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# ST2 and IL-33 polymorphisms and the development of childhood asthma: a prospective birth cohort study in Finnish children

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The ST2/IL-33 signaling pathway has an important role in the host inflammatory response. Here we aimed to study the association of *ST2* and *IL-33* polymorphisms with serum soluble (s) ST2 and IL-33 concentrations in healthy Finnish children and, in addition, their association with childhood asthma. In total, 146 children were followed from birth to the age 7 years for the development of asthma. Single-nucleotide polymorphisms (SNPs) in *ST2* and *IL-33* were determined, and associations of the SNP variants with serum levels of sST2 and IL-33 at age of 13 months and with recurrent wheezing and childhood asthma at 7 years of age were analyzed. Children with *ST2* rs1041973 AC/AA genotypes had significantly lower level of serum sST2 (2453 pg/mL; IQR 2265) than those with CC genotype (5437 pg/mL; IQR 2575; $p = < 0.0001$). Similar difference was also observed with *ST2* rs13408661. No differences were observed between subjects with studied *IL-33* SNPs. Children who carried genetic variants of *ST2* rs1041973 or rs13408661 seemed to have a higher risk of asthma. In contrast, children who carried genetic variants of *IL-33* rs12551268 were less often diagnosed with asthma. Even though these SNPs seemed to associate with asthma, the differences were not statistically significant.

Key words: Asthma; ST2; sST2; IL-33; polymorphism.

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Asthma is a chronic lung disease that often starts at childhood and causes airway hyperresponsiveness, mucus secretion, and airway remodeling [1]. It is a complex disease which is typically associated with allergy and T helper (Th) 2 cell responses. These allergen-specific Th2 cells produce Type 2 cytokines like interleukin (IL)-4, IL-5, IL-9, and IL-13, leading to the synthesis of immunoglobulin E (IgE), accumulation of eosinophils, and mucus overproduction [2]. In recent years, there has been increased interest in the ST2/IL-33 axis in the asthma research [3].

Suppression of tumorigenicity 2 (ST2) protein, also known as IL-1 receptor-like 1 protein, is a member of the IL-1 receptor family and a receptor for IL-33 [4]. ST2 has four different splice isoforms: a membrane receptor ST2, sST2 which is a soluble form of ST2 and two less known variants; ST2V and ST2LV. ST2 and sST2 have different exon I and C-terminal sequence and, in addition, sST2 lacks the transmembrane and cytoplasmic domain of ST2 [5]. ST2 is expressed on cardiomyocytes [6] and variety of immune cells such as innate lymphoid cell type 2 (ILC2), regulatory T cells (Tregs), and eosinophils [3].

IL-33 is a member of IL-1 cytokine family. It is constitutively and abundantly expressed in many human cells including epithelial and endothelial cells. IL-33 is a so-called alarming cytokine. Under stable conditions, it is mostly stored in the cell nucleus and upon tissue injury, cell necrosis or infection its bioactive form is released into the extracellular environment and its binding to ST2 will promote pro-inflammatory response [7].

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Stimulation of ST2 via its ligand IL-33 leads to MyD88-dependent signaling and further downstream activation of NF-κB, JNK, p38, and ERK pathways leading to inflammatory responses and production of pro-inflammatory cytokines such as IL-4, IL-5, and IL-13, depending on the target cells [3]. SolubleST2 acts as a decoy receptor of IL-33 and inhibits ST2/IL-33 signaling. ST2/IL-33 signaling pathway is linked with asthma susceptibility [3]. Several genome-wide association studies (GWAS) and cohort studies of asthma have shown that *ST2* is one of the most highly replicated susceptibility loci for asthma. Especially three genetic variants in the *ST2* gene; rs1041973, rs13408661, and rs13431828, are associated with asthma in different populations [8–10]. Two of these SNPs, rs13408661 and rs13431828, are co-segregating in Finnish population [11]. In addition, three *IL-33* SNPs, rs1342326, rs12551256, and rs12551268, were included in this study, because literature has shown them to associate either with an altered IL-33 expression [12] and an increased risk for allergy or atopic diseases, such as hay fever [13] or allergic rhinitis [14], or decreased risk for asthma [15].

We have previously shown with the same cohort that low concentration of serum sST2, but not IL-33, at early age (13 months) is associated with a higher risk for development of asthma at 7 years of age in Finnish children [16]. In addition, we found that subjects who carry a 23 bp deletion in the promoter of *TLR2* (rs111200466) produced lower level of serum IL-33 compared to those who carry the insertion [17]. This finding suggests that children with the polymorphism of *TLR2* may have impaired production of IL-33, which could contribute to the protection against asthma development. However, we did not find similar correlation between *TLR2* rs111200466 polymorphism and serum sST2 levels [17]. Here we wanted further to study the effects of these SNPs of *ST2* and *IL-33* on serum sST2 and IL-33 levels and in addition, to investigate whether these polymorphisms are associated with the susceptibility to recurrent wheezing or asthma.

