

Genetic Predisposition to Natural Rubber Latex Allergy Differs Between Health Care Workers and High-Risk Patients

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BACKGROUND: In health care workers, the natural rubber latex (NRL) allergy phenotype has been shown to be associated with promoter polymorphisms in interleukins 13 and 18 (*IL13* and *IL18*) when compared with nonatopic controls. However, it is not known whether high-risk patient populations, such as those born with neural tube defects or genitourinary abnormalities, demonstrate a heightened propensity toward the same genetic/immunologic risk factors that have been reported for health care workers. In this study, we tested the hypothesis that single-nucleotide polymorphisms in genes encoding *IL13* and *IL18* occur at an increased frequency in NRL allergic patients with spina bifida (SB) or bladder exstrophy (BE).

METHODS: One hundred twenty subjects (40 SB, 40 BE, and 40 control) were screened using a clinical history questionnaire and NRL-specific immunoglobulin E (IgE) antibody measurements in the blood. Genomic DNA was extracted from peripheral blood lymphocytes and analyzed for single-nucleotide polymorphisms in candidate genes of interest. Univariate and multivariate analyses were performed to identify significant variables with significance defined as $P < 0.05$.

RESULTS: Sensitization (IgE antibody positivity) to NRL allergens was associated with atopic history and number of prior operations and was prevented by the avoidance of NRL beginning at birth. However, unlike health care workers, the NRL allergy phenotype was not significantly associated with promoter polymorphisms in *IL13* or *IL18* when comparing NRL allergic SB and BE patients with nonsensitized patients and with atopic and nonatopic controls.

CONCLUSIONS: In patients born with SB or BE, environmental factors seem to play a greater role in the development of NRL sensitization and overt allergic symptoms than the IL polymorphisms in *IL13* and *IL18* previously shown to be associated with NRL allergy in health care workers. (Anesth Analg 2010;110:1310–7)

Allergy to products containing natural rubber latex (NRL) emerged in the 1980s as an important medical condition because of an increase in the use of NRL-containing barrier protection products. NRL is produced from a sap-like extract of the *Hevea brasiliensis* tree and contains at least 13 known allergenic proteins (Hev b 1–13). Some of the earliest reported cases of NRL allergy, including life-threatening anaphylactic reactions that were managed by anesthesiologists, occurred in patients with spina bifida (SB) while undergoing surgical procedures. At the peak of the epidemic in the mid to late

1990s, 25% to 50% of children with SB and/or genitourinary anomalies developed NRL sensitization or allergy if not treated with NRL avoidance practices.^{1–4} This prevalence is reportedly higher than NRL sensitization in health care workers with chronic occupational exposure (17%),^{5,6} in populations of atopic children (<10%),⁷ and in the general population (<1%).^{8,9}

Although allergen exposure associated with the use of NRL-containing products seems to be necessary for sensitization,¹⁰ exposure alone is not sufficient. Several other factors contribute to the NRL-sensitive phenotype, including an atopic history, concomitant food allergies, and a delayed-type hypersensitivity response to certain chemicals in NRL-containing products.^{6,11} A role for genetic background in an individual's susceptibility to allergic diseases has been reported. Polymorphisms in >30 genes located on 15 different chromosomes have been associated with human allergy.^{12–18} Major histocompatibility complex–coded molecules have an important role in regulating immune responses by binding processed antigens during recognition by T cells and causing the release of cytokines that drive immunoglobulin E (IgE) production. Focusing specifically on NRL allergy, Rihs et al.¹⁹ demonstrated a significantly increased frequency of the human leukocyte antigen (HLA) class II alleles DQB1 and DRB1 in NRL-allergic health care workers. NRL-sensitized patients with SB showed an increased DQB1 HLA frequency, but this result did not reach the level of significance.¹⁹

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Subsequently, we demonstrated a connection in health care workers between interleukin (*IL*)18 and *IL*13 single-nucleotide polymorphisms (SNPs) and allergic response. These cytokine-producing genes are of particular interest because they play an important role in the promotion of allergic responses. IL-18, the protein product of the *IL*18 gene, can act by stimulating interferon production¹⁷ and by enhancing cytokine and IgE production in mice,¹⁸ whereas IL-13 activates isotype switching of B cells to IgE production.¹⁶ In keeping with these observations, SNPs in these genes have been postulated to influence immunologic functions that are important in the development of atopy.^{13,14} We observed both a significant association in health care workers between *IL*18 (−607 and −656) SNPs and atopic status, and, more specifically, an association between NRL allergy status and promoter polymorphisms in *IL*13 (−1055).²⁰ This polymorphism was more than twice as frequent in NRL allergic health care workers compared with control patients who were not allergic to NRL, and it suggests a potential location for genetic control in the development of NRL allergy in atopic individuals.

