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Cross-sensitization profiles of edible nuts in a birch-endemic area

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Abstract

Background: Sensitization to birch pollen causes cross-sensitization to nuts, but rarely leads to clinical nut allergy. The aim is to study sensitizations to nuts in individuals sensitized to birch pollen and examine cross-reactivities between birch and nut species.

Methods: All subjects with skin prick tests (SPT) for birch pollen conducted during 1997-2013 in the Skin and Allergy Hospital in Helsinki (n = 114 572) and their available SPTs for nuts (n = 50 606) were included. Nut sensitizations were analyzed both with and without co-sensitization to birch and stratified into age-categories. Cross-reactivities were analyzed with hierarchical clustering. One group of 1 589 patients was surveyed for symptoms. Data were

gathered also from Lapland to examine sensitizations in an area with less birch-pollen exposure.

Results: Of subjects with birch sensitization, 84% were co-sensitized to hazelnut, 71% to almond, and 60% to peanut. In a subgroup without birch sensitization, young children (<5 years) were most commonly nut sensitized (8 to 40%); and this prevalence decreased in adolescents and further in adults (4 to 12%). Cashew and pistachio, ($\rho = 0.66$; $P < .001$) and pecan and walnut ($\rho = 0.65$; $P < .001$) correlated the strongest. The majority of nut-sensitized patients (71% hazelnut, 83% almond, 73% peanut) reported no or mild symptoms. Co-sensitizations between nuts and birch were similar in Lapland with its lower birch-pollen exposure.

Conclusion: Birch-sensitized individuals are frequently co-sensitized to hazelnut, almond, and peanut. Among the birch-negatives, prevalences of nut sensitizations decrease from early childhood to adolescence. Cashew and pistachio, and pecan and walnut cross-react the most.

Key words

Birch pollen, birch sensitization, cross-reactivity, nut hypersensitivity, oral allergy syndrome

Abbreviations used:

SPT: Skin prick test

CI: Confidence interval

OAS: Oral allergy syndrome

PR: Prevalence ratio

IQR: Interquartile range

PR-10 protein: Pathogenesis-related protein 10

INTRODUCTION

Nut allergy develops at an early age and often persists into adulthood (1) (2), causing the majority of food-induced anaphylactic reactions. (3) Allergy-screening tests even in young children can show sensitization to multiple nut species, (4) but not all nut sensitizations indicate severe or any symptoms. (5)

In northern Europe, pollen from the birch tree is the most important sensitizer, with one-fourth of young adults affected. (6) Birch sensitization commonly leads to cross-reactivities to nuts and other plant-derived foods. (7) Hazelnut is especially considered birch-pollen related, and the major allergen in birch pollen, Bet v 1, has a 80.5 to 83% sequence similarity to hazelnut Cor a 1. (8) Many individuals have a mild nut allergy which is merely pollen-related and without severe symptoms. In birch-endemic areas, birch-pollen sensitization may occur also in patients who suffer a severe nut allergy which is unrelated to pollen. To determine the nature of nut allergy is crucial in reducing unnecessary diet restrictions and yet keeping severely allergic patients safe.

Avoidance of all nuts is common advice for patients diagnosed as allergic to one nut species, because of cross-reactivity between species (4) (9) (10), the possibility of cross-contamination, and difficulties in recognizing species. (11) Certain species such as peanut and cashew, some suggest, cause more severe reactions. (12)

Although the effect of birch pollen sensitization on simultaneous nut sensitizations is strong, the extent of the phenomenon and the specific characteristics of various nut species remain unknown. The aim of this study was to examine nut sensitizations from a large registry of skin prick tests in a population that is very highly exposed to birch pollen.

METHODS

We gathered data on SPTs from the Skin and Allergy Hospital (Helsinki) database between 1997 and 2013, selecting all SPTs to birch pollen and available nut testing. The database includes all SPTs conducted in the hospital, and indications comprise respiratory allergy, allergic rhinitis, atopy and food allergy screening, and history of food allergy. The nuts were walnut, pecan, pistachio, cashew, coconut, Brazil nut, macadamia, almond, hazelnut, and peanut. If an individual was tested several times, we included only the most recent result. In cross-reactivity analyses, we included only those subjects with all ten nut species examined. To assess age-differences in sensitization prevalences, we formed three age-groups. Included in the analysis were only those subjects with at least four nut species examined.

Survey of symptoms was according to Haahtela et al (13) in 1 307 subjects sensitized to hazelnut, in 1 158 to peanut, and in 1 032 to almond. The symptoms were collected using a structured interview conducted by nurses on the site. Symptoms were categorized as follows: conjunctival, oral allergy syndrome (sneezing, rhinitis, congestion, pruritus of the throat or ears), lower airway, skin, contact urticaria, gastrointestinal (diarrhea or vomiting), and anaphylaxis. Anaphylaxis was defined according to the Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network. (14) Helsinki, which served as the study center in southern Finland, is located at 60° N, 24° E. The Skin and Allergy Hospital in Helsinki provides skin prick diagnostics for 1 603 388 regional inhabitants. (15)

To assess co-sensitizations in an area with less exposure to birch pollen, we gathered data from northern Finland from an archive of skin prick tests conducted in Lapland Central Hospital (Rovaniemi). The central city of Lapland, Rovaniemi, is located on the Arctic Circle at 66° N, 25° E. Its hospital provides skin prick diagnostics for 118 145 area inhabitants. (15) We selected subjects who were sensitized to almond, hazelnut, or peanut and had been tested

for birch pollen. Prevalence of birch sensitization in the north was assessed from a population of 359 subjects tested for birch pollen during 2011-2012.

