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ORIGINAL ARTICLE GASTROINTESTINAL DISEASES

The EuroPrevall outpatient clinic study on food allergy: background and methodology

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cross-sectional; EuroPrevall; food allergy; methods; outpatient clinic.

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Abstract

Background: The EuroPrevall project aimed to develop effective management strategies in food allergy through a suite of interconnected studies and a multidisciplinary integrated approach. To address some of the gaps in food allergy diagnosis, allergen risk management and socio-economic impact and to complement the EuroPrevall population-based surveys, a cross-sectional study in 12 outpatient clinics across Europe was conducted. We describe the study protocol.

Methods: Patients referred for immediate food adverse reactions underwent a consistent and standardized allergy work-up that comprised collection of medical history; assessment of sensitization to 24 foods, 14 inhalant allergens and 55 allergenic molecules; and confirmation of clinical reactivity and food thresholds by standardized double-blind placebo-controlled food challenges (DBPCFCs) to milk, egg, fish, shrimp, peanut, hazelnut, celeriac, apple and peach.

Results: A standardized methodology for a comprehensive evaluation of food allergy was developed and implemented in 12 outpatient clinics across Europe. A total of 2121 patients (22.6% <14 years) reporting 8257 reactions to foods were studied, and 516 DBPCFCs were performed.

Conclusions: This is the largest multicentre European case series in food allergy, in which subjects underwent a comprehensive, uniform and standardized evaluation including DBPCFC, by a methodology which is made available for

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further studies in food allergy. The analysis of this population will provide information on the different phenotypes of food allergy across Europe, will allow to validate novel *in vitro* diagnostic tests, to establish threshold values for major allergenic foods and to analyse the socio-economic impact of food allergy.

Despite important advances in the knowledge of food allergy in the last two decades, gaps in epidemiology, diagnosis, treatment and socio-economic impact hamper an effective disease management.

The prevalence of food allergy is not well established. Recent studies (1–3) have shown marked heterogeneity in prevalence estimates depending on the definition and methodology used. The higher estimates (3–35%) are found in studies using reported reactions, but when food sensitization or oral food challenges are used, the estimate is significantly reduced. Sound methodological studies are missing to assess risk factors for food allergy, differences in prevalence, pattern of foods involved and severity in different geographical areas.

In the diagnosis of IgE-mediated food allergy, sensitization to the food is assessed by skin prick tests (SPTs) and/or measurements of serum-specific IgE, but to establish their clinical relevance, oral challenges are (frequently) needed. Although challenges provide the definitive diagnosis of food allergy, they carry an inherent risk of potentially severe reactions, are time-consuming and have an important healthcare cost, and as a result, they are not performed in many clinical settings. The consequence of inadequate diagnostic procedures is predominantly an overdiagnosis of food allergy (4–6).

The current management of food allergy is based on food avoidance and rescue medication in case of accidental reactions, including self-injectable adrenaline in patients at risk of anaphylaxis. Recently, food immunotherapy has shown promise in the management of patients, but it is not yet a standard care option (6–8). Therefore, the management of patients with food allergies still relies on food avoidance. The stress and anxiety about possible reactions has a profound negative impact on their quality of life (QOL) (4, 5, 9, 10).

Within this context, the EU-funded integrated project EuroPrevall aimed to deliver the information and tools necessary for policymakers, regulators, food industry, clinicians and allergic consumers (patients), to effectively manage different aspects of food allergy and improve the QOL of food-allergic patients (11).

Rationale and objectives of the outpatient clinic study of EuroPrevall

Three complementary epidemiological studies were conducted in EuroPrevall (11): a birth cohort study conducted in nine countries (12), a general population survey in schoolchildren and adults in eight countries (13) and a cross-sectional study in allergy clinics in 12 countries (Fig. 1), which is the subject of this manuscript. Countries were chosen to give a geographic spread across Europe, reflecting different climatic and cultural regions. In six of them (Greece, Iceland, Lithuania, the Netherlands, Poland and Spain), all three studies were performed (11).