## MATERIAL AND METHODS

### Study design and sample collection

The present study is part of a Finnish population-based birth cohort study called Steps to the Healthy Development and Well-being of Children (the STEPS Study) [18]. The aims of the study are to search for the precursors and causes of problems in child health and well-being. The child cohort consists of 1827 children born in the Hospital District of Southwest Finland, January 2008 to April 2010. Of these, 923 children were intensively followed for respiratory tract infections from birth to 2 years of age and after that with annual questionnaires and collection of health registry data. Wheezing illnesses were diagnosed by a physician and recurrent wheezing was defined as two or more wheezing illnesses during the follow-up time [19]. Physician-diagnosed asthma at 7 years of age was defined as a diagnosis of asthma in the medical records or an electronic prescription of inhaled corticosteroids for asthma [20] (data available from 910 (99%) children). Nasopharyngeal (NP), blood, and serum samples were collected at scheduled participant visits at age 2, 13, and 24 months [18]. In this study, we included a subgroup of 146 children. The selection was based on availability of samples and data; we included all children whose genetic data, serum samples (13 months) and 7-year follow-up data were available.

The STEPS study was approved by the Ministry of Social Affairs and Health and the Ethics Committee of the Hospital District of Southwest Finland (February 27, 2007) and was found ethically acceptable by the Ministry of Social Affairs and Health (STM 1575/2008, STM 1838/2009) and the Ethics Committee of the Hospital District of Southwest Finland (19.2.2008 §63, 15.4.2008 §134, 19.4.2011 §113). All participants or parents of participating children gave their written informed consent.

### Genetic analyses and cytokine measurements

Genomic DNA was extracted from peripheral blood samples [21]. Two *ST2* SNPs (rs1041973 and rs13408661/rs13431828) and three *IL-33 SNPs* (rs1342326, rs12551256, and rs12551268) were analyzed with Sanger sequencing. Invitrogen Platinium Taq DNA polymerase (Thermo Fisher Scientific Inc.MA, USA) was used for PCR according to the manufacturer's instructions. The primers were designed using a Primer-Blast tool (National Center for Biotechnology Information, NCBI, Bethesda, MA, USA) and produced by Eurofins Genomics (Ebersberg, Germany; Table S1). Prior the sequencing, PCR products were purified enzymatically with Exonuclease FastAP and Exo I (Thermo Fisher Scientific Inc, Waltham, MA, USA). Purified PCR products were sent for sequencing to Eurofins Genomics, Ebersberg.

Serum sST2 levels were measured with Human sST2 ELISA kit (Elabscience Biotechnology, Wuhan, China) and IL-33 levels by multiplex immunoassay (Bio-PLex 200, Bio-Rad Laboratories, Hercules, CA, USA) with MILLIPLEX Th17 Kit (Merck & C0., Kenilworth, NJ, USA) according to the instructions of the manufacturers. The detection limits of IL-33 and sST2 were 6.3 and 190 pg/mL, respectively [16].

### Statistical analysis

Statistical analyses were performed using SPSS software, version 28.0 (IBM Corp. in Armonk, NY, USA). Two-sided chi-squared tests were used to identify differences in allele frequency distributions between the groups. In the haplotype analyses, the Bonferroni correction was used for determining adjusted p-values (Pa). The non-normally distributed data were compared by Mann–Whitney *U*-test. Deviations from the Hardy–Weinberg equilibrium (HWE) were studied with the chi-squared test. Two-tailed $p < 0.05$ was considered significant.

 

## RESULTS

### Demographics of the patients included

The detailed characteristics of study subjects (n = 146) are presented in Table 1. The gender distribution was almost equal, 47.3% were male and 52.7% were female. Most of the children (82.9%) were born vaginally and 61.0% were exclusively or partially breast fed until 6 months of age. Twenty-three (15.8%) children were diagnosed with atopy at time of sampling (13 months) and a physician-diagnosed asthma was documented in 15 (10.3%) children at age 7 years.

### Relationships between the SNPs in the two genes and susceptibilities to asthma or recurrent wheezing

Frequencies of studied polymorphisms; *ST2* rs1041973 and rs13408661/rs13431828 and *IL-33* SNPs rs1342326, rs12551256, and rs12551268 and their distributions between subjects with and without asthma or recurrent wheezing are presented in Table 2. All studied SNPs were in HWEs (all p-values = > 0.05).

As shown in Table 2, the variant types of *ST2*, either rs1041973 (50.0%) or rs13405661 (41.7%) were more frequent in children diagnosed with asthma compared to those without asthma (34.5% and 26.6%, respectively). Opposite to this, the variant type (CA or AA) of *IL-33* rs12551268 was less frequent among children diagnosed with asthma compared to those without asthma. However, these differences were not statistically significant. There were no significant differences observed between studied SNPs and recurrent wheezing. Although the *IL-33* rs12551268 variant type appeared to be less frequent among children with recurrent wheezing, it did not reach to be statistically significant (Table 2).