Our skin prick-testing method utilized raw nuts with a prick-prick technique. Nuts were ground up unpeeled and mixed with 0.9% saline. Birch pollen was tested with a commercial extract (ALK-Abelló; Hørsholm, Denmark or Allergopharma; Reinbek, Germany). Histamine hydrochloride (10 mg/ml) served as a positive control, and allergen solvent as a negative control. The skin was pricked with a single-headed lancet on the volar forearm. Results were read after 15 minutes, and wheal sizes 3 mm or larger documented in millimeters, with sizes below 3 mm documented as zero. We considered a wheal size of at least 3 mm to be positive. All the results from Rovaniemi were analyzed as dichotomous variables because before mid-2009, results were reported as 0 through 4+ presenting the wheal size arbitrarily. A wheal size at least 1+ was considered positive.

The seasonal pollen counts in two study regions were provided by the University of Turku, Aerobiology Unit, Turku, Finland. Median seasonal birch pollen count in Helsinki was 15 000 grains/m³ (range 2 047-97 453) (years 1995-2012) and in Rovaniemi 7 300 grains/m³ (range 665-22 770) (years 2002-2012) ($P = .04$).

The study was approved by the ethics committee of Helsinki and Uusimaa Hospital District.

Data were analyzed with Chi square and Fisher exact tests, Spearman rank-order correlation, binomial logistic regression, and hierarchical clustering with euclidean distance and method of average linkage were selected for clustering. Trends in age-differences were analyzed with the Linear-by-Linear association test and Poisson regression. All tests were done two-sided,

with P value $<.05$ considered significant. We used IBM SPSS Statistics 22 (IBM; Armonk, NY) and R-program 3.0.2 (R Project for Statistical Computing, <http://www.r-project.org/>).

RESULTS

Co-sensitizations to birch and nuts

Skin prick tests with birch were available from 114 572 subjects, of whom 35 941 (31.4% [95% CI, 31.1-31.7%]) were sensitized. At least one nut was tested in each of 50 606 subjects (Fig 1).

Of the subjects with sensitization to birch, 84.1% (95% CI, 83.5-84.7%) were sensitized to hazelnut, 70.8% (95% CI, 70.1-71.5%) to almond, and 59.9% (95% CI, 59.2-60.6%) to peanut. In subjects without sensitization to birch, sensitization to all nuts was significantly less common ($P <.001$ for all species) (Fig 2).

Hazelnut was most strongly linked to birch, with a 15.9-fold prevalence in birch-sensitized subjects compared to subjects without birch sensitization (PR 15.9 [95% CI, 15.03 to 16.74]). Cashew was least linked to birch, with a 2.25-fold prevalence in birch-sensitized subjects (PR 2.25 [95% CI, 1.84 to 2.76]) (Table I).

Subjects with larger wheal sizes to birch were sensitized to more nut species (Spearman $\rho = 0.63$, $P < .001$) (Fig 3a).

With increasing wheal sizes to birch, wheal sizes to nuts also increased, this correlation being strongest with birch and hazelnut (Spearman $\rho = 0.84$, $P < .001$; vs. birch and almond, Spearman $\rho = 0.74$; $P < .001$ vs. birch and peanut Spearman $\rho = 0.61$, $P < .001$) (Fig 3b).

Symptoms related to nut sensitizations

With increasing wheal size to a nut, the proportion of symptomatic patients increased (Fig 4).

Of 1 307 patients sensitized to hazelnut, 900 (69%) reported symptoms, with 518 (58%) of those reporting oral allergy syndrome (OAS). Of 1 099 patients sensitized to almond, 569 (52%) reported symptoms, of whom 378 (66%) showed OAS. Of 1 159 peanut-sensitized patients, 737 (64%) reported symptoms, of whom 421 (57%) had OAS. Anaphylaxis occurred in 19 (1.6%) patients sensitized to peanut, in 8 (0.6%) sensitized to hazelnut, and in 2 (0.2%) sensitized to almond.

Of the 1 589 patients who answered the symptom questionnaire 1 520 had results available for birch. Those patients who reported symptoms from nuts had larger birch wheal sizes than did asymptomatic patients. For peanut, the birch wheal size was 6 mm (median, IQR 5-7 mm) vs. 5 mm (4-6) ($P < .001$), for hazelnut, 6 mm (5-7) vs. 5 mm (5-6) ($P < .001$), and for almond, 6 mm (5-7) vs. 5 mm (5-7) ($P = .001$).

Cross-sensitizations between nut species

All ten nut species were examined in 780 subjects. In hierarchical clustering, cashew and pistachio formed a pair. Birch, almond, hazelnut, and peanut fell into a single cluster. Other species formed the third cluster, which included paired pecan and walnut (Fig 5).

The strongest bivariate correlation appeared between hazelnut and almond (Spearman $\rho = 0.80$, $P < .001$). Pecan and walnut, and cashew and pistachio also correlated strongly (Spearman $\rho = 0.66$, $P < .001$; Spearman $\rho = 0.65$, $P < .001$).

Of 326 subjects sensitized to peanut and tested for all nine tree nuts, 303 (92.9% [95% CI, 89.6-95.3%]) were sensitive to at least one tree nut. In a subgroup of 264 birch-sensitized subjects, the co-sensitivity of peanut and at least one tree nut was 99.2% (95% CI, 97.3-99.8%), whereas in a subgroup of 62 without birch sensitization, 66.1% (95% CI, 53.7-76.6%) had co-sensitization to peanut and tree nuts ($P < .001$).

Nut sensitizations by age-group

At least four nut species were examined in 1 769 subjects. In subjects without birch sensitization, prevalences of sensitizations to all nut species decreased with age (Fig 6 and Table S1). In subjects with birch sensitivity, their hazelnut, almond, and peanut sensitizations remained high (Fig 6 and Table S1).

