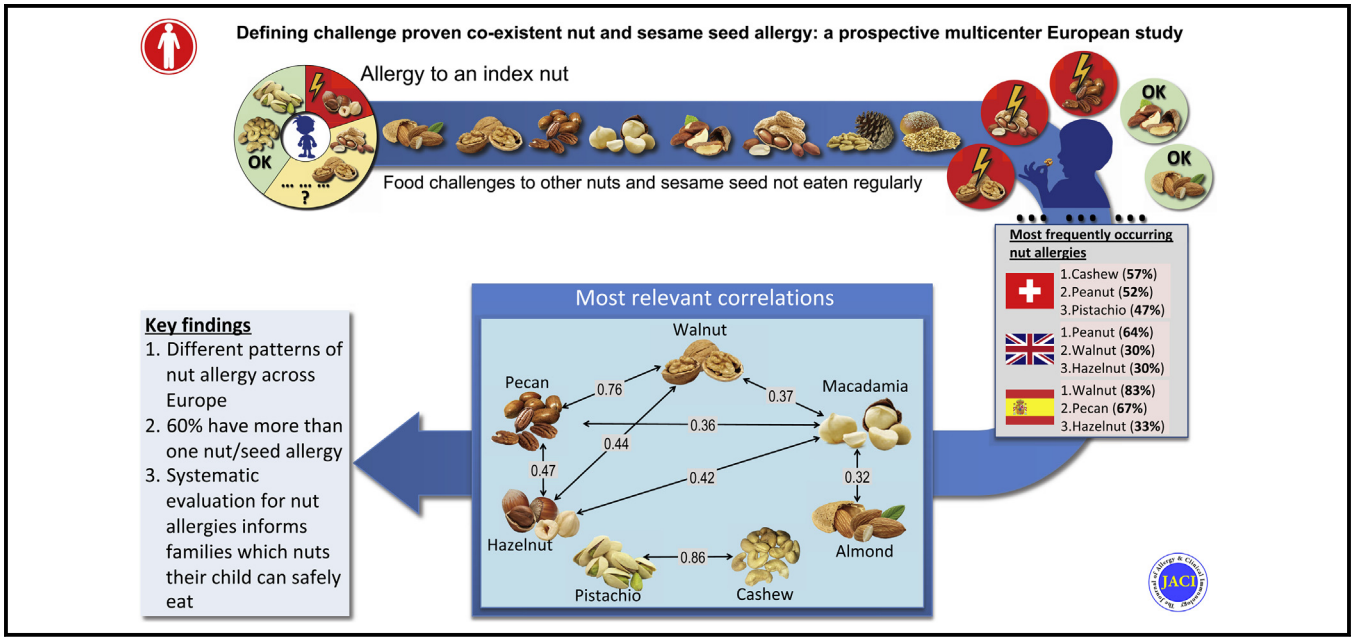
Defining challenge-proven coexistent nut and sesame seed allergy: A prospective multicenter European study

Helen A. Brough, FRCPCH, PhD,[a,b,c]* Jean-Christoph Caubet, MD,[d]* Angel Mazon, MD, PhD,[e] Diab Haddad, MRCPCH,[f] Marcel M. Bergmann, MD,[d] Jacqueline Wassenberg, MD,[g] Valentina Panetta, MSc,[h] Rosalynd Gourgey, RD, MSc,[a,b,c] Suzana Radulovic, MD,[a,b,c] Maria Nieto, MD,[e] Alexandra F. Santos, MD, PhD,[a,b,c,i] Antonio Nieto, MD, PhD,[e] Gideon Lack, MB BChir,[a,b,c] and Philippe A. Eigenmann, MD[d]

London and Chertsey, United Kingdom, Geneva, Switzerland, Valencia, Spain, Lausanne, Switzerland, and Rome, Italy

GRAPHICAL ABSTRACT



Background: Peanut, tree nut, and sesame allergies are responsible for most life-threatening food-induced allergic reactions. Rates of coexistent allergy between these foods have been from mostly retrospective studies that include only a limited number of tree nuts or were not based on oral food challenges.

Objective: The Pronuts study is a multicenter European study (London, Geneva, and Valencia) assessing the challenge-proven rate of coexistent peanut, tree nut, and/or sesame seed allergy.

Methods: Children aged 0 to 16 years with at least 1 confirmed nut or sesame seed allergy underwent sequential diagnostic food challenges to all other nuts and sesame seed.

Results: Overall, the rate of coexistent peanut, tree nut, and sesame seed allergy was 60.7% (n = 74/122; 95% CI, 51.4% to 69.4%). Peanut allergy was more common in London, cashew and pistachio nut allergies were more common in Geneva, and walnut and pecan allergies were more common in Valencia. Strong correlations were found between cashew-pistachio, walnut-pecan, and walnut-pecan-hazelnut-macadamia clusters. Age (>36 months) and center (Valencia > Geneva > London) were associated with an increased odds of multiple nut allergies. By pursuing the diagnostic protocol to demonstrate tolerance to other nuts, participants were able to introduce a median of 9 nuts.

Conclusion: We found a higher rate of coexistent nut and sesame seed allergies than previously reported. Performing sequential food challenges was labor intensive and could result in severe allergic reactions; however, it reduced dietary restrictions. Age was a significant predictor of multiple nut allergies, and thus the secondary spread of nut allergies occurred in older children. (J Allergy Clin Immunol 2020;145:1231-9.)