### Serum levels of sST2 and IL-33 among different genotypes of the SNPs of the two genes

The serum levels of sST2 varied from 85 to 13 626 pg/mL, with a median of 4567 pg/mL (IQR3397) and levels of IL-33 from 3.1 to 2608 pg/mL, with a median of 12.4 pg/mL (IQR41.4). No correlation was observed between serum IL-33 and sST2. As seen in Fig. 1, children who carried *ST2* rs1041973 variant genotypes CA or AA had significantly lower level of serum sST2 (2453 pg/mL;IQR 2265) than those with CC genotype (5437 pg/mL; IQR 2575; p = < 0.001). Same was observed with *ST2* SNP rs13408661, children with the variant genotype (GA or AA) had significantly lower levels of sST2 (2442 pg/mL; IQR 2239) than those with GG genotype (5337 pg/mL; IQR 2705; p = < 0.001). No such association was observed when the *ST2* SNPs were compared with serum IL-33 levels (Fig. 1C,D). No differences were found between children with different *IL-33* genotypes in serum sST2 (Fig. 2A–C) or IL-33 (Fig. 2D–F) levels at age of 13 months.

**Table 1.** Characteristics of study subjects

| Variables | Subjects with available serum sample, n = 146 (%) | Asthma at age of 7 years | | |
|---|---|---|---|---|
| | | Yes, n = 15 (%) | No, n = 131 (%) | p-Value |
| Sex | | | | |
| Female | 69 (52.7) | 6 (40.0) | 63 (48.1) | 0.376 |
| Male | 77 (47.3) | 9 (60.0) | 68 (51.9) | |
| Mode of delivery | | | | |
| Vaginal | 121 (82.9) | 8 (53.3) | 113 (86.3) | 0.005* |
| Cesarean section | 25 (17.1) | 7 (46.7) | 18 (13.7) | |
| Exclusive or partial breast feeding ≥6 m | 86 (61.0) | 7 (41.7) | 79 (61.2) | 0.537 |
| Missing data | 5 (3.4) | 3 (20.0) | 2 (1.5) | |
| Older siblings | 60 (41.1) | 7 (46.7) | 53 (40.5) | 0.422 |
| Atopy at age of 13 months | 23 (15.8) | 2 (13.3) | 21 (16.0) | 0.577 |
| Missing data | 8 (5.5) | 1 (6.7) | 7 (5.3) | |
| Recurrent wheezing | 21 (14.4) | 8 (53.3) | 13 (9.9) | 0.001* |
| Missing data | 1 (1.4) | 0 (0.0) | 1 (0.8) | |
| Parental asthma | 18 (12.3) | 4 (26.7) | 14 (10.7) | 0.092 |

Data are presented as numbers (n) of children and valid percentages (%), without missing observations. The percentages of missing values are presented as percent of all observations. The diagnoses of asthma at age of 6.5–7.5 years were retrieved from medical records including electronic prescriptions of the Hospital District of Southwest Finland. Atopy was defined as doctor-diagnosed atopy by age 13 months. Wheezing illnesses were diagnosed by a physician and recurrent wheezing was defined as two or more wheezing illnesses during the follow-up time. p-values <0.05 were considered significant (*).

