

pedra dust is IgE-mediated. This is the first case of occupational asthma by Angelim pedra reported and the first wood allergen in which the N-terminal amino acid composition is known.

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REFERENCES

1. NETbureau Comunicação. Angelim pedra. Available at: http://www.net-bureau.com.br/forex/espanhol/angelim_pedra. Accessed January 31, 2005.
2. Hinojosa M, Moneo I, Dominguez J, Delgado E, Losada E, Alcover R. Asthma caused by African maple (*Triplochiton scleroxylon*) wood dust. *J Allergy Clin Immunol* 1984;74:782-6.
3. Hinojosa M, Subiza J, Moneo I, Puyana J, Diez ML, Fernandez-Rivas M. Contact urticaria caused by obeche wood (*Triplochiton scleroxylon*): report of eight patients. *Ann Allergy* 1990;64:476-9.
4. Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975;112:829-59.
5. Moneo I, Caballero ML, Gómez F, Ortega E, Alonso MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol* 2000;106:177-82.
6. European Bioinformatics Institute. Program FASTA. Available at: <http://www.ebi.ac.uk>. Accessed January 31, 2005.
7. Swiss Institute of Bioinformatics. Swiss-Prot database. Available at: <http://www.isb-sib.ch>. Accessed January 31, 2005.

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SPINK5 polymorphism is associated with disease severity and food allergy in children with atopic dermatitis

To the Editor:

Serine protease inhibitor Karzal type 5 (SPINK5) is a protease inhibitor protein and is encoded by the gene *SPINK5*.¹ Several mutations in the *SPINK5* gene can cause Netherton syndrome, a rare autosomal recessive disorder characterized by defective cornification of the skin and T_H2-skewed immunologic alterations resembling atopic dermatitis (AD).² SPINK5 regulates proteolysis in epithelia formation and keratinocyte terminal differentiation, and its defects lead to overdesquamation of corneocytes and skin barrier dysfunction.³ SPINK5 is also expressed in the thymus, and its defects have been suggested to cause abnormal maturation of T lymphocytes and lead to T_H2 responses, such as increased IgE levels and eosinophilia.²

Walley et al⁴ recently reported an association between AD and the G1258A (Glu420Lys) polymorphism in *SPINK5* in a white population, thus establishing a link between a rare and a common skin disease. Thereafter, the association of this polymorphism with AD in 2 independent Japanese populations^{5,6} and with bronchial asthma (BA) in a German population⁷ has been reported. However, there have been no reports as to whether the single nucleotide polymorphism (SNP) is associated with the severity of AD. We therefore aimed to determine the association of the G1258A (Glu420Lys) polymorphism in *SPINK5* with AD severity and other allergic diseases in children with AD.

Japanese children more than 5 years of age with AD (n = 118), whose symptoms started in infancy and who are still being followed up at our clinic, were enrolled in the study. The diagnosis of AD was made according to the criteria set out by Haniffin and Rajka.⁸ After written informed consent was obtained, blood was collected for serum IgE measurement, eosinophil counts, CAP-RAST score of 10 selected allergens, and DNA extraction to genotype the study population for the G1258A (Glu420Lys) polymorphism in *SPINK5*, as described elsewhere.⁴ The study was approved by the ethics committee of the Graduate School of Kyoto University. The severity of AD (mild, moderate, severe, or most severe) was determined on the basis of the guidelines established by the Study Group founded by the Japanese Ministry of Health, Labour, and Welfare. The prevalence and severity of BA was determined on the basis of the guidelines established by the Japanese Society of Pediatric Allergy and Clinical Immunology. The details of the severity determination of both AD and BA can be provided on request. Food allergy was defined as an episode of immediate allergic reaction (skin, oral, respiratory, and/or gastrointestinal symptoms) within 1 hour after eating at least one causative food, which was confirmed on the basis of positive CAP-RAST results.

Genomic frequencies of the *SPINK5*/1258 polymorphism site were 41 (34.7%) for AA, 51 (43.2%) for AG, and 26 (22.0%) for GG. Distribution of age, IgE level, eosinophil count, and number of CAP-RAST-positive allergens did not differ significantly among genotypes (data not shown).

A comparison of the distributions of mild-to-moderate and severe to most severe cases indicates that both the AA and AG groups showed significantly higher rates of more than severe cases (Table I; odds ratio, 95% CI, and *P* values of 4.00, 1.26-12.65, and .02 for AA and 3.45, 1.13-10.58, and 0.03 for AG, respectively) than did the GG group. When the distributions of mild-to-severe cases and most severe cases were compared, the AA group proved to have significantly higher rates of most severe cases (Table II; odds ratio, 95% CI, and *P* value of 6.22, 1.28-30.2, and .02, respectively) than did the GG group. These data clearly show that the polymorphism is associated not only with disease occurrence but also with disease severity in children with AD. It has been well established that AD is a multifactorial disease and that not only its atopic status

TABLE I. Distribution of AD severity among different genotypes (comparison between mild-to-moderate and severe to most severe symptoms)

Genotype	Mild to moderate	Severe to most severe	OR	95% CI	P value
All subjects					
AA	21	20	4.00	1.26-12.65	.02
AG	28	23	3.45	1.13-10.58	.03
GG	21	5	1.00	Reference	—
Subjects aged 5-9 y					
AA	13	12	7.38	1.40-39.08	.02
AG	17	20	9.41	1.89-46.89	<.01
GG	16	2	1.00	Reference	—
Subjects aged ≥10 y					
AA	8	8	1.67	0.29-9.45	.68
AG	11	3	0.45	0.07-3.09	.62
GG	5	3	1.00	Reference	—

OR, Odds ratio.

but also genetically acquired skin conditions, such as dry skin and skin barrier disruption, contribute to the development of the disease.⁹ Disruption of the *SPINK5* gene leads to the Netherton syndrome because of the loss of serine protease inhibitor activity in the skin, which results in disturbance of the maintenance of corneal structure.³ The site of the protein is important for its inhibitory effect, and the change in site from G to A might cause minor dysfunction of the inhibitory activity and lead to the barrier disruption commonly seen in patients with AD.⁴ Our findings therefore suggest that although there are many other aggravating factors for AD, *SPINK5* dysfunction is one of the important factors among children with AD.