From [a]the Paediatric Allergy Group, Department of Women and Children's Health, King's College London; [b]the Paediatric Allergy Group, Peter Gorer Dept of Immunobiology, School of Immunology & Microbial Sciences, King's College London, Guys' Hospital; [c]the Children's Allergy Service, Evelina Children's Hospital, Guy's and St. Thomas' Hospital NHS Foundation Trust, London; [d]the Department of Pediatrics Gynecology and Obstetrics, Medical School of the University of Geneva, University Hospitals of Geneva; [e]the Unit of Pediatric Allergy and Pneumology, Children's Hospital La Fe, Institute of Health Research La Fe, Valencia; [f]St Peter's Hospital, Chertsey; [g]the Unit of Pediatric Allergy and Rheumatology, Department of Pediatrics, University Hospital of Lausanne; [h]L'altrastatistica srl, Consultancy & Training, Biostatistics Office, Rome; and [i]the Asthma UK Centre in Allergic Mechanisms of Asthma, London.

*These authors contributed equally to this work and are joint first authors.

Key words: *Peanut allergy, tree nut allergy, sesame seed allergy, multiple nut allergies, predictor, age, London, Geneva, Valencia*

Abbreviations used
AD: Atopic dermatitis
IQR: Interquartile range
OFC: Oral food challenge
OR: Odds ratio

Peanut and tree nut allergies are among the most common food allergies across ages (1.4%) and have increased in children in the United States (2.1%) and United Kingdom (3%, including sesame) over the last 3 decades.[1-3] According to a recent systematic review, the challenge-proven prevalence of tree nut allergy is 0% to 1.4%, and that of probable tree nut allergy is 0.05% to 4.9%.[4] In Europe there are clear geographic differences in the rate of sensitization to several nuts in adults: hazelnut sensitization was 1.3% in Iceland, 6.0% in Spain, and 17.8% in Switzerland, and walnut sensitization was 0.1% in Iceland, 5.6% in Switzerland, and 7.7% in Spain.[5] The EuroPrevall rate of challenge-proven tree nut allergy is currently being assessed.[6] Sensitization to nuts is affected by pollen sensitization because of cross-reactive allergens, and only a proportion of children sensitized to nuts will truly be allergic. Pollen sensitization also varies with geographic location.[7,8]

Anaphylactic and fatal food allergy reactions are most commonly caused by peanuts and tree nuts.[9-12] Sesame seed allergy is also associated with severe reactions.[13] Typically, children have allergy to peanut or a single tree nut early in life and then have multiple nut and seed allergies over time.[14-17] Data from a United Kingdom tertiary allergy center comprising 784 children showed a 23.5-fold increase in multiple nut allergies over a 12-year period (47% at age 14 years vs 2% in children aged from 0-2 years).[18] Research suggests that children with multiple food allergies are more likely to experience an allergic reaction: from 0.2 reactions per year in children with only 1 food allergy to 0.7 reactions per year in children with 2 food allergies and 3.4 reactions per year in children with 3 or more food allergies.[18] Thus having tree nut and sesame seed allergies, as well as peanut allergy, would clearly increase the risk of more frequent allergic reactions. Besides this, one of the major risk factors for life-threatening asthma is multiple food allergy.[19-21] Peanut allergy significantly reduces quality of life.[22,23] Having multiple versus single food allergies reduces health-related quality of life even further.[24]

Coexistent peanut and tree nut allergy was reported to be between 20% and 50% based on self-reported questionnaires, IgE test results (by specific IgE measurements or skin prick testing), or both.[25-28] However, questionnaire-based data and IgE test results might overestimate the rate of clinical allergy by overreporting allergic symptoms and cross-reactivity between peanut and tree nuts. The rate of coexistent peanut and tree nut allergy based on oral food challenges (OFCs) has been cited as less than 30%[29,30]; however, these studies were mainly conducted retrospectively[29,30] and included only a limited number of tree nuts.[31] Sesame seed allergy also often coexists with peanut allergy, 58% to 84% of children with sesame seed sensitization or reported sesame seed allergy were also sensitized or had reported allergic reactions to peanut,[2,13] and 25% of children with peanut allergy were reported to have sesame seed allergy.[2]

The primary aim of the Pronuts study was to prospectively determine the challenge-proven rate of coexistent allergy to peanut, 9 tree nuts, and sesame seed in children with at least 1 proven nut or seed allergy from 3 geographically distinct populations in Europe (London, Geneva, and Valencia) and to determine regional differences between the 3 centers. Our secondary aims addressed the feasibility and safety of performing challenges to up to 11 nuts/sesame seed in children. We also

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Corresponding author: Helen A. Brough, FRCPCH, PhD, Consultant in Paediatric Allergy, Children's Allergy Service, Evelina London, Guys' and St. Thomas' Hospital, South Wing, Stairwell B, Level 2, Westminster Bridge Rd, London SE1 7EH, United Kingdom. E-mail: Helen.Brough@gstt.nhs.uk.

