects were residents of the mainland of Japan, the population of which belongs to the genetically homogeneous Hondo cluster,[39] and also on the results of genomic control analysis[40] that showed the populations from the Kinki and Kanto regions (where we recruited our samples and controls) do not differ in the allele frequency of the null marker. In terms of stratification determined by an individual's socioeconomic position, we would refer to the specific egalitarian characteristic of the Japanese society in support of our claim.[41]

In conclusion, our findings and previous studies suggest that *IL13*, *TNF*, *IL4RA*, *ADAM33* and *TBXA2R* might represent the major asthma and asthma-related traits genes common across populations. *GSTM1*, *GSTP1*, *LTC4S*, *AAA1*, *NOS1* and *CCL5* along with *MMP9*, *IL12B*, *C3* and *SOCS1* might be additional susceptibility genes, which have stronger effects in the Japanese population. Despite our failure to replicate the other genes, our results were not strong enough to eliminate them from the candidate gene list because we did not investigate all known variations in these genes and we did not consider

the effects of environmental factors. Replication studies of genotype–phenotype associations with sample sizes ranging from several hundred to several thousand are not exempt from inconsistencies in findings and have low replication rates. Given the present limited availability of biobanks, methodologically irreproachable studies that integrate more detailed clinical information and that explore the effects of genes in their entirety by dissecting the direct and interactive effects from environmental factors and other genes are required to improve the power and reproducibility of genetic association studies.

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