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Article type : Original Article: Experimental Allergy and Immunology

## CRD and beyond: multivariable regression models to predict severity of hazelnut allergy

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13328

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*Funding:* This work was funded by the European Commission under the 6th Framework Programme through EuroPrevall (FP6- FOOD-CT-2005-514000) and iFAAM (Grant agreement no. 312147), respectively.

*Abbreviations used*

CRD: Component-resolved diagnosis

DBPCFC: Double-blind placebo controlled food challenge

LTP: Lipid transfer protein

OAS: Oral allergy syndrome

OR: odds ratio

SPT: Skin prick test

Key words: EuroPrevall, iFAAM, allergen components, Cor a 9, Cor a 14, hazelnut allergy, severity, prediction

Key message:

Severe allergic symptoms to hazelnut are reproducibly associated with high IgE against Cor a 9 and 14 and low against Cor a 1, but predictive accuracy of stand-alone CRD is poor. A model that combines sensitization to Cor a 14 with atopic dermatitis (risk factor), pollen allergy (protective) and IgE against walnut (risk factor) significantly improves severity risk assessment.

### Acknowledgements:

We would like to thank all the patients for their participation in the study. We would like to thank ALK Abello (Madrid, Spain) for their generous gift of SPT reagents. We acknowledge the support by the 6<sup>th</sup> and 7<sup>th</sup> Framework Programs of the EU, for EuroPrevall (FP6-FOOD-CT-2005-514000) and iFAAM (Grant agreement no. 312147), respectively.

### Author contributions

|                                                                                  |                                                                                      |
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| ENCM, RvR                                                                        | Coordination of EuroPrevall project                                                  |
| MFR                                                                              | Coordinator EuroPrevall outpatient clinic survey Design of survey, protocol writing  |
| MRF, BBW                                                                         | Training of the clinical partners, anaphylaxis board                                 |
| BBW                                                                              | Coordination of the food challenges in EuroPrevall                                   |
| RA, FdB, SB, MC, RD, PF, DG, MJC, ACK, MLK, TZK, TML, TAP, AP, IR, SLS, MFR, BBW | Recruitment and clinical investigation of the patients<br>Food challenge performance |
| KHS                                                                              | Coordination allergen bank                                                           |
| JL                                                                               | Generation of reagents and experimental IgE assays                                   |
| LJ, SAV                                                                          | Performance of serological analyses (CRD)                                            |
| SV                                                                               | ImmunoCAP IgE testing (extract based)                                                |
| MFR, LB, CFP                                                                     | Database design, data collection and data cleaning                                   |
| LB, CFP, NdP, MFR                                                                | Monitoring data collection and data cleaning                                         |
| MD                                                                               | Statistical data analyses                                                            |
| AHZ                                                                              | Supervision of the statistical data analyses                                         |
| MD                                                                               | Writing of the manuscript                                                            |
| RvR, RA, MC, CFP, KHS, LJ, TML, JL, NGP, TAP, SJS, SV, AHZ, ENCM, MFR, BBW       | Critical review of the manuscript                                                    |

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## Abstract

**Background:** Component-resolved diagnosis (CRD) has revealed significant associations between IgE against individual allergens and severity of hazelnut allergy. Less attention has been given to combining them with clinical factors in predicting severity.

**Aim:** To analyze associations between severity and sensitization patterns, patient characteristics and clinical history, and to develop models to improve predictive accuracy.

**Methods:** Patients reporting hazelnut allergy (n=423) from 12 European cities were tested for IgE against individual hazelnut allergens. Symptoms (reported and during DBPCFC) were categorized in mild, moderate and severe. Multiple regression models to predict severity were generated from clinical factors and sensitization patterns (CRD- and extract-based). Odds ratios (OR) and areas under receiver operating characteristic (ROC) curves (AUC) were used to evaluate their predictive value.

**Results:** Cor a 9 and 14 were positively (OR 10.5 and 10.1 respectively), and Cor a 1 negatively (OR 0.14) associated with severe symptoms during DBPCFC, with AUCs of 0.70-0.73. Combining Cor a 1 and 9 improved this to 0.76. A model using a combination of atopic dermatitis (risk), pollen allergy

(protection), IgE against Cor a 14 (risk) and walnut (risk), increased the AUC to 0.91. At 92% sensitivity, the specificity was 76.3% and the positive and negative predictive values 62.2% and 95.7%, respectively. For reported symptoms, associations and generated models proved to be almost identical but weaker.

**Conclusion:** A model combining CRD with clinical background and extract-based serology is superior to CRD alone in assessing the risk of severe reactions to hazelnut, particular in ruling out severe reactions.

## Introduction

In the EuroPrevall project, symptoms and sensitization to hazelnut was most frequently reported and observed. (1–3) Symptoms can vary from mild oral symptoms to more severe symptoms such as bronchospasm and, in some cases, life-threatening anaphylaxis. An area that has drawn much attention in predicting the risk of severe reactions is the IgE recognition profile of individual allergen molecules in foods. These profiles, usually referred to as component-resolved diagnosis (CRD), may facilitate better distinction of patients with severe reactions from those with milder reactions, or reactive from tolerant among sensitized patients.

There is growing evidence that the seed storage proteins Cor a 9 (11S globulin) and Cor a 14 (2S albumin) are associated with more severe reactions(4–6) similarly to lipid transfer protein (LTP) Cor a 8 in the Mediterranean area.(7–9) In Northern and Central Europe, sensitization to the Bet v 1-homologue Cor a 1 is typically seen in adolescents and adults with sensitization to birch pollen and oral symptoms.(9–11) Less is known about the clinical relevance of profilin (Cor a 2) (12,13), Cor a 11 (vicilin-like protein) (3,14), and the oleosins Cor a 12 and Cor a 13.(15)

Although multiple studies have shown an association between severity and sensitization to specific hazelnut allergens (4–6,16,17), these reports are mostly in children and limited to patient populations with specific geographical background. To establish broader applicability of CRD, it is important to confirm these associations in larger populations with different geographical backgrounds. Additionally, it is relevant to evaluate the accuracy in classifying between mild-to-moderate allergy and severe allergy because markers associated with clinical outcomes, such as severity, are not necessarily good diagnostic tools as well.(18)

In clinical practice, a thorough clinical history is the starting point. Here we aim to investigate whether a combination of a detailed anamnesis and molecular and/or extract-based sensitization patterns can lead to better prediction of the risk of severe reactions. The EuroPrevall outpatient-clinic survey, in which detailed clinical histories and sensitization profiles were recorded in twelve European cities, allowed us to investigate this and validate factors related to severe hazelnut allergy

in a large and demographically diverse study population.(3,19) We evaluated clinical and serological data (24 foods, 12 inhalants and latex) on 731 outpatients that reported allergic symptoms to hazelnut and that were enrolled across Europe, using a standardized protocol. Molecular diagnostics using rCor a 1, rCor a 2, rCor a 8, nCor a 9, nCor a 11, rCor a 12, rCor a 14 and CCD was performed for 423 subjects. For 124 of these, a double-blind placebo-controlled food challenge (DBPCFC) was performed, allowing us to establish whether associations with severity during challenge were similar to those found for reported food allergy.

## METHODS

### *Patient selection*

In the outpatient clinic survey of the EuroPrevall project(19), subjects reporting allergic reactions to food were prospectively recruited between 2006 and 2009 in 12 outpatient clinics: Athens (Greece), Łódź (Poland), Madrid (Spain), Manchester (United Kingdom), Milan (Italy), Prague (Czech Republic), Reykjavik (Iceland), Sofia, (Bulgaria), Strasbourg (France), Utrecht (The Netherlands), Vilnius (Lithuania) and Zürich (Switzerland). Details are presented elsewhere.(3,19,20)

Here, we evaluate 731 subjects reporting immediate adverse reactions ( $\leq 2$ hrs) to hazelnut. Of these patients 652 were recruited in the outpatient clinics, the remaining 79 patients selected for detailed clinical evaluation during the EuroPrevall general population surveys.(3) There was no difference in reported symptom severity between both groups, and therefore the groups were analyzed together. For two patients, data concerning symptoms were missing, and they were excluded from statistical analyses. Of the remaining 729 patients, 120 (16.5%) were children ( $< 18$  years). Sera of 423 patients (12.5% children) were available for CRD, as detailed elsewhere.(3) Patients evaluated by CRD were older and had lower frequencies of an atopic family background and atopic dermatitis (AD) than those that were not. They also showed some differences in the severity classification (table S1).

### *Clinical evaluation and double blind placebo-controlled food challenges (DBPCFC)*

The protocol used for clinical evaluation has been described in detail elsewhere(19). In short, allergy specialists used standardized case-record forms for a detailed description of hazelnut-induced symptoms (type, age of onset, and duration), family members with atopy (father, mother, sibling) and of co-existing non-food-induced atopic co-morbidities (current or ever) including asthma, allergic rhinitis and AD. Patients were classified as pollen-, house dust mite- and/or latex-allergic when they reported respiratory symptoms to that source supported by matching sensitization.

All patients were asked to undergo a DBPCFC for hazelnut and 124 patients (18 <18 years) consented to do so. Hazelnut challenges were performed by trained physicians using the same protocol and challenge meals in all clinics.(21) Details are provided in this article's online repository. The backgrounds of patients undergoing DBPCFC were comparable to those not undergoing the procedure, except that they had a lower frequency of AD and slightly higher matching sensitization to hazelnut (table S2). Patients with positive DBPCFC (n=87) and patients with a convincing history of severe anaphylaxis(19) to hazelnut (n=22, excluded from DBPCFC) were considered true hazelnut allergic patients. From 1/87 patients, clinical data was not recorded and this patient was excluded in the statistical analyses. We classified symptoms (reported and during the challenge) as follows: isolated symptoms of the oral cavity as mild, symptoms of skin, upper airway and gastro-intestinal as moderate, and laryngeal, bronchial, cardiovascular and neurological symptoms as severe. The patients classified as "anaphylaxis" were included in the severe group.

#### *Specific IgE antibody measurements*

Hazelnut sensitization was evaluated by skin prick testing (SPT) and specific IgE (sIgE) in serum. Additionally, sIgE to 23 other foods, 12 inhalant allergens and latex was also measured. The descriptive outcomes of these analyses were reported previously.(3) In addition, CRD was performed for the following purified natural (n) or recombinant (r) hazelnut components produced and purified as described elsewhere(15,22–24): rCor a 1 (Bet v 1 homologue), rCor a 2 (profilin), rCor a 8 (LTP), nCor a 9 (11S globulin), rCor a 11 (7S vicilin), nCor a 12 (oleosin) and rCor a 14 (2S albumin). All sIgE measurements were performed by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). IgE levels  $\geq 0.35$  kU<sub>A</sub>/L were considered positive.