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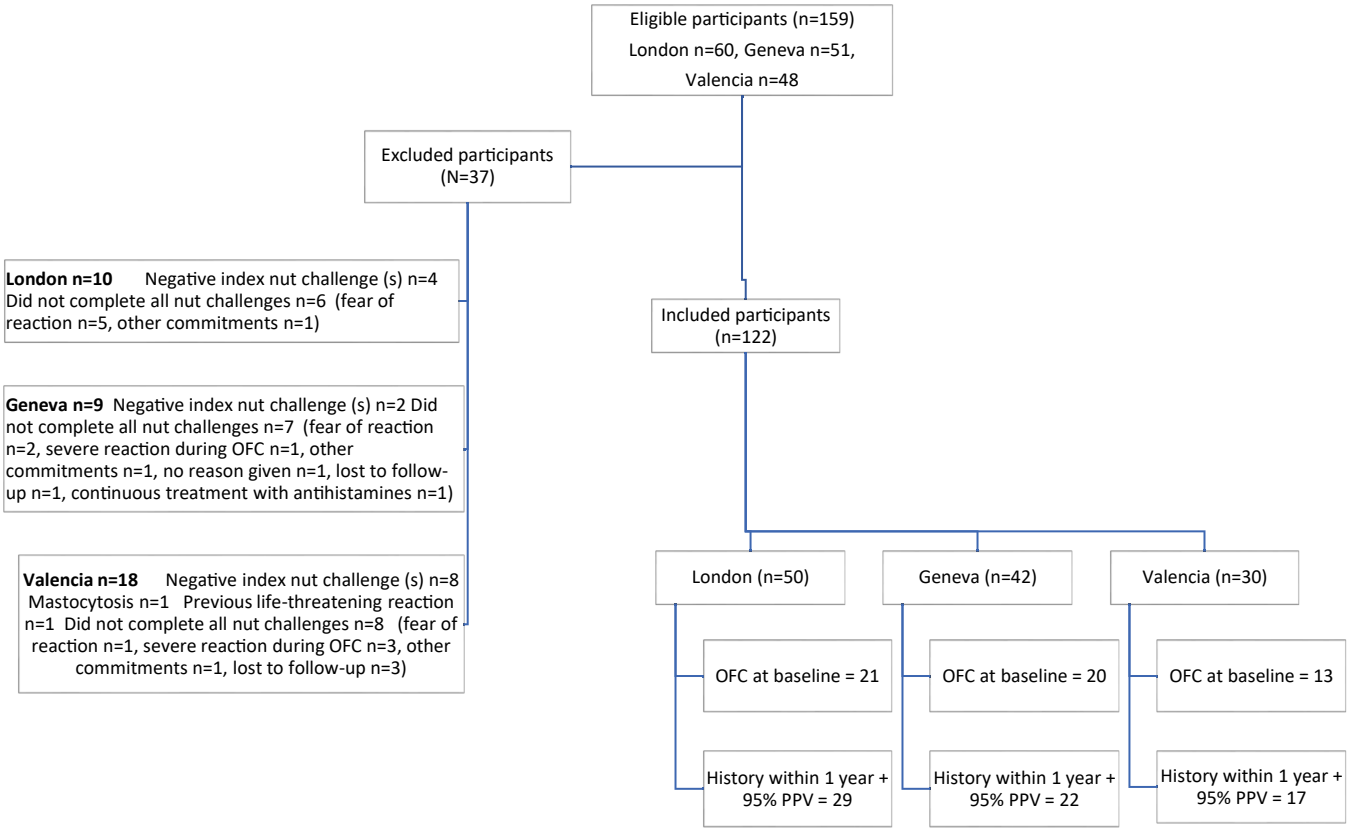

FIG 1. CONSORT figure for participants recruited to the Pronuts study. *PPV*, Positive predictive value.

assessed factors associated with multiple nut allergies and the severity and characteristics of reactions during food challenges.

METHODS

Study population

Under the auspices of the iPAC (international Pediatric Allergy and Asthma Consortium) research consortium,[32] we created the Pronuts research group from London, Geneva, and Valencia (ClinicalTrials.gov Identifier: NCT01744990). Ethical approval for the Pronuts study was obtained in London (14/LO/0066), Geneva (CER 12-020PS), and Valencia (2012/0108). Children aged 6 months to 16 years with 1 or more nut or seed allergies were recruited from allergy centers, and informed written consent was obtained. We recruited children with a positive open OFC result or convincing history of a systemic IgE-mediated allergic reaction to at least 1 index nut (peanut, hazelnut, walnut, almond, cashew, pistachio, pecan, Brazil nut, macadamia, and pine nut) or sesame seed in the last 12 months and diagnostic test results of greater than the 95% positive predicting value for the "index" nut or seed allergy.[33-36] Exclusion criteria were uncontrolled asthma, chronic urticaria, chronic systemic disease, daily antihistamine, or oral allergy syndrome only to the index nut. Children with a history of anaphylaxis to a nut or seed were included in the study but not those with a history of life-threatening anaphylaxis as defined by documented desaturation of less than 89%, hypotension (20% decrease in systolic blood pressure), or admission to a pediatric intensive care unit.[37]

Skin prick and specific IgE tests

Skin prick tests were performed with commercial extracts for peanut, hazelnut, almond, cashew, pistachio, pecan, Brazil nut, macadamia, pine nut (Stallergenes, Antony, France), fresh walnut, and tahini (sesame) paste (Meridien Foods, Winchester, United Kingdom) by using Stallerpoint plastic lancets with the standard method described (Stallergenes). Maximum wheal diameter and flare were recorded after 15 minutes. Specific IgE levels were quantified to all nuts and sesame seed extract by using ImmunoCAP (Thermo Scientific, Uppsala, Sweden).

OFCs

After confirmation of at least 1 nut or seed allergy, children underwent sequential OFCs to all other nuts and sesame seed unless they met the criteria outlined in Fig E1 in this article's Online Repository at www.jacionline.org. OFCs were performed based on the PRACTALL OFC guidelines, although starting at 30 mg up to a cumulative dose of 4.43 g of nut protein for each nut (3.43 g for children <36 months, see Table E1 in this article's Online Repository at www.jacionline.org).[38] For further information on doses and material used during OFC, see this article's Online Repository at www.jacionline.org. We used a severity score for allergic symptoms during OFCs published by Sampson[39] and modified to fit the PRACTALL OFC criteria. Symptoms qualifying for each organ system (skin, upper respiratory tract, lower respiratory tract, gastrointestinal, and cardiovascular/neurological) were taken from the PRACTALL OFC outcome scoring criteria.[38]