When the subjects were divided into 2 groups according to age (5-9 years vs ≥10 years), these statistically significant results could still be seen in subjects aged 5 to 9 years (Tables I and II), whereas the results became no longer significant in subjects aged 10 years or older (Tables I and II). This might be because the SNP we have observed influences the AD severity mainly among younger patients, and some other factors might become more important in older patients. Alternatively, it is possible that statistical significance could not be seen in older groups because of the relatively small numbers of subjects. Further evaluation would thus be necessary to answer this question by increasing the number of subjects.

When the same analysis was performed for the prevalence and severity of BA, there was no statistically significant deviation in prevalence or severity according to genotype. In contrast to our findings, Kabesch et al⁷ found a significant association of this polymorphism with asthma phenotypes. Differences in sampling or ethnicity might be the reason for the discrepancy. Nishio et al,⁵ on the other hand, studied Japanese atopic families and found a significant association of this polymorphism with AD but not with asthma, which agrees with our data. Further

TABLE II. Distribution of AD severity among different genotypes (comparison between mild-to-severe and most severe symptoms)

Genotype	Mild to severe	Most severe	OR	95% CI	P value
All subjects					
AA	27	14	6.22	1.28-30.2	.02
AG	43	8	2.23	0.44-11.37	.48
GG	24	2	1.00	Reference	—
Subjects aged 5-9 y					
AA	16	9	9.56	1.09-84.25	.03
AG	30	7	3.97	0.45-35.03	.25
GG	17	1	1.00	Reference	—
Subjects aged ≥10 y					
AA	11	5	3.18	0.30-33.26	.62
AG	13	1	0.54	0.03-9.99	.99<
GG	7	1	1.00	Reference	—

OR, Odds ratio.

study is needed to determine whether *SPINK5* is associated with asthma.

When prevalence of food allergy among genotypes was observed, patients with the AA or AG genotype displayed a significantly higher prevalence of food allergy (20/91 subjects) than did those with the GG genotype (1/26 subjects; AA + AG vs GG, $P = .03$), indicating that the SNP is also associated with food allergy. The distribution of AD severity in children with food allergy did not differ significantly from that in children without food allergy ($P = .42$), indicating that AD severity per se did not affect the prevalence of food allergy. Children with Netherton syndrome often have severe atopic phenotypes, such as food allergy, suggesting that *SPINK5* is important to maintain barrier function not only of the skin but also of the gut. Our results are in agreement with these observations and indicate that a higher risk of food allergy should be taken into consideration for children with AD with either the AA or AG genotype.

Although there have been hundreds of studies evaluating the association of gene polymorphisms and atopic disease, no polymorphism study results have thus far been applied to the actual conditions of the allergy clinic and used for diagnosis and treatment. For this reason, it is tempting to speculate that the findings for the polymorphism we have studied can be used to predict the course of the disease and accompanying food allergies for infants with AD. However, because ours is a retrospective study targeting only patients who have continued to visit our clinic for more than 5 years since the initial occurrence of the disease in infancy, status of the SNP among those who outgrew the disease in less than 5 years and have not been followed up remains unknown. Thus to confirm the validity of this genotype analysis for the prediction of the disease course, it is important to examine it in a prospective manner in children with AD who visit the clinic during infancy. Also, because our study targeted only Japanese children, it would be necessary to see whether the

association we have observed in this study can be applied to children with AD of other ethnicities.

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REFERENCES

1. Magert HJ, Standker L, Kreutzmann P, Zucht HD, Reinecke M, Sommerhoff CP, et al. LEKT1, a novel 15-domain type of human serine proteinase inhibitor. *J Biol Chem* 1999;274:21499-502.
2. Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;25:141-2.
3. Komatsu N, Takata M, Otsuki N, Ohka R, Amano O, Takehata K, Saijoh D. Elevated stratum corneum hydrolytic activity in Netherton syndrome suggests an inhibitory regulation of desquamation by SPINK5-derived peptides. *J Invest Dermatol* 2002;118:436-43.
4. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubni B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. *Nat Genet* 2001;29:175-8.
5. Nishio Y, Noguchi E, Shibasaki M, Kamioka M, Ichikawa E, Ichikawa K, et al. Association between polymorphisms in the SPINK5 gene and atopic dermatitis in the Japanese. *Genes Immun* 2003;4:515-7.
6. Kato A, Fukai K, Oiso N, Hosomi N, Murakami T, Ishii M. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *Br J Dermatol* 2003;148:665-9.
7. Kabesch M, Carr D, Weiland SK, von Mutius E. Association between polymorphisms in serine protease inhibitor, karzai type 5 and asthma phenotypes in a large German population sample. *Clin Exp Allergy* 2004;34:340-5.
8. Haniffin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92:44-7.
9. Leung DYM, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651-7.

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