The birth cohort and the population survey in schoolchildren and adults aimed to study prevalence patterns, and factors associated with food sensitization and confirmed food allergy across Europe (9, 11). To complement these population-based studies, a cross-sectional study was implemented to characterize patients referred for adverse reactions to foods to 'expert' allergy clinics. In this outpatient clinic study, patients underwent a consistent and standardized allergy work-up. It comprised assessment of sensitization to 24 foods, 14 inhalant allergens and latex and 55 allergenic molecules; and confirmation of clinical reactivity by standardized double-blind placebo-controlled food challenges (DBPCFCs) to milk, egg, fish, shrimp, peanut, hazelnut, celeriac, apple and peach. Additionally, the allergy clinics in Athens, Lodz, Madrid, Reykjavik, Utrecht, Vilnius and Zürich performed a full clinical evaluation including DBPCFC in cases selected within the population surveys of schoolchildren and adults (13).

This highly characterized population was used to establish a serum bank (14, 15) and was a core platform for many of the project activities: (i) standardization of DBPCFC (16); (ii) provision of threshold values for major allergenic foods (17); (iii) assessment of the impact of food allergy in QOL (9); (iv) evaluation of the costs of food allergy (18, 19); (v) authentication of the EuroPrevall allergen library and characterization of novel allergens (20–22); (vi) validation of novel *in vitro* diagnostic methods (component-resolved diagnosis [CRD], protein chip diagnostics and cell-based tests that measure biological activity) (11); and (vii) investigation of the effect of the food matrix and digestion on food allergenicity (23).

Methods

Study design and setting

The outpatient clinic study was conducted as a collaborative multicentre cross-sectional study carried out in 12 allergy clinics across Europe: Reykjavik (Iceland), Manchester (UK), Utrecht (the Netherlands), Lodz (Poland), Prague (Czech Republic), Sofia (Bulgaria), Strasbourg (France), Vilnius (Lithuania), Zürich (Switzerland), Milano (Italy), Athens (Greece) and Madrid (Spain) (Fig. 1, Table 1).

Abbreviations

BP, blood pressure; CRD, component-resolved diagnosis; DBPCFC, double-blind placebo-controlled food challenge; NOAEL, no observed adverse effect level; OFC, open food challenge; PEF, peak expiratory flow; QOL, quality of life; SAE, severe adverse event; SOP, standard operating procedures; SPT, skin prick test.

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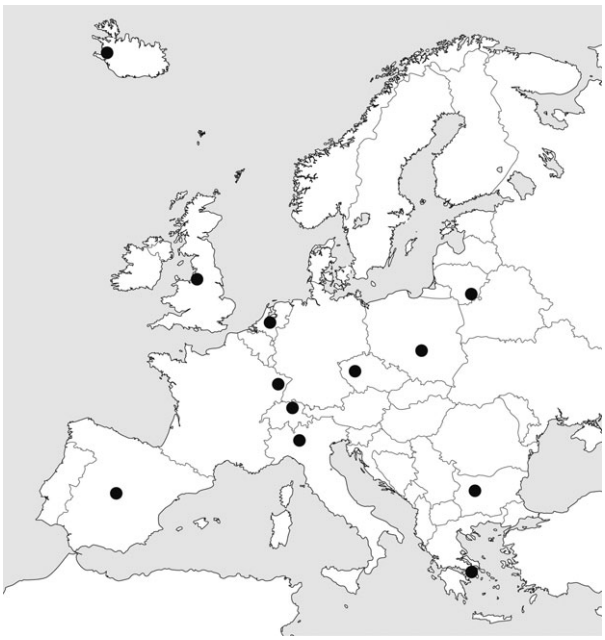


Figure 1 Centres participating in the outpatient clinic study of EuroPrevall: Reykjavik (Iceland), Manchester (UK), Utrecht (the Netherlands), Lodz (Poland), Prague (Czech Republic), Sofia (Bulgaria), Strasbourg (France), Vilnius (Lithuania), Zürich (Switzerland), Milano (Italy), Athens (Greece) and Madrid (Spain).

