

# Association Between *TSLP* Polymorphisms and Eczema in Japanese Women: the Kyushu Okinawa Maternal and Child Health Study

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**Abstract**—We examined the association between thymic stromal lymphopoietin (*TSLP*) single nucleotide polymorphisms (SNPs) and eczema in young adult Japanese women. Cases were 188 women who met the criteria of the International Study of Asthma and Allergies in Childhood (ISAAC) for eczema. Controls were 565 women without eczema according to the ISAAC criteria, who had not been diagnosed with asthma, atopic eczema, and/or allergic rhinitis by a doctor and who had no asthma as defined by the European Community Respiratory Health Survey criteria and no rhinoconjunctivitis according to the ISAAC criteria. Compared with women with the TT genotype of SNP rs1837253, those with the TC or CC genotype had a significantly increased risk of eczema after adjustment for age and smoking, although this association was not significant in crude analysis. There were no relationships between SNP rs3806933 or rs2289276 and eczema. The TC and CC genotypes combined of SNP rs1837253 may be significantly positively associated with eczema.

**KEY WORDS:** eczema; Japanese women; polymorphism; *TSLP*.

## INTRODUCTION

Thymic stromal lymphopoietin (*TSLP*) is an epithelial cell-derived cytokine expressed in the skin, gut, lungs, and thymus [1]. Several cellular targets of *TSLP* have been identified, including dendritic cells, lymphocytes, and granulocytes [2]. *TSLP* plays a critical role in Th2 cell differentiation [1, 2]. It is highly expressed in epithelial cells, especially keratinocytes from patients with atopic eczema [3]. The numbers of cells within the bronchial epithelium and submucosa expressing mRNA for *TSLP* are significantly increased in asthmatics as compared with control subjects, and *TSLP* expression is correlated with airway obstruction [4].

The human *TSLP* gene is located on chromosome 5q22.1, near the atopic cytokine cluster on 5q31. Several

genetic association studies have examined the relationships between the *TSLP* single-nucleotide polymorphisms (SNPs) and allergic disorders, but the results have been inconsistent [5–12]. To our knowledge, only one study has investigated the association between *TSLP* SNPs and eczema [11], and only one other study has assessed the interactions between *TSLP* SNPs and smoking with respect to asthma [8].

In view of the lack of epidemiological information regarding the association between *TSLP* SNPs and eczema in non-Western populations, the current case-control study examined this issue in young adult Japanese women using data from the Kyushu Okinawa Maternal and Child Health Study (KOMCHS). In addition, we performed haplotype analyses and looked for the possibility of an interaction between one SNP and smoking.

## METHODS

### Study Population

The KOMCHS is a prospective prebirth cohort study. Details of the baseline survey of the KOMCHS have been described elsewhere [13]. Eligible women were those who

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became pregnant while living in one of the seven prefectures on Kyushu island in southern Japan, with a total population of approximately 13.26 million, or in Okinawa Prefecture, an island chain in the southwest of Japan, with a total population of nearly 1.37 million. Between April 2007 and March 2008, we requested that 423 obstetric hospitals in the abovementioned eight prefectures provide as many pregnant women as possible with a set of leaflets explaining the KOMCHS, an application form to participate in the study, and a self-addressed and stamped return envelope. Pregnant women who were willing to participate in the study returned the application form to the data management center. In total, 1757 pregnant women between the 5th and 39th weeks of pregnancy gave their written informed consent to participate in the KOMCHS and completed the baseline survey. Around 4 months after delivery, 1492 women gave informed consent to genotyping. The ethics committee of the Faculty of Medicine, Fukuoka University approved the KOMCHS.

### Selection of Cases and Control Subjects

Based on the questions in the International Study of Asthma and Allergies in Childhood (ISAAC) [14], affirmative answers to the following three questions were required to indicate the presence of eczema: ‘Have you ever had an itchy rash which was coming and going for at least 6 months?’, ‘Have you had this itchy rash at any time in the last 12 months?’ and ‘Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?’ Among the 1492 women whose DNA samples were available, 188 cases of eczema were identified. Among the 1304 remaining women who were eligible to serve as control subjects, 668 women were excluded who were not considered to have eczema as defined by the ISAAC criteria but who had answered ‘yes’ to one or more of the following three questions: ‘Have you ever been diagnosed by a physician as having asthma?’, ‘Have you ever been diagnosed by a physician as having atopic eczema?’, and/or ‘Have you ever been diagnosed by a physician as having allergic rhinitis?’ In addition, we excluded 70 women who had never been diagnosed by a physician as having asthma, atopic eczema, or allergic rhinitis but who were considered to have asthma as defined according to the questions in the European Community Respiratory Health Survey [15] or rhinoconjunctivitis as defined according to the questions in the ISAAC [16]; the presence of asthma was defined as the existence of either of two conditions: an asthma attack