It is not known whether high-risk patient populations, such as those born with neural tube defects or genitourinary abnormalities, demonstrate a heightened propensity toward the same genetic/immunologic risk factors that have been reported for health care workers. Direct NRL contact exposure through surgery causes children with SB to preferentially develop IgE antibody responses to Hev b 1 and 3, which are attached to rubber particles, in addition to the soluble Hev b 5 and 6 aeroallergens to which health care workers become sensitized.²¹ Notably, the frequency of candidate IL polymorphisms has not been evaluated in these patients. The goal of this current study, therefore, was to determine whether there is an association between IL promoter polymorphisms and the incidence of atopic disease, specifically NRL allergy, in high-risk patients as has been demonstrated in health care workers with chronic occupational exposure to NRL.²⁰

METHODS

Population

After IRB approval and written informed consent, 120 patients (40 with SB, 40 with bladder exstrophy [BE], and 40 healthy controls) presenting to the Johns Hopkins Medical Institutions and Kennedy Krieger Institute for medical care were screened using a clinical history questionnaire. The questionnaire examined the participants' history of atopic disease (seasonal rhinitis, asthma, eczema, and food allergies), symptoms associated with exposure to NRL-containing devices, frequency of surgical procedures, use of bladder catheters, onset of NRL avoidance procedures where appropriate, and the frequency of any suggestive delayed-type or immediate-type hypersensitivity reactions to NRL-containing products (Appendix 1).

Serology

Whole blood (3 mL) was collected by venipuncture from each subject using synthetic gloves and tourniquet. The specimen was clotted for 30 minutes, centrifuged, and

the serum was separated. Samples were coded and stored at −20°C. Each serum specimen was analyzed for the presence and quantity of IgE antibody specific to NRL allergens using the Phadia ImmunoCAP System® (Kalamazoo, MI), which uses a solid matrix with covalently attached NRL allergen. Results are reported in kilo international unit of allergen-specific IgE antibody per liter (kIUa/L) with a positive result being defined conservatively as >0.35 kIUa/L. All NRL-specific IgE measurements were repeated for confirmation of results. The reported diagnostic sensitivity and specificity of the NRL-allergen IgE ImmunoCAP System assay using the Hev b 5–enriched NRL allergosorbent have been reported at 74.8% and 93.8%, respectively.²²

Genotyping

An additional 3 mL whole blood was drawn from each subject into a tube containing 15% (K3) EDTA and stored frozen at 80°C. Genomic DNA was isolated using the Flexigene DNA Kit from Qiagen (Valencia, CA). After precipitation, DNA was spooled onto glass rods, rinsed with 75% ethanol, briefly air dried at room temperature, and then resuspended in sterile TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) with gentle rotation. Quantitation of DNA was performed using a NanoDrop spectrophotometer (ThermoFisher, Asheville, NC).

Subjects were genotyped for 5 specific SNPs in 2 genes of interest by allelic discrimination using TaqMan SNP genotyping assays (Applied Biosystems [AB], Foster City, CA). These assays contained allele-specific 6FAM- and VIC-labeled TaqMan MGB Probes. The following assays were used: *IL*18 −656 (rs1946519, assay ID C_2898459_20), *IL*18 −607 (rs1946518, assay ID C_2898460_10), and *IL*13 −1055 (rs1800925, C_8932056_10). Assays for *IL*18 +113 and +127 were designed and optimized as previously described.²⁰ All reactions contained 1× assay mix, 1× TaqMan Genotyping MasterMix (AB), and 20 ng DNA in a 25-μL volume. Cycling was performed in the AB 7500 Real Time PCR system with the following conditions: 95°C for 10 minutes and 40 cycles of amplification at 95°C for 15 seconds and 60°C for 1 minute. After amplification, plate reads were performed in the 7500 Real Time polymerase chain reaction (PCR) system. Genotypes were determined by clustering using 7500 system software version 1.2.3 (AB).