#### *Statistical analysis*

Differences in patient characteristics (age, sex, family member with atopy, atopic dermatitis and allergy to pollen, house dust mite and latex) of the 731 patients between mild, moderate and severe symptoms were evaluated by chi-square test and ANOVA.

Differences in IgE levels were analyzed using non-parametric tests (Mann-Whitney *U* and Kruskal-Wallis test). Multinomial logistic regression models were used to determine associations between severity and clinical characteristics, and sensitization to hazelnut (extract and allergens). Having mild symptoms was the reference category.

The ability of sIgE levels against hazelnut allergens to discriminate between mild-to-moderate and severe symptoms was evaluated by receiver operating characteristic (ROC) analysis and sensitivity, specificity, positive and negative predictive values (PPV and NPV) were determined. Because the main focus was prediction of severe reactions, patients with mild and moderate

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reactions to hazelnut were grouped together. Additionally, we generated four multivariate logistic regression models. In each model, only variables that were univariate associated with severity ( $p < 0.1$ ) were added, followed by backward selection. The first model (model 1) tested the predictive accuracy of combinations of sensitization to allergen molecules as a comparison to the use of single allergens. In the next step, we used clinical variables coming from medical history (model 2) sensitization data (model 3 and 4). Model 2 was generated using clinical variables only. Model 3 included selected clinical variables from model 2 to which hazelnut sensitization markers (SPT and sIgE to hazelnut extract and to the allergens) were added. Model 4 was built from the remaining variables in model 3 and sIgE against 23 food, 12 inhalant and latex extracts.

We performed these analyses for both reported symptom severity and for symptom severity recorded during the DBPCFC. The predictive accuracy of the models was quantified with the AUC and AUCs were compared using DeLong tests. Specificity, PPV and NPV at the threshold at which 95% sensitivity was obtained, or the maximum sensitivity that could be achieved. Prediction formulas are provided in this article's online repository. All statistical analyses were done in R software version 3.2.4.

## RESULTS

### *Patient characteristics associated with severe symptoms*

Table 1 summarizes all patient characteristics. Mild symptoms (Isolated OAS) were reported by 350 patients, moderate by 263 and severe by 116. Severe reactions were unevenly distributed over distributed over the 12 centers (Fig. S1).

AD was more frequently seen in patients with moderate (34%) and severe (37%) reactions to hazelnut, as compared to those with mild symptoms (20%), with an odds ratio (OR) of 2.1 and 2.3, respectively (Fig 1A). Latex allergy was more frequently seen in patients with severe (11%) compared to patients with mild and moderate symptoms (5% and 4%, respectively) with an OR of 2.7 (95%CI 1.2-6.0). In contrast, pollen allergy was significantly (around two-fold) less often reported by patients with moderate and severe symptoms.

Of the 86 DBPCFC-positive patients, 36 experienced mild oral symptoms, and 40 skin, gastrointestinal or upper airway symptoms (moderate). The severe group included 10 patients with lower respiratory and neurological symptoms during challenge, and 22 patients with a convincing history of anaphylactic reaction to hazelnut. Except for latex allergy, similar associations between the severity of reactions observed during a DBPCFC with patient characteristics as for reported reactions, although with wider confidence intervals (Fig. 1B).

### *Sensitization to hazelnut allergens univariately associated with severe symptoms*

Of patients reporting severe symptoms, 21% were negative on all seven hazelnut allergens investigated, compared to 9% of patients reporting mild symptoms ( $p=0.061$ ). Sensitization to the seed storage proteins Cor a 9 and Cor a 14 was positively associated with severe symptoms compared to mild symptoms (Fig. 1A) with odds ratios (OR) of 2.9 (95% CI 1.3-6.3) when sensitized to Cor a 9 and 4.7 (95% CI 1.8-12.4) when sensitized to Cor a 14. Sensitization to Cor a 1 and to Cor a 2 were negatively associated with severe symptoms (OR 0.4, 95% CI 0.20-0.64 and 0.5, 95% CI 0.2-1.0, respectively). Although the majority (176/309) of Cor a 1-sensitized patients had mild symptoms, 41 reported severe symptoms of which 26 had no detectable IgE to the other tested allergens.

In sera of patients sensitized to Cor a 9 ( $\geq 0.35$  kU<sub>A</sub>/L), specific IgE levels were significantly higher in those with severe symptoms (median 11.3 kU<sub>A</sub>/L) than in patients with moderate and mild symptoms (median 1.68 and 0.73 kU<sub>A</sub>/L, respectively; see Fig. 2). A similar difference was observed for Cor a 14 although not significant. No significant differences in IgE levels against hazelnut extract and the other hazelnut allergens were observed between patients of different severity. Compared to reported symptoms, Cor a 9 and Cor a 14 showed even greater ORs for severe symptoms observed during challenge (10.5, 95% CI 1.2-91.4 and 10.1, 95% CI 1.1-91.5, respectively, Fig. 1B). Also for Cor a 1 sensitization, a stronger negative association was found (OR 0.14, 95% CI 0.03-0.55), this was not observed for Cor a 2 and hazelnut extract.

### *Impact of inhalant sensitization on reported severity*

We investigated whether milder symptoms in pollen-sensitized cross-reactive patients were also observed when there was co-sensitization to non-pollen associated hazelnut allergens. We compared severity reported by patients sensitized to hazelnut storage proteins (Cor a 9, 11 and 14), oleosin and/or LTP that were either co-sensitized to (birch) pollen-associated Cor a 1, Cor a 2 and/or CCD or not. In addition the same analysis was performed for presence or absence of co-sensitization to birch pollen, to any pollen or to HDM, cat and/or dog (indoor allergen sensitization). Severity was being less frequently reported in patients co-sensitized to pollen-associated allergens, to birch pollen or to any pollen, but this was not the case when co-sensitization was to indoor allergens (Fig.3). IgE levels to non-pollen associated hazelnut allergens were not significantly different between patients co-sensitized to pollen-associated allergens and those without that cross-reactive response.

### *Models to improve the discriminatory ability to assess the risk of severe reactions*

AUCs of the ROC curves of single hazelnut allergens in discriminating between mild-to-moderate and severe symptoms were poor, i.e. between 0.57 and 0.62 (Fig. 4 and see also Table S3 in the online repository). For symptoms recorded during DBPCFC, the AUCs were higher (0.70-0.73). AUCs were also higher when a sub-analysis was performed for reported symptoms of children (n=53) in case of Cor a 9 (AUC 0.70, 95%CI 0.48-0.92) and Cor a 14 (AUC 0.70, 95%CI 0.53-0.88), but not for Cor a 1 (AUC 0.58, 95%CI 0.39-0.72). We evaluated whether combining IgE responses to different allergens in a model would improve the predictive accuracy (model 1). Although some improvement was achieved, the best combination of Cor a 1 (protective) and Cor a 9 (risk) only marginally increased the AUC from 0.62 to 0.66, ( $p < 0.05$ ).

In model 2 AD, latex allergy (both risk factors) and pollen allergy (protective factor) were included, resulting in an AUC of 0.62 (95%CI: 0.57-0.68). For severity in DBPCFC, the AUC of model 2 was 0.75 (95%CI: 0.66-0.85), without latex allergy being included. In model 3, adding IgE against Cor a 14 resulted in a significantly higher AUC for reported (0.70, 95%CI 0.63-0.77) and challenge-recorded symptoms (AUC 0.86, 95%CI 0.77-0.95) as compared to model 1 and 2. Finally, in model 4 sIgE to walnut, cat and milk (for details, see table S4) slightly increased the AUC for reported symptoms (from 0.70 to 0.72). For symptoms during challenge, just sIgE to walnut increased the AUC to 0.91 (95%CI: 0.84-0.97).

The predictive probability on severe allergic reactions generated from the models are presented in the online repository in table S5 and illustrated in figure S2 for models 3 and 4. Using model 4, a probability of 8% or higher on severe symptoms, based on DBPCFCs, corresponded to 96% sensitivity, 76% specificity and a PPV and NPV of 62% and 96%, respectively (see also table S6 in this article's online repository). All patients in which severe symptoms were excluded (probability < 8%), were pollen allergic and had no AD, latex allergy or detectable IgE against Cor a 14.

## **DISCUSSION**

Measurement of specific IgE against individual allergen molecules is now widely recognized as a valuable tool in the diagnosis of food allergy.(5,16) In the present study, we had the unique opportunity to evaluate associations between IgE responses to seven individual hazelnut allergens and symptom severity in patients in twelve European centers with very diverse climatic and cultural backgrounds. Moreover, around 30% of patients were also undergoing a DBPCFC, allowing comparison of associations with severity for both reported and challenge-recorded symptoms. Our study confirmed that IgE responses against Cor a 1 are associated with mild symptoms and against Cor a 9 and Cor a 14 (and to a lesser extent Cor a 11 and Cor a 12) with severe symptoms to

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hazelnut, both reported and challenge-induced. Although responders to Cor a 1 mostly suffer from mild symptoms, 60% of the patients with severe symptoms also recognize Cor a 1, of which a significant number exclusively. Also, it is important to note that overall 12% of the patients were negative for all seven hazelnut allergens tested, and that this percentage was significantly higher in those with severe symptoms (21%) than mild (9%), suggesting that one or more allergens with relevance for severity may still be missing.

Although associations with severity for Cor a 9 and 14 were quite strong (OR reported: 2.9 and 4.7; OR DBPCFC: 10.5 and 10.1, respectively), the predictive accuracy of isolated serological tests for these allergens was relatively poor, with AUCs at best around 0.60 for reported symptoms, increasing to 0.70 for children, and around 0.7 for DBPCFC symptoms (almost only adults). Similar to our results, Masthoff et al.(5) found AUC in adults for Cor a 9 and Cor a 14 of 0.66 and 0.67, respectively. For children, the AUCs were higher (0.87 and 0.80, Cor a 9/Cor a 14) than we observed, but their analysis was based on DBPCFC which in our analyses for the whole population also resulted in higher AUCs compared to reported symptoms.