during the last 12 months or current use of asthma medication, and the presence of rhinoconjunctivitis was defined as a positive response to both of the following two questions: ‘In the last 12 months, have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or flu?’ and ‘In the last 12 months, has this nose problem been accompanied by itchy-watery eyes?’ One woman with incomplete data on smoking was also excluded. The final analyses thus comprised 188 cases and 565 control subjects.

### DNA Extraction and Genotyping

Genomic DNA from buccal specimens collected with BuccalAmp swabs (Epicenter BioTechnologies, Madison, WI, USA) was extracted using a QIAamp DNA Mini Kit (Qiagen, Inc., Valencia, CA, USA). Three *TSLP* SNPs in the promoter region, rs1837253 (–5717T/C), rs3806933 (–847C/T), and rs2289276 (–82C/T), were genotyped using TaqMan SNP Genotyping Assays on the StepOnePlus machine (Applied Biosystems, Foster City, CA, USA), according to the manufacturer’s instructions. These SNPs have previously been shown to be significantly associated with asthma in two Japanese studies [7, 9]. Table 1 shows the genotype frequencies of these SNPs in the Japanese population according to the HapMap and the 1000 Genomes Project.

### Statistical Analysis

Calculations of Hardy-Weinberg equilibrium were performed among the control subjects using the chi-square test. Linkage disequilibrium was examined using HaploView software version 4.2 [17]. Using logistic regression analysis, crude odds ratios (ORs) and 95 % confidence intervals (CIs) for eczema associated with the SNPs under investigation were calculated, with the reference category being the homozygote of the major allele. Multiple logistic regression analysis was used to adjust for age and smoking. The statistical power calculation was performed using Quanto version 1.2 [18]. Haplotypes and their frequencies were inferred by means of the expectation maximization algorithm. For differences in haplotype frequency between the cases and control groups, crude ORs and 95 % CIs were estimated based on the frequency of each haplotype relative to all other haplotypes combined. The multiplicative interaction was estimated by introducing a multiplicative term into a multiple logistic regression model. Three measures for the additive interaction were calculated using the Excel sheet provided by Andersson *et al.* [19]: (1) relative excess risk due to interaction (RERI),

**Table 1.** Genotype Frequencies of *TSLP* Polymorphisms in the Japanese Population According to the HapMap and the 1000 Genomes Project

| SNP       | Genotype | HapMap | 1000 Genomes |
|-----------|----------|--------|--------------|
| rs1837253 | TT       | 0.360  | 0.449        |
|           | TC       | 0.523  | 0.438        |
|           | CC       | 0.116  | 0.112        |
| rs3806933 | CC       | 0.465  | 0.539        |
|           | CT       | 0.453  | 0.382        |
|           | TT       | 0.081  | 0.079        |
| rs2289276 | CC       | 0.500  | 0.573        |
|           | CT       | 0.453  | 0.371        |
|           | TT       | 0.047  | 0.056        |

(2) attributable proportion due to interaction (AP), and (3) synergy index (S). RERI is the excess risk due to an interaction relative to the risk without exposure. AP refers to the attributable proportion of disease among individuals exposed to both factors that is due to the factors' interaction. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without an interaction. RERI=0, AP=0, or S=1 means no interaction or strict additivity; RERI>0, AP>0, or S>1 means a positive interaction or more than additivity; RERI<0, AP<0, or S<1 means a negative interaction or less than additivity [20]. If any of the null values (0 in RERI and AP or 1 in S) falls outside the 95 % CI of its respective measurement, then the additive interaction is considered statistically significant. Excluding the calculation of linkage disequilibrium and statistical power, all statistical analyses were performed using STATA/SE software version 13.1 (StataCorp, College Station, TX, USA).

## RESULTS

Compared with control subjects, cases were more likely to be younger (Table 2). There was no difference between cases and control subjects with regard to smoking.