All genotypes for *IL*18 −607 were confirmed by PCR and restriction fragment length polymorphism analysis. Amplifications and digests were performed as previously described.²⁰ PCR–restriction fragment length polymorphism was also used to validate a subset of genotypes for *IL*18 −656. Aliquots of the same amplicons generated for *IL*18 −607 were digested with CviKI 1 (New England Biolabs, Beverly, MA) and resolved by capillary electrophoresis using DNA1000 LabChips (Agilent Technologies, Wilmington, DE).

Statistical Analyses

Statistical analyses were performed using JMP 7.0.1 software (SAS Institute, Cary, NC). Participants were categorized into 3 groups after their clinical evaluation: patients with SB, patients with BE, and healthy control patients.

Table 1. Study Population Demographics

	Spina bifida (n = 39)	Bladder exstrophy (n = 40)	Spina bifida or bladder exstrophy (n = 79)	Controls
Gender (male/female)	15/24	26/14	41/38	18/21
Age, mean $\pm$ SD (y)	21.8 $\pm$ 10.8	18.3 $\pm$ 10.6	20 $\pm$ 10.7	20.7 $\pm$ 11.3
Caucasian (%)	28 (72)	34 (85)	62 (78)	30 (77)
African American (%)	8 (21)	1 (3)	9 (11)	5 (13)
Asian American (%)	0 (0)	1 (3)	1 (1)	1 (1)
Hispanic American (%)	2 (5)	2 (5)	4 (5)	1 (1)
Other race (%)	1 (3)	1 (3)	2 (3)	2 (3)
Latex-specific IgE positive (%)	17 (44)	17 (43)	34 (43)	3 (8)

IgE = immunoglobulin E.

Table 2. Study Population Characteristics

	Control atopic (n = 16)	Control nonatopic (n = 23)	Bladder exstrophy latex IgE (+) (n = 17)	Bladder exstrophy latex IgE (–) (n = 23)	Spina bifida latex IgE (+) (n = 17)	Spina bifida latex IgE (–) (n = 22)
Age, mean $\pm$ SD (y)	22.2 $\pm$ 9.1	19.7 $\pm$ 12.6	19.7 $\pm$ 7.5	17.2 $\pm$ 12.4	20.8 $\pm$ 5.7	22.6 $\pm$ 13.5
Gender (male/female)	9/7	9/14	10/7	16/7	7/10	8/14
History of food allergies	1 (6.3) ^a	0 (0)	2 (12)	2 (9)	6 (35)	6 (27)
Use of bladder catheters	0 (0)	1 (4)	9 (53)	12 (52)	15 (88)	16 (73)
Age at first surgery (mean)	9.7 y	12.1 y	0.4 mo	4 mo	0.7 mo	1 mo
No. of Prior surgeries						
None	8 (50)	5 (22)	0 (0)	0 (0)	0 (0)	0 (0)
<2	7 (44)	10 (43)	0 (0)	2 (9)	0 (0)	1 (5)
3–5	1 (6)	4 (17)	3 (18)	8 (35)	1 (6)	6 (27)
5–10	0 (0)	3 (13)	5 (29)	6 (26)	3 (18)	4 (18)
>10	0 (0)	1 (4)	9 (31)	7 (30)	13 (76)	11 (50)
Median no. of surgeries	<2	<2	5 to 10	5 to 10	>10	5 to 10
History of atopy	16 (100)	0 (0)	17 (100)	9 (39)	17 (100)	10 (45)
History of atopy to nonlatex triggers	16 (100)	0 (0)	11 (65)	9 (39)	11 (65)	9 (41)
History of reaction to latex- containing products	0 (0)	0 (0)	14 (82)	3 (13) ^b	13 (76)	10 (45) ^c
No. of patients born after 1993	2 (13)	7 (30)	2 (12)	11 (48)	1 (6)	5 (23)
No. patients born after 1993 practicing latex avoidance since birth	0 (0)	0 (0)	0 (0)	5 (22)	0 (0)	3 (14)
No. of patients currently practicing latex avoidance	1 (6)	0 (0)	15 (88)	15 (65)	16 (94)	16 (73)
Latex-specific IgE serology positive	3 (19) ^d	0 (0)	17 (100)	0 (0)	17 (100)	0 (0)
Latex-specific IgE range (kIUa/L)	<0.35–1.86	<0.35	0.36–43.6	<0.35	0.43–66.2	<0.35
Median/average latex- specific IgE (kIUa/L)	<0.35/0.42	<0.35	0.68/9.6	<0.35	3.0/11.9	<0.35