**Table 2.** SNP frequencies

| SNP | Whole study cohort, n = 146 (%) | Asthma at age of 7 years | | | Recurrent wheezing | | |
|---|---|---|---|---|---|---|---|
| | | Yes, n = 15 (%) | No, n = 131 (%) | OR (95% Cl), p-value | Yes, n = 21 (%) | No, n = 124 (%) | OR (95% Cl), p-value |
| *ST2* | | | | | | | |
| rs1041973 | | | | | | | |
| CC | 86 (64.2) | 6 (50.0) | 80 (65.6) | | 11 (55.0) | 73 (65.2) | |
| CA | 44 (32.8) | 5 (41.7) | 39 (32.0) | | 9 (45.0) | 35 (31.3) | |
| AA | 4 (2.7) | 1 (8.3) | 3 (2.5) | | 0 (0.0) | 4 (3.6) | |
| CA/AA | 48 (35.5) | 6 (50.0) | 42 (34.5) | 1.91 (0.58–6.27), 0.222 | 9 (45.0) | 39 (34.8) | 1.53 (0.59–4.01), 0.383 |
| rs13408661 | | | | | | | |
| GG | 97 (71.9) | 7 (58.3) | 90 (73.2) | | 15 (75.0) | 80 (70.8) | |
| GA | 37 (27.4) | 5 (41.7) | 32 (26.0) | | 5 (25.0) | 32 (28.3) | |
| AA | 1 (0.7) | 0 (0.0) | 1 (0.8) | | 0 (0.0) | 1 (0.9) | |
| GA/AA | 38 (28.1) | 5 (41.7) | 33 (26.6) | 1.95 (0.58–6.57), 0.317 | 5 (25.0) | 33 (29.2) | 0.80 (0.27–2.40), 0.701 |
| *IL-33* | | | | | | | |
| rs1342326 | | | | | | | |
| AA | 106 (74.6) | 11 (73.3) | 95 (74.8) | | 16 (80.0) | 89 (74.2) | |
| AC | 34 (23.9) | 4 (26.7) | 30 (23.6) | | 3 (15.0) | 30 (25.0) | |
| CC | 2 (1.4) | — | 2 (1.6) | | 1 (5.0) | 1 (0.8) | |
| AC/CC | 36 (25.3) | 4 (26.7) | 32 (25.2) | 1.23 (0.36–4.16), 0.750 | 4 (20.0) | 31 (25.8) | 0.72 (0.22–2.31), 0.577 |
| rs12551256 | | | | | | | |
| GG | 41 (29.3) | 4 (26.7) | 37 (39.6) | | 5 (26.3) | 36 (30.3) | |
| GA | 69 (49.3) | 10 (66.7) | 59 (47.2) | | 9 (47.4) | 58 (48.7) | |
| AA | 30 (21.4) | 1 (6.7) | 29 (23.2) | | 5 (26.3) | 25 (21.0) | |
| GA/AA | 99 (70.7) | 11 (73.3) | 88 (70.4) | 1.17 (0.35–3.91), 0.999 | 14 (73.7) | 83 (69.7) | 1.21 (0.41–3.63) |
| rs12551268 | | | | | | | |
| CC | 92 (65.7) | 13 (86.7) | 79 (63.2) | | 16 (84.2) | 75 (63.0) | |
| CA | 44 (31.4) | 2 (13.3) | 42 (33.6) | | 3 (15.8) | 40 (33.6) | |
| AA | 4 (2.9) | — | 4 (3.2) | | 0 (0.0) | 4 (3.4) | |
| CA/AA | 48 (34.3) | 2 (13.3) | 46 (36.8) | 0.27 (0.06–1.25), 0.089 | 3 (15.8) | 44 (37.0) | 0.32 (0.08–1.16), 0.07 |

Data are presented as numbers (n) of children and valid percentages (%), without missing observations. The diagnoses of asthma at age of 6.5–7.5 years were retrieved from medical records including electronic prescriptions of the Hospital District of Southwest Finland. Wheezing illnesses were diagnosed by a physician and recurrent wheezing was defined as two or more wheezing illnesses during the follow-up time. Asthma data were available from all children (n = 146) and wheezing data from 145 (99.3%) children. The missing data rates of SNPs data varied between 8.2% and 2.7%. Comparisons were performed by use of two-sided chi-squared tests. p-values < 0.05 were considered significant (*).

Haplotype analyses strengthened our finding on the effect of *ST2* rs1041973 variant types (CA or AA) on serum sST2 levels. As seen in Fig. 3A, children who carried both wild types of *ST2* rs1041973 (CC) and *ST2* rs13408661 (GG) had significantly higher levels of sST2 than those who had variant type of *ST2* rs1041973 (CA or AA) with wild (GG) or variant type (GA or AA) of *ST2* rs13408661 (p = < 0.001, ap = 0.002). When the haplotype analyses were done with *ST2* rs1041973 and *IL*-33 rs1342326 (Fig. 4A) or with *IL-33* rs12551268 (Fig. 4B) findings were parallel. Children carrying the *ST2* rs1041973 variant genotypes (CA or AA) had generally lower serum sST2 levels than those who carried CC genotype (p = < 0.001, ap = 0.002), regardless of the carried *IL-33* genotype. No statistically significant differences were observed between haplotypes and serum IL-33 levels. Detailed analyses are presented in Tables S2 and S3.

## DISCUSSION

In this study, we aimed to evaluate the gene polymorphisms of *ST2* and *IL-33* and serum concentrations of sST2 and IL-33 in healthy Finnish children and in addition, investigate whether these polymorphisms are associated with asthma or recurrent wheezing susceptibility. This study has found that two *ST2* polymorphisms were associated with a decreased concentration of circulating serum *sST2* but they have no effect on serum IL-33 concentration. Both of studied *ST2* SNPs were more frequent

 