Among the control subjects, the genetic distributions of *TSLP* SNPs rs1837253, rs3806933, and rs2289276 did not deviate from the Hardy-Weinberg equilibrium

**Table 2.** Characteristics of the Study Population

| Variable                     | Cases<br>(N=188) | Controls<br>(N=565) | P value <sup>a</sup> |
|------------------------------|------------------|---------------------|----------------------|
| Age, years, mean±SD          | 30.7±4.6         | 31.4±4.3            | 0.040                |
| Having ever smoked,<br>n (%) | 63 (33.5)        | 175 (31.0)          | 0.517                |

<sup>a</sup>  $\chi^2$  test or *t* test

( $P=0.78$ ,  $0.29$ , and  $0.21$ , respectively). All SNPs pairs were in high linkage disequilibrium ( $D'>0.80$ ), and one haplotype block was identified between SNPs rs3806933 and rs2289276 ( $D'=0.98$ ,  $r^2=0.84$ ) (Table 3).

In crude analysis, using women with the TT genotype of *TSLP* SNP rs1837253 as a reference group, a positive association between the combination of TC and CC genotypes and eczema fell just short of the significance level under the dominant model (Table 4). After adjustment for age and smoking, however, women with the TC or CC genotype had a significantly increased risk of eczema: the adjusted OR was 1.41 (95 % CI 1.004–1.99,  $P=0.048$ ). No such significant association was found in the co-dominant or additive model. There were no significant relationships between SNPs rs3806933 or rs2289276 and eczema in any genetic model. With regard to the association between rs2289276 and eczema under the dominant model (adjusted OR=1.33 [95 % CI 0.96–1.86,  $P=0.09$ ]), the statistical power calculation revealed that, using our sample size, we could detect the gene-disease association for an OR of 1.606 with an accuracy of more than 80 % at a significance level of 0.05 with a two-sided alternative hypothesis under the dominant model.

When haplotypes with a frequency of less than 1 % in either cases or control subjects were deleted, five haplotypes remained; none of these were significantly associated with eczema (Table 5).

No significant multiplicative or additive interaction was observed between *TSLP* SNP rs1837253 and smoking with respect to eczema (Table 6).

## DISCUSSION

To our knowledge, the present study is the first to find that the TC and CC genotypes combined of *TSLP* SNP rs1837253 is significantly associated with an increased risk of eczema. On the other hand, *TSLP* SNPs rs3806933 and rs2289276 were not materially related to eczema in the current study. Gao *et al.* showed that there were no significant associations between SNPs rs1837253, rs3806933, or

**Table 3.** Pairwise Linkage Disequilibrium of *TSLP* Polymorphisms ( $r^2$  below and  $D'$  Above the Diagonal)

|           | rs1837253 | rs3806933 | rs2289276 |
|-----------|-----------|-----------|-----------|
| rs1837253 |           | 0.81      | 0.90      |
| rs3806933 | 0.48      |           | 0.98      |
| rs2289276 | 0.52      | 0.84      |           |

**Table 4.** Odds ratios (ORs) and 95 % Confidence Intervals (CIs) for Eczema Associated with *TSLP* Polymorphisms in Japanese Women