^a Number of patients (% of patients).^b Three of 3 patients reported rash only to latex.^c Five of 10 patients reported rash only to latex; 4 of 10 patients reported respiratory and/or cardiovascular symptoms.^d All control patients with latex-specific IgE positive serology were born before 1993.

Unpaired *t* tests were used to compare continuous variables (NRL-specific IgE antibody), and χ^2 tests were used to compare categorical and binomial variables (polymorphisms). Univariate and multivariate analyses were performed to identify significant variables. All *P* values are 2-tailed and those <0.05 were considered significant.

RESULTS

Population Characteristics

Of 120 patients initially enrolled, 2 patients were withdrawn from data analysis as a result of insufficient DNA obtained for genetic testing. Demographic data for the

remaining 118 patients are presented in Tables 1 and 2. In all, 37 of 118 individuals studied (31% total; 43.5% SB, 42.5% BE, and 7.7% control) were identified as sensitized to NRL allergens based on a positive NRL-specific IgE (range, 0.36–66.2 kIUa/L). Of these 37 patients, 27 (23% total; 76% SB positives and 82% BE positives) reported a history of NRL allergy symptoms, whereas 10 denied any NRL-associated symptoms (4 SB, 3 BE, and 3 control). An additional 13 patients (16% total; 10 SB and 3 BE) reported a positive clinical history for NRL allergy but were seronegative. Of these 13, 8 reported only NRL-associated skin reactions, whereas 4 reported associated

Table 3. Minor Allele Numbers and Corresponding Frequencies (f) of *IL13* and *IL18* Polymorphisms Among Controls, Spina Bifida (SB), and Bladder Exstrophy (BE) Patients

Polymorphism	Allele number (f)					
	All controls (n = 39)	Atopic controls (n = 16)	Nonatopic controls (n = 23)	BE/SB latex IgE (+) (n = 34/33 ^a)	BE/SB latex IgE (–) (n = 45/44 ^a)	BE/SB latex IgE (–) and negative clinical history (n = 32/31 ^a)
<i>IL13</i> –1055T	17 (0.22)	7 (0.22)	10 (0.22)	14 (0.21)	16 (0.18)	13 (0.20)
<i>IL18</i> –607T	28 (0.36)	10 (0.31)	18 (0.39)	28 (0.41)	35 (0.39)	24 (0.38)
<i>IL18</i> –656T	28 (0.36)	10 (0.31)	18 (0.39)	28 (0.41)	35 (0.39)	24 (0.38)
<i>IL18</i> +113G	14 (0.18)	5 (0.16)	9 (0.20)	23 (0.35)	18 (0.20)	13 (0.21)
<i>IL18</i> +127T	13 (0.17)	5 (0.16)	8 (0.17)	22 (0.33)	18 (0.20)	13 (0.21)

^a 34, 45, or 32 SB and BE patients were genotyped for *IL13* –1055, *IL18* –607, and *IL18* –656; 33, 44, or 31 SB and BE patients were genotyped for *IL18* +113 and *IL18* +127.

respiratory and/or cardiovascular symptoms when exposed to NRL-containing products. There was no additional testing (e.g., skin testing or glove provocation testing) available to clarify the true clinical status of these subjects.

