Functional and clinical translation of asthma and allergy associated genetic variants in IL33 and IL1RL1

Ketelaar, Marlies

DOI:
10.33612/diss.171580070

Chapter 8

Predictive value of serum sST2 in preschool wheezers for development of asthma with high FeNO

M E Ketelaar 1 2 , K D van de Kant 3 , F N Dijk 1 , E M Klaassen 4 , N S Grotenboer 1 2 , M C Nawijn 2 , E Dompeling 3 , G H Koppelman 1

1 Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, Groningen Research Institute for Asthma and COPD (GRIAC), University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
2 Department of Pathology and Medical Biology, Laboratory of Experimental Pulmonology and Inflammation Research (EXPIRE), Groningen Research Institute for Asthma and COPD (GRIAC), University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.
3 Department of Pediatric Pulmonology, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.
4 Department of General Practice, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands.

Published: Allergy. 2017 Nov;72(11):1811-1815.

Abstract

Wheezing is common in childhood. However, current prediction models of pediatric asthma have only modest accuracy. Novel biomarkers and definition of subphenotypes may improve asthma prediction. Interleukin-1-receptor-like-1 (IL1RL1 or ST2) is a well-replicated asthma gene and associates with eosinophilia. We investigated whether serum sST2 predicts asthma and asthma with elevated exhaled NO (FeNO), compared to the commonly used Asthma Prediction Index (API). Using logistic regression modeling, we found that serum sST2 levels in 2-3 years-old wheezers do not predict doctors' diagnosed asthma at age 6 years. Instead, sST2 predicts a subphenotype of asthma characterized by increased levels of FeNO, a marker for eosinophilic airway inflammation. Herein, sST2 improved the predictive value of the API (AUC=0.70, 95% CI 0.56-0.84), but had also significant predictive value on its own (AUC=0.65, 95% CI 0.52-0.79). Our study indicates that sST2 in preschool wheezers has predictive value for the development of eosinophilic airway inflammation in asthmatic children at school age.

Keywords

childhood asthma; fraction of exhaled NO; prediction; preschool wheezers; serum sST2.

Abbreviations

ADEM study	Asthma DEtection and Monitoring study
API	Asthma Prediction Index
FeNO	Fraction of exhaled nitric oxide
IL-1RL1	Interleukin-1 receptor-like 1
PIAMA study	Prevalence and Incidence of Asthma and Mite Allergy study
SNP	Single nucleotide polymorphism

To the editor

Approximately 40% of all preschool children (aged <6 years [y]) encounter one or more episodes of respiratory symptoms, such as wheezing, coughing, and dyspnoea. However, only some (20%-40%) of these preschool children with respiratory problems will develop asthma at school age. (257) Current prediction models such as the asthma prediction index [API] are based on familiar predisposition, history of eczema, and presence of eosinophils or sensitization and have only modest accuracy in predicting asthma. (257,258) To enable better prediction of asthma development at young age, novel biomarkers associated with asthma are required, which could include expression levels of well-replicated asthma genes. (258)

One potential biomarker for asthma is soluble interleukin-1-receptor-like 1 [IL-1RL1-a or sST2], which is encoded by the IL-1RL1 gene (chr 2) and can be detected in serum. IL-1RL1 is an asthma susceptibility gene identified in genetic studies of pediatric and adult asthma patients. (26) IL-1RL1 has also been linked to blood eosinophilia, IgE (sensitization), eczema, and hay fever. (26,259) ST2 is the receptor for interleukin-33 (IL-33), a cytokine thought to initiate and amplify a Th-type-2 response in inflammatory diseases such as asthma. (260) Soluble ST2 has been proposed to act as a decoy receptor, sequestering IL-33, thereby preventing its role in the induction of an immune response, and particularly its modulation of a Th-type-2 reaction. (82,260)

Previously, we found that IL-1RL1 SNPs associate with sST2 levels in childhood asthma in a Dutch birth cohort, the Prevalence and Incidence of Asthma and Mite Allergy [PIAMA] cohort. In this study, asthma-risk alleles were consistently associated with lower serum sST2 levels, indicating a putative protective effect of high sST2. (27) Moreover, IL-1RL1 SNPs were associated with intermediate-onset and late-onset wheezing phenotypes. (28) These children start to wheeze at age 2-3 years, often have allergen sensitization at age 4 years, and are at high risk of subsequent asthma development at school age. However, the expression levels of IL-1RL1 in preschool wheezers are unknown, as well as whether these levels could identify those children who will eventually develop asthma.