Patients

The selection of patients and their clinical evaluation followed a standardized protocol to which all centres agreed, and that was submitted to local ethics committees. Once ethical approval was obtained, the recruitment started by inviting all new patients seen in the clinics who reported adverse reactions to any food in the 2 h following ingestion. Oral and written information was given to all the subjects (or their legal representatives). Only those who gave written consent were included, and submitted to a thorough clinical evaluation comprising a detailed medical history, SPT, and blood sampling for *in vitro* measurements. Double-blind placebo-controlled food challenge were performed in patients who reported reactions to either milk, egg, fish, shrimp, peanut, hazelnut, peach, apple or celeriac.

Standardized collection of medical history

A detailed clinical history was collected by means of a standardized questionnaire divided in two forms (online supplement material):

- Background form: including demographic data, family and personal history of atopy.
- Food form: including a detailed information of the severest (if more than one) reaction induced by the food intake. For each patient, one food form was completed for each food involved in adverse reactions.

Skin prick tests

Skin prick tests were performed in one session to improve patients' compliance, and four different panels were tested according to the patients' age (Table 2): priority 1 and 2 foods in children <4 years; the 24 EuroPrevall foods in children 4–6 years old; the 24 foods and four inhalant allergens – grass, birch and mugwort pollens, and mites – in children 7–12 years old; and the 24 foods, 14 inhalant allergens and latex in subjects ≥13 years. Anisakis (a parasitic nematode of fish) was only tested in patients who reported reactions to fish. Saline and histamine were used as negative and positive controls, respectively. Extracts were generously provided by ALK Abelló SA (Madrid, Spain). Although the whole panel of foods, inhalants and latex was not tested in children <13 years of age, all subjects were tested to the whole panel of allergens by means of serum IgE determinations.

The 24 EuroPrevall foods (Table 2) comprise all the foods included in the European allergen labelling directive, and some additional foods that deserved to be investigated on a broad European basis (i.e. fruits, walnut, soya bean, buckwheat, banana or poppy seed) (2, 3, 11). Priority 1 foods were those selected for DBPCFC, and the remaining foods were classified in two priorities for obtaining information on clinical reactivity, sensitization and allergen characterization (11–13, 20–22).

Skin prick tests were performed on the volar aspect of the forearms or on the back, and the location of each allergen extract was predefined. The mean wheal diameter (mm) was calculated (largest diameter + perpendicular diameter in its centre/2). The ratio of the allergen mean wheal divided by the histamine mean wheal (skin index) was calculated for each allergen. Skin prick tests with a mean wheal diameter at least 3 mm larger than the saline control, or with a skin index ≥0.5 were considered positive (24).

In vitro measurements

A blood sample was obtained from all the subjects. Total IgE, specific IgE to the 24 foods, 12 inhalant allergens and latex, and tryptase were measured using the ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). When the serum sample was <2 ml (48/2121, 2.26%; 38 patients <14 years, and 10 subjects ≥14 years), sera were first screened with ImmunoCAP tests containing five different food allergen mixes, that together covered the 24 foods studied. Two of the mixes were commercially available: fx5 (egg, milk, soya, peanut, wheat, fish) and fx6 (sesame seed, wheat, buckwheat, corn, rice). Three mixes were custom-made: mix 1 (hazelnut, tomato, walnut, carrot and celery), mix 2 (shrimp, poppy seed, lentil, mustard and sunflower seed) and mix 3 (apple, kiwi, melon, banana and peach). Samples testing positive for a mix (≥0.35 kU_A/l) were subsequently tested for the individual foods of that mix. All testing was performed using the ImmunoCAP 250 analyzer. Sensitivity of the mixes had previously been assessed by submitting a subset of mix-negative samples to testing with single allergens, without finding any false mix negatives (J. Lidholm, personal communication).