No patient who was born during or after 1993 and who reported NRL avoidance practices instituted at birth developed detectable levels of NRL-associated IgE in their blood ($n = 8$; 3 SB and 5 BE). An additional 8 patients born from 1993 onward were not treated with NRL avoidance from birth. Of these, 4 (50%; 3 BE and 1 SB) became sensitized as evidenced by seroconversion, whereas 4 did not. Two of these patients were highly sensitized as evidenced by a positive clinical history of NRL allergy and detectable NRL-specific IgE antibody (1 BE and 1 SB, NRL-specific IgE values: 43.6 and 49.1 kIUa/L, respectively).

In SB and BE patients, the prevalence of atopic symptoms that were not related to NRL exposure was higher in those patients who were NRL sensitized compared with the nonsensitized SB and SE patients (65% vs 40%, $P = 0.04$). The lower prevalence in the nonsensitized SB and BE patients was comparable with the prevalence of atopic symptoms in the control group (41%). SB patients reported a higher incidence of food allergies (31% total; 35% seropositive and 27% seronegative) than either BE or control patients (10% and 2.5%, respectively). The use of a urinary catheter was not associated with an increased prevalence of NRL sensitization for either the SB group ($P = 0.43$) or BE group ($P = 1.0$).

Whereas the mean age of patients in the 3 groups was not different ($P = 0.34$), age at the time of first surgery was different among the groups. As expected, most patients born with SB or BE underwent surgery in infancy. The average age at first surgery was 2.5 months, 1 month, and 11.3 years for the BE, SB, and healthy control patients, respectively ($P < 0.001$ for the healthy controls versus the other 2 groups). The number of prior operations as reported by families was none, ≤ 2 , 3–5, 5–10, or >10 . There were significantly more reported operations among the SB and BE patients compared with the control patients ($P < 0.001$). In addition, among SB and BE patients, there was an association between increasing number of operations and NRL sensitization ($P = 0.04$).

Finally, we fit a multiple logistic regression model with the absence or presence of NRL-specific IgE (NRL seronegative or seropositive) as the dependent variable and the

number of operations, atopy, and diagnosis (BE, SB, or healthy control) as the independent variables. We found that atopic history ($P = 0.007$) and the number of operations (0.04) were statistically significant, whereas diagnosis ($P = 0.31$) was not.

Allele Frequencies

Genotypes were determined in 118 individuals for 3 polymorphisms in 2 genes (*IL13* –1055, *IL18* –607, and *IL18* –656) and 116 individuals for 2 polymorphisms in 1 gene (*IL18* +113 and +127). Allele numbers and frequencies were calculated based on the results of the genotype analysis (allele frequency = $[\# \text{ heterozygotes} + 2 \times \# \text{ minor allele homozygotes}] / 2 \times \# \text{ subjects genotyped}$) and are shown in Table 3. Genotype distributions fit predictions for Hardy-Weinberg equilibrium. Unlike the health care workers previously genotyped, there was no significant difference between the allelic frequencies for *IL18* –607 and –656 or *IL13* –1055 between seropositive SB and BE patients, seronegative SB and BE patients, and control subjects ($P > 0.05$). This association remained the case when control patients were stratified into atopic and nonatopic subgroups and when seronegative SB and BE patients born after 1993 who had never been NRL sensitized were excluded. Restricting the study groups further to only those SB and BE patients who demonstrated both a positive history and detectable NRL-specific IgE or a negative history and serology did not alter this finding. Likewise, multivariate analysis in which NRL-IgE serology and number of prior operations were both included showed no association between SNP status and NRL allergy status. Interestingly, there was an association between the *IL18* +113 and *IL18* +127 minor frequency allele and NRL-IgE-positive serology. However, this association did not persist in our multiple logistic regression model when number of prior operations was considered.

DISCUSSION

The field of genome-based medicine, which attempts to identify genetic factors that individuals possess that may protect them or create problems when they undergo medical interventions, is rapidly evolving and affecting all fields of medical practice. However, once associations are identified, it is often unknown whether they will remain relevant across different classes of patients. We have previously used SNP analysis to study the effect of

cytokine polymorphisms on NRL allergy risk in health care workers. Prospectively extending these findings to patients with genitourinary and neural tube defects, who are at the highest documented risk of developing NRL allergy, we found that environmental factors seem to play a greater role in the development of NRL sensitization and overt allergic symptoms than the previously identified polymorphisms.