Sensitization to birch pollen among nut-sensitized populations between south and north

Overall prevalence of birch pollen sensitization was lower in the north (24.0% [95% CI, 19.6-28.4%] vs. 31.4% [95% CI, 31.1-31.7%] $P = .003$).

In the north, 141 subjects were positive for hazelnut, almond, or peanut and were tested also for birch pollen. In the south, similar population (hazelnut, almond, or peanut sensitization and available result for birch) comprised 18 558 subjects (Table S2).

In hazelnut-sensitized subjects, prevalence of birch sensitization was 91.3% (95% CI, 90.8-91.8%) in the south, and 89.7% (95% CI, 84.2-95.2%) in the north ($P = .51$). Of almond-sensitized subjects, 89.6% (95% CI, 89.1-90.1%) were birch sensitized in the south, and 84.9% (95% CI, 78.1-91.7%) in the north ($P = .15$), and of peanut-sensitized subjects, 82.8% (95% CI, 82.2-83.4%) in the south and 82.7% (95% CI, 74.1-91.2%) in the north ($P = 1.0$) had concurrent birch-pollen sensitization.

DISCUSSION

This is, to our knowledge, the largest study to date to examine cross-sensitization to nuts in population as high as 50 000 birch-sensitized and non-sensitized patients. Prevalence of sensitization to nuts is usually high in those with birch sensitization, but this study has the power to show that with higher levels of birch sensitization, an increasing proportion of tested subjects were sensitive to multiple nuts. Moreover, with increasing wheal size to birch pollen, nut wheal size increased. The most prominent co-sensitizations with birch pollen were hazelnut, almond, and peanut. A highly interesting finding was that non-birch-induced sensitization to nuts commonly begins in young children, with prevalences decreasing with age.

Hazelnut is an important symptom-eliciting food in birch-allergic individuals (16), supporting our finding of close linkage between these sensitizations. In the Finnish population, birch sensitization is mainly directed toward the pathogenesis-related 10 (PR-10) protein Bet v 1, which drives other sensitizations toward PR10-proteins. (17) (18) Among nuts, PR-10 proteins are characterized in hazelnut, almond, and peanut. (19)

The vast majority of our study subjects reported no symptoms or merely oral allergy syndrome from hazelnut, almond, and peanut. Monosensitization to Bet v 1-homologues Cor a 1 in hazelnut and Ara h 8 in peanut usually causes mild or no symptoms. (20) (21) Sensitization to seed storage proteins Cor a 9 and 14 in hazelnut and Ara h 2 and 6 in peanut associates with severe symptoms. (22) (23) Our symptom profile indicates that nut sensitization in birch-endemic areas is mostly pollen-related and without severe symptoms.

Our study is limited by the fact that we have evaluated mere sensitizations, with their clinical relevance assessed only in a subgroup of patients and based on reported symptoms without confirmation by challenge. Some patients may possibly experience more severe symptoms when challenged to high doses of nuts, though even the severe reactions reported were not confirmed by challenge. Further, our study population included subjects with clinical suspicion of birch and nut allergies and our data are thus subject to overestimation of those co-sensitizations. The findings of age-related changes can be regarded only as suggestive and may be skewed by any possible differences in patient selection or changes in population rather than at an individual level. Our research extends to the year 1997 and was designed to study cross-sensitizations in skin prick tests. We are limited by the lack of serum samples, and due to the large sample size we would be unable to analyze serum-specific IgE. In clinical use, however, component resolved diagnostics is adapted to discriminate cross-sensitization and true allergy.

Young children without birch sensitization had the highest sensitization prevalences to all nuts, but this decreased by age, perhaps because nut sensitization develops early and already during the first years of life begins to decrease. (24)

Subjects sensitized to birch showed, however, no decrease in sensitization prevalences to most nut species during childhood and adolescence, perhaps due to an increase in their pollen sensitization and in their subsequent cross-sensitizations to nuts. Asymptomatic sensitization to peanut increases until adulthood, whereas clinical reactivity increases only during early childhood. (25) (26) This suggests that asymptomatic peanut sensitization is secondary to pollen sensitization. Pollen-related allergy to hazelnut is also more common in adults than in children. (21) (27)

Our most prominent co-sensitizations were pecan and walnut from the family *Juglandaceae*, and cashew and pistachio from *Anacardiaceae* (28); skin prick tests and specific IgEs between these species link strongly. (29) (10) (30)

Almond, hazelnut, and peanut formed a group with birch. Sensitizations to seed storage proteins in hazelnut and peanut are mostly independent in a birch-endemic region; meaning that the cross-reactivity of these species relies on pan-allergens and primary pollen sensitization. (31)

Knowledge of cross-reactivities allows more detailed patient advising, although further studies with food challenges will be necessary.

In the north, sensitization to birch pollen was less common, in accordance with the lower exposure in Lapland. Southern Finland is an area with very high birch-pollen exposure, with markedly higher pollen counts than in most of Europe and the USA. (34) (35) For individuals with sensitivity to nuts, concurrent sensitization to birch was common in both regions. We found no significant differences between south and north, though our finding is affected by the small northern sample size. Peanut and hazelnut allergies associate more strongly with birch pollen sensitization in northern Europe than in southern Europe or the USA. (32) (33) (27) That sensitization to hazelnut and almond was slightly higher in the southern Finland may suggest that in the north, linkages between the most birch-pollen related nuts and birch are weaker.