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Table 1 Characteristics of the 12 centres involved in the outpatient clinics study of EuroPrevall

Centre	Public/ private	University hospital	Covered population	Patients' referral system*	Patients' age	Hospital Department
Athens	Public	Yes	Athens; Central and Southern Greece	1,2,3,4	Any	Allergy
Lodz	Public	Yes	800 000 inhabitants	1,2,3	Any	Immunology, Rheumatology and Allergy
Madrid	Public	Yes	750 000 inhabitants of Madrid area	1,2,3	Any	Allergy
Manchester	Public	Yes	Manchester area; Northwest UK	1,2,3	Any	Allergy & Clinical Immunology
Milano	Private	No	Milano	1,2,3,4	≥8 years	Allergy
Prague	Public	Yes	NA	1,2,3,4	≤19 years	Pediatrics
Sofia	Public	Yes	NA	1,2,3,4	Any	Allergy
Strasbourg	Public	Yes	Alsace; East France	1,2,3,4	Any	Pulmonology
Reykjavik	Public	Yes	Whole Iceland (320 000 inhabitants)	1,2,3,4	Any	Allergy & Pulmonology
Utrecht	Public	Yes	Utrecht area	1,3	≥16 years	Dermatology
Vilnius	Public	Yes	Vilnius area	1,2,3,4	Any	Allergy
Zürich	Public	Yes	Zürich area	1,3	Adults	Dermatology

*Patients' referral system to allergy clinics: (1) by general practitioners; (2) by paediatricians; (3) by other specialties; and (4) patients take the appointment directly.
NA, not available.

Table 2 Panel of foods, inhalant allergens and latex used in skin prick tests and serum IgE determinations

Priority 1 foods	Priority 2 foods	Priority 3 foods	Inhalant allergens & latex
Milk	Soya bean	Sunflower seed	Grass pollen*
Egg	Walnut	Carrot	Birch pollen
Fish (cod)	Sesame seed	Buckwheat	Olive pollen
Shrimp	Mustard seed	Banana	Ash pollen†
Peanut	Kiwi	Lentil	Cypress pollen
Hazelnut	Wheat	Tomato	Mugwort pollen
Peach		Poppy seed	Plane tree pollen
Apple		Corn	Parietaria judaica pollen
Celery		Melon	Chenopodium album pollen
			Ragweed pollen
			Dermatophagoides spp‡
			Lepidoglyphus destructor†
			Cat dander
			Dog dander
			Latex

*Grass pollen sensitization was assessed to a mixture of grasses in skin prick tests (SPT) and to *Phleum pratense* in serology.
†Ash pollen and Lepidoglyphus destructor were only used in SPT.
‡Mite sensitization was assessed to a mixture of *Dermatophagoides pteronyssinus* and f*arinae* in SPT and to *Dermatophagoides pteronyssinus* in serology.

Allergens from the EuroPrevall allergen library (20–22) were tested in a custom-made microarray technically resembling the ImmunoCAP ISAC test (Table 3) (25), and in ImmunoCAPs for CRD in selected food allergies (e.g. kiwifruit, hazelnut, celeriac, peanut) (26).

DBPCFC tests

Foods, dosing and blinding

Double-blind placebo-controlled food challenge with milk, egg, hazelnut, peanut, celeriac, fish, shrimp, apple and peach were undertaken (Table S1, supplement material). To ensure that the challenge meals used were the same in all clinical centres, the meals (except apple, peach and shrimp) were prepared centrally, using a common matrix with a 6-month shelf life validated for the purposes of DBPCFC. The challenge matrix consisted on an allergen-free dry chocolate dessert that could be shipped at ambient temperature, and stored prior to rehydration at the point of use. The ability of the dessert formulation to blind each food was assessed by a tasting panel (16). The microbiological safety and allergen content were confirmed before materials were sent out to the clinical centres.

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Table 3 Individual allergens included in the EuroPrevall microarray