Allergic disease is mediated by allergen-specific IgE antibody that is produced as a result of T-cell recognition of foreign molecules and their resultant cytokine release. Genetic factors in the form of major histocompatibility complex-coded molecules are centrally involved in regulating this adaptive immune response through binding processed antigens for recognition by T cells and causing the release of defined cytokine profiles that drive IgE production. However, although numerous studies have suggested the importance of this genetic component in the development of allergic disease, susceptibility genes have been difficult to identify given the multigenic nature of this effect. Two genes that have been of interest with regard to NRL allergy are *IL13* and *IL18*. *IL-13*, along with *IL-4*, is critical for the promotion of allergic responses. *IL-13* plays an important role in mediating airway hyperresponsiveness in asthma.²³ Binding of *IL-4* and *IL-13* to the α chain of the *IL-4* receptor activates germline transcription of the ϵ heavy-chain gene locus and isotype switching of B cells to IgE production.¹⁶ In addition, *IL-18* can stimulate interferon production¹⁷ or enhance cytokine and IgE production in mice.¹⁸ SNPs in these genes have been postulated to influence physiologic functions that are important in the development of atopy.^{13,14} We have previously demonstrated a significant association between NRL allergy status and promoter polymorphisms in the *IL13* and *IL18* genes when comparing NRL-sensitized health care workers to nonatopic controls.²⁰ This association was not seen when these patients were genotyped for SNPs in other immunomodulatory genes, including *IL4*, tumor necrosis factor- α and - β , CC chemokine receptors 2 and 5, and toll-like receptor 4 (unpublished data). This led us to hypothesize that these polymorphisms might, likewise, play a role in the development of NRL allergy among high-risk patients such as children who undergo multiple operations and other exposure to NRL early in life.

The SB and BE patients who were studied in the current investigation were typical in their demographic characteristics to those previously described in the literature. The prevalence of a positive NRL-specific IgE antibody response was 43% in both groups. This is consistent with previously quoted prevalence rates of NRL sensitization ranging between 22% and 50%.^{1,2,4} In the current study, we found, as had previously been reported, that increasing numbers of surgical procedures was a risk factor associated with the development of NRL sensitization.^{3,4} In general, NRL-sensitized SB and BE patients were more likely to be atopic (exclusive of NRL allergy) than those who were NRL-specific IgE antibody negative. Patients with SB had an increased prevalence of food allergies compared with BE and

control patients. Finally, implementation of NRL avoidance beginning at birth in those patients born during or after 1993 (the time at which our institution began targeted NRL avoidance practices in high-risk patients in the operating room setting as well as a transition to nonlatex urinary catheters) was effective in preventing NRL sensitization to *H brasiliensis* allergens. However, we found no genetic association in these patients between their underlying disease or NRL sensitization status and their *IL13* or *IL18* SNP status. This is in contrast to the NRL allergic health care workers previously studied in whom we were able to show this association.²⁰

Although this negative gene result was unexpected, there are a number of possible explanations. First, we considered methodological differences between the 2 studies. One difference between this study and our prior study involving health care workers is that in the earlier study both the controls and NRL allergic subjects were health care workers who had been challenged by a comparable exposure to NRL in the operating room. In general, health care workers are exposed to NRL as a result of a loss of a protective skin barrier (e.g., eczema) or an inhaled challenge from breathing air containing aerosolized particles from powered gloves. However, most of our current study patients were exposed to NRL-containing products at the mucus membrane level as a result of an invasive surgical procedure within the first weeks of life, and then were subsequently rechallenged with additional operations. In addition, our control group for this study, which we used for a population baseline, had a much lower NRL glove exposure than our SB and BE patients. Most had not had multiple prior operations, and their first surgery was at a much older age. Thus, these groups were much less homogeneous in terms of latex exposure than those previously studied, which may have affected our results.