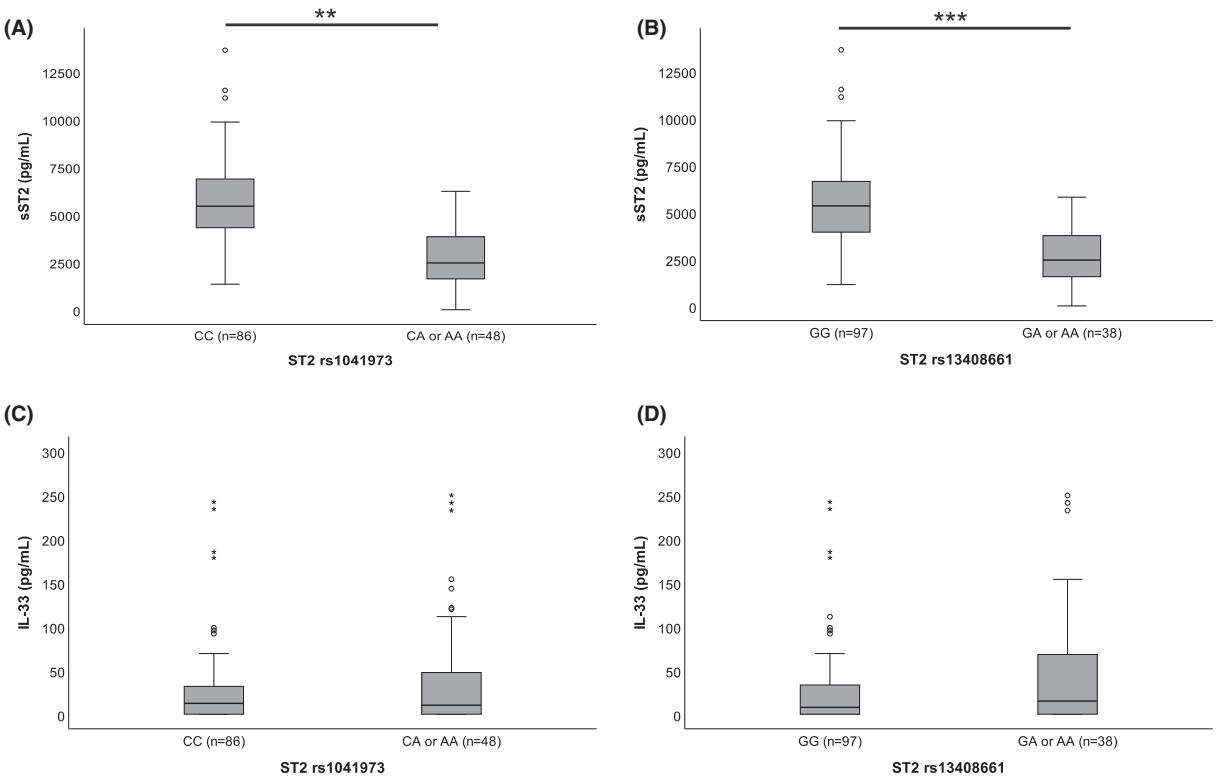


**Fig. 1.** Serum sST2 and IL-33 levels in individuals with and without *ST2* rs1041973 or *ST2* rs13408661 polymorphisms. (**p = < 0.001 and ***p = < 0.001)

in children diagnosed with asthma than those without diagnosed asthma; however, due to the small number of subjects this finding was not statistically significant. In addition, there were no significant differences observed between studied SNPs and recurrent wheezing.

Allergic asthma is a chronic inflammatory disorder that typically starts in the childhood, in which the allergic inflammation is an important mechanism, mainly regulated by Th2 cells [21,22]. Inflammation in the airways is associated with the activation of immune cells such as T cells, ILC2s, eosinophils, and mast cells and further over production of inflammatory cytokines like IL-4, IL-5, IL-9, and IL-13 [23]. The ST2/IL-33 signaling pathway plays a significant role in regulating the production of these cytokines and contributes to the pathogenesis of asthma [3].

In asthma, increased amount of IL-33 is released from lung epithelial cells in response to allergens, microbes, or viruses [24]. IL-33 acts as a cytokine through the ST2 and stimulates the production of Th2 cytokines [3]. These cytokines further promote the various key features of asthma such as tissue eosinophilia (IL-5), goblet cell metaplasia (IL-4 and IL-13), and bronchial hyperresponsiveness (IL-13) [25]. It is known that the sST2 can regulate Th2 responses by neutralizing the IL-33 activity [3]. Soluble ST2 is produced spontaneously in the lung, kidney, heart, and small intestine cells [26] but also during allergic inflammation after the activation of IL-33 in mast cells [27]. Elevated levels of serum IL-33 [24] and sST2 [28] have been reported in asthma.

To date, several GWAS and cohort studies of asthma have shown that *ST2* is susceptibility loci for asthma, especially three genetic variants in the *ST2* gene; rs1041973, rs13408661, and rs13431828, [8–10]. Here we found, that both studied *ST2* SNPs rs1041973 and rs13408661 were more frequent in children diagnosed with asthma compared to those without asthma; and, in addition, the variant type (CA or AA) of *IL-33* rs12551268 was less frequent among children diagnosed with asthma. However, the differences were not statistically significant. In this study cohort only 15 children were diagnosed with asthma, which explains in part why the finding was not significant.