Patients sensitized to birch pollen or pollen-related allergens (like Cor a 1) and to hazelnut storage proteins less frequently reported severe symptoms than those without co-sensitization to pollen, although this difference did not reach significance. Interestingly, there was no difference in IgE levels against the hazelnut allergens between both groups. A protective phenomenon by pollen co-sensitization was for the first time described amongst Pru p 3 sensitized peach allergic patients that displayed less severe phenotypes if co-sensitized to birch pollen.(25) It is still unclear why, amongst patients becoming sensitized to hazelnut storage proteins, severe reactions would be less frequent if they also have IgE against pollen-related allergens. A number of co-sensitizations to other foods were univariate associated ( $p < 0.1$ ) with severity of reactions to hazelnut: soybean, walnut, sesame seed, poppy seed and buckwheat (table S4). It has been reported that patients with broader spectra of sensitization to tree nuts, seeds and legumes have more severe phenotypes.(26) In the models developed in the present study, only walnut co-sensitization significantly contributed to the prediction of severity. Whether recognition of cross-reactive epitopes on walnut and hazelnut storage proteins or a propensity to develop co-sensitization to multiple tree nuts explains the association with severity remains to be established. Currently, walnut ImmunoCAP is quite insensitive for picking up typical Bet v 1-related cross-reactivity.(27) Positive walnut IgE tests are therefore most likely dominated by IgE against walnut storage protein. In the past hazelnut ImmunoCAP had similar problems and is therefore now spiked with rCor a 1. If a similar approach would be taken for walnut, the association with severity of hazelnut allergy may disappear.

We aimed to improve the predictive accuracy by combining clinical history, CRD and extract-based serology. Interestingly, AD and latex allergy were positively related with severe symptoms whereas pollen allergy proved to have a negative association. It is well-established that AD is a risk factor for the development of food allergy in young children(28) and that mutations in the filaggrin gene predispose for the development of both AD and food allergy.(29) It is interesting to speculate that this route of sensitization, associated with AD and skin permeability, might be a risk factor for severe food allergy.

In the multivariable regression models combining clinical background, CRD and other (extract-based) sensitizations, a combination of AD, pollen allergy and sIgE against Cor a 14 and walnut resulted in the best predictive accuracy of severity with an AUC of 0.91. The model performs particularly well in excluding the risk of severe reactions with a NPV of 96% at a sensitivity of 92%. Specificity (76.3%) and PPV (62.2%) are less favourable. The prevalence of severity is however relatively low (16%), which impacts sensitivity and PPV. Therefore a relatively large number of patients are falsely indicated as being severely allergic. All patients that are classified as 'severe' will thus need additional testing. Nevertheless, the model(s) developed perform better than CRD alone and will now be validated in different study populations of hazelnut allergic patients that have been studied in the EU-funded project iFAAM (<http://research.bmh.manchester.ac.uk/iFAAM>).

It is important to realize that we evaluated patients from tertiary clinics heavily dominated by the typical Northern and Central European adult patients with birch pollen-associated hazelnut allergy. Removing Spanish and Greek patients from the analyses did not significantly change the predictive accuracy of the models (data not shown). The number of patients from Madrid and Athens was too small (38 of which 6 reported severe symptoms) to reliably evaluate the performance the models generated in the present study.

The strength of the present study is the evaluation and confirmation of previously reported CRD findings in more selected populations, but now in a much larger number of patients with a diverse pan-European background. On top of that, not only serological tests but also patients' characteristics were evaluated, showing that this approach significantly improves the identification of patients with increased risk on experiencing severe reactions. A limitation of our study is that only a subset of the patients volunteered to undergo a DBPCFC which could introduce bias resulting in less generalizable results. Additionally, stopping criteria makes DBPCFC less accurate for establishing real-life severity, our associations with severity found in reported and challenge-recorded symptoms were similar. These findings however, need to be further validated. In summary, we have confirmed the important role of Cor a 9 and Cor a 14 in severe hazelnut allergy, and have developed models

incorporating clinical background, molecule and extract-based sensitizations to more accurately predict the risk of severe reactions. A patient sensitized to Cor a 14 and walnut, with a history of AD but no pollen allergy, should be further evaluated for the risk on severe reactions, whereas this risk is very low in pollen and hazelnut allergic patients without sensitization to Cor a 14 and walnut.

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## Tables

Table 1

TABLE 1: Characteristics in patients reporting mild, moderate and severe symptoms to hazelnut

|                                      | Severity classification <sup>†</sup> |      |                  |      |                |      | P      |
|--------------------------------------|--------------------------------------|------|------------------|------|----------------|------|--------|
|                                      | Mild (n=350)                         |      | Moderate (n=263) |      | Severe (n=116) |      |        |
| Age (mean, sd)                       | 33.1                                 | 13.4 | 30.9             | 16.5 | 32.8           | 14.8 | 0.426  |
| < 18 years (n/N, %)                  | 42/350                               | 12.0 | 63/263           | 24.0 | 15/116         | 12.9 | <0.001 |
| Female gender (n/N, %)               | 228/350                              | 65.1 | 158/263          | 60.1 | 73/116         | 62.9 | 0.438  |
| Atopy family (n/N, %)                | 247/350                              | 70.6 | 183/263          | 69.6 | 84/116         | 72.4 | 0.856  |
| Atopic diseases (n/N, %)             |                                      |      |                  |      |                |      |        |
| Atopic dermatitis (ever)             | 68/339                               | 20.1 | 86/253           | 34.0 | 42/114         | 36.8 | <0.001 |
| Pollen allergy <sup>‡</sup>          | 301/350                              | 86.0 | 206/263          | 78.3 | 82/116         | 70.7 | 0.001  |
| House dust mite allergy <sup>‡</sup> | 170/350                              | 48.6 | 105/263          | 39.9 | 57/116         | 49.1 | 0.073  |
| Latex allergy <sup>‡</sup>           | 14/314                               | 4.5  | 10/236           | 4.2  | 12/108         | 11.1 | 0.019  |
| Cat / dog sensitization              | 146/346                              | 42.0 | 100/252          | 39.7 | 53/114         | 46.5 | 0.471  |
| Hazelnut sensitization (n/N, %)      |                                      |      |                  |      |                |      |        |
| iPT                                  | 291/345                              | 84.3 | 191/256          | 74.6 | 89/115         | 77.4 | 0.011  |
| ImmunoCAP                            | 297/337                              | 88.1 | 195/246          | 79.3 | 92/114         | 80.7 | 0.010  |
| Single hazelnut molecules* (n/N, %)  |                                      |      |                  |      |                |      |        |
| Any                                  | 202/223                              | 90.6 | 107/127          | 84.3 | 56/71          | 78.9 | 0.045  |
| rCor a 1                             | 176/219                              | 80.4 | 92/127           | 72.4 | 41/69          | 59.4 | 0.002  |
| rCor a 2                             | 55/222                               | 24.8 | 18/127           | 14.2 | 10/71          | 14.1 | 0.024  |
| rCor a 8                             | 24/220                               | 10.9 | 15/127           | 11.8 | 9/70           | 12.9 | 0.899  |
| nCor a 9                             | 16/222                               | 7.2  | 13/127           | 10.2 | 13/71          | 18.3 | 0.025  |
| nCor a 11                            | 7/222                                | 3.2  | 2/127            | 1.6  | 6/71           | 8.5  | 0.047  |
| nCor a 12                            | 26/221                               | 11.8 | 19/127           | 15.0 | 15/71          | 21.1 | 0.142  |
| rCor a 14                            | 8/217                                | 3.7  | 7/125            | 5.6  | 10/66          | 15.2 | 0.006  |
| CCD                                  | 23/216                               | 10.6 | 13/127           | 10.2 | 7/69           | 10.1 | 0.989  |

<sup>†</sup> Missing data 2 patients; <sup>‡</sup> Reported symptoms + matching sensitization by SPT or ImmunoCAP

\* In 423 patients, IgE against hazelnut allergen molecules were tested by component- resolved diagnosis (CRD). Not all patients had complete data for all allergens measured. Age < 18 years, atopic dermatitis, pollen allergy and Cor a 1, remained significant after Bonferroni correction. For the sake of clarity, positive associations (risk) with severity are highlighted in red, negative (protection) in green.

Figure legend text

**FIG 1: Clinical factors and hazelnut sensitization (IgE  $\geq$  0.35 kUA/L) associated with severe symptoms in the univariate analysis.**

The x-axis represents odds ratios (OR). The symbols indicate the OR on moderate (triangle) and severe (square) symptoms compared to mild symptoms when sensitized to an allergen. The lines present the 95% confidence interval (CI). CI lines that cross the hatched line indicate no significant association, upper limits were truncated at 20.

**FIG 2. IgE levels to hazelnut in subjects with mild moderate and severe symptoms.**

The dots present the level of IgE to hazelnut measured by hazelnut extract, Cor a 1, Cor a 2, Cor a 8, Cor a 9, Cor a 11, Cor a 12 and CCD. The y-axis presents the IgE in kUA/L on a common log scale. The horizontal red lines represent the median and interquartile IgE levels for each symptom group within those that are positively sensitized ( $\geq$  0.35 kUA/L). The black top lines show the significant difference in IgE levels between groups \*( $P \leq 0.05$ ) \*\*( $P \leq 0.001$ ). The numbers indicate the total number of sensitized subjects within the groups.

**Fig 3. Severe symptoms to hazelnuts and inhalant allergen co-sensitization in patients sensitized to hazelnut storage proteins, LTP and/or oleosin.**

The bars show the proportion of patients reporting severe symptoms. All patients are sensitized to Cor a 8, Cor a 9, Cor a 11, Cor a 12 and/or Cor a 14. Orange bars indicate co-sensitization either against Cor a 1, Cor a 2 and/or CCD, or against birch pollen, or against any pollen or against indoor allergens (house dust mite, cat and/or dog). The white bars indicate absence of co-sensitization to these allergen sources.

**FIG 4. Receiver operating characteristic (ROC) curves.**

(A) Results from patients reporting symptoms to hazelnut and tested with CRD (N=423). (B) A subset (n=124) of patients that underwent a DBPCFC for hazelnut. Area under the curves (AUC) are shown for hazelnut extract, Cor a 1, Cor a 9, Cor a 14 and the combination of Cor a 1 and 14 (model 1). The other models were built from atopic dermatitis (AD), pollen allergy and latex allergy (model 2); AD, pollen allergy, latex allergy and Cor a 14 (model 3); AD, pollen allergy, latex allergy, Cor a 14, and IgE against walnut, cat and milk (model 4).