One strength of our study is the great number of individuals examined. This allowed detailed assessment of levels of sensitization and their linkages and examination of individuals of all ages. The wide selection of nut species gives a comprehensive picture of the nuts most commonly consumed. Further, the data from Northern Finland make our results more

generalizable to other regions as we show the strong linkage between birch pollen and nut sensitizations in an area with less exposure and sensitization to birch pollen.

In conclusion, in birch-endemic areas, sensitization to birch pollen associates strongly with nut sensitizations, mostly with hazelnut, almond, and peanut. A major proportion of subjects sensitized to these nut species experience only mild or no symptoms. The strongest linkages among edible nuts occurred between cashew and pistachio, and pecan and walnut. Our results imply that patients sensitized to some nuts may be able to receive more specific directions about which nuts can be safely consumed and which should be avoided. Further clinical studies, preferably with double-blind controlled nut challenges, are warranted.

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References

- (1) Mullins RJ, Dear KB, Tang ML. Characteristics of childhood peanut allergy in the Australian Capital Territory, 1995 to 2007. *J Allergy Clin Immunol* 2009;**123**:689-693.
- (2) Fleischer DM, Conover-Walker MK, Matsui EC, Wood RA. The natural history of tree nut allergy. *J Allergy Clin Immunol* 2005;**116**:1087-1093.

(3) Worm M, Moneret-Vautrin A, Scherer K, Lang R, Fernandez-Rivas M, Cardona V, et al. First European data from the network of severe allergic reactions (NORA). *Allergy* 2014;**69**:1397-1404.

(4) Clark AT, Ewan PW. The development and progression of allergy to multiple nuts at different ages. *Pediatr Allergy Immunol* 2005;**16**:507-511.

(5) Flinterman AE, Hoekstra MO, Meijer Y, van Ree R, Akkerdaas JH, Bruijnzeel-Koomen CA, et al. Clinical reactivity to hazelnut in children: association with sensitization to birch pollen or nuts?. *J Allergy Clin Immunol* 2006;**118**:1186-1189.

(6) Warm K, Backman H, Lindberg A, Lundback B, Ronmark E. Low incidence and high remission of allergic sensitization among adults. *J Allergy Clin Immunol* 2012;**129**:136-142.

(7) Osterballe M, Hansen TK, Mortz CG, Bindslev-Jensen C. The clinical relevance of sensitization to pollen-related fruits and vegetables in unselected pollen-sensitized adults. *Allergy* 2005;**60**:218-225.

(8) Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Rumpold H, et al. Four recombinant isoforms of Cor a I, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993;**212**:355-362.

(9) de Leon MP, Drew AC, Glaspole IN, Suphioglu C, Rolland JM, O'Hehir RE. Functional analysis of cross-reactive immunoglobulin E antibodies: peanut-specific immunoglobulin E sensitizes basophils to tree nut allergens. *Clin Exp Allergy* 2005;**35**:1056-1064.

(10) Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *J Allergy Clin Immunol* 2008;**122**:145-151.

(11) Hostetler TL, Hostetler SG, Phillips G, Martin BL. The ability of adults and children to visually identify peanuts and tree nuts. *Ann Allergy Asthma Immunol* 2012;**108**:25-29.

(12) Johnson J, Malinovschi A, Alving K, Lidholm J, Borres MP, Nordvall L. Ten-year review reveals changing trends and severity of allergic reactions to nuts and other foods. *Acta Paediatr* 2014;**103**:862-867.

(13) Haahtela T, Burbach GJ, Bachert C, Bindslev-Jensen C, Bonini S, Bousquet J, et al. Clinical relevance is associated with allergen-specific wheal size in skin prick testing. *Clin Exp Allergy* 2014;**44**:407-416.

(14) Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: Summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium *J Allergy Clin Immunol* 2006;**117**:391-7.

(15) Official Statistics of Finland (OSF): Population structure [e-publication]. ISSN=1797-5395. Helsinki: Statistics Finland [referred: 22nd June 2015]. Access method: http://tilastokeskus.fi/til/vaerak/tau_en.html.

(16) Geroldinger-Simic M, Zelniker T, Aberer W, Ebner C, Egger C, Greiderer A, et al. Birch pollen-related food allergy: clinical aspects and the role of allergen-specific IgE and IgG4 antibodies. *J Allergy Clin Immunol* 2011;**127**:616-22.e1.

(17) Moverare R, Westritschnig K, Svensson M, Hayek B, Bende M, Pauli G, et al. Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol* 2002;**128**:325-335.

(18) Tolkki L, Alanko K, Petman L, Skydtsgaard MB, Milvang PG, Seppala U, et al. Clinical characterization and IgE profiling of birch (*Betula verrucosa*)-allergic individuals suffering from allergic reactions to raw fruits and vegetables. *J Allergy Clin Immunol in Practice* 2013;**1**:623-31.e1.

(19) Masthoff LJ, Hoff R, Verhoeckx KC, van Os-Medendorp H, Michelsen-Huisman A, Baumert JL, et al. A systematic review of the effect of thermal processing on the allergenicity of tree nuts. *Allergy* 2013;**68**:983-993.

(20) Asarnoj A, Nilsson C, Lidholm J, Glaumann S, Östblom E, Hedlin G, et al. Peanut component Ara h 8 sensitization and tolerance to peanut. *J Allergy Clin Immunol* 2012;**130**:468-472.

(21) De Knop KJ, Verweij MM, Grimmelikhuijsen M, Philipse E, Hagendorens MM, Bridts CH, et al. Age-related sensitization profiles for hazelnut (*Corylus avellana*) in a birch-endemic region. *Pediatr Allergy Immunol* 2011;**22**(1 Pt 2):e139-49.

(22) Masthoff LJ, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J Allergy Clin Immunol* 2013;**132**:393-399.