Our results led us to speculate that our negative genetic association finding may have resulted from an insufficient power to detect a significant difference that may have truly existed. Our a priori power calculation was based on our previous study in which we found the prevalence of the minor allele SNP *IL13* -1055T to be >2-fold higher in latex allergic subjects than in controls.²⁰ Given these results, we hypothesized that subjects with SB or BE who have been at high risk of developing NRL allergy might be genetically predisposed to develop latex allergy as a result of an increased minor allele SNP *IL13* -1055T frequency. Assuming an α value of 0.05 and a power = 0.9, we calculated that 40 patients with BE, 40 patients with SB, and 40 controls would be sufficient to allow us to determine whether a comparable 2-fold increase was present in the BE/SB patients compared with controls. However, unlike our prior study, in this study, we found <4% difference in SNP *IL13* -1055T prevalence between healthy control subjects and those patients born with SB or BE (0.22 vs 0.18 vs 0.20, respectively). Thus, in light of these results and assuming an α value of 0.05 and a power = 0.9, the number of control and study patients that would be needed to show a statistical difference would be in the

thousands and up to tens of thousands. Clearly, these SNPs do not seem to be major risk factors for these groups.

We next attempted different stratification strategies in an attempt to limit our confounding variables but still found no increased incidence of the *IL13* -1055T SNP in any subgroup of high-risk patients. When comparing NRL-specific IgE-positive and -negative high-risk patients, most of whom had significant and comparable NRL exposure, we found only a slight difference in *IL13* -1055T prevalence (0.22 vs 0.18). We also postulated that our results might have been influenced by inclusion of patients who had symptoms of NRL allergy but were NRL IgE negative; however, our *IL13* -1055T prevalence was 0.21 for NRL-specific IgE-positive SB/BE patients compared with 0.20 for NRL-specific IgE-negative SB/BE patients who denied signs or symptoms of NRL allergy. Finally, we considered whether our results had been affected by the inclusion of patients who were protected from NRL from birth in our NRL seronegative group. However, removing these patients from the analysis did not affect our results. Taken together, these results again suggest that whether latex allergic or not, our high-risk patients had a SNP frequency that was almost identical to both our control population and the previously reported health care worker controls, and, again, that thousands of patients would need to be screened to demonstrate or refute a minor association between SNP *IL13* -1055T prevalence and NRL allergy status. However, the need to expand our study to this degree to detect a statistically significant difference implies that the clinical effect of this SNP is too small to be considered a major risk factor for the development of NRL allergy in these high-risk populations.

Another caveat is the limited number of SNPs we studied. However, the primary objective of this study was to determine whether there was an obvious association between allergy to NRL and *IL13* SNP status, which was relevant in these populations as had been previously observed. Interestingly, in evaluating *IL18* SNP status, a higher minor allele frequency did initially suggest a genetic predisposition to NRL allergy determined by the *IL18* +113 and +127 alleles. However, multivariate analysis revealed that surgical exposure had a predominant role in these patients. These findings suggest that although we cannot exclude the role of other genes in the development of NRL allergy in these high-risk groups, future studies would require a significantly larger and more homogeneous study group to minimize the effect of confounding variables.

Our results are in keeping with reports of an increased frequency of the DQB1 and DRB1 HLA haplotypes in NRL-allergic health care workers but no significant association in NRL-sensitized patients with SB.¹⁹ Therefore, we postulate that these differences may be more directly related to major differences between the time and mode of exposure to NRL-containing products in these patients compared with health care workers. Because NRL epitopes differ in size and solubility, sensitization to some may require direct contact of NRL with blood or mucosal surfaces as occurs with surgery,

whereas others may allow sensitization via inhalation of allergen particles.²¹ Hev b 1 and 3, hydrophobic epitopes that SB patients are frequently sensitized to, are attached to rubber particles, whereas Hev b 5 and 6, the hydrophilic epitopes that health care workers are often sensitized to, are aeroallergens. Interestingly, it has also been shown that concentrations of Hev b 1 and 3 are higher on the external surfaces of latex gloves, which would promote mucus membrane exposure, whereas levels of Hev b 5 and 6.02 are higher on the internal surfaces of the gloves where they might more easily sensitize the glove wearer.²⁴ Thus, these 2 factors may both have a role in explaining the variability seen in NRL-specific IgE antibody profiles and risk factors when comparing health care workers with patients with BE and SB.²¹

In addition, exposure of mucus membranes to NRL allergen may also result in a different and possibly more potent antigenic stimulus than that seen by health care workers. Mast cells, crucial mediators of allergic response, migrate to and terminally differentiate in distinct body sites (mucosal versus connective tissue) where they display characteristic profiles of intracellular mediators.²⁵ Hence, activation of "connective tissue" mast cells by peritoneal exposure to NRL during surgery might result in a different sensitivity profile and end effect than that seen after stimulation of "mucosal" mast cells in the lung after inhalation of NRL particles. Thus, changes in immune response that are important for sensitization in health care workers may be less relevant in the setting of a different method of antigen presentation.