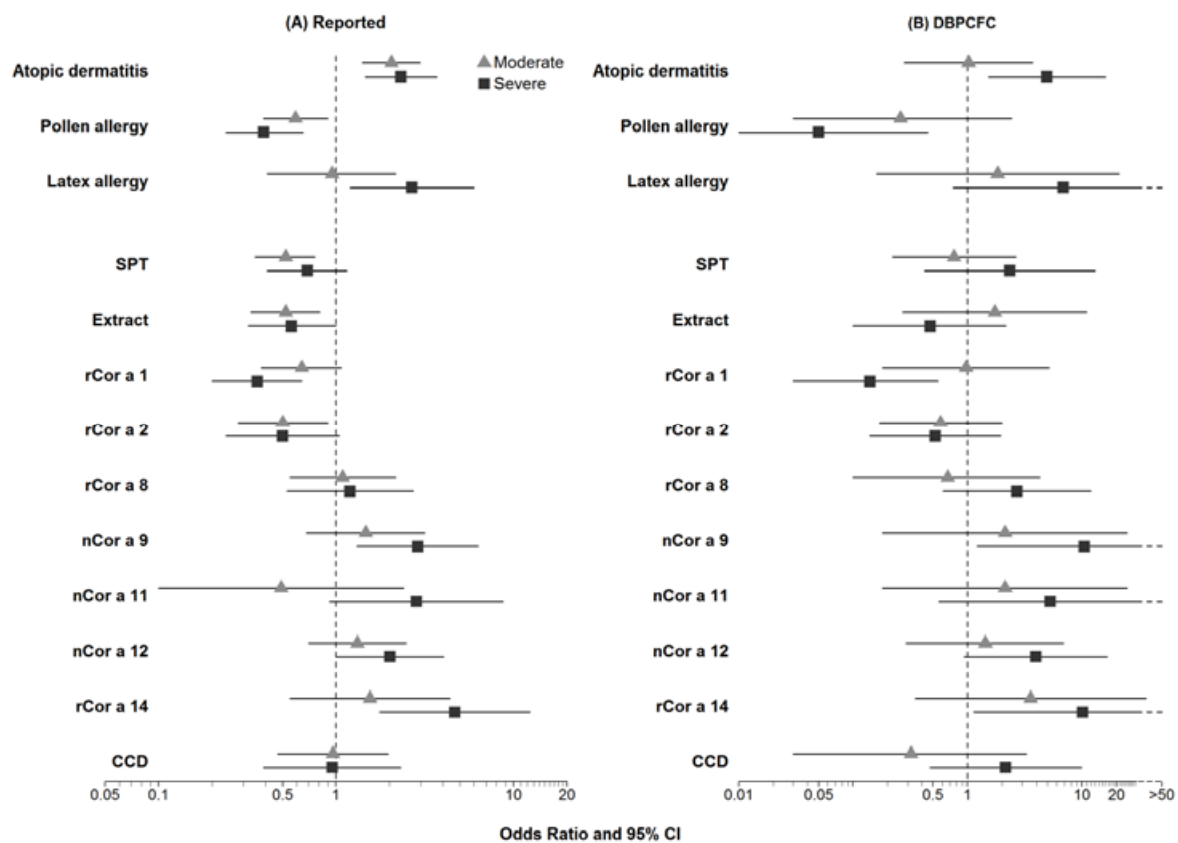
**FIG S1: Severity of hazelnut allergy in European cities.**

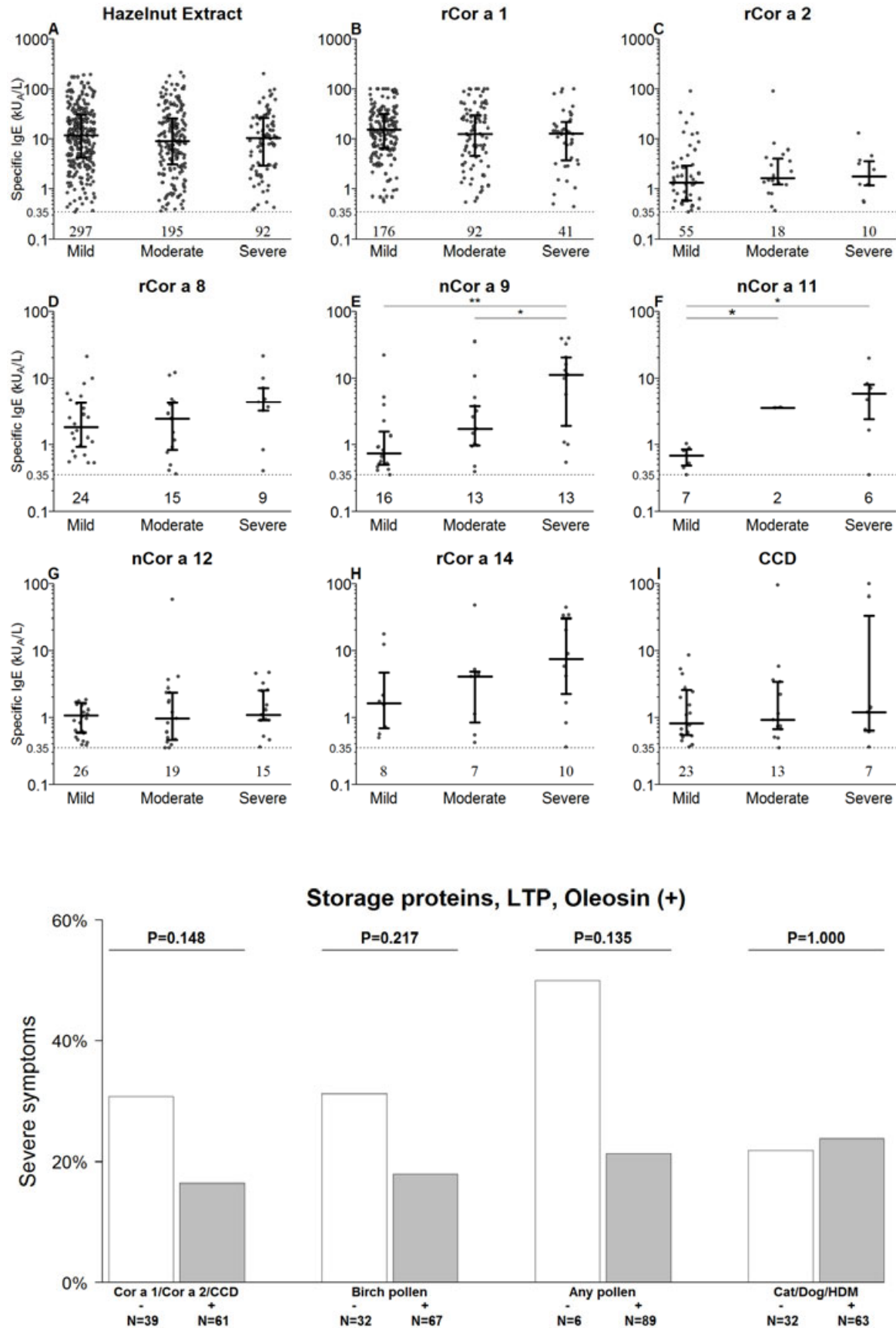
Frequency of severe reported symptoms and 95%CI. Hatched line shows the mean frequency of all the cities.

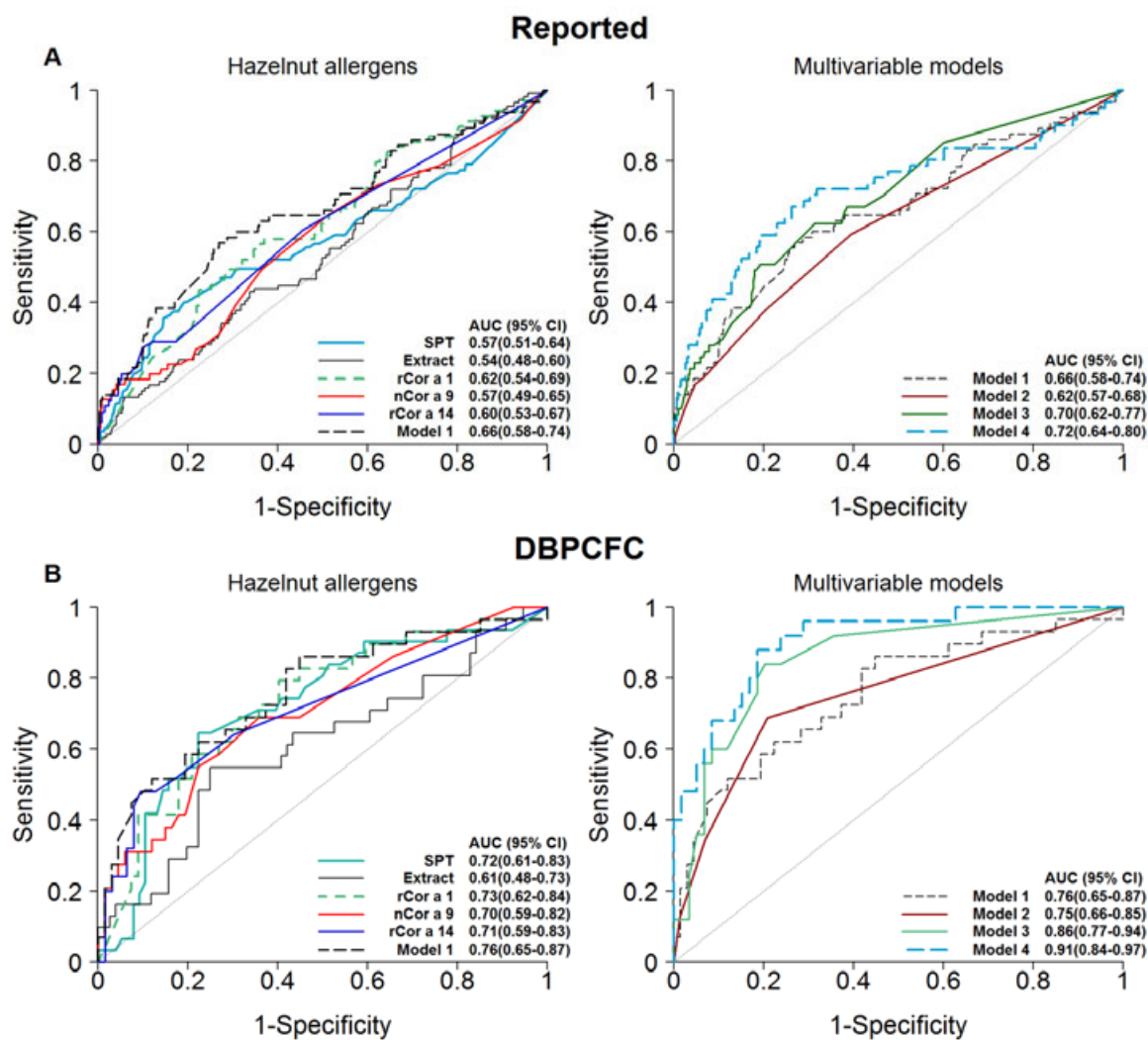
**FIG S2. Probability of having severe allergic reactions to hazelnut based on (A) reported symptoms and (B) symptoms during a challenge.**

The red line represents the probability and 95% confidence interval on severe allergic symptoms to hazelnut. For reported symptoms, model 3 severity was estimated including atopic dermatitis (yes/no), pollen allergy (yes/no), latex allergy (yes/no), sIgE levels to Cor a 14. Model 4 also included sIgE levels to walnut, cat and milk. For challenged symptoms, model 3 included atopic dermatitis (yes/no), pollen allergy (yes/no), sIgE levels to Cor a 14 and model 4 additionally included sIgE levels to walnut.