(23) Klemans RJB, Knol EF, Bruijnzeel-Koomen CAFM, Knulst AC. The diagnostic accuracy of specific IgE to Ara h 6 in adults is as good as Ara h 2. *Allergy* 2014;**69**:1112-1114.

(24) Peters RL, Allen KJ, Dharmage SC, Koplin JJ, Dang T, Tilbrook KP, et al. Natural history of peanut allergy and predictors of resolution in the first 4 years of life: A population-based assessment. *J Allergy Clin Immunol* 2015;**135**:1257-66.e1-2.

(25) Arshad SH, Venter C, Roberts G, Dean T, Kurukulaaratchy R. The natural history of peanut sensitization and allergy in a birth cohort. *J Allergy Clin Immunol* 2014;**134**:1462-1463.e6.

(26) Wickman M, Asarnoj A, Tillander H, Andersson N, Bergstrom A, Kull I, et al. Childhood-to-adolescence evolution of IgE antibodies to pollens and plant foods in the BAMSE cohort. *J Allergy Clin Immunol* 2014;**133**:580-582.

(27) Datema MR, Zuidmeer-Jongejan L, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Hazelnut allergy across Europe dissected molecularly: A EuroPrevall outpatient clinic survey. *J Allergy Clin Immunol* 2015;**136**:382-91.

(28) THE ANGIOSPERM PHYLOGENY GROUP (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 2009;**161**:105-121.

(29) O'Sullivan MD, Somerville C. Cosensitization to orange seed and cashew nut. *Ann Allergy Asthma Immunol* 2011;**107**:282-283.

(30) Goetz DW, Whisman BA, Goetz AD. Cross-reactivity among edible nuts: double immunodiffusion, crossed immunoelectrophoresis, and human specific IgE serologic surveys. *Ann Allergy Asthma Immunol* 2005;**95**:45-52.

(31) Masthoff LJ, van Hoffen E, Mattsson L, Lidholm J, Andersson K, Zuidmeer-Jongejan L, et al. Peanut allergy is common among hazelnut-sensitized subjects but is not primarily the result of IgE cross-reactivity. *Allergy* 2015;**70**:265-274.

(32) Vereda A, van Hage M, Ahlstedt S, Ibanez MD, Cuesta-Herranz J, van Odijk J, et al. Peanut allergy: Clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol* 2011;**127**:603-607.

(33) Hansen KS, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009;**123**:1134-41, 1141.e1-3.

(34) Spieksma FTM, Corden JM, Detandt M, Millington WM, Nikkels H, Nolard N, et al. Quantitative trends in annual totals of five common airborne pollen types (*Betula*, *Quercus*, Poaceae, *Urtica*, and *Artemisia*), at five pollen-monitoring stations in western Europe. *Aerobiologia* 2003;**19**:171-184.

(35) Zhang Y, Bielory L, Georgopoulos PG. Climate change effect on *Betula* (birch) and *Quercus* (oak) pollen seasons in the United States. *Int J Biometeorol* 2014;**58**:909-919.

TABLE I. Prevalence ratios of nut sensitization in subjects with and without sensitization to birch pollen.

	Prevalence ratios	95% CI for prevalence ratios
Hazelnut	15.86	15.03 to 16.74
Almond	12.96	12.28 to 13.68
Peanut	8.31	7.97 to 8.66
Coconut	4.63	3.26 to 6.57
Macadamia	4.37	3.26 to 5.85
Pistachio	3.90	3.28 to 4.63
Walnut	3.78	3.08 to 4.63
Pecan	2.44	1.89 to 3.15
Brazil nut	2.30	1.76 to 3.01
Cashew	2.25	1.84 to 2.76

CI, Confidence interval

Figure legends

Figure 1. Flow chart of the study. All subjects with available results for birch and nut skin prick tests were selected. Age- and cross-reactivity analyses included subjects with at least four or all ten nut species examined. A subgroup of nut-sensitized patients was compared to a similar population from Lapland. A symptom survey was conducted in a separate group of nut-sensitized patients.

Figure 2. Sensitization prevalences to nut species stratified by birch pollen sensitization. $P < .001$ for difference between the two groups in all nut species.