Statistical analysis

We hypothesized a 40% rate of coexistent peanut, tree nut, and sesame seed allergy with a range of 95% CIs (using exact CIs) based on a sample size of 100 to 150 (see Table E2 in this article's Online Repository at www.jacionline.org). Percentages and 95% exact CIs were reported for all samples and by center. The rate of coexistent peanut, tree nut, and sesame seed allergy was compared by using the χ^2 test. The χ^2 test was also used to compare all categorical variables. Mann-Whitney and Kruskal-Wallis tests were used to compare age, age at onset, and number of nuts between 2 or more than 2 groups, respectively. Median values and interquartile ranges (IQRs) were

TABLE I. Baseline demographics

Clinical characteristics	All participants (n = 122)	London (n = 50)	Geneva (n = 42)	Valencia (n = 30)	*P* value
Age (y), median (IQR)	5.5 (3-10)	4.5 (2-9)	6.0 (4-10)	7.3 (5-12)	**.014**
Male sex, no. (%)	69 (56.6)	28 (56.0)	22 (52.4)	19 (63.3)	.649
Current AD, no. (%)	58 (47.5)	31 (62)	25 (59.5)	2 (6.7)	**.001**
Life-time history of AD,* no. (%)	83 (70.3)	39 (81.3)	36 (87.8)	8 (27.6)	**<.001**
AD age of onset (mo), median (IQR)	4.0 (2-6)	3.0 (2-6)	5.0 (3-6)	18.0 (12-36)	**.029**
Allergic rhinoconjunctivitis, no. (%)	52 (42.6)	22 (44)	22 (52.4)	8 (26.7)	.091
Asthma, no. (%)	38 (31.1)	13 (26)	18 (42.9)	7 (23.3)	.125
Allergic to a nonpeanut/tree nut/sesame food, no. (%)	46 (37.7)	23 (46)	15 (35.7)	8 (26.7)	.213

Values in boldface indicate statistical significance.
*Missing data in 4 patients

reported. Pearson correlation was calculated to evaluate different nut allergy clusters. Univariate logistic regression was used to compare risk factors for 1 nut allergy versus more than 1 nut allergy. In case of separability, penalized logistic regression was used. A logistic mixed model was used to evaluate the relationship between age in class and 1 nut allergy versus 1 or more nut allergies considering center as a random factor. Because of multiple OFCs in the same patient, the comparison between centers of severity and treatment of allergic reaction was made by using mixed models considering multiple data for the same patient (patients as random factor).

SAS 9.4 software (SAS Institute, Cary, NC) was used for all analyses. A *P* value of less than .05 was considered statistically significant.

RESULTS

We identified 159 potentially eligible participants (London, n = 60; Geneva, n = 51; Valencia, n = 48). After a baseline OFC, 16 children were excluded: 14 children were found not to be allergic to an index nut, and 2 children had mastocytosis or a previous life-threatening reaction (Fig 1). During the subsequent OFCs to other nuts and sesame seed, 21 children dropped out of the study because of fear of severe reactions (n = 8), severe reactions during OFCs (n = 4), other commitments (n = 3), continuous antihistamine use (n = 1), loss to follow-up (n = 4), and no reason given (n = 1). Thus dropout rates were 6 (11%) of 56 in London, 7 (14%) of 49 in Geneva, and 8 (27%) of 30 in Valencia. In total, 866 OFCs were performed; results of 238 (27.5%) were positive. In Valencia, after several severe allergic reactions to pecan and walnut, OFCs were performed to only either of these nuts, with the second OFC being withheld. Walnut and pecan allergies were therefore imputed based on allergy test results in Valencia (see Table E3 and the Results section in this article's Online Repository at www.jacionline.org). The median duration for conducting OFCs ranged from 3 to 6 months between centers (see Table E4 in this article's Online Repository at www.jacionline.org). Results are also displayed for positive OFCs for each nut overall (see Fig E2 in this article's Online Repository at www.jacionline.org), each nut within each center (see Table E5 in this article's Online Repository at www.jacionline.org) and per participant (see Fig E3 in this article's Online Repository at www.jacionline.org). Skin prick test responses and specific IgE results in children who underwent OFCs for the nonindex nut are displayed in Tables E6 and E7 in this article's Online Repository at www.jacionline.org, respectively.

Demographics

Median age was lower in London (4.5 years), followed by Geneva (6 years) and Valencia (7.3 years, Table I). In Valencia children had a significantly lower rate of lifetime atopic dermatitis (AD; $P < .001$), and in Geneva they had a trend toward higher asthma rates ($P = .125$). There were no differences in sex, allergic rhinoconjunctivitis, or other food allergies. Children less than 6 years of age were more likely to have AD, and children 6 years or older were more likely to have asthma and allergic rhinitis (see Table E8 in this article's Online Repository at www.jacionline.org).

Primary end point

Overall, the proportion of participants with more than 1 nut or sesame seed allergy was 60.7% (n = 74/122; 95% CI, 51.4% to 69.4%). The proportion of participants with more than 1 nut allergy was 48% in London (n = 24/50; 95% CI, 33.6% to 62.6%), 66.7% in Geneva (n = 28/42; 95% CI, 50.4% to 80.4%), and 73.3% in Valencia (n = 22/30; 95% CI, 54.1% to 87.7%). The median (IQR) number of nut/seed allergies in London was 1 (IQR, 1-3) versus 2 (IQR, 1-4) in Geneva and 2 (IQR, 1-3) in Valencia. The maximum number of nut or seed allergies was 9. Single nut allergy was present in 39.3% (n = 48/122) of the whole cohort, ranging from 52% (n = 26/50) in London to 26.7% (n = 8/30) in Valencia.