Source	Latin name	Allergen
Cow's milk	*Bos domesticus*	nBos d 4 (α-lactalbumin)
		nBos d 5 (β-lactoglobulin)
		nBos d 8 (total casein)
Goat's milk	*Capra hircus*	nCap h (total casein)
Hen's egg	*Gallus domesticus*	nGal d 1 (ovomucoid)
		nGal d 2 (ovalbumin)
		nGal d 3 (conalbumin, ovotransferin)
		nGal d 4 (lysozyme)
		nGal d 5 (α-livetin)
Carp	*Cyprinus carpio*	rCyp c 1 (parvalbumin)
Cod	*Gadus callarias*	rGad m 1 (parvalbumin)
Shrimp	*Penaeus aztecus*	rPen a 1 (tropomyosin)
Peanut	*Arachis hypogea*	nAra h 1 (7S globulin)
		nAra h 2, 6 (2S albumin)
		nAra h 3, 4 (11S globulin)
		rAra h 8 (PR-10)
Hazelnut	*Corylus avellana*	rCor a 1.04 (PR-10)
		rCor a 2 (profilin)
		rCor a 8 (nsLTP)
		nCor a 9 (11S globulin)
		nCor a 11 (7S globulin)
Peach	*Prunus persica*	rPru p 1 (PR-10)
		nPru p 3 (nsLTP)
Apple	*Malus domesticus*	rMal d 1 (PR-10)
		nMal d 2 (thaumatin-like protein)
		nMal d 3 (nsLTP)
		rMal d 4 (profilin)
Celery	*Apium graveolens*	rApi g 1.01 (PR-10)
		rApi g 4 (profilin)
		nApi g 5 (FAD-containing oxidase)
Kiwi fruit	*Actinidia chinensis*	nAct c 1 (actinidin)
Mustard	*Sinapis alba*	nSin a 1 (2S albumin)
Sesame	*Sesamum indicum*	nSes i 1, nSes i 2 (2S albumin)
		nSes i 3 (7S globulin)
Soya	*Glycine max*	rGly m 4 (PR-10)
		nGly m 5 (ß-conglycinin, 7S globulin)
		nGly m 6 (Glycinin, 11 S globulin)
Walnut	*Juglans regia*	nJug r 2 (7S vicilin)
		nJug r 4 (11S globulin)
Wheat	*Triticum aestivum*	rTri a 12 (profilin)
		nTri a 19 (total gliadins/glutenins)
Sunflower	*Helianthus annuus*	nHel a 2S albumin
		nHel a 3 (nsLTP)
Carrot	*Daucus carota*	rDau c1.0103; rDau c1.0201 (PR-10)
		rDau c 4 (profilin)
Tomato	*Lycopersicon esculentum*	nLyc e 3 (nsLTP)
Birch pollen	*Betula verrucosa*	rBet v 1 (PR-10)
		rBet v 2 (profilin)
Timothy grass	*Phleum pratense*	rPhl p 5
		rPhl p 6
		rPhl p 7 (polcalcin)
Latex	*Hevea brasiliensis*	rHev b 8 (profilin)
Pineapple	*Ananas comosus*	nAna c 2 (bromelain)

N, natural; r, recombinant.

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The aims of the DBPCFCs were to provide threshold information and be a diagnostic tool. Thus, it was necessary to start at a low dose and gradually increase until the amount of food in a typical serving was reached. All doses were assessed in terms of the amount of protein provided. Data from the literature suggested that a first dose of 3 μg of protein should be low enough to provide no observed adverse effect levels (NOAELs) (17, 27). From there, doses used were 30 and 300 μg; 3, 30, 100 and 300 mg; and 1 and 3 g. The top dose for peanut and hazelnut was delivered in a chocolate bar to maintain blinding. Because of the large volume required to deliver the top dose and the cumulative amount of Tween-60 contained, the dessert was unsuitable for use in children below 29 kg weight (16). Challenges in bottle-fed infants were performed by adding milk or egg powder to the formula taken by the baby. In older children, foods were masked in a vanilla-orange pudding according to a recipe developed for the EuroPrevall birth cohort (12).

For fish, the maximum amount that was adequately blinded in the chocolate matrix was 1 g of protein, equivalent to 5 g cooked fish. For this reason, six blinded doses containing 3, 60 and 600 μg; 12 and 120 mg; and 1 g fish protein were followed by the open administration of three doses of cooked Atlantic cod of 10, 30 and 60 g, equivalent to 2, 6 and 12 g protein. The cumulative dose of fish was 101 g.

We were not able to blind shrimp in the chocolate matrix; so instead, this was prepared in a shrimp burger recipe. It was made with chicken meat, dried oregano, dried onion and ground black pepper, to which varying amounts of whole frozen shrimp were added before mincing, forming into burgers and cooking. The shrimp burgers were prepared according to a protocol in the certified hospital kitchen at each clinical centre. The maximum amount of cooked shrimp adequately blinded was 16 g, equivalent to 3 g protein. For this reason, seven blinded doses containing 3, 60 and 600 μg; 12 and 120 mg; and 1 and 3 g shrimp protein were followed by the open administration of two doses of cooked shrimp of 30 and 50 g, equivalent to 6 and 9 g protein. The cumulative dose of shrimp was 102 g.