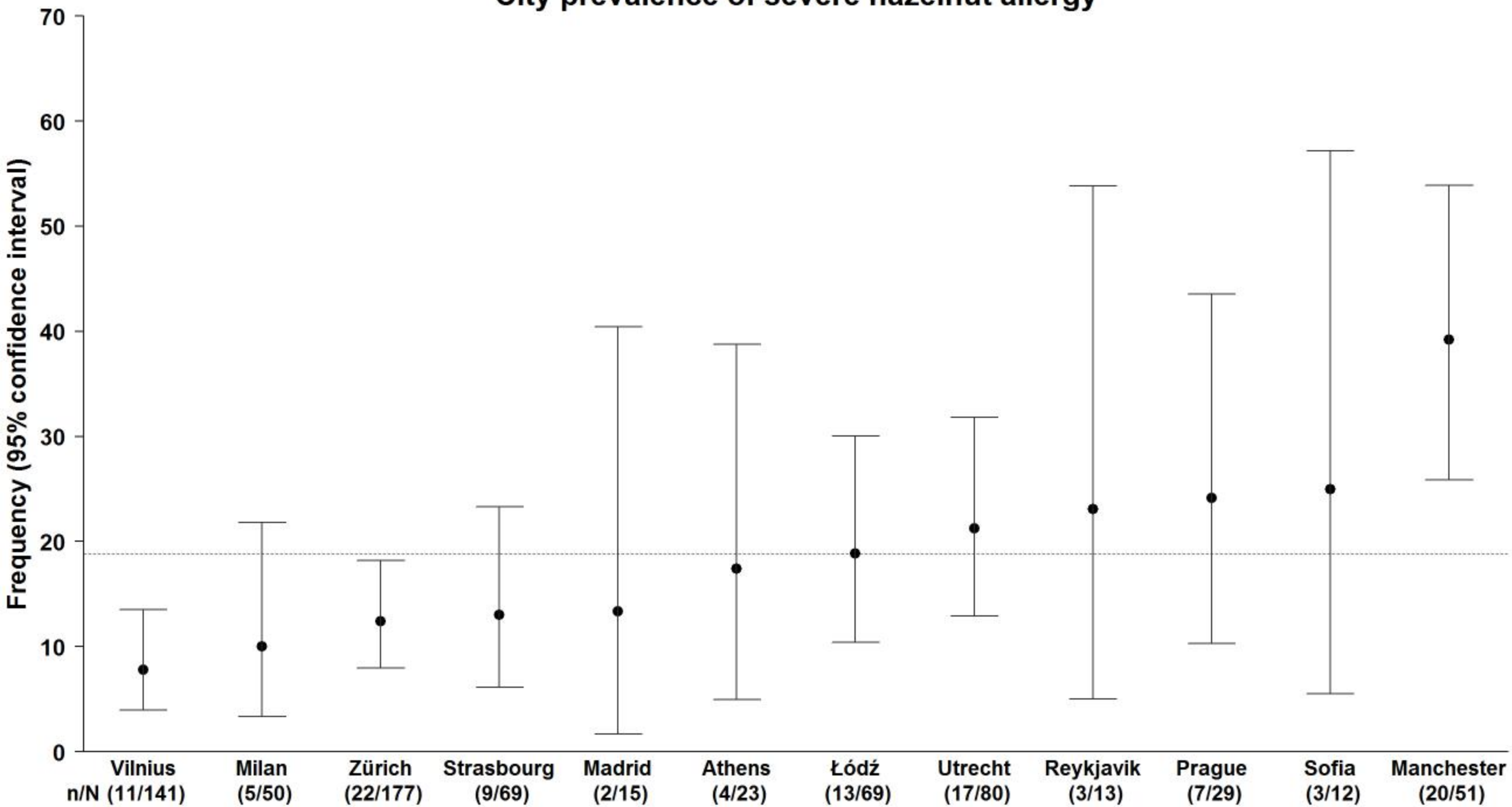
The combination of the weight of the variables is indicated by the x-axis and the distribution of this score is depicted in the histogram. The hatched lines indicate the predictive probability that gives the highest possible sensitivity to classify severe patients. The numbers show the classification of the patients using that probability as at cut-point; TP (true positive), FP (false positive), TN (true negative), FN (false negative).







## City prevalence of severe hazelnut allergy



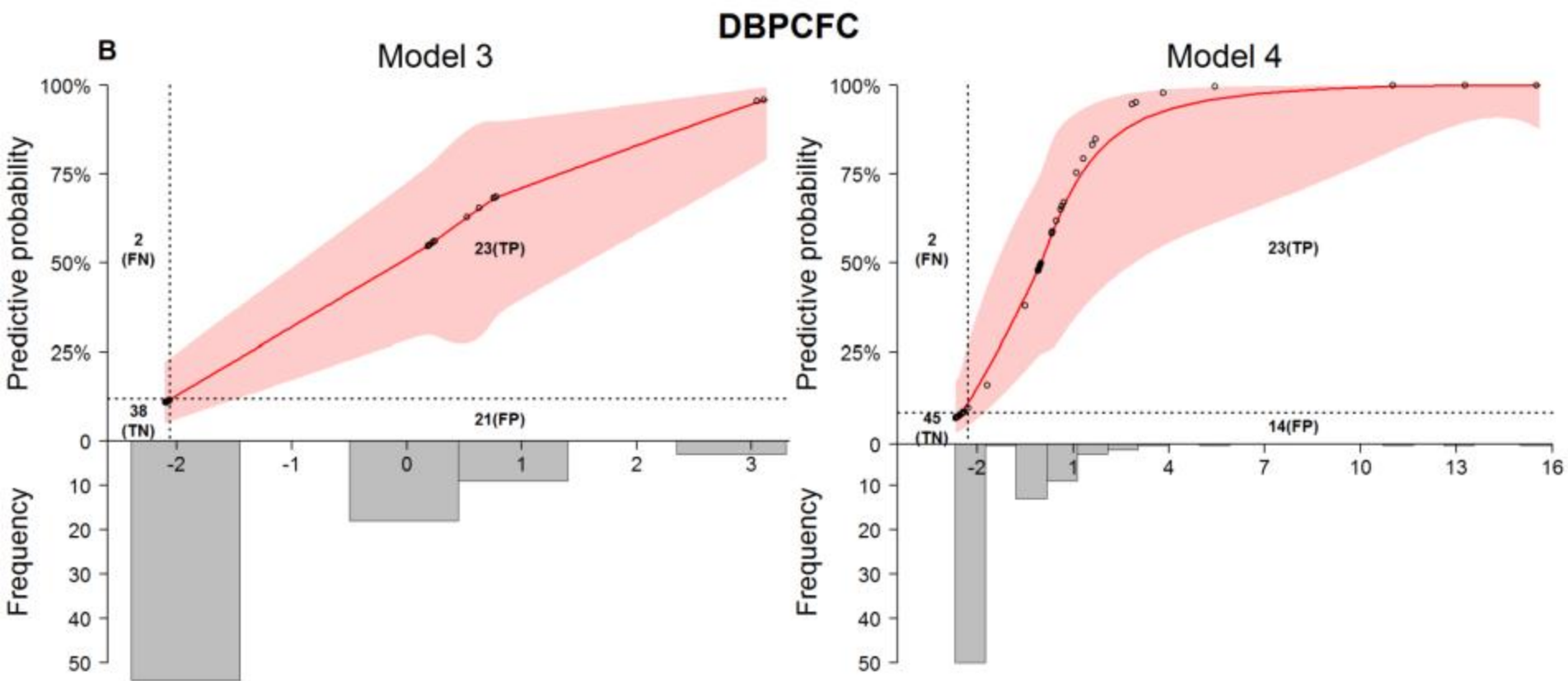
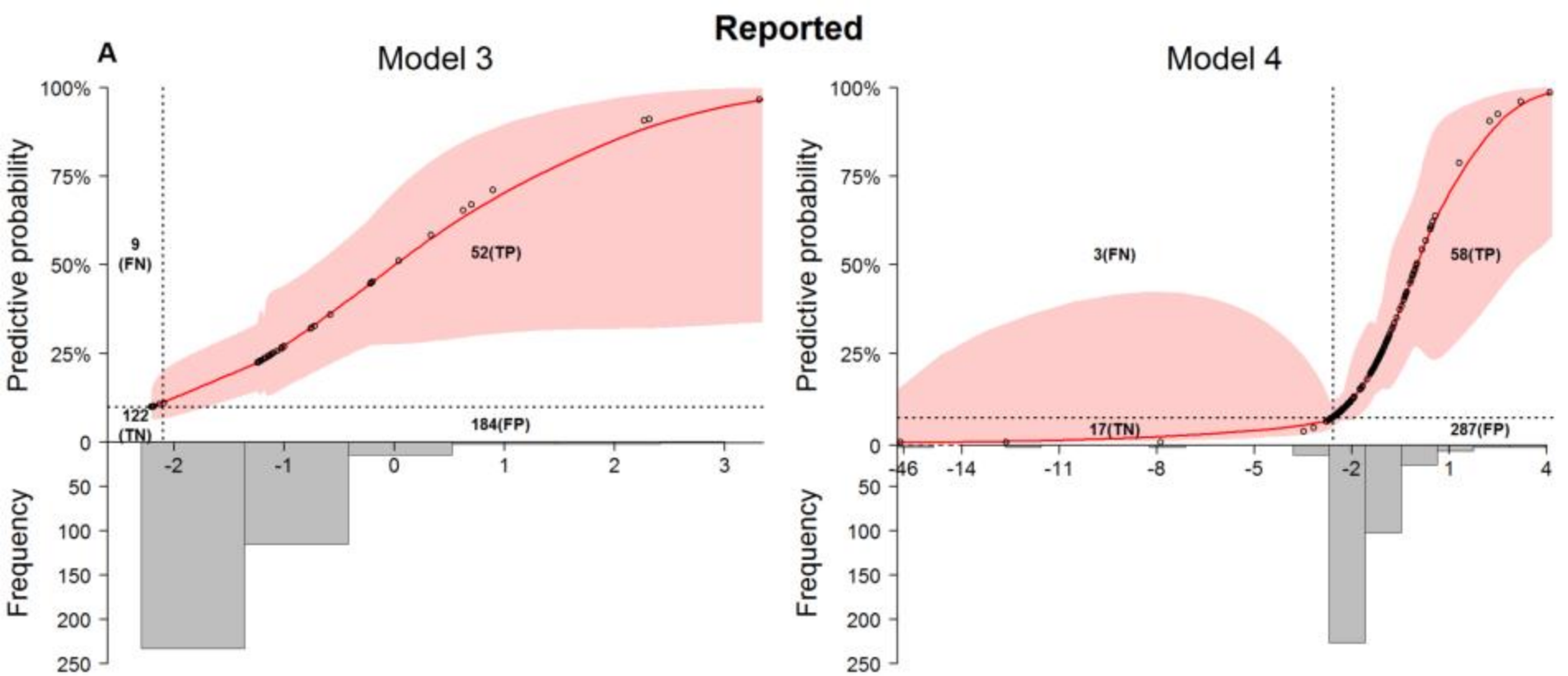