We had previously shown with the same cohort that children who developed asthma at age of 7 years had significantly lower levels of serum sST2 at age of 13 months [16]. Ketelaar *et al.* [29] did a

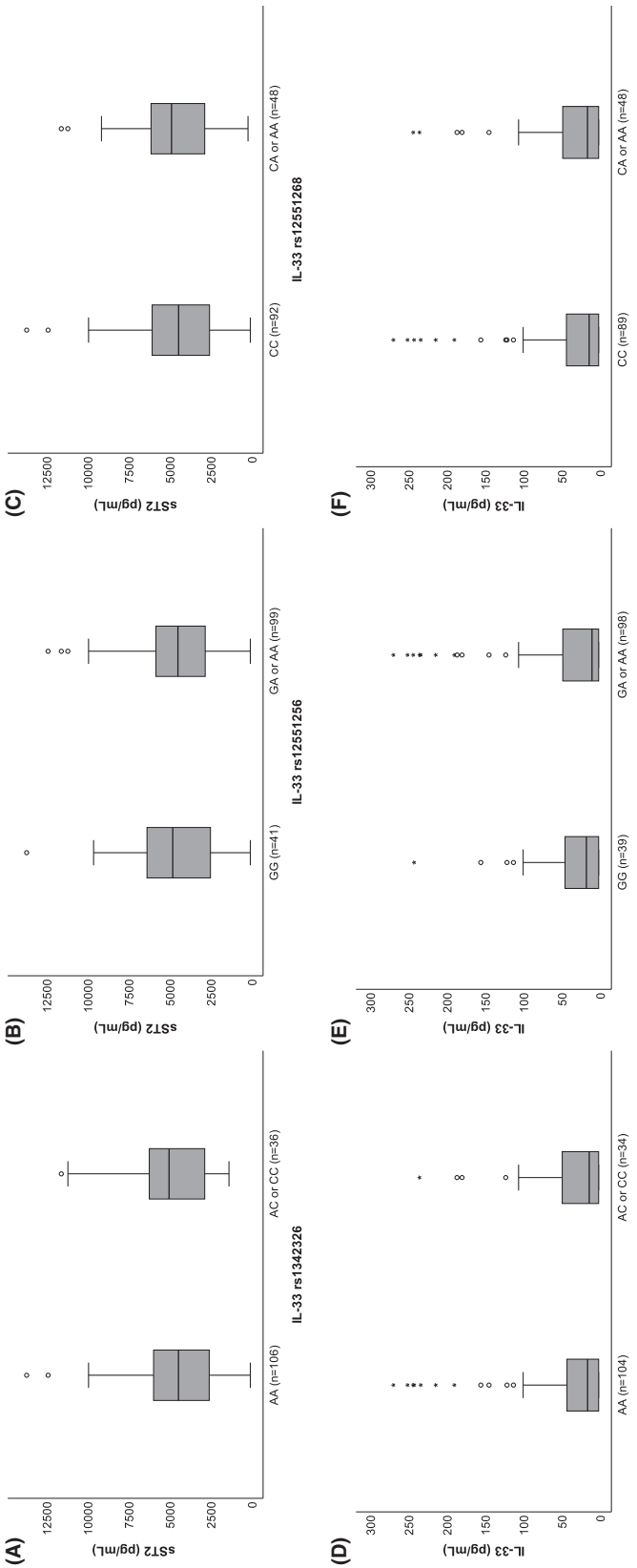


**Fig. 2.** Serum sST2 and IL-33 levels in individuals with and without *IL-33* rs1342326, *IL-33* rs12551256, or *IL-33* rs12551268 polymorphisms.