Although apple and peach could be blinded in the chocolate matrix as freeze-dried powder, the allergenicity of labile allergens was reduced. Thus, these fruits were prepared at the centres using a smoothie style recipe in a matrix containing orange and pineapple juice, coconut yoghurt, soluble hydrolysed cereals, instant coffee and ground coconut. In the apple and peach DBPCFC, only three blinded doses (1/8, 2/8 and 5/8 of a whole fruit with peel) were given. As a result, the data were not suitable for obtaining thresholds. The recipe had already proved to be useful as a diagnostic tool in patients sensitized to profilin and lipid transfer proteins (28, 29). Pilot challenges performed in Swiss patients with birch pollen-related apple ($n = 6$) and peach ($n = 6$) allergies confirmed that the recipe was also useful in this population. Nine of the 12 DBPCFCs were positive with oral itching, and four of 12 with mild objective symptoms. Two of the three patients with negative DBPCFC were also tolerant in an open challenge with one raw apple, and one patient developed mild oral itching after the open intake of one raw peach.

Inclusion and exclusion criteria

Patients of any age with a case history of an immediate adverse reaction to either milk, egg, fish, shrimp, hazelnut, peanut, celeriac, apple or peach, and providing signed informed consent were included. Exclusion criteria are shown in Table 4. Additionally, for shrimp, apple and peach challenges, allergy to any of the ingredients used for masking these foods was an exclusion criterium. Patients with severe life-threatening anaphylaxis occurring within 2 h after ingestion of the food in question, involving drop of blood pressure (BP), severe bronchospasm or laryngeal oedema, leading to emergency treatment, were excluded. The principal investigators in Madrid, Utrecht and Zürich reviewed these severe reactions and had to agree before excluding the subject from DBPCFC.

Table 4 Exclusion criteria for double-blind placebo-controlled food challenge

Anaphylaxis	Life-threatening anaphylaxis with the study food, involving drop of blood pressure, severe bronchospasm or laryngeal oedema, leading to emergency treatment
Pregnancy	
Contraindication to adrenaline	Ischaemic heart disease, poor controlled hypertension or cardiac arrhythmia
Unstable/poor controlled asthma	($FEV_1 < 70\%$ of predicted)
Unstable atopic dermatitis	
Chronic urticaria	
Mastocytosis	
Drugs	Systemic corticosteroids in the two previous weeks Antihistamines in the previous 3 days, except hydroxyzine and dexclorfeniramine in the previous 10 days. Beta-blocking agents in the previous day Angiotensin-converting enzyme (ACE) inhibitors in the previous 2 days Tricyclic antidepressants in the previous 5 days Monoamine oxidase (MAO) inhibitors in the previous 2 weeks Immunosuppressive treatments
Seasonal allergy (transient contraindication)	
Acute infection (transient contraindication)	
Any clinically significant disease that can affect patient's safety or can make implementation of the protocol or interpretation of the results difficult	

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Pre- and postchallenge assessments

Patients were carefully examined before undergoing DBPCFC. Pulse rate, BP, lung auscultation, baseline peak expiratory flow (PEF) and, in subjects with a case history of asthma or a bronchospasm within an allergic reaction to the food under investigation, FEV_1 were assessed. The oral cavity and the skin were carefully inspected. In case of a positive reaction or at the end of the DBPCFC session, all parameters were reassessed. Intravenous lines were installed in patients with a history of systemic reactions to the studied food or when the responsible physician thought this appropriate. After intake of the last dose, patients were kept under observation for at least 2 h. At discharge, patients were provided with emergency drugs (antihistamines, corticosteroids, inhaled beta-2-mimetics) and advised to report any late-onset reactions occurring within 48 h after the challenge.