Table S1

**TABLE S1.** Differences in characteristics between subset with and without CRD

|                                      | CRD (n=423)* |      | No CRD (n=308) |      | P-value          |
|--------------------------------------|--------------|------|----------------|------|------------------|
|                                      | Mean         | SD   | Mean           | SD   |                  |
| Age (mean, sd)                       | 33.7         | 14.2 | 30.3           | 15.5 | <b>0.003</b>     |
| < 18 years (n/N, %)                  | 53/423       | 12.5 | 67/308         | 21.8 | 0.096            |
| Female gender (n/N, %)               | 278/423      | 65.7 | 183/308        | 59.4 | 0.096            |
| Atopy family (n/N, %)                | 284/423      | 67.1 | 231/308        | 75.0 | <b>0.027</b>     |
| Atopic diseases (n/N, %)             |              |      |                |      |                  |
| Atopic dermatitis                    | 87/410       | 21.2 | 109/298        | 36.6 | <b>&lt;0.01</b>  |
| Pollen allergy <sup>‡</sup>          | 391/423      | 92.4 | 280/308        | 90.9 | 0.545            |
| House dust mite allergy <sup>‡</sup> | 193/423      | 45.6 | 139/308        | 45.1 | 0.954            |
| Latex allergy <sup>‡</sup>           | 19/394       | 4.8  | 17/266         | 6.4  | 0.487            |
| Symptom classification (n/N, %)*     |              |      |                |      |                  |
| Mild                                 | 223/421      | 53.0 | 127/308        | 41.2 | <b>&lt;0.001</b> |
| Moderate                             | 127/421      | 30.2 | 136/308        | 44.2 |                  |
| Severe                               | 71/421       | 16.9 | 45/308         | 14.6 |                  |
| Hazelnut sensitization (n/N, %)      |              |      |                |      |                  |
| sPT                                  | 342/422      | 81.0 | 230/296        | 77.7 | 0.622            |
| ImmunoCAP                            | 353/421      | 83.8 | 232/278        | 83.5 | 0.973            |

\*Missing data 2 patients

Table S2

**TABLE S2.** Differences in characteristics of subjects who received a DBPCFC\*

|                                      | DBPCFC (n=124) |      | No DBPCFC (n=585) |      | P-value      |
|--------------------------------------|----------------|------|-------------------|------|--------------|
|                                      | Mean           | SD   | Mean              | SD   |              |
| Age (mean, sd)                       | 33.1           | 14.1 | 32.2              | 15.0 | 0.527        |
| < 18 years (n/N, %)                  | 18/124         | 14.5 | 98/585            | 16.8 | 0.633        |
| Female gender (n/N, %)               | 82/124         | 66.1 | 366/585           | 62.6 | 0.519        |
| Atopy family (n/N, %)                | 86/124         | 69.4 | 413/585           | 70.6 | 0.867        |
| Atopic diseases (n/N, %)             |                |      |                   |      |              |
| Atopic dermatitis                    | 23/119         | 19.3 | 165/567           | 29.1 | <b>0.039</b> |
| Pollen allergy <sup>‡</sup>          | 119/124        | 96.0 | 532/585           | 90.9 | 0.071        |
| House dust mite allergy <sup>‡</sup> | 55/124         | 44.4 | 268/585           | 45.8 | 0.844        |
| Latex allergy <sup>‡</sup>           | 4/115          | 3.5  | 28/523            | 5.4  | 0.488        |
| Symptom classification               |                |      |                   |      |              |
| Mild                                 | 61/123         | 49.6 | 289/584           | 49.5 | 0.333        |
| Moderate                             | 41/123         | 33.3 | 222/584           | 38.0 |              |
| Severe                               | 21/123         | 17.1 | 73/584            | 12.5 |              |
| Hazelnut sensitization (n/N, %)      |                |      |                   |      |              |
| sPT                                  | 107/123        | 87.0 | 445/573           | 77.7 | 0.056        |
| ImmunoCAP                            | 111/123        | 90.2 | 439/534           | 82.2 | <b>0.041</b> |

\*22 subjects with a convincing history of anaphylaxis were excluded for a DBPCFC.

Table S3

Table S3: Predictive accuracy individual hazelnut allergens and multivariable models

|                  | Reported |           | DBPCFC |   |   |      |           |   |
|------------------|----------|-----------|--------|---|---|------|-----------|---|
|                  | AUC      | CI95%     |        |   |   | AUC  | CI95%     |   |
| <b>SPT</b>       | 0.57     | 0.51-0.64 | §      | † | ‡ | 0.72 | 0.61-0.83 | ‡ |
| <b>Extract</b>   | 0.54     | 0.48-0.60 | §      | † | ‡ | 0.61 | 0.48-0.73 | † |
| <b>rCor a 1</b>  | 0.62     | 0.54-0.69 | §      | † | ‡ | 0.73 | 0.62-0.84 | ‡ |
| <b>rCor a 2</b>  | 0.54     | 0.46-0.61 | §      | † | ‡ | 0.54 | 0.41-0.67 | § |
| <b>rCor a 8</b>  | 0.51     | 0.44-0.58 | §      | † | ‡ | 0.62 | 0.50-0.74 | † |
| <b>nCor a 9</b>  | 0.57     | 0.49-0.65 | §      | † | ‡ | 0.70 | 0.59-0.82 | ‡ |
| <b>nCor a 11</b> | 0.50     | 0.42-0.57 | §      | † | ‡ | 0.48 | 0.34-0.62 | § |
| <b>nCor a 12</b> | 0.52     | 0.43-0.60 | §      | † | ‡ | 0.55 | 0.41-0.68 | § |
| <b>rCor a 14</b> | 0.60     | 0.53-0.67 | §      | † | ‡ | 0.71 | 0.59-0.83 | † |
| <b>CCD</b>       | 0.55     | 0.47-0.62 | §      | † | ‡ | 0.53 | 0.41-0.65 | § |
| <b>Model 1</b>   | 0.66     | 0.58-0.74 |        |   | ‡ | 0.76 | 0.65-0.87 | ‡ |
| <b>Model 2</b>   | 0.62     | 0.57-0.68 |        |   |   | 0.75 | 0.66-0.85 | † |
| <b>Model 3</b>   | 0.70     | 0.63-0.77 |        |   |   | 0.86 | 0.77-0.94 |   |
| <b>Model 4</b>   | 0.72     | 0.64-0.80 |        |   |   | 0.91 | 0.84-0.97 |   |

§ significantly different from model 2

† significantly different from model 3

‡ significantly different from model 4

Model 1 included: Cor a 1 and Cor a 9

Model 2 included: Atopic dermatitis, pollen allergy, latex allergy (Reported); Atopic dermatitis, pollen allergy (DBPCFC);

Model 3 included: Atopic dermatitis, pollen allergy, latex allergy, Cor a 14(Reported); Atopic dermatitis, pollen allergy, Cor a 14 (DBPCFC);

Model 4 included: Atopic dermatitis, pollen allergy, latex allergy, Cor a 14, walnut, milk, cat(Reported); Atopic dermatitis, pollen allergy, Cor a 14, walnut (DBPCFC);