Types of nut allergies overall and between centers

Overall, peanut allergy (49%) was the most common allergy, followed by walnut (42.6%) and cashew nut (34.4%, Table II and see Fig E4 in this article's Online Repository at www.jacionline.org); however, this might be due to London having the largest number of participants in the study and peanut allergy being more common in London. Cashew and pistachio nut allergy were the most common nut allergies in Geneva, and walnut and pecan allergy were more frequent in Valencia. If all centers contributed the same number of patients (n = 50), assuming that the proportion of different nuts would stay the same, then walnut allergy would have been the most frequent nut allergy (47.2%), followed by peanut (45.4%) and then pecan allergy (35.7%). There were no cases of sesame seed allergy in Valencia. No significant differences were found in the type of nut allergy in older versus younger children (using 6- and 3-year cutoffs). The most frequent index nut allergy (nut or seed allergy to which the child presented to the study) was peanut, followed by walnut, hazelnut, and then cashew (see Table E9 in this article's Online Repository at www.jacionline.org). Sesame seed, Brazil nut, pistachio, almond, macadamia, and pine nut (in descending order) were seldom the index nut allergy, and pecan was never the index nut allergy.

TABLE II. Comparison of all nut allergies in the 3 centers

	All participants (n = 122)	London (n = 50)	Geneva (n = 42)	Valencia (n = 30)	*P* value
Peanut	60 (49.2)	32 (64)	22 (52.4)	6 (20)	**<.001**
Walnut	52 (42.6)	15 (30)	12 (28.6)	25 (83.3)	**<.001**
Cashew nut	42 (34.4)	14 (28)	24 (57.1)	4 (13.3)	**<.001**
Pecan	39 (32)	12 (24)	7 (16.7)	20 (66.7)	**<.001**
Hazelnut	39 (32.0)	15 (30)	14 (33.3)	10 (33.3)	.927
Pistachio	36 (29.5)	12 (24)	20 (47.6)	4 (13.3)	**.004**
Macadamia nut	14 (11.5)	5 (10)	4 (9.5)	5 (16.7)	.588
Sesame seed	12 (9.8)	7 (14)	5 (11.9)	0	.084
Brazil nut	10 (8.2)	5 (10)	4 (9.5)	1 (3.3)	.533
Pine nut	6 (4.9)	1 (2)	2 (4.8)	3 (10)	.277
Almond	5 (4.1)	1 (2)	2 (4.8)	2 (6.7)	.574

Data are shown as numbers (percentages) for each center. Values in boldface indicate statistical significance.

TABLE III. Comparison of nut(s) ingested regularly before inclusion between centers

Nut(s) ingested regularly before inclusion	All participants (n = 122)	London (n = 50)	Geneva (n = 42)	Valencia (n = 30)	*P* value
Sesame seed	56%	46%	40%	93%	<.001
Almond	39%	20%	48%	60%	.227
Hazelnut	35%	12%	45%	60%	<.001
Pine nut	28%	26%	12%	53%	<.001
Peanut	25%	8%	26%	53%	<.001
Pistachio	16%	8%	14%	33%	.011
Cashew nut	16%	16%	10%	23%	.279
Walnut	11%	8%	17%	10%	.412
Pecan	6%	8%	2%	7%	.498
Brazil nut	3%	4%	0%	7%	.227
Macadamia nut	1%	0%	2%	0%	.590

Nuts or sesame seed consumed before study entry

Sesame seed was the most highly consumed nut or seed before study entry (55.7%), followed by almond, hazelnut, and pine nut (Table III and Fig E5 in this article's Online Repository at www.jacionline.org). Pecan, Brazil nut, and macadamia nut were eaten infrequently before study entry in all centers, which reflects the limited representation of these nuts as index nuts. In Valencia more than 50% of participants were already eating almond, pine nut, peanut, and hazelnut before study entry, and 93% were already eating sesame seed. London had the lowest rate of nut consumption before study entry except for sesame seed, pine nut, and almond. Sesame seed was more commonly consumed before study entry in older children, and there was a trend for peanut, pecan, pistachio, and pine nut to be consumed more often in older children (Table E10 in this article's Online Repository at www.jacionline.org).

Predictors of multiple nut allergies

Age (>36 months) and center (Valencia > Geneva > London) were associated with an increased odds of multiple nut allergies (Table IV); the relationship between age (>36 months) and multiple nut allergies was still significant if we considered center as a random factor in a logistic mixed model (OR, 2.94; 95% CI, 1.04-8.32; $P = .042$). There were no significant associations between the number of nut allergies and the sex of the child, AD, allergic rhinoconjunctivitis, or asthma. In Geneva having more nonnut allergies was associated with multiple nut allergies, but this was not found in other centers. The most common nut allergy in children with a single nut allergy was peanut (n = 31), followed by hazelnut (n = 5). In our study population macadamia nut, pistachio, and pecan allergy were only present in children with 3 or more nut allergies. Children with 3 or more nut allergies often had hazelnut or walnut as their index nut allergy. If children were already eating cashew, peanut, walnut, or pecan before study entry, they were likely to be allergic to fewer nuts.

Nut clusters

The number of coexistent nut allergies in the study population is compared in Fig E6 in this article's Online Repository at www.jacionline.org, and the coexistent nut allergies if a participant already had 1 nut allergy is described in percentages (see Table E11 in this article's Online Repository at www.jacionline.org), ORs (Table V), and correlation coefficients (Fig 2). Overall, cashew and pistachio were the most highly correlated nut allergies ($r = 0.86$; OR, 585), followed by walnut and pecan allergy ($r = 0.76$; OR, 150.6). Almost all (97% [n = 35/36]) children with pistachio allergy were allergic to cashew, but only 83.3% of children allergic to cashew were allergic to pistachio. Almost all (97% [n = 38/39]) children with pecan allergy were allergic to walnut, but only 75% of children with walnut allergy were allergic to pecan (further information is provided in the Results section in this article's Online Repository at www.jacionline.org). There was a cluster between macadamia nut, pecan, walnut, and hazelnut (see Fig E7 in this article's Online Repository at www.jacionline.org), with correlation ranging from an OR of 56 for macadamia nut–hazelnut to an OR of 11.5 for walnut-hazelnut (Table V). Peanut allergy was inversely related to hazelnut, walnut, pecan, and Brazil nut allergy. Correlations between cashew-pistachio and walnut-pecan were higher in London (see Fig E8, *A*, in this article's Online Repository at www.jacionline.org) than in Geneva (see Fig E8, *B*) and Valencia (see Fig E8, *C*).