Therefore, we hypothesized that serum sST2 levels measured in wheezing preschool children contribute to the prediction of asthma at school age. Moreover, as IL-1RL1 was previously associated with blood eosinophilia, our second aim was to determine whether serum sST2 levels predict exhaled NO, as a marker of eosinophilic asthma at school age.

We investigated our hypotheses in children of the ADEM [Asthma DEtection and Monitoring] study (clinicaltrial.gov: NCT 00422747). The ADEM study is a unique longitudinal cohort designed to study the added value of biomarkers to clinical information (API) for an early asthma diagnosis. A detailed study protocol has been published previously. (261) This study included 202 wheezing children and 50 healthy controls who were enrolled from primary care practices in The Netherlands at age 2-3 years and were followed up annually until age 6 years. At age 6 years, a final asthma diagnosis was made based on symptoms, use of asthma medication, and lung function by experienced pediatricians in the field of

respiratory medicine and by a computer algorithm. Corticosteroids were stopped 4 weeks before measurements when applicable. Wheezing at preschool age was defined as two or more wheezing episodes before inclusion, according to the questionnaire developed by the International Study of Asthma and Allergies in Childhood. sST2 serum levels at age 2-3 years were quantified using a commercially available ELISA (R&D Systems Quantikine ELISA kit #DST200, Abingdon, UK), which was selected after a series of validation steps comparing specificity, sensitivity, assay recovery, and interassay variability (Methods S1).

For the current study, in analogy to earlier analyses of the ADEM study, (258) a logistic regression model was built to predict asthma diagnosis at age 6 years comparing the API, sST2, and sST2 combined with API as predictors. Model performance was assessed by testing the contribution of a predictor to the model (F-test) and by quantifying discrimination (area under the curve, AUC) using a receiver operating characteristics (ROC) curve. In the ADEM cohort, 40% of the preschool wheezers developed asthma at age 6 years, while the remainder were transient wheezers. Predictive analyses were performed in the group with available sST2 levels at age 2-3 years, which were 171 of the 202 preschool wheezers. The subgroup of children with available sST2 serum levels did not significantly differ from the overall group in general characteristics. sST2 levels were square-root-transformed to meet normality criteria.

A negative association was found between IL-1RL1 genotype (using rs1420101, representing a major LD block in IL-1RL13) and sST2 protein expression in serum (ANOVA P<.001). Carriers of the asthma-risk allele (A) had lower sST2 levels, which is in the same direction as previously reported in the PIAMA cohort. (27)

However, serum levels of sST2 measured at age 2-3 years could not distinguish which of the preschool wheezing children eventually developed asthma at school age (AUC=0.50 [95CI 0.41-0.59, P=.98], B=−0.002 [OR=0.998, P=.89]). No difference in serum sST2 levels at age 2-3 years was found between children with transient wheeze, true asthmatics, or healthy controls at age 6 years (P=.881, ANOVA). Consequently, serum sST2 levels at 2-3 years did not significantly add to the prediction of an asthma diagnosis of the commonly used API (API alone: AUC= 0.60 [95% CI 0.52-0.68, P=.02]; API+IL1-RL1-a: AUC= 0.57 [95CI 0.49-0.66, P=.12]). These results show that, although IL-1RL1 SNPs may affect IL-1RL1 expression levels, serum sST2 levels in wheezing children at 2-3 years do not have added value in the prediction of doctors' diagnosed asthma as general phenotype at school age.

Possible reasons for this finding is the heterogeneity of the asthma phenotype in childhood or the fact that our cohort of children was derived from a primary care setting, likely leading to an a priori lower asthma risk compared to a hospital setting. As sST2 serum levels had previously been associated with blood eosinophil numbers in childhood asthma, (262) we hypothesized that sST2 levels at 2-3 years may predict measures of eosinophilic asthma rather than a general diagnosis of asthma at school age. In the ADEM cohort, levels of nitric oxide in exhaled breath (FeNO), considered a surrogate marker of eosinophilic airway inflammation in asthma patients, (263) were measured at 6 years (NIOX®; Aerocrine, Solna, Sweden). Interestingly, we found that serum levels of sST2 measured at age 2-3 years,