Table S4

TABLE S4: Sensitization to food and pollen and other inhalant sources

|                         | Severity classification |                  |             |                   |              |
|-------------------------|-------------------------|------------------|-------------|-------------------|--------------|
|                         | Mild/moderate           |                  | Severe      |                   | p-value      |
|                         | Median                  | IQR              | Median      | IQR               |              |
| Peach                   | 1.41                    | 0.31-4.58        | 1.40        | 0.11-4.26         | 0.101        |
| Apple                   | 0.73                    | 0.16-2.65        | 0.69        | 0.05-2.37         | 0.192        |
| Kiwi                    | 0.24                    | 0.04-0.8         | 0.21        | 0.04-1.14         | 0.960        |
| Banana                  | 0.11                    | 0.04-0.33        | 0.13        | 0.04-0.41         | 0.688        |
| Melon                   | 0.09                    | 0.05-0.25        | 0.09        | 0.05-0.31         | 0.967        |
| Carrot                  | 0.36                    | 0.07-1.35        | 0.29        | 0.05-1.36         | 0.385        |
| Celery                  | 0.45                    | 0.09-1.74        | 0.44        | 0.03-1.68         | 0.220        |
| Corn                    | 0.13                    | 0.06-0.44        | 0.17        | 0.06-0.79         | 0.181        |
| Tomato                  | 0.16                    | 0.04-0.75        | 0.23        | 0.04-1.23         | 0.319        |
| Lentil                  | 0.08                    | 0.02-0.31        | 0.10        | 0.02-0.78         | 0.174        |
| <b>Soybean*</b>         | <b>0.09</b>             | <b>0.02-0.33</b> | <b>0.15</b> | <b>0.03-0.67</b>  | <b>0.039</b> |
| Peanut                  | 0.25                    | 0.06-1.33        | 0.50        | 0.06-1.47         | 0.394        |
| <b>Walnut**</b>         | <b>0.08</b>             | <b>0.02-0.36</b> | <b>0.10</b> | <b>0.02-0.88</b>  | <b>0.071</b> |
| <b>Sesame seed*</b>     | <b>0.23</b>             | <b>0.11-0.74</b> | <b>0.29</b> | <b>0.12-1.66</b>  | <b>0.028</b> |
| Sunflower               | 0.10                    | 0.05-0.36        | 0.12        | 0.05-0.72         | 0.147        |
| <b>Poppy seed*</b>      | <b>0.07</b>             | <b>0.03-0.24</b> | <b>0.11</b> | <b>0.03-0.66</b>  | <b>0.015</b> |
| Mustard                 | 0.04                    | 0.02-0.11        | 0.05        | 0.02-0.19         | 0.212        |
| Wheat                   | 0.17                    | 0.07-0.56        | 0.19        | 0.07-0.58         | 0.450        |
| <b>Buckwheat*</b>       | <b>0.10</b>             | <b>0.04-0.31</b> | <b>0.15</b> | <b>0.05-0.60</b>  | <b>0.021</b> |
| Fish                    | 0.00                    | 0-0.03           | 0.00        | 0.00-0.03         | 0.790        |
| Shrimp                  | 0.04                    | 0.01-0.16        | 0.05        | 0.02-0.17         | 0.401        |
| Egg                     | 0.06                    | 0.04-0.1         | 0.06        | 0.05-0.14         | 0.135        |
| <b>Milk**</b>           | <b>0.06</b>             | <b>0.04-0.1</b>  | <b>0.06</b> | <b>0.05-0.16</b>  | <b>0.045</b> |
| <b>Birch*</b>           | <b>14.38</b>            | <b>1.96-41.6</b> | <b>5.62</b> | <b>0.47-29.75</b> | <b>0.006</b> |
| Grass                   | 2.4                     | 0.1-13.08        | 2.81        | 0.14-13.56        | 0.624        |
| Mugwort                 | 0.27                    | 0.06-1.49        | 0.23        | 0.07-1.33         | 0.987        |
| Parietaria              | 0.14                    | 0.04-0.61        | 0.14        | 0.06-0.83         | 0.506        |
| Plane tree              | 0.21                    | 0.06-1.36        | 0.19        | 0.05-1.27         | 0.564        |
| Ragweed                 | 0.29                    | 0.07-1.58        | 0.29        | 0.07-1.12         | 0.422        |
| Chenopodium             | 0.14                    | 0.04-0.71        | 0.15        | 0.04-0.95         | 0.572        |
| Cypress                 | 0.12                    | 0.04-0.43        | 0.08        | 0.03-0.39         | 0.328        |
| Olive                   | 0.59                    | 0.11-2.74        | 0.53        | 0.07-1.61         | 0.103        |
| <b>Cat**</b>            | <b>0.11</b>             | <b>0.03-1.18</b> | <b>0.29</b> | <b>0.03-2.24</b>  | <b>0.068</b> |
| Dog                     | 0.33                    | 0.1-1.38         | 0.35        | 0.12-1.86         | 0.497        |
| <b>House dust mite*</b> | <b>0.14</b>             | <b>0.04-1.73</b> | <b>0.40</b> | <b>0.05-5.69</b>  | <b>0.041</b> |
| Latex                   | 0.11                    | 0.07-0.48        | 0.11        | 0.07-0.39         | 0.864        |

The p-value indicates the difference between mild-to-moderate and severe patients.

\*Variables included in the multivariable logistic regression model ( $p < 0.1$ , indicated in bold); \*\* variables selected in the final model after a backwards selection method (indicated in bold and italic). For the sake of clarity, positive associations (risk) with severity are highlighted in red, negative (protection) in green. None of the markers remained significant after Bonferroni correction ( $P < 0.0013$ ).

Table S5

Table S5. Multivariable logistic regression model

| REPORTED SYMPTOMS                                                                                                                                                                                                          |        |           |                         |       |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-----------|-------------------------|-------|
| Model 1: hazelnut allergens                                                                                                                                                                                                | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                                                                                            |        |           | Lower                   | upper |
| Cor a 1                                                                                                                                                                                                                    | -0.016 | 0.98      | 0.97                    | 0.99  |
| Cor a 9                                                                                                                                                                                                                    | 0.117  | 1.12      | 1.05                    | 1.20  |
| Intercept                                                                                                                                                                                                                  | -1.528 |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                                                                                     |        |           |                         |       |
| $1/(1 + \frac{e^{-( -1.528 + (\text{Cor a 1} * -0.016) + (\text{Cor a 9} * 0.117) )}}{1}))$                                                                                                                                |        |           |                         |       |
| Model 2: Clinical characteristics                                                                                                                                                                                          | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                                                                                            |        |           | Lower                   | upper |
| Atopic dermatitis                                                                                                                                                                                                          | 0.591  | 1.81      | 1.14                    | 2.86  |
| Pollen allergy                                                                                                                                                                                                             | -0.846 | 0.43      | 0.26                    | 0.70  |
| Latex allergy                                                                                                                                                                                                              | 0.953  | 2.60      | 1.22                    | 5.50  |
| Intercept                                                                                                                                                                                                                  | -1.201 |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                                                                                     |        |           |                         |       |
| $1/(1 + \frac{e^{-( -1.201 + (\text{atopic dermatitis} * 0.591) + (\text{pollen allergy} * -0.846) + (\text{latex allergy} * 0.953) )}}{1}))$                                                                              |        |           |                         |       |
| Model 3: Clinical characteristics + sIgE against Cor a 14                                                                                                                                                                  | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                                                                                            |        |           | Lower                   | upper |
| Atopic dermatitis                                                                                                                                                                                                          | 1.026  | 2.79      | 1.45                    | 5.35  |
| Pollen allergy                                                                                                                                                                                                             | -0.964 | 0.38      | 0.20                    | 0.72  |
| Latex allergy                                                                                                                                                                                                              | 0.013  | 1.01      | 0.29                    | 3.49  |
| Cor a 14                                                                                                                                                                                                                   | 0.103  | 1.11      | 1.01                    | 1.22  |
| Intercept                                                                                                                                                                                                                  | -1.242 |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                                                                                     |        |           |                         |       |
| $1/(1 + \frac{e^{-( -1.242 + (\text{atopic dermatitis} * 1.026) + (\text{pollen allergy} * -0.964) + (\text{latex allergy} * 0.013) + (\text{Cor a 14} * 0.103) )}}{1}))$                                                  |        |           |                         |       |
| Model 4: Clinical characteristics + sIgE against Cor a 14, walnut, milk, cat                                                                                                                                               | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                                                                                            |        |           | Lower                   | upper |
| Atopic dermatitis                                                                                                                                                                                                          | 1.161  | 3.19      | 1.61                    | 6.33  |
| Pollen allergy                                                                                                                                                                                                             | -1.088 | 0.34      | 0.17                    | 0.66  |
| Latex allergy                                                                                                                                                                                                              | 0.350  | 1.42      | 0.38                    | 5.27  |
| Cor a 14                                                                                                                                                                                                                   | 0.075  | 1.08      | 0.97                    | 1.19  |
| Walnut                                                                                                                                                                                                                     | 0.068  | 1.07      | 1.00                    | 1.14  |
| Milk                                                                                                                                                                                                                       | -1.323 | 0.27      | 0.07                    | 0.98  |
| Cat                                                                                                                                                                                                                        | 0.025  | 1.03      | 1.01                    | 1.04  |
| Intercept                                                                                                                                                                                                                  | -1.231 |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                                                                                     |        |           |                         |       |
| $1/(1 + \frac{e^{-( -1.231 + (\text{atopic dermatitis} * 1.161) + (\text{pollen allergy} * -1.088) + (\text{latex allergy} * 0.350) + (\text{Cor a 14} * 0.075) + (\text{Walnut} * 0.068) + (\text{Cat} * 0.025) )}}{1}))$ |        |           |                         |       |

| DBPCFC SYMPTOMS                                                                                                                                        |        |           |                         |       |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-----------|-------------------------|-------|
| Model 1: hazelnut allergens                                                                                                                            | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                        |        |           | Lower                   | upper |
| Cor a 1                                                                                                                                                | -0.031 | 0.97      | 0.94                    | 1.00  |
| Cor a 9                                                                                                                                                | 0.079  | 1.08      | 1.00                    | 1.18  |
| Intercept                                                                                                                                              | -0.631 |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                 |        |           |                         |       |
| $1/(1 + \frac{e^{-0.631}}{e^{-0.631 + (Cor\ a\ 1 + -0.031) + (Cor\ a\ 9 + 0.079)}})$                                                                   |        |           |                         |       |
| Model 2: Clinical characteristics                                                                                                                      | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                        |        |           | Lower                   | upper |
| Atopic dermatitis                                                                                                                                      | 1.699  | 5.47      | 1.98                    | 15.13 |
| Pollen allergy                                                                                                                                         | -2.076 | 0.125     | 0.04                    | 0.44  |
| Intercept                                                                                                                                              | 0.378  |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                 |        |           |                         |       |
| $1/(1 + \frac{e^{0.378}}{e^{0.378 + (atopic\ dermatitis + 1.699) + (pollen\ allergy + -2.076)}})$                                                      |        |           |                         |       |
| Model 3: Clinical characteristics + sIgE against Cor a 14                                                                                              | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                        |        |           | Lower                   | upper |
| Atopic dermatitis                                                                                                                                      | 2.290  | 9.87      | 2.66                    | 39.69 |
| Pollen allergy                                                                                                                                         | -2.862 | 0.06      | 0.01                    | 0.28  |
| Cor a 14                                                                                                                                               | 0.010  | 1.01      | 0.94                    | 1.08  |
| Intercept                                                                                                                                              | 0.757  |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                 |        |           |                         |       |
| $1/(1 + \frac{e^{0.757}}{e^{0.757 + (atopic\ dermatitis + 2.290) + (pollen\ allergy + -2.862) + (Cor\ a\ 14 + 0.010)}})$                               |        |           |                         |       |
| Model 4: Clinical characteristics + sIgE against Cor a 14 and walnut                                                                                   | Beta   | Exp(Beta) | 95% CI around exp(beta) |       |
|                                                                                                                                                        |        |           | Lower                   | Upper |
| Atopic dermatitis                                                                                                                                      | 2.574  | 13.11     | 3.04                    | 56.54 |
| Pollen allergy                                                                                                                                         | -3.005 | 0.05      | 0.01                    | 0.28  |
| Cor a 14                                                                                                                                               | -0.074 | 0.93      | 0.80                    | 1.08  |
| Walnut                                                                                                                                                 | 0.400  | 1.49      | 1.10                    | 2.01  |
| Intercept                                                                                                                                              | 0.338  |           |                         |       |
| Probability of a severe allergic reaction to hazelnuts                                                                                                 |        |           |                         |       |
| $1/(1 + \frac{e^{0.338}}{e^{0.338 + (-0.338 + (atopic\ dermatitis + 2.574) + (pollen\ allergy + -3.005) + (Cor\ a\ 14 + -0.074) + (Walnut + 0.400)}})$ |        |           |                         |       |

Results from the multivariable regression model in predicting severe allergic reaction to hazelnuts.