| SNP       | Model    |             | Cases, <i>n</i> (%) | Controls, <i>n</i> (%) | Crude OR (95 % CI) | Adjusted OR (95 % CI) <sup>a</sup> |
|-----------|----------|-------------|---------------------|------------------------|--------------------|------------------------------------|
| rs1837253 | Genotype | Co-dominant | ( <i>N</i> =188)    | ( <i>N</i> =565)       |                    |                                    |
|           |          |             | TT 68 (36.2)        | 250 (44.3)             | 1.00               | 1.00                               |
|           |          |             | TC 94 (50.0)        | 249 (44.1)             | 1.39 (0.97–1.99)   | 1.41 (0.98–2.01)                   |
|           |          |             | CC 26 (13.8)        | 66 (11.7)              | 1.45 (0.85–2.45)   | 1.44 (0.85–2.45)                   |
|           |          | Additive    |                     |                        | 1.25 (0.98–1.59)   | 1.25 (0.98–1.59)                   |
|           |          |             |                     |                        | 1.40 (0.996–1.97)  | 1.41 (1.004–1.99)                  |
|           | Allele   | Dominant    | T 230 (61.2)        | 749 (66.3)             | 1.00               |                                    |
| rs3806933 | Genotype | Co-dominant | ( <i>N</i> =188)    | ( <i>N</i> =565)       |                    |                                    |
|           |          |             | C 146 (38.8)        | 381 (33.7)             | 1.25 (0.97–1.60)   |                                    |
|           |          |             | CC 89 (47.3)        | 290 (51.3)             | 1.00               | 1.00                               |
|           |          |             | CT 84 (44.7)        | 237 (42.0)             | 1.15 (0.82–1.63)   | 1.16 (0.82–1.63)                   |
|           |          |             | TT 15 (8.0)         | 38 (6.7)               | 1.29 (0.68–2.45)   | 1.32 (0.69–2.52)                   |
|           |          | Additive    |                     |                        | 1.14 (0.88–1.49)   | 1.15 (0.89–1.50)                   |
|           |          |             |                     |                        | 1.17 (0.84–1.63)   | 1.18 (0.85–1.64)                   |
| rs2289276 | Genotype | Co-dominant | ( <i>N</i> =188)    | ( <i>N</i> =564)       |                    |                                    |
|           |          |             | C 262 (69.7)        | 817 (72.3)             | 1.00               | 1.00                               |
|           |          |             | T 114 (30.3)        | 313 (27.7)             | 1.14 (0.87–1.48)   |                                    |
|           |          | Additive    | CC 92 (48.9)        | 316 (56.0)             | 1.00               | 1.00                               |
|           |          |             | CT 85 (45.2)        | 220 (39.0)             | 1.33 (0.94–1.87)   | 1.33 (0.94–1.87)                   |
|           |          |             | TT 11 (5.9)         | 28 (5.0)               | 1.35 (0.65–2.81)   | 1.37 (0.65–2.86)                   |
|           |          | Dominant    |                     |                        | 1.25 (0.95–1.64)   | 1.25 (0.95–1.65)                   |
|           |          |             |                     |                        | 1.33 (0.96–1.85)   | 1.33 (0.96–1.86)                   |
|           | Allele   |             | C 269 (71.5)        | 852 (75.5)             | 1.00               |                                    |
|           |          |             | T 107 (28.5)        | 276 (24.5)             | 1.23 (0.93–1.61)   |                                    |

<sup>a</sup> Adjusted for age and smoking

rs2289276 and eczema in a case-control study of European American subjects, while SNP rs 2289976, but not rs1837253 or rs3806933, was significantly related to eczema in a case-control study of African American subjects [11]. Our results regarding rs1837253 are at variance with these findings, although the present results in relation to rs3806933 and rs2289276 are in partial agreement with these findings. In genome-wide association studies of US

individuals [6], Japanese individuals [7], and subjects from Australia, the USA, and the UK [5], SNP rs1837253 was associated with asthma at a genome-wide significance level. There were no associations between SNPs rs1837253 or rs2289276 and asthma in a case-control study of US individuals [8]. These results are in partial agreement with our findings. In another case-control study, SNPs rs3806933 and rs2289276 were significantly associated with both childhood atopic asthma and adult asthma in Japanese individuals [9]. These findings are inconsistent with the present results.

A study by Harada *et al.* on normal human bronchial epithelial cells found that SNP rs3806933 created a binding site for the transcription factor activating protein (AP)-1 and enhanced AP-1 binding to the regulatory element, and that the functional variant increased promoter-receptor activity of long-form *TSLP* in response to polyriboinosinic:polyribocytidylic acid stimulation [21].

In the abovementioned case-control study in the USA, a significant association between *TSLP* SNP rs1898671 and asthma was found in ex-smokers while SNP rs1898671 was not related to asthma in never-smokers [8]. No significant interaction affecting eczema was observed between SNP rs1837253 and smoking in the present study.

**Table 5.** Haplotype Analysis of Three *TSLP* Polymorphisms Associated with Eczema in Japanese Women

| Haplotype <sup>a</sup> | <i>n</i> (%)            |                             | Crude OR (95 % CI) <sup>b</sup> |
|------------------------|-------------------------|-----------------------------|---------------------------------|
|                        | Cases (2 <i>N</i> =376) | Controls (2 <i>N</i> =1130) |                                 |
| TCC                    | 220 (58.5)              | 706 (62.5)                  | 0.85 (0.66–1.08)                |
| TTC                    | 4 (1.1)                 | 26 (2.3)                    | 0.46 (0.12–1.33)                |
| TTT                    | 6 (1.6)                 | 16 (1.4)                    | 1.13 (0.36–3.07)                |
| CCC                    | 42 (11.2)               | 108 (9.6)                   | 1.19 (0.80–1.76)                |
| CTT                    | 101 (26.9)              | 257 (22.7)                  | 1.25 (0.94–1.64)                |

Rare haplotypes (frequency less than 1 % in either cases or controls) were deleted