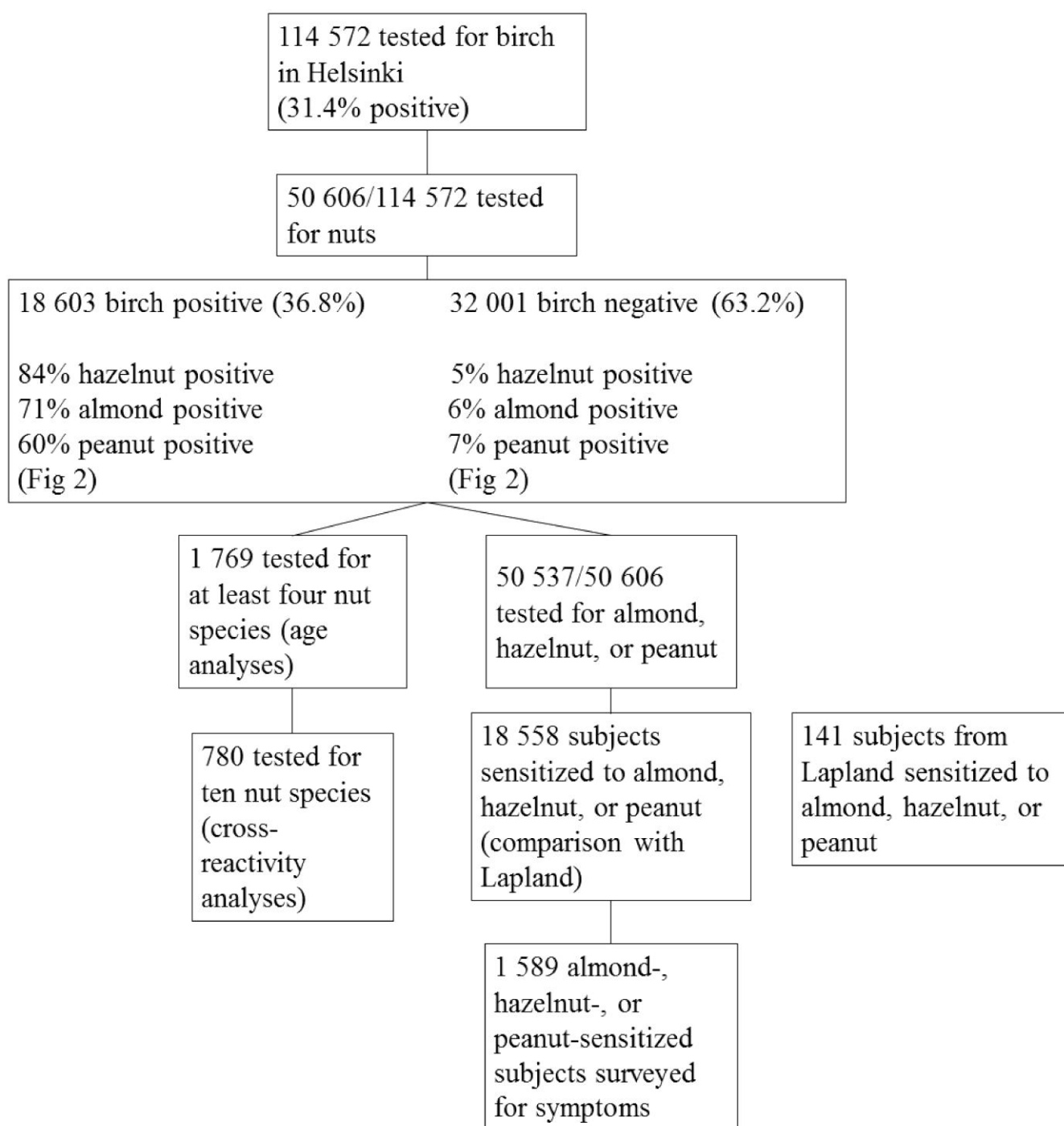
Figure 3. a) Wheal size for birch and percentage of subjects with at least three nut sensitizations. Wheal sizes below 3 mm are reported as zero.

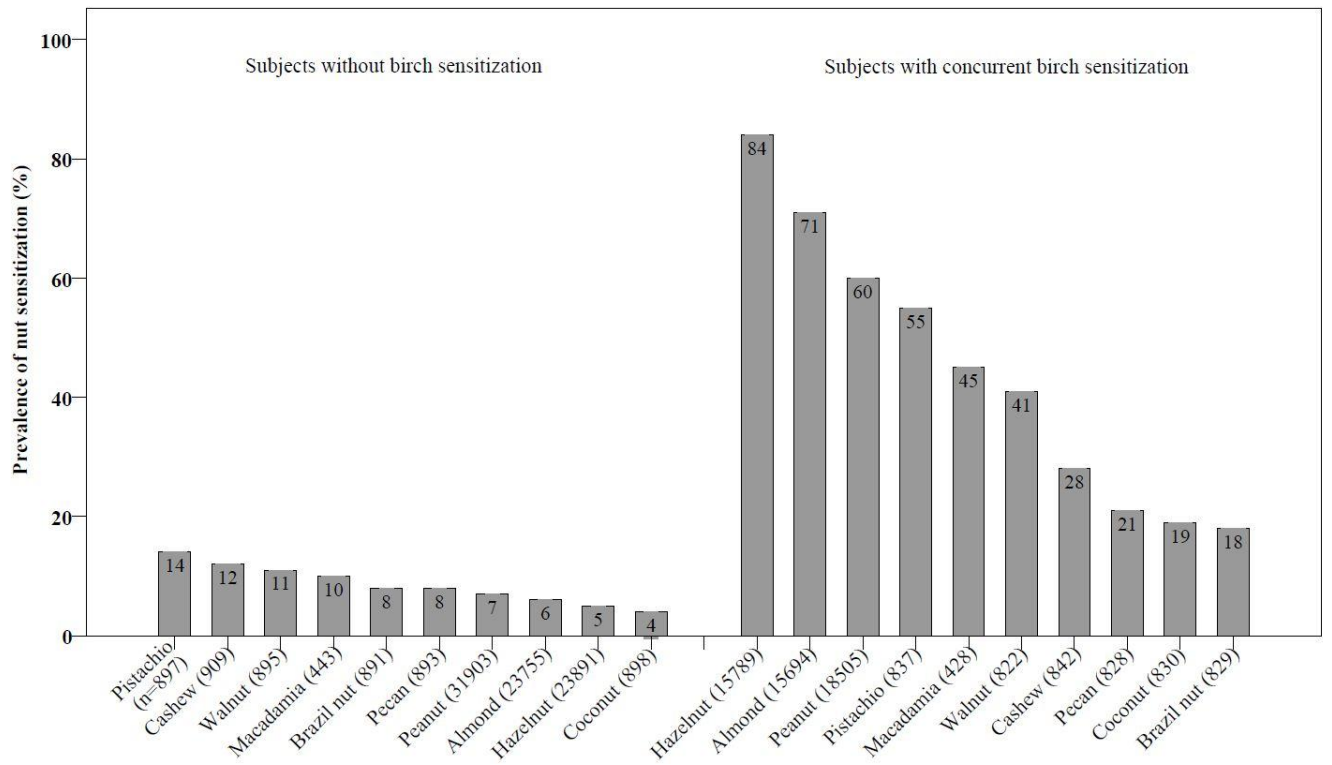
3. b) Mean wheal sizes for hazelnut, almond, and peanut at different levels of birch sensitization. Correlations with birch: hazelnut, Spearman $\rho = 0.84$, $P < .001$; almond, Spearman $\rho = 0.74$ $P < .001$, and peanut, Spearman $\rho = 0.61$, $P < .001$.