Calculations are based on a model combining atopic dermatitis (0/1), pollen allergy (0/1), latex allergy (0/1), Cor a 14 (IgE in kU/L), walnut (IgE in kU/L), Milk (IgE in kU/L), Cat (IgE in kU/L).

For the quantitative variables of serology, do the models show the effect of a change in 1 kU/L?

Table S6

| Reported  |                      |        |                   |          |                      |              |                      |             |             |           |              |            |
|-----------|----------------------|--------|-------------------|----------|----------------------|--------------|----------------------|-------------|-------------|-----------|--------------|------------|
| Allergens | Positivity threshold |        | Mild/<br>Moderate | Severe   | Sensitivity (95% CI) |              | Specificity (95% CI) |             | PPV(95% CI) |           | NPV (95% CI) |            |
| Extract   | ≥ 0.35               | +<br>- | 492<br>91         | 92<br>22 | 80.7                 | 72.2 - 87.5  | 15.6                 | 12.8 - 18.9 | 15.8        | 12.9-19   | 80.5         | 72.0-87.4  |
| CRD (any) | ≥ 0.35               | +<br>- | 309<br>41         | 56<br>15 | 78.9                 | 67.6-87.7    | 11.7                 | 8.54-15.5   | 15.3        | 11.8-19.5 | 73.2         | 59.7-84.2  |
| rCor a 1  | ≥ 0.35               | +<br>- | 268<br>78         | 41<br>28 | 59.4                 | 46.9 - 71.1  | 22.5                 | 18.2 - 27.3 | 13.3        | 9.7-17.6  | 73.6         | 64.1-81.7  |
| rCor a 2  | ≥ 0.35               | +<br>- | 73<br>276         | 10<br>61 | 14.1                 | 7.0 - 24.4   | 79.1                 | 74.4 - 83.2 | 12.1        | 5.9-21.0  | 81.9         | 77.4-85.9  |
| rCor a 8  | ≥ 0.35               | +<br>- | 39<br>308         | 9<br>61  | 12.9                 | 6.0 - 23.0   | 88.8                 | 85.0 - 91.9 | 18.8        | 8.9-32.6  | 83.5         | 79.3-87.1  |
| nCor a 9  | ≥ 0.35               | +<br>- | 29<br>320         | 13<br>58 | 18.3                 | 10.1 - 29.3  | 91.7                 | 88.3 - 94.4 | 30.9        | 17.6-47.1 | 84.7         | 80.6-88.1  |
| nCor a 11 | ≥ 0.35               | +<br>- | 9<br>340          | 6<br>65  | 8.4                  | 3.2 - 17.4   | 97.4                 | 95.2- 98.8  | 40.0        | 16.3-67.7 | 84.0         | 80.0-87.4  |
| nCor a 12 | ≥ 0.35               | +<br>- | 45<br>303         | 15<br>56 | 21.1                 | 12.3 - 32.4  | 87.1                 | 83.1 - 90.4 | 25.0        | 14.7-37.9 | 84.4         | 80.2-88.0  |
| rCor a 14 | ≥ 0.35               | +<br>- | 15<br>327         | 10<br>56 | 15.2                 | 7.5 - 26.1   | 95.6                 | 92.9 - 97.5 | 40.0        | 21.1-61.3 | 85.4         | 81.4-88.8  |
| CCD       | ≥ 0.35               | +<br>- | 36<br>307         | 7<br>62  | 10.1                 | 4.2 - 19.8   | 89.5                 | 85.8 - 92.5 | 16.3        | 6.8-30.7  | 83.2         | 79.0-86.9  |
| Models    | Positivity threshold |        | Mild/<br>Moderate | Severe   | Sensitivity (95% CI) |              | Specificity (95% CI) |             | PPV(95% CI) |           | NPV (95% CI) |            |
| Model 1   | ≥ 6%                 | +<br>- | 316<br>18         | 62<br>3  | 95.4                 | 87.1-99.0    | 5.4                  | 3.2-8.4     | 16.4        | 12.8-20.5 | 85.7         | 63.7-97.0  |
| Model 2   | ≥ 19%                | +<br>- | 210<br>322        | 63<br>43 | 59.4                 | 49.5-68.9    | 60.5                 | 56.2-64.7   | 23.1        | 18.2-28.5 | 88.2         | 84.5-91.3  |
| Model 3   | ≥ 10%                | +<br>- | 184<br>122        | 52<br>9  | 85.3                 | 73.8-93.0    | 39.9                 | 34.3-45.6   | 22.0        | 16.9-27.9 | 93.1         | 87-36-96.8 |
| Model 4   | ≥ 7%                 | +<br>- | 287<br>17         | 58<br>3  | 95.1                 | 86.3-99.0    | 5.6                  | 3.3-8.8     | 16.8        | 13.0-21.2 | 85.0         | 62.1-96.8  |
| DBPCFC    |                      |        |                   |          |                      |              |                      |             |             |           |              |            |
| Allergens | Positivity threshold |        | Mild/<br>Moderate | Severe   | Sensitivity (95% CI) |              | Specificity (95% CI) |             | PPV(95% CI) |           | NPV (95% CI) |            |
| Extract   | ≥ 0.35               | +<br>- | 71<br>5           | 26<br>5  | 83.9                 | 66.3 - 94.55 | 6.6                  | 2.2 - 14.7  | 26.8        | 18.3-36.8 | 50.0         | 18.7-81.3  |
| CRD (any) | ≥ 0.35               | +<br>- | 65<br>2           | 25<br>4  | 86.2                 | 68.3-96.1    | 3.0                  | 0.4-10.4    | 27.8        | 18.9-38.2 | 33.3         | 4.3-77.7   |
| rCor a 1  | ≥ 0.35               | +<br>- | 61<br>6           | 17<br>12 | 58.6                 | 38.9 - 76.5  | 9.0                  | 3.4 - 18.5  | 21.8        | 13.2-32.6 | 33.3         | 13.3-59.0  |
| rCor a 2  | ≥ 0.35               | +<br>- | 13<br>54          | 4<br>25  | 13.8                 | 3.9- 31.7    | 80.6                 | 69.1 - 89.2 | 23.5        | 6.8-49.9  | 68.3         | 56.9-78.4  |
| rCor a 8  | ≥ 0.35               | +<br>- | 5<br>62           | 6<br>23  | 20.7                 | 8.0 - 39.72  | 92.5                 | 83.4 - 97.5 | 54.5        | 23.4-83.2 | 72.9         | 62.2-82.0  |
| nCor a 9  | ≥ 0.35               | +<br>- | 3<br>64           | 7<br>22  | 24.1                 | 10.3 - 43.5  | 95.5                 | 87.5 - 99.1 | 70.0        | 34.8-93.3 | 74.4         | 63.9-83.2  |
| nCor a 11 | ≥ 0.35               | +<br>- | 3<br>64           | 4<br>25  | 13.8                 | 3.9 - 31.7   | 95.5                 | 87.5 - 99.1 | 57.1        | 18.4-90.1 | 71.9         | 61.4-80.9  |
| nCor a 12 | ≥ 0.35               | +<br>- | 7<br>60           | 8<br>21  | 27.6                 | 12.7 - 47.2  | 89.5                 | 79.6 - 95.7 | 53.3        | 26.6-78.7 | 74.1         | 63.1-83.2  |
| rCor a 14 | ≥ 0.35               | +<br>- | 4<br>59           | 6<br>19  | 24.0                 | 9.4 - 45.1   | 93.7                 | 84.5 - 98.2 | 60.0        | 26.2-87.8 | 75.6         | 64.6-84.7  |
| CCD       | ≥ 0.35               | +<br>- | 4<br>307          | 5<br>62  | 17.2                 | 5.8 - 35.8   | 94.0                 | 85.4 - 98.3 | 55.6        | 21.2-86.3 | 72.4         | 61.8-81.5  |
| Models    | Positivity threshold |        | Mild/<br>Moderate | Severe   | Sensitivity (95% CI) |              | Specificity (95% CI) |             | PPV(95% CI) |           | NPV (95% CI) |            |
| Model 1   | ≥ 22%                | +<br>- | 46<br>21          | 27<br>2  | 93.1                 | 77.2-99.2    | 31.3                 | 20.6-43.8   | 37.0        | 26.0-49.1 | 91.3         | 72.0-98.9  |
| Model 2   | ≥ 50%                | +<br>- | 15<br>57          | 22<br>10 | 68.8                 | 50.0-83.9    | 79.2                 | 68.0-87.8   | 59.5        | 42.1-75.2 | 85.1         | 74.3-92.6  |
| Model 3   | ≥ 11%                | +<br>- | 21<br>38          | 23<br>2  | 92.0                 | 72.0-98.9    | 64.4                 | 50.9-76.5   | 52.3        | 36.7-67.5 | 95.0         | 83.1-99.4  |
| Model 4   | ≥ 8%                 | +<br>- | 14<br>45          | 23<br>2  | 92.0                 | 76.5-99.1    | 76.3                 | 63.4-86.4   | 62.2        | 44.8-77.5 | 95.7         | 85.5-99.5  |

Model 1: Cor a 1 and Cor a 9

model 2: *Reported* : Atopic dermatitis, pollen allergy, latex allergy; *DBPCFC*: Atopic dermatitis, pollen allergy;model 3: *Reported*: Atopic dermatitis, pollen allergy, latex allergy, Cor a 14; *DBPCFC*: Atopic dermatitis, pollen allergy, Cor a 14;model 4: *Reported*: Atopic dermatitis, pollen allergy, latex allergy, Cor a 14, walnut, milk, cat; *DBPCFC*: Atopic dermatitis, pollen allergy, Cor a 14, walnut;