Severity of allergic reactions

There was no significant difference in severity between centers by using the modified PRACTALL severity scoring system, although there was a trend toward Valencia having less severe reactions ($P = .119$). Conversely, epinephrine was used during 21 (40.4%) OFCs with positive results in Valencia, 20 (22%) in Geneva, and 2 (2%) in London (Table VI). In Valencia epinephrine was used for 12 grade 2 reactions, 5 grade 3 reactions, and 4 grade 4 reactions, whereas in London and Geneva epinephrine was used only for grade 4 reactions, apart from 1 grade 3 reaction. In London a dry frequent cough with normal oxygen saturation (grade 4) was treated with salbutamol, whereas in Geneva and Valencia this was treated with epinephrine. The rate of repeated epinephrine used in grade 4 reactions was 9.1% (n = 1/11) in London, 19% (n = 4/21) in Geneva, and 20% (n = 1/5) in Valencia.

Mean ± SD severity scores were highest for Brazil nut (3.1 ± 0.9) and lowest for pine nut (2.5 ± 0.7), hazelnut (2.5 ± 0.7), and almond (2.5 ± 0.5, see Table E12 in this article's Online Repository at www.jacionline.org); however, given the low number of OFCs for Brazil nut (n = 7), pine nut (n = 2), and almond (n = 6), no statistical comparisons were made. Nuts that most

TABLE IV. Predictors of multiple nut allergies (>1 nut allergy) in a univariate logistic regression model

	One nut allergy (n = 48)	More than 1 nut allergy (n = 74)	OR (95% CI)	*P* value
Male sex	56.3%	56.8%	1.02 (0.49-2.12)	.956
Current AD	52.1%	44.6%	0.74 (0.36-1.53)	.419
History of AD	52.4%	41.9%	0.65 (0.23-1.87)	.428
Age of AD onset	5.0 (2.0-9.0)	4.0 (2.0-6.0)	1.00 (0.95-1.04)	.860
Allergic rhinitis	43.8%	41.9%	0.93 (0.44-1.93)	.839
Asthma	27.1%	33.8%	1.37 (0.62-3.05)	.436
Nonnut allergy	37.5%	37.8%	1.01 (0.48-2.14)	.978
Age >6 y	39.6%	51.4%	1.61 (0.77-3.36)	.204
Age >3 y	70.8%	87.8%	2.97 (1.17-7.57)	**.022**
London (reference center)	52%	48%	1	
Geneva	33.3%	66.7%	2.17 (0.93-5.06)	.074
Valencia	26.4%	73.3%	2.98 (1.12-7.96)	**.029**

Values in boldface indicate statistical significance.

TABLE V. ORs for coexistent nut allergies for all centers

Nut combination	OR	95% CI	*P* value
Cashew nut–pistachio	585.2	31.7->9999.9	<.0001
Walnut-pecan	150.6	18.5-1228.3	<.0001
Hazelnut–macadamia nut	56.2	3.1-1024.0	.0007
Walnut–macadamia nut	20.4	2.4-171.3	.005
Pecan–macadamia nut	15.2	2.9-79.2	<.0001
Pecan–hazelnut	14.9	5.1-44.1	<.0001
Walnut-hazelnut	11.5	4.4-30.2	<.0001
Sesame–pine nut	9.1	1.15-71.8	.036
Macadamia nut–sesame seed	8.8	1.96-39.5	.005
Sesame seed–Brazil nut	4.8	1.0-22.7	.046
Walnut–Brazil nut	4.6	1.08-19.8	.039
Hazelnut–sesame seed	3.6	1.05-12.4	.04
Peanut-walnut	0.42	0.18-0.96	.04
Peanut-pecan	0.38	0.15-0.95	.039
Peanut-hazelnut	0.28	0.12-0.67	.004

commonly required epinephrine during a positive OFC were almond (33.3%, n = 2/6), macadamia nut (31.3%, n = 5/16), and walnut (30.6%, n = 11/36, see Table E13 in this article's Online Repository at www.jacionline.org); almond OFC epinephrine was used solely in Valencia, where 2 (67%) of 3 children with a positive almond OFC result required epinephrine. Pine nut was the only nut for which epinephrine was not administered during OFCs. Valencia had a higher rate of epinephrine use for walnut-, peanut-, and hazelnut-positive OFC results. Age, sex, asthma, allergic rhinitis, current AD, and increased number of nut or seed allergies were not associated with the severity of reactions on OFCs. The number of organ systems affected during allergic reactions on OFCs was highest in London, and sesame seed had the highest number of organ systems involved (see Table E14 in this article's Online Repository at www.jacionline.org).

DISCUSSION

The Pronuts study aim was to determine the challenge-proven rate of coexistent peanut, tree nut, and sesame seed allergies in 3 geographically distinct regions. We found that 60.7% of children had more than 1 nut or seed allergy (n = 74/122; 95% CI, 51.4% to 69.4%). This is significantly higher than shown in previous studies; however, previous studies were retrospective (thus the patients selected for OFCs could have been to rule out allergy)[29] or included only a subset of tree nuts[30] and potentially were therefore more biased toward a population with a lower risk of coexistent nut allergies. Pursuing the diagnostic protocol to demonstrate tolerance to other nuts helped relax the dietary restrictions with a median of 9 nuts introduced. Performing multiple OFCs in children allergic to peanut, tree nut, and sesame seed was feasible, and analysis of the nut butters used for OFCs showed that there was unlikely to be contamination of the nut to which the child was allergic in sufficient concentration to cause an allergic reaction on OFCs (see the Methods section in this article's Online Repository at www.jacionline.org). However, OFCs carried the risk of anaphylaxis, and 8.2% (n = 12/145) of children dropped out because of fear of or presence of previous severe reactions. There were important regional differences between our 3 centers, including numbers and clusters of nut allergies and severity of reactions during challenges, age, and history of AD (see the Discussion section in this article's Online Repository at www.jacionline.org). Age and study center were the most important predictors of multiple nut allergies.