although modestly, were negatively correlated with FeNO levels at 6 years in children who had developed asthma (Pearson's R=-0.24, P=.046, N=59), while no significant correlation was observed in transient wheezers (Pearson's R=.08, P=.47, N=89), see Figure 1. This suggests that serum levels of sST2 at preschool age predict increased FeNO levels as a marker of eosinophilic airway inflammation in those children who will develop asthma. To investigate whether sST2 levels indeed could be a predictive biomarker for asthma with elevated FeNO levels, we next divided our population of asthmatic children at age 6 years into a group with likely eosinophilic airway inflammation (FeNO≥20ppb, n=15) and asthmatics unlikely to have eosinophilic airway inflammation (FeNO<20ppb, n=60), based on the ATS guideline of FeNO. (263) We then performed logistic predictive modeling of asthma with elevated FeNO (Y/N) in wheezing children. Indeed, sST2 serum levels negatively predicted asthma with high FeNO in preschool wheezers (OR=0.96, P=.04, Figure 2A,B), having a predicted AUC of 0.65 (95CI 0.52-0.79). When sST2 serum levels were combined with the API, the predictive model slightly and significantly improved to distinguish preschool wheezers who developed asthma with elevated FeNO at school age (predicted AUC of 0.70, 95% CI 0.56-0.84). We acknowledge that the sample size of the group with likely eosinophilic airway inflammation at age 6 years (FeNO ≥20 ppb) of this analysis is limited and propose that our findings should be replicated in future studies with larger sample size.

FeNO levels in 2- to 3-y-old wheezers did not have predictive value for FeNO at school age in our cohort (data not shown), nor for asthma development. (264) Moreover, although FeNO levels have previously been found useful in prediction of management of established asthma, (265,266) FeNO could not predict treatment response in preschool wheezers. (267) Given the time span (3-4 years) between the measurement of sST2 and FeNO, and the negative correlation, it is tempting to speculate that sST2 levels could have a protective effect on the development of eosinophilic airway inflammation in asthmatic children. However, no data on eosinophil counts were available in the current cohort to further study this relationship. Nevertheless, in a previous study, an inverse relationship between sST2 levels and blood eosinophil counts has been reported during exacerbations of childhood asthma. (262) Further evidence indicating a potential protective effect of sST2 in (eosinophilic) asthma is more experimental (murine) model studies of asthma, wherein delivering sST2 (respectively, intraperitoneally/intranasally) significantly decreased inflammatory airway disease, including reduced eosinophil counts in BAL and methacholine-induced airway hyper-responsiveness. (268,269) That a protective effect of sST2 might be rather disease specific is indicated by findings that sST2 levels positively predict other conditions, including mortality in cardiovascular disease (270,271) and disease activity in autoimmune diseases such as juvenile arthritis. (272)

In summary, we show that sST2 serum levels in preschool wheezers do not add to the prediction of doctors' diagnosed asthma as general phenotype at school age. However, sST2 serum levels at age 2-3 years inversely correlate with FeNO levels in asthmatic children at 6 years. Likewise, sST2 serum levels in preschool wheezers contributed to the prediction of a subtype of asthma with elevated FeNO at age 6 years, showing a negative direction of

effect. Therefore, our study indicates that sST2 might play a protective role in the development of eosinophilic airway inflammation in children who experience asthma at school age. Our findings suggest that sST2 has potential to be further explored as a biomarker in wheezing children to predict the development of asthma with predominant eosinophilic inflammation. A combination of several markers is likely necessary to accurately predict asthma on an individual level. So far exhaled volatile organic compounds demonstrated great potential for the prediction of asthma at age 6 years. (258) Furthermore, in future biomarker studies investigating the role of sST2, other measures likely relevant in the development of eosinophilic inflammation should be considered, including sputum and blood eosinophil counts.

To take home

- Prediction of asthma in childhood may require definition of specific subphenotypes, potentially IL-1RL1 serum protein could add to the prediction of asthma characterized by high FeNO as marker of eosinophilic airway inflammation.