Although our search for genetic factors as a primary cause for the development of NRL sensitization was admittedly limited, it was focused on genes in which we had previously demonstrated an association between polymorphism status and positive NRL-specific IgE antibody response. However, our results suggest that the role of this specific genetic predisposition for NRL allergy may be limited at best. An atopic genotype may be sufficient to predispose an individual to the risk of developing NRL allergy. However, exposure is also required. In our regression model, the number of operations and an atopic history were significantly associated with a positive IgE antibody. As expected, a simple analysis comparing the prevalence of NRL-specific IgE antibody among the BE, SB, and healthy controls showed a significantly greater rate of NRL sensitization in the SB and BE patients compared with the controls ($P = 0.0001$). However, when controlling for the number of operations and a history of atopy, the diagnosis of SB or BE was no longer statistically significant ($P = 0.31$). Furthermore, among the SB and BE patients, the number of NRL IgE antibody-positive patients was higher in the atopic group compared with the nonatopic group in every surgery number category.

In conclusion, our current findings combined with confirmation that in our patients NRL avoidance instituted before their first surgery was effective in preventing sensitization, emphasizes that, at least for now, in patients with SB and BE, environmental factors may play a greater role in the development of NRL sensitization and overt allergic symptoms than specific genetic predisposition. ■■

Appendix 1: Latex Allergy Questionnaire

Date: ____/____/____

Subject #: _____

Patient MR #: _____

Age: _____

Gender ☐ M ☐ F

Race ☐ Native American
☐ Asian/Pacific Islander
☐ African American (black)
☐ Hispanic American
☐ Caucasian, not Hispanic American
☐ Other (specify) _____

Medications being taken: _____

ALLERGY HISTORY:

Does your child have seasonal allergies? Y N

If YES, during which season do they occur? _____

Are the symptoms SEVERE or MILD?

Does your child take medicine to treat these symptoms? Y N

If YES, which medicines? _____

Does your child have asthma? Y N

Does your child have eczema? Y N

Is your child allergic to avocado, kiwi, or banana? Y N

Is your child allergic to any other foods? Y N

If YES, please list the foods your child is allergic to and the type of reaction that occurs: _____

LATEX HISTORY

Has your child had previous surgery? Y N

If YES, how many prior surgeries? ≤2 3-5 5-10 >10

Age at first surgery? _____

Does your child require a catheter to drain urine from the bladder? Y N

Are latex avoidance precautions taken when your child is cared for at the doctor's office, hospital, or dentist's office? Y N

If YES, when were "latex precautions" first used in the care of your child? _____

Has your child ever been tested for latex allergy? Y N

If YES, what tests were done and did they demonstrate latex allergy? _____

Where was the testing done? _____

When was the testing done? _____

Has your child ever had a reaction to rubber/latex?

☐ Never
☐ Once
☐ More than once

If your child has experienced a reaction, where and when did this reaction occur? _____

If your child has had a reaction, please check the symptoms your child has experienced when exposed to latex:

Skin: ☐ hives ☐ itching
☐ rash ☐ redness
☐ swelling ☐ irritation

Eyes: ☐ itchy
☐ tearing/watering
☐ redness

Breathing: ☐ runny nose ☐ throat swelling
☐ itchy/tight throat ☐ sneezing
☐ asthma, wheezing ☐ wheezing cough
☐ shortness of breath ☐ chest discomfort

Gastrointestinal: ☐ nausea
☐ vomiting

Cardiovascular ☐ chest pain
☐ irregular heart rhythm
☐ low blood pressure
☐ light-headedness
☐ rapid heart rate

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