

# Serum zonulin levels are higher in pediatric allergic patients than those in healthy children

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**To the Editor** In allergic diseases, not only ‘allergic inflammation’, but also ‘epithelial barrier impairment’ play important roles in their development<sup>1</sup>. Allergen penetration through epithelium to the body is essential for allergen sensitizations, which are the most critical risk factors for the development of allergic diseases. In children with atopic dermatitis (AD), in which epithelial barrier impairment is one of its famous features, the prevalences of allergen sensitization and food allergy (FA) are known to be very high<sup>1</sup>. Concerning about the assessment of epithelial barrier function, while transepidermal water loss (TEWL) is a clinically useful marker in AD skin, it is quite difficult to evaluate barrier function in gastrointestinal tracts.

Zonulin (pre-haptoglobin 2), an epithelial tight-junction regulator, plays an important role in the regulation of epithelial barrier function<sup>2,3</sup>. Recently, zonulin was reported to play a pathogenic role in celiac disease and other chronic inflammatory diseases<sup>23</sup>. Sturgeon et al reported that zonulin transgenic mice, as a result of zonulin-dependent small intestinal barrier impairment and altered gut permeability, increased morbidity and mortality in the DSS colitis model<sup>4</sup>.

Because allergen sensitization and chronic inflammation are important in the pathogenesis of allergic diseases, zonulin has potential to play significant roles in allergic diseases. However, there are few reports about zonulin levels in pediatric allergic patients. So, the objectives of this study are to assess whether i) serum zonulin levels in allergic children are higher than those in children without allergic diseases and ii) serum zonulin levels are different among allergic diseases.

To evaluate them, we measured serum zonulin levels in infants and school-age children (with or without allergic diseases) using zonulin enzyme-linked immunosorbent assay kit. All infants (9 months old) were selected from Katsushika cohort study<sup>5</sup>. Allergic infants (‘AD infants’) were defined as infants who had doctor-diagnosed AD, and whose total IgE levels were above normal levels and Eczema area and severity index (EASI) were above zero. ‘Healthy infants’ were defined as infants who did not have AD, FA, bronchial asthma (BA), nor allergic rhinitis (AR), and whose serum total IgE levels were below measureable limits and EASI were zero. There were significant differences in total IgE levels and EASI score between groups (AD infants vs. healthy infants) (Table S1). Allergic school-age children were recruited in our outpatient clinic in Chiba University hospital and were defined as children who had doctor-diagnosed FA or BA or both, and whose total IgE levels were above normal levels. ‘healthy school-age children’<sup>6</sup> were defined as school-age children who did not have AD, FA, nor BA and their serum total IgE levels were below measureable limits. The details of study subjects are shown in online supplementary methods and table S1-S3.

Our first question was whether or not serum zonulin levels in allergic children are higher than those in children without allergic diseases. In infants, median zonulin levels in AD infants and healthy infants were 28.1 ng/ml (interquartile range (IQR): 23.8-32.3) and 15.3 ng/ml (IQR: 10.0-23.7), so serum zonulin levels were significantly higher in allergic infants than those in healthy infants ( $p < 0.01$ ) (Figure 1). To confirm this result in other age group, we evaluated zonulin levels in school-age children. Same as in infants, serum zonulin levels in school-age children were significantly higher in allergic children with FA and BA than those

in healthy children without FA, BA, nor AD (Median (IQR): 29.5 ng/ml (22.6-41.9) and 10.6 ng/ml (9.4-12.2),  $p < 0.001$ ) (Figure 2a). Those results suggest that zonulin levels are higher in allergic children than in non-allergic children, regardless of their age.

To assess our second question, we compared serum zonulin levels in BA patients with those in FA patients. In this analysis, we selected asthmatic patients as children who had BA but not FA, and FA patients as children who had FA but not BA, and there were no significant differences in total IgE and age between groups (Table S3). Serum zonulin in FA patients was significantly higher than those in BA patients (Median (IQR): 38.5 ng/ml (35.0-46.7) and 31.0 ng/ml (13.6-34.0),  $p < 0.05$ ) (Figure 2b). Those results suggest that zonulin levels are higher in FA patients than those in BA patients.

Sheen et al reported that Serum zonulin levels were elevated in children with AD<sup>7</sup>, and this result was confirmed in our present paper. On the other hand, zonulin primarily reflects epithelial permeability in the gastrointestinal tract, it is important to examine Zonulin levels in other age groups and in patients with other allergic diseases. As we showed in this paper, in school-age children, zonulin levels are higher in allergic children than in healthy children, and higher in food allergy children than in asthmatic children. In general, many AD infants also have FA and intestinal permeability is reported to be increased in younger children with AD but not in older children with AD<sup>8</sup>. Those facts might influence the serum zonulin levels in infants with AD.

Zonulin regulates intestinal barrier function through the regulation of tight junctional<sup>2</sup>. This suggests that zonulin may affect allergen permeability in epithelia of the intestinal tract. Zonulin may contribute to the development of allergic diseases through allergen sensitization and affect the appearance of allergic symptoms via their effect on allergen exposure.

There were several limitations in this study. First, the number of study subjects was small. And secondly, we did not compare zonulin levels in allergic children to those in children with other non-allergic diseases. So it is not clear whether serum zonulin is an “allergic marker”, and zonulin plays a role in the “development” of allergic diseases.

In conclusion, serum zonulin levels are significantly higher in allergic children than in healthy children. In addition, those levels are significantly higher in food allergy patients than in asthmatic patients. Based on the knowledge about the role of zonulin in the intestinal epithelial barrier regulation, zonulin may play a role in the development of allergic diseases, especially in FA.

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## **Title**

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The authors declare no conflict of interest.

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## **Figure legends**

### **Figure 1.**

Comparison of serum zonulin levels between healthy infants and AD infants.

### **Figure 2.**

Serum Zonulin levels in school-age children.

a) Comparison of serum zonulin levels between healthy children and allergic children. b) Comparison of serum zonulin levels between BA children without FA and FA children without BA

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