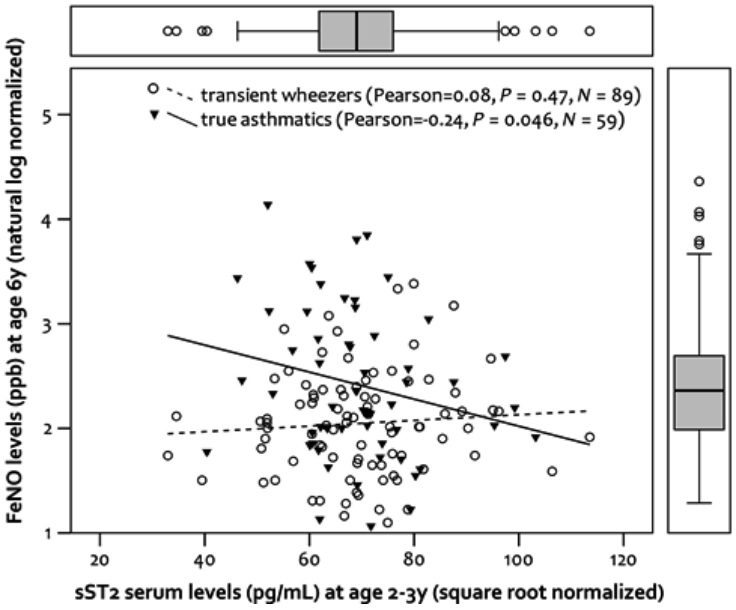
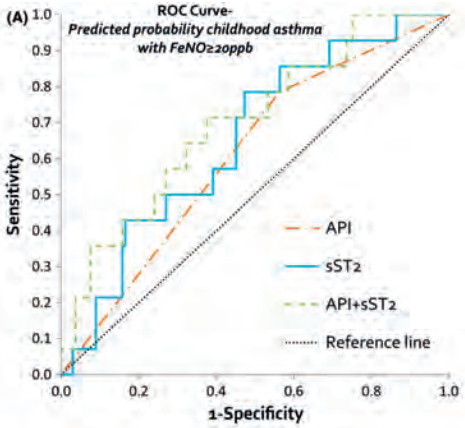


Figure 1.
Correlation of sST2 serum levels (2-3 y) and FeNO (6 y). sST2 serum levels measured in wheezers at age 2-3 y negatively correlate with fraction of exhaled nitric oxide levels (FeNO, online method) in children who have developed asthma at age 6 y. Boxplots show the quartiles and outliers of the distribution of sST2 (x-axis) and FeNO (y-axis). ppb, parts per billion



(B)

	MODEL: sST2 only or API only				MODEL: sST2 and API combined							
	Pred AUC (95CI)	*B*	*OR*	*P value (OR)*	*Pred AUC (95CI)*	*B*	*OR*	*P value (OR)*	*PPV*	*NPV*	*Sens*	*Spec*
Constant	-	0.24	1.28	*P* = 0.85	-	-0.07	0.93	*P* = 0.89				
Serum sST2	0.65 (0.52-0.79) (P=0.059)	-0.04	0.96	***P* = 0.04**	0.65 (0.52-0.79) (P=0.059)	-0.05	0.96	***P* = 0.03**	0.11	0.96	0.86	0.33
API	0.62 (0.49-0.76) P=0.11)	1.18	3.26	*P* = 0.053	0.61 (0.47-0.76) (P=0.17)	1.23	3.43	***P* = 0.049**	0.12	0.96	0.80	0.45
API+ Serum sST2	-	-	-	-	0.70 (0.56-0.84) **(P=0.01)**	-	-	-	0.14	0.95	0.71	0.52

Figure 2.
Prediction of asthma with elevated FeNO in childhood using sST2 serum levels and the API. (A) Receiver operating characteristics (ROC) curve comparing the predictive value of serum sST2 levels, the Asthma Prediction Index (API), and their combined predictive value for development of childhood asthma with FeNO≥20ppb to the Reference Line (representing the 0-hypothesis of a predicted area under the curve (AUC)= 0.5). (B) Calculations of the AUC and parameters of the logistic regression model of the prediction of asthma with FeNO≥20 ppb. sST2 serum levels in 2- to 3-y-old wheezers (n=171) significantly predict this eosinophilic subtype of asthma at age 6 y (n=15, P=.04) with an average AUC of 0.65 (95% CI 0.52-0.79, P=.059). The combined logistic model of API+sST2 serum levels has an average AUC of 0.70 (95% CI 0.56-0.84, P=.01). API, Asthma Prediction Index (based on parental asthma, eczema, allergic rhinitis, wheezing apart from cold, atopy as determined by Phadiatop). Pred AUC, predicted area under the curve. 95% CI, 95% confidence interval. B, regression coefficient. NPV, negative predictive value. OR, Odds ratio. PPV, positive predictive value. Sens, sensitivity. Spec, specificity. Note: P-values of the AUC are compared to the Reference Line (0-hypothesis AUC=0.5).