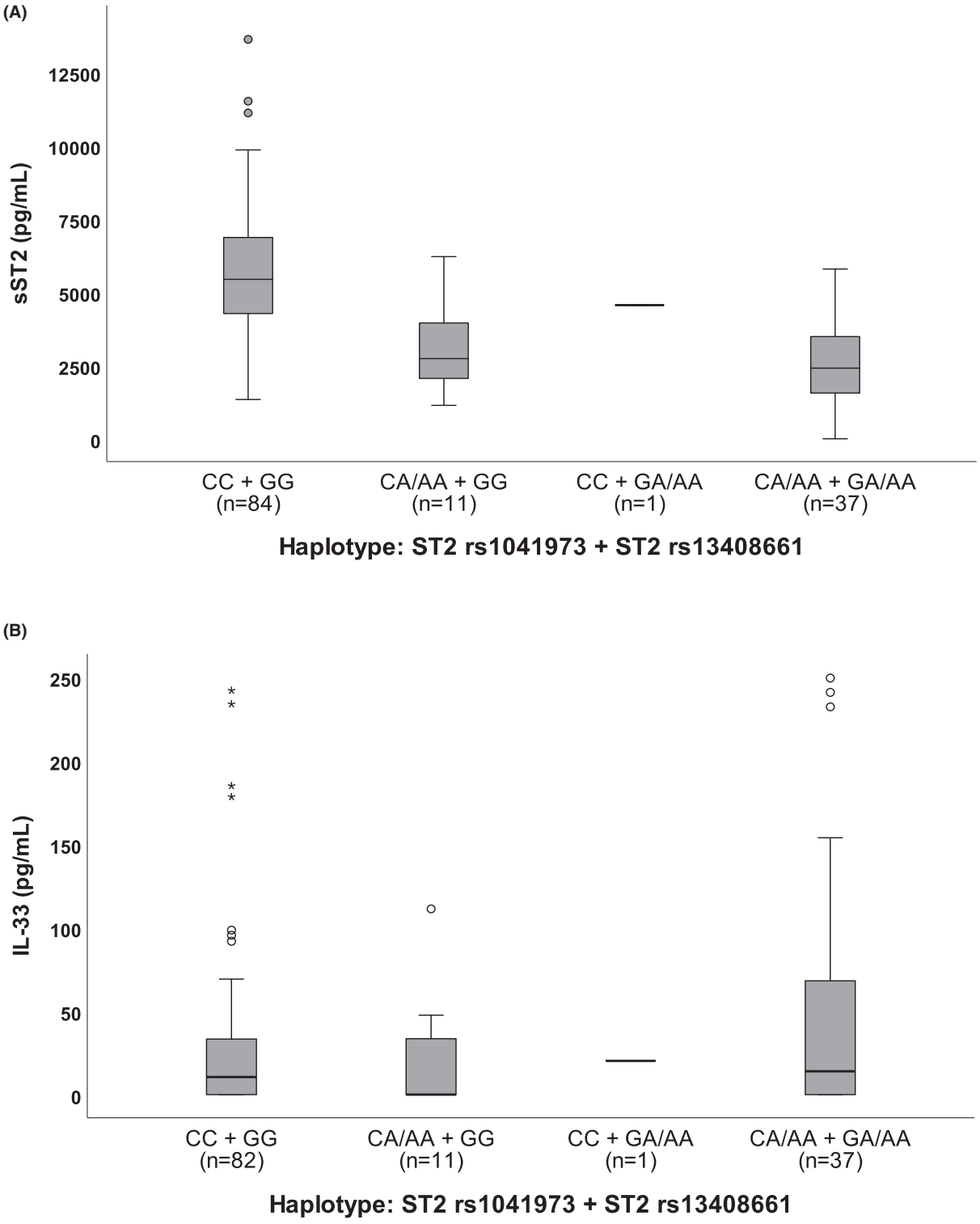


**Fig. 3.** Serum sST2 and IL-33 levels in individuals with different haplotypes of *ST2* rs1041973 and *ST2* rs13408661 polymorphisms.