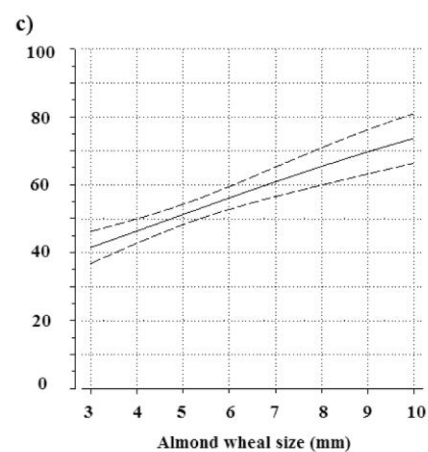
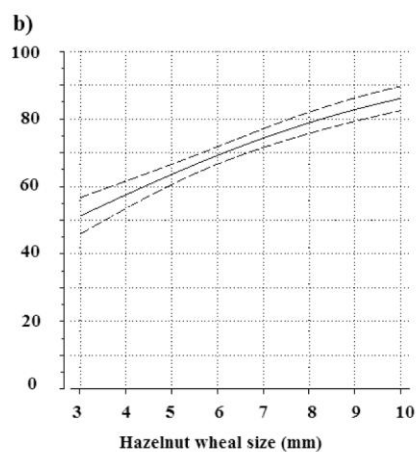
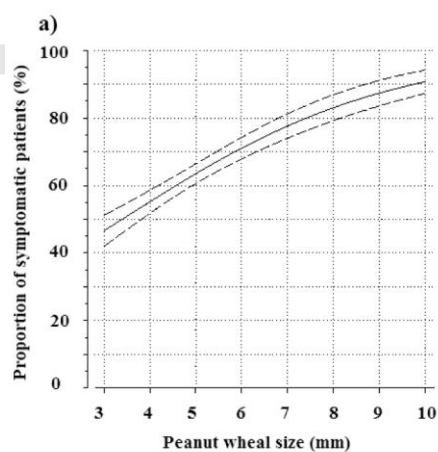
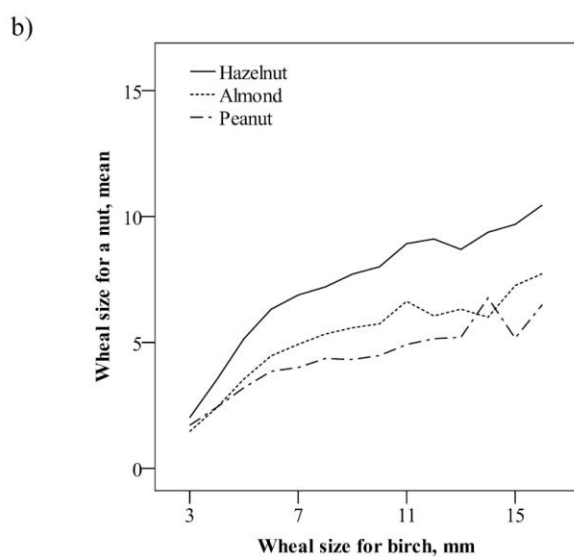
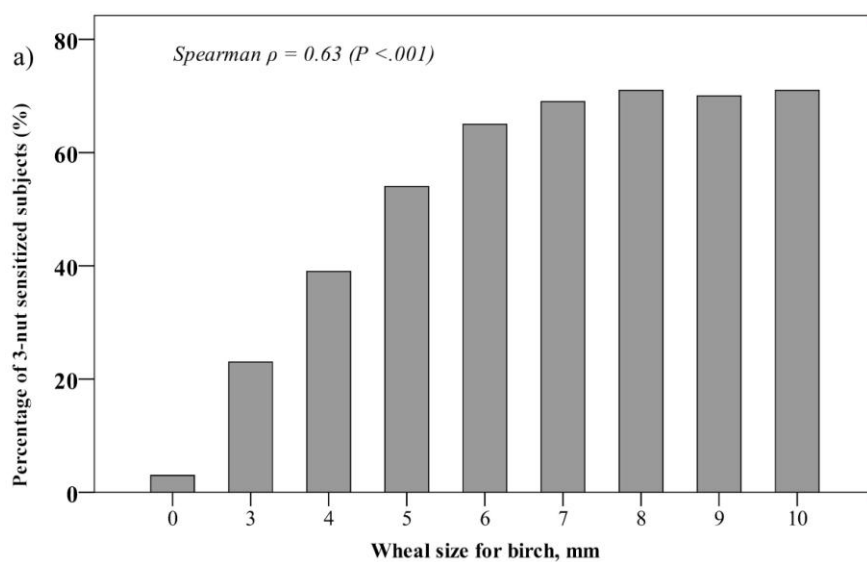
Figure 4. Skin prick wheal sizes and the proportion of symptomatic patients (%) sensitized to a) peanut, b) hazelnut, or c) almond. Confidence interval of 95% is shown as a dashed line.