DBPCFC procedure

Active and placebo provocations were randomly assigned and performed on two different days with doses administered at 20-min intervals. Challenges were discontinued after the dose, leading to the first objective reaction or ingestion of the whole meal. Objective symptoms considered for discontinuation were blisters of the oral mucosa, skin flushing, urticaria, angioedema, rhinitis, conjunctivitis, drop of BP ≥20 mmHg, drop of FEV_1 >12% or PEF ≥20%, laryngeal oedema, diarrhoea and emesis. Challenges were also stopped in case of severe persistent (>45 min) subjective symptoms such as severe itching of palms, soles, head or severe gastric/abdominal pain.

Open food challenges

In case of a negative DBPCFC, patients underwent an open food challenge (OFC) with a normal helping (e.g. one hen's egg, one apple, 20 shelled raw hazelnuts) that was administered in three doses (1/8, 2/8 and 5/8) at 30-min intervals. The same pre- and postchallenge assessments and criteria for positivity as described for DBPCFC were applied.

Reporting of severe adverse events (SAEs) during challenge procedures

In case of SAE during oral challenges, a report had to be sent to the study coordinator within 24 h of the event. The specific SAE reporting form and the DBPCFC and OFC forms are included in the online supplement material.

Study management

Training in standard operating procedures (SOP)

Before starting the recruitment of patients, a training session was organized for revision of questionnaires, and SOPs

Table 5 Figures of the final data set of the EuroPrevall outpatient clinic study

Centre	Patients *N* (% <14 years)	SPT	Serology	Foods	Priority 1 foods	DBPCFC	OFC
Outpatient clinics study							
Reykjavik	131 (1.5)	131	129	352	170	65	12
Manchester	124 (17.7)	123	118	538	245	0	0
Utrecht	133 (0)	133	133	782	233	28	6
Lodz	251 (33.9)	251	248	1205	528	49	11
Prague	110 (68.2)	109	98	283	148	24	6
Sofia	157 (46.5)	156	143	386	226	24	15
Strasbourg	174 (8.1)	173	169	726	343	38	1
Vilnius	206 (15.5)	205	183	683	403	1	0
Zürich	285 (2.5)	285	282	1372	579	106	24
Athens	186 (15.6)	186	185	525	175	44	22
Milano	119 (1.7)	119	119	751	229	17	2
Madrid	245 (56.7)	244	238	654	264	120	43
Total	2121 (22.6)	2115	2045	8257	3543	516	142
Population-based survey in schoolchildren and adults							
Reykjavik	13 (53.9)	13	13	25	18	13	0
Utrecht	67 (25.4)	66	67	377	154	24	5
Lodz	4 (50)	4	4	9	7	3	0
Zürich	46 (8.7)	46	46	167	101	46	4
Athens	5 (80)	5	5	12	5	3	1
Madrid	17 (47.1)	17	17	46	21	8	1
Total	152 (27.6)	151	152	636	306	97	11

Patients, skin prick test (SPT) and serology: figures correspond to the number of patients included and for whom SPT and serology (ImmunoCAP) were performed. Under 'Patients', the total number of subjects included per centre (N) and the per cent of those below 14 years of age (%<14 years) are given.

Foods: number of different foods involved in adverse reactions.

Priority 1 foods: number of adverse reactions to milk, egg, fish shrimp, hazelnut, peanut, celeriac, apple and peach.

DBPCFC, OFC: number of challenges performed to priority 1 foods.

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including SPT, blood sampling, DBPCFC and OFC. Further training sessions were organized at the project meetings. Partners with limited experience on food challenges undertook 2-week training at either Madrid or Zürich clinics. Additionally, before starting DBPCFC, all centres had to ensure that they fulfilled basic requirements in terms of medical skills and equipment (Table 2S, supplement material). Site visits at most centres were conducted to ensure uniform implementation of the procedures, to guarantee that requirements to perform DBPCFCs were available and to document and correct any deviations.

Database and quality control of data

The database was set up as a relational and central web-based database that included 5 tables corresponding to the case record forms (background, food, SPT, DBPCFC and OFC) and related by the subject identification code, which was used to link the serological data. Prior to data collection, all investigators attended a training workshop, and further training sessions were held every 6 months during the project meetings. A manual of procedures was distributed to ensure standardization in both data collection and data entry. Data were entered locally in the 12 centres, and quality control of data was carried out throughout the project on a monthly basis and when the data set was complete.