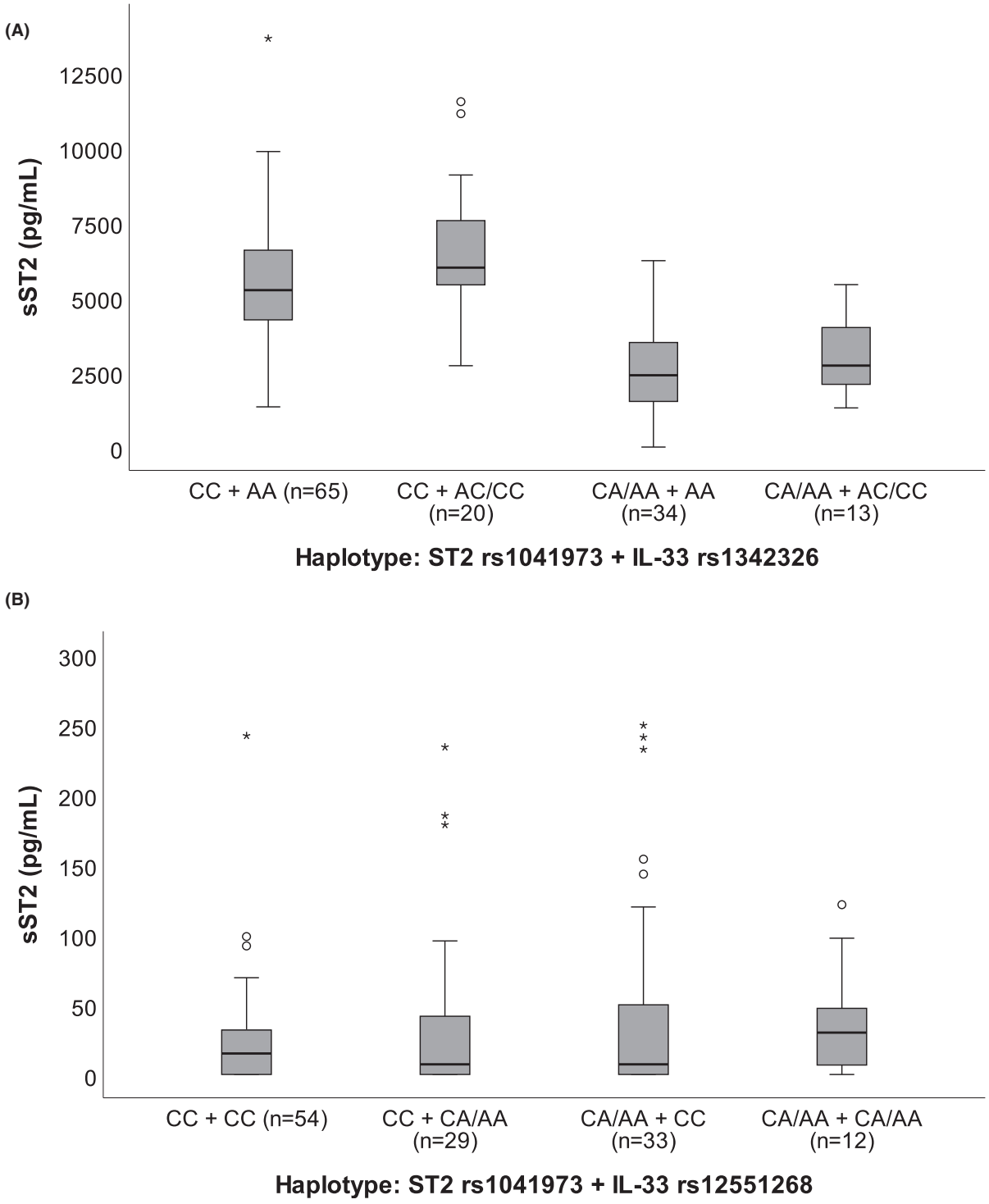


**Fig. 4.** Serum sST2 levels in individuals with different haplotypes of *ST2* rs1041973 and *IL-33* rs1342326, or *IL-33* rs12551268 polymorphisms.

 

similar observation in their study where they showed that sST2 was negatively associated with asthma with high FeNO in preschool wheezers (2–3 years).

It is well known that there is a large interindividual variation in cytokine production after the stimulation by different antigens [30,31]. The difference is partly caused by genetic variation of host [8,32]. Here we found that children who carry *ST2* rs1041973 CA or AA had significantly lower level of serum sST2 than those with CC genotype. Similar finding was also reported by Savenije *et al*, in which they showed that children who carry allele A of *ST2* rs1041973 had significantly lower serum sST2 levels than those who have allele C [9]. Later, Queiroz *et al*. reported a lower level of serum sST2 in atopic subjects with AA genotype for *ST2* SNP rs1041973 compared with individuals with AC and CC genotypes. In the same study, they showed with expression quantitative trait loci (eQTL) analysis that rs1041973 and rs873022 regulate the expression of *ST2* gene to be lower in subjects having the A and T alleles for rs1041973 and rs873022, respectively [15]. In another case–control study, performed in asthmatic patients, the A allele of *ST2* rs1041973 was associated with lower expression of ST2L mRNA and sST2 BAL protein expression levels [33].

When examining the association of *IL-33* SNPs rs1342326, rs12551256, and rs12551268 with asthma susceptibility or levels of serum sST2 and IL-33, no significant differences were observed between different genotypes and groups. In previous studies, *IL-33* rs1342326 variant genotypes (AC or CC) have been associated with both an increased [34] and decreased risk for asthma [35]. In this present study we did not observe any association between asthma and *IL-33* rs1342326 genotypes. The finding is in agreement with a previous study conducted with a different Finnish cohort, in which Korppi *et al*. showed that there was no significant associations of this gene variant with previous or current asthma at the mean ages of 6.4 or 11.7 years. However, they reported that the variant *IL-33* rs1342326 genotype was associated with allergic rhinitis at 6.4 years [14].

Some limitations of the present study should be considered when interpreting the results. First, the number of study subjects was limited, which affected especially our ability to study genotype associations with asthma. Second, the number of functional SNPs studied in the *ST2* gene was only three. Finally, even though *IL-33* SNPs which were included in the study did not appear to be related to serum IL-33 levels, we cannot rule out the genetic factors affecting the serum IL-33 production and further ST2/IL-33 signaling pathway efficiency. Clearly further research is needed to better understand the mechanisms how the studied *ST2* SNPs affect the production or sST2 or pathophysiology of childhood asthma.

Although the current study was based on a small sample of participants, the findings clearly suggest that polymorphisms of *ST2* were associated with a decreased concentration of circulating serum sST2. The *IL-33* variants studied were not associated with altered levels of sST2 or IL-33. Our results warrant further studies on the role of blood sST2 on development of asthma in different populations.

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## CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Primers used in the study.

**Table S2.** Serum sST2 and IL-33 levels in individuals with and without *ST2* and *IL-33* polymorphisms.

**Table S3.** Serum sST2 and IL-33 levels in individuals with different haplotypes of *ST2* and *IL-33* polymorphisms.