The most common nut allergies (in descending order) in London were peanut, followed by walnut, hazelnut, and cashew; those in Geneva were cashew, peanut, pistachio, and hazelnut; and those in Valencia were walnut, pecan, hazelnut, and peanut. Sesame seed allergy was not found in Valencia, which might be explained by the high rate (93%) of children already eating sesame seed before study entry. Pecan was never the index nut allergy. The frequency of index nut allergies is a function of the nut allergy prevalence of the region and frequency at which the child is likely to eat the nut. Given that pecan nut allergy was not infrequent, the fact that pecan nut was never the index nut is more likely due to the lack of opportunity to eat it in the region tested.

We found clustering of cashew and pistachio and walnut and pecan allergies across all study centers, as shown in previous studies.[31] Interestingly, walnut and pecan also clustered with hazelnut and macadamia. This could have important ramifications with regard to cross-tolerance, potentially because of homologous proteins. Importantly, cashew and walnut seemed to be the dominant allergen with respect to their partners pistachio and pecan, as in other studies.[40]

Despite geographic differences, age (>36 months) was a significant predictor of multiple nut allergies, possibly explaining

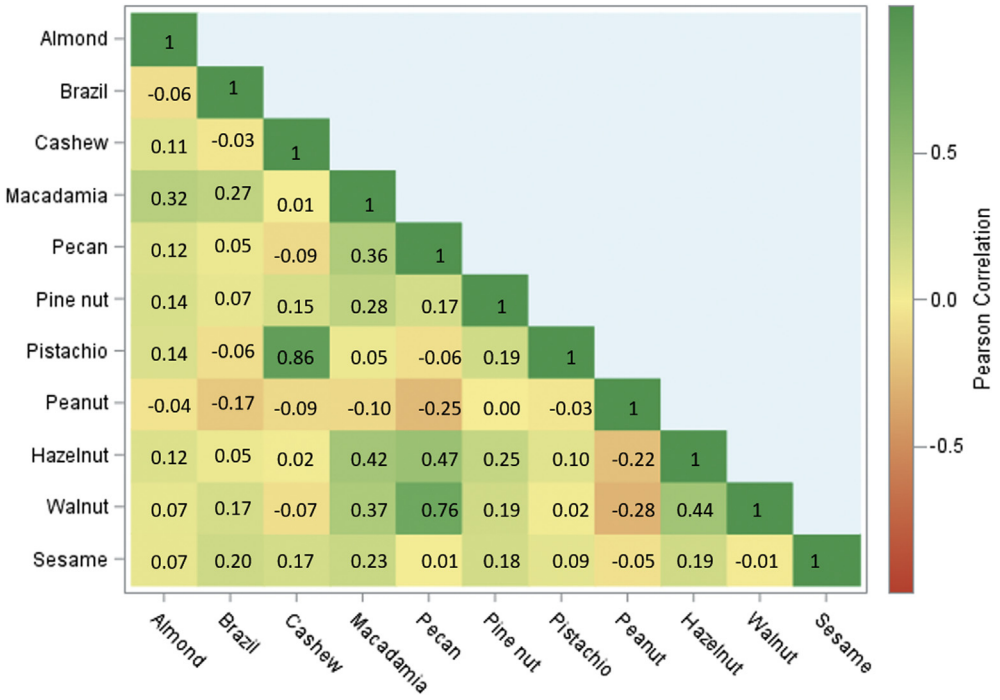

FIG 2. Correlation coefficients between nut and sesame seed allergies in all centers.

the lower rate of multiple nut allergy in London, which had the youngest participants. Cross-sectional and longitudinal studies have also demonstrated the secondary spread of nut allergies over time; in a tertiary London allergy clinic,[5] all infants were monosensitized to peanut (SPT ≥ 3 mm), but from the teenage years, none were monosensitized to peanut. The HealthNuts longitudinal study found an increase in tree nut allergy from parent-reported tree nut allergy (0.1%; 95% CI, 0.04% to 0.2%) at age 1 year to challenge-confirmed tree nut allergy (3.3%; 95% CI, 2.8% to 4.0%) at age 6 years.[4] In the Pronuts study asthma was not associated with multiple nut allergies, which was contrary to previous studies showing that asthma is associated with a higher rate of multiple food allergies.[41] Center was another important predictor of multiple nut allergies; this might be partly because in centers with higher rates of coexistent allergies, the most common nut allergies were those that usually occur in pairs. These were walnut allergy in Valencia and cashew nut allergy in Geneva, whereas in London peanut allergy was more likely to occur on its own. The difference might also be due to differences in eating habits, environmental exposure to nuts, and age of introduction to nuts.

Use of epinephrine in the management of allergic reactions during OFCs has been noted to vary between centers previously, ranging from 1.6% to 39.2%,[42] and, indeed, we also observed this in the Pronuts study. Use of epinephrine during OFCs was higher in Valencia, followed by Geneva and then London, whereas there was a trend toward less severe OFC reactions in Valencia. In Valencia children with lower severity scores (grade 2 and 3) were administered epinephrine if they had had a previous severe reaction to a nut (particularly walnut and pecan) and if they had generalized urticaria or significant abdominal pain, even in the absence of other symptoms. Additionally, the PRACTALL OFC guideline allows clinicians to repeat doses during OFCs when there might not be a clear allergic reaction. In London OFC doses were repeated more frequently (in 54 OFCs) than in Geneva (in 27 OFCs), and doses were repeated only once in Valencia, which could have also influenced the pattern of reaction and response to treatment.