Final data set

The final population comprises 2121 patients selected in 12 outpatient clinics (Tables 5, S3). Additionally, 152 subjects from the general population surveys in children and adults (13) underwent the same evaluation (Tables 5, S3). This is the largest multicentre European case series in food allergy, in which subjects underwent a comprehensive, uniform and standardized evaluation including DBPCFC for nine major foods, namely milk, egg, fish, shrimp, hazelnut, peanut, celeriac, apple and peach. The analysis of this population will provide information on the different phenotypes of food allergy across Europe, as well as their severity and socio-economic impact. This highly characterized population will allow to establish threshold dose distributions for major allergenic foods in Europe and to validate novel *in vitro* diagnostic tests. Altogether, the information generated by this study will contribute to the improvement of diagnosis and management of food allergy and to health policy formulation in the European Union.

Authors contributions

MFR, BBW, RVR, ENCM and PB initiated the project, and conceived and designed the study. MFR, PF, SVC, MJC, MLK, MC, DG, AS, EK, TML, ACK, AP, FB, TK, TP, RA, SB, SJS, RD and BBW performed the clinical studies. ARM, RC and ENCM provided materials for oral challenges. JL, KHS, MB, SV, ENCM and RVR provided allergens and performed serological analysis. LB, CFP and MFR monitored data collection, and cleaned and analysed the data. MFR, LB, ARM and BBW wrote the manuscript. All authors revised the manuscript and approved the final version.

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Conflicts of interest

Dr. Knulst reports grants from European Commission, for conducting the study. Dr. Lidholm is employed by Thermo Fisher Scientific and received funding from EU project FP6-FOOD-CT-2005-514000. Dr. BURNEY reports grants from European Commission, during the conduct of the study; Dr. Ballmer-Weber reports grants from EU, Brussels, during the conduct of the study; personal fees from ThermoFisher, outside the submitted work; Dr. van Ree reports grants from European Commission, during the conduct of the study; personal fees from HAL Allergy, outside the submitted work; Prof. Dr. S. Vieths reports personal fees from Food Allergy Resource and Research Program, Lincoln, NE USA, personal fees from Medical University of Vienna, Austria, grants from Monsanto Company, personal fees from American Academy of Asthma, Allergy and Immunology, personal fees from Deutsche Dermatologische Gesellschaft, personal fees from Spanish Society of Allergy and Clinical Immunology, personal fees from Westdeutsche Arbeitsgemeinschaft für pädiatrische Pneumologie und Allergologie e.V., Köln, Germany, personal fees from Gesellschaft für pädiatrische Allergologie und Umweltmedizin, personal fees from Ärzteverband Deutscher Allergologen, personal fees from Schattauer Allergologie Handbuch, personal fees from Elsevier Nahrungsmittelallergien und Intoleranzen, non-financial support from German Research Foundation, non-financial support from Federal Institute for Risk Assessment, non-financial support from Austrian Society for Allergology and Immunology, non-financial support from European Directorate for the Quality of Medicines and Health Care, non-financial support from European Academy of Allergy and Clinical Immunology, non-financial support from World Allergy Organization, non-financial support from Association Monégasque pour le Perfectionnement des Connaissances des Médicins, non-financial support from Federal Office of Consumer Protection and Food Safety, non-financial support from German Chemical Society (GDCh), non-financial support from Austrian Society for Dermatology and Venerology, non-financial support from AKM Allergiekongress, non-financial support from Austrian Food Chemical Society, outside the submitted work. Dr. Fernandez-Rivas reports grants from European Commission, during the conduct of the study.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

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Table S1. List of foods used in EuroPrevall DBPCFC and the matrix used to deliver the food.

Table S2. Basic clinical requirements for the implementation of food challenges.

Table S3. Patients tested by double-blind placebo-controlled food challenges (DBPCFC).

Organisational structure of the EuroPrevall outpatient clinic study.

Case record form files: Background, Food, DBPCFC, Open food challenge, Serious event report.

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