<sup>a</sup> Haplotype order is rs1837253, rs3806933, and rs2289276

<sup>b</sup> Crude OR for each haplotype is relative to all other haplotypes combined

**Table 6.** Interaction Between *TSLP* SNP rs1837253 and Smoking History Affecting Eczema in Japanese Women

| Genotype                                                                  | Smoking history       |                                    |                       |                                    |
|---------------------------------------------------------------------------|-----------------------|------------------------------------|-----------------------|------------------------------------|
|                                                                           | No                    |                                    | Yes                   |                                    |
|                                                                           | No. of cases/controls | Adjusted OR (95 % CI) <sup>a</sup> | No. of cases/controls | Adjusted OR (95 % CI) <sup>a</sup> |
| TT                                                                        | 46/171                | 1.00                               | 22/79                 | 1.00 (0.56–1.78)                   |
| TC+CC                                                                     | 79/219                | 1.34 (0.36–5.01)                   | 41/96                 | 1.57 (0.96–2.56)                   |
| <i>P</i> for multiplicative interaction=0.68                              |                       |                                    |                       |                                    |
| Measures of additive interaction <sup>b</sup>                             |                       |                                    |                       |                                    |
| Relative excess risk due to interaction (RERI)=0.22 (95 % CI –1.66–2.11)  |                       |                                    |                       |                                    |
| Attributable proportion due to interaction (AP)=0.14 (95 % CI –1.05–1.34) |                       |                                    |                       |                                    |
| Synergy index (S)=1.64 (95 % CI 0.01–369.56)                              |                       |                                    |                       |                                    |

<sup>a</sup> Adjusted for age

<sup>b</sup> Statistically significant when the 95 % CI of RERI>0, the 95 % CI of AP>0, or the 95 % CI of S>1, indicating additive interaction

The present study's limitations should be considered. Selection bias could have influenced our results. For example, the distribution of educational status among participants differed considerably from that among the general population. According to the 2000 population census of Japan, the proportions of women aged 30 to 34 years in Fukuoka Prefecture with <13, 13–14, ≥15, and unknown years of education were 52.0, 31.5, 11.8, and 4.8 %, respectively [22]. The corresponding figures for the control group in the current study were 20.7, 33.3, 46.0, and 0.0 %, respectively. Nevertheless, the distribution of the three SNPs under study was consistent with Hardy-Weinberg equilibrium, and any selection bias based on genotype distribution would be negligible.

The definition of eczema was based on the questions in the ISAAC questionnaire. The possibility of non-differential outcome misclassification would introduce a bias toward the null. The current study size was rather small for a valid genetic association study, although a significant association was found between SNP rs1837253 and eczema. The lack of significant associations between the other SNPs and eczema might be due to an insufficient statistical power. Although age and smoking were adjusted for, residual confounding effects could not be ruled out.

The current study suggests that the combination of TC and CC genotypes of *TSLP* SNP rs1837253 is significantly associated with an increased risk of eczema and that there are no relationships between *TSLP* SNP rs3806933 or rs2289276 and eczema. We could not provide evidence for an interaction between SNP rs1837253 and smoking affecting eczema. Given the scarcity of epidemiological information on this topic, further evidence from well-designed genetic association studies is needed. Functional studies are also necessary.

## ACKNOWLEDGMENTS

The authors would like to thank the Kyushu Branch of the Japan Allergy Foundation, the Fukuoka Association of Obstetricians and Gynecologists, the Okinawa Association of Obstetricians and Gynecologists, the Miyazaki Association of Obstetricians and Gynecologists, the Oita Association of Obstetricians and Gynecologists, the Kumamoto Association of Obstetricians and Gynecologists, the Nagasaki Association of Obstetricians and Gynecologists, the Kagoshima Association of Obstetricians and Gynecologists, the Saga Association of Obstetricians and Gynecologists, the Fukuoka Society of Obstetrics and Gynecology, the Okinawa Society of Obstetrics and Gynecology, the Fukuoka City government, and the Fukuoka City Medical Association for their valuable support, as well as Mrs. Yukari Hayashi for her technical assistance. This study was supported by JSPS KAKENHI Grant Numbers 19590606, 20791654, 21590673, 22592355, 22119507, 24390158, 25463275, and 25670305, by Health and Labour Sciences Research Grants for Research on Allergic Disease and Immunology and Health Research on Children, Youth and Families from the Ministry of Health, Labour, and Welfare of Japan and by the Takeda Science Foundation.

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