Brazil nut, sesame, and macadamia nut OFCs had the highest symptom severity scores and had proportionally more involvement of the lower respiratory tract and/or cardiovascular/neurological system than other nuts (see Fig E9 in this article's Online Repository at www.jacionline.org). Pine nut, hazelnut, and almond allergic reactions were associated with the lowest severity scores. However, it should be noted that the low number of OFCs for Brazil nut, sesame, almond, and pine nut make these findings less certain, and more research should be performed to confirm this. A previous United Kingdom study showed that a larger proportion of children with Brazil nut allergy had more severe reactions compared with those with peanut allergy.[35] Comorbidities, such as asthma and other food allergies, were not associated with increased severity of reactions, in contrast with previous studies.[43]

Strengths of the Pronuts study include that it was a prospective study evaluating the challenge-proven rate of peanut, tree nut, and sesame seed allergy. In contrast to previous studies evaluating coexistent nut allergies, the Pronuts study assessed peanut, all 9 tree nuts, and sesame. Additionally, OFCs used large cumulative and top doses in all ages, which provided more certainty of the allergic and nonallergic status of the child. Our study was conducted in different European centers by using the same study protocol, which enabled us to compare regional differences.

A main limitation of this study was that, because of safety concerns, our center in Valencia did not perform OFCs to pecan and walnut in all cases, and walnut and pecan allergies were

TABLE VI. Comparison of severity and treatment received for allergic reactions during challenges across centers

Positive OFC result	Total (n = 238)	London (n = 97)	Geneva (n = 90)	Valencia (n = 51)	*P* value*
Severity parameters					
Organs affected (mean ± SD)	2.6 ± 1.0	2.9 ± 0.8	2.5 ± 1.0	2.0 ± 0.7	**<.001**
1	31 (13.0)	6 (6.2)	15 (16.7)	10 (19.6)	
2	86 (36.1)	19 (19.6)	35 (38.9)	32 (62.7)	
3	79 (33.2)	51 (52.6)	21 (23.3)	7 (13.7)	
4	39 (16.4)	19 (19.6)	18 (20)	2 (3.9)	
5	3 (1.3)	2 (2.1)	1 (1.1)	0	
Severity score (mean ± SD)	2.7 ± 0.7	2.8 ± 0.6	2.7 ± 0.8	2.5 ± 0.7	.119
1 (mild)	2 (0.8)	1 (1)	0	1 (2.0)	
2 (moderate)	104 (43.7)	27 (27.8)	48 (53.3)	29 (56.9)	
3 (moderate-severe)	95(39.9)	58 (59.8)	21 (23.3)	16 (31.4)	
4 (severe)	37(15.5)	11 (11.3)	21 (23.3)	5 (9.8)	
Medication					
Epinephrine	43 (18.1)	2 (2.1)	20 (22.2)	21 (41.2)	**<.001**
Antihistamines	210 (88.2)	95 (97.9)	77 (85.6)	38 (74.5)	**<.001**
Corticosteroids	23 (9.7)	3 (3.1)	12 (13.3)	8 (15.7)	**.026**
Salbutamol	29 (12.2)	17 (17.5)	10 (11.1)	2 (3.9)	.119
Oxygen	2 (0.8)	2 (2.1)	0	0	.233
Intravenous fluids	1 (0.4)	0	1 (1.1)	0	.457
None	22 (9.2)	0	13(14.4)	9 (17.6)	**<.001**

Data are shown as means ± SDs or numbers (percentages) for the rest of the variables. Values in boldface indicate statistical significance.
**P* values are from mixed models, taking into account multiple data for the same patient.

imputed based on allergy testing. Indeed, walnut had a high rate of epinephrine use (30.6%), particularly in Valencia (61.5%) compared with Geneva (20%) and London (7.7%). The Pronuts study was conducted in 3 tertiary centers, and therefore a higher rate of coexistent nut allergies might have been found due to more complex allergic patients being included in the study. We excluded children with life-threatening anaphylaxis, which might have biased our population against more severe allergic reactions on OFCs. Additionally, we did not capture ethnicity, which has an important role in the predisposition to allergy; however, considering ethnic differences between centers, further differences might have been difficult to interpret.[44]

The Pronuts study demonstrated a higher number of children with more than 1 nut allergy (60.7%) than originally hypothesized (40%). The process of sequential diagnostic OFCs was time and resource intensive, which has important implications for clinical practice. Importantly, OFCs carry the risk of a severe allergic reaction. Nevertheless, by performing sequential OFCs, we were able to demonstrate tolerance to a median of 9 nuts and/or sesame seed in a child with a pre-existing nut and sesame seed allergy. Age was a significant predictor of multiple nut allergies, and thus the secondary spread of nut allergies would seem to occur in older children. This opens up the possibility of preventing the spread of nut allergies through a randomized controlled study in toddlers who present with an index nut allergy. Indeed, peanut oral immunotherapy was more successful for desensitization and sustained unresponsiveness in toddlers.[45] Current practice in many centers is avoidance of all nuts in children with a known nut allergy. We are following up compliance with selective nut introductions to determine whether participants manage to regularly consume nuts to which they are deemed not to be allergic based on OFCs and will be monitoring the safety of selective nut eating in terms of whether this increases the risk of reactions to established nut allergies.

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Clinical implications: Selective nut eating is growing in momentum. The Pronuts study provides data on clinically relevant cross-reactivity between nuts and sesame seed and severity of reactions based on OFCs.

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