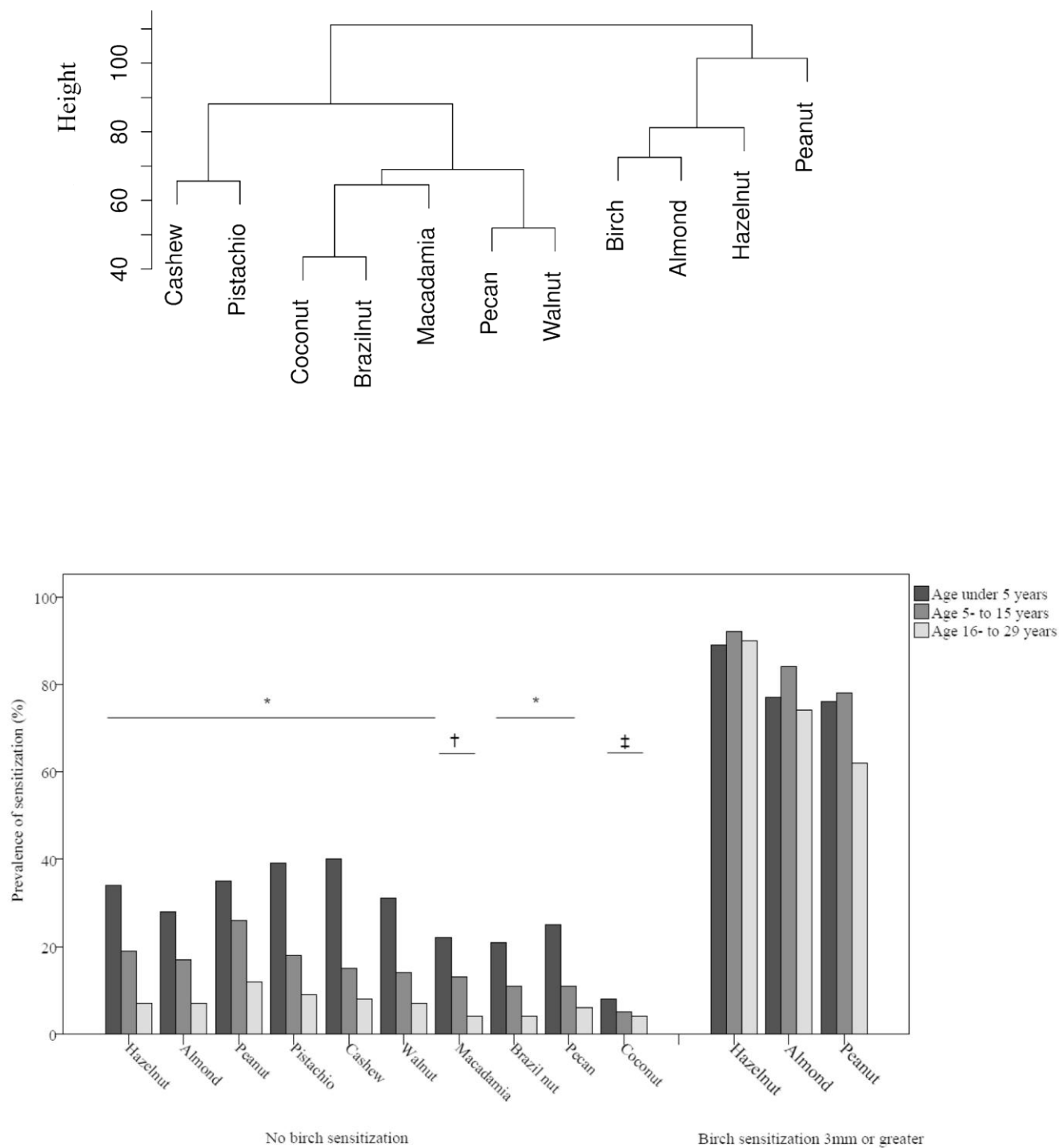
Figure 5. Hierarchical cluster analysis of nut species and birch pollen based on results of skin prick tests.

Figure 6. Prevalences of sensitizations to nut species in age groups stratified by birch pollen sensitization. * $P < .001$; † $P = .02$; ‡ $P = .40$. P values for trend, detailed information in Table S1.









		Under 5 years		5- to 15 years		16- to 29 years		P value*
		%	n	%	n	%	n	
No birch sensitization	Hazelnut	34	41	19	26	7	11	<.001
	Almond	28	31	17	22	7	11	<.001
	Peanut	35	46	26	38	12	20	<.001
	Pistachio	39	51	18	27	9	16	<.001
	Cashew	40	54	15	23	8	14	<.001
	Walnut	31	41	14	21	7	12	<.001
	Macadamia	22	18	13	11	4	3	.02
	Brazil nut	21	28	11	16	4	7	<.001
	Pecan	25	33	11	17	6	10	<.001
	Coconut	8	10	5	7	4	7	.40
		%	n	%	n	%	n	
Birch sensitization ≥ 3mm	Hazelnut	89	71	92	195	90	201	.95
	Almond	77	52	84	173	74	163	.15
	Peanut	76	64	78	182	62	139	.001

TABLE S 1. Prevalences of nut sensitizations by age-group

*P value for trend

1 **TABLE S 2.** Characteristics of nut-sensitized populations in two regions with differing birch pollen
 2 exposure

3

	South, n=18 558	North, n=141	P value
Age, y; median (IQR)	23 (11 -36)	24 (16 -33)	.13
Female, (%)	62	73	.01
Birch sensitization, n (%)	15446 (83)	118 (84)	.99
Hazelnut sensitization, n (%)	14547 (88)	117 (85)	.36
Almond sensitization, n (%)	12402 (75)	106 (79)	.48
Peanut sensitization, n (%)	13385 (72)	75 (54)	<.001

4 IQR, Interquartile range

