

Immunoglobulin E Sensitization to Cross-Reactive Carbohydrate Determinants: Epidemiological Study of Clinical Relevance and Role of Alcohol Consumption

Allan Linneberg^a Runa Vavia Fenger^a Lise-Lotte N. Husemoen^a Carmen Vidal^b
Luis Vizcaino^b Arturo Gonzalez-Quintela^b

^aResearch Centre for Prevention and Health, Glostrup University Hospital, Copenhagen, Denmark;

^bDepartment of Medicine, Complejo Hospitalario Universitario, Santiago de Compostela, Spain

Key Words

Alcohol • Alcohol dehydrogenase • Cross-reactive carbohydrate determinants • Immunoglobulin E • Polymorphism

Abstract

Background: The determinants and biologic significance of IgE-mediated sensitization to cross-reactive carbohydrate determinants (CCDs) are not entirely known. An association between alcohol consumption and CCD sensitization has been reported in studies from Spain and Portugal. **Objective:** To investigate the relationship of alcohol consumption with CCD sensitization, the role of alcohol dehydrogenase gene polymorphisms, and the clinical consequences of CCD sensitization. **Methods:** Serum-specific IgE sensitization (≥ 0.1 kU/l) to a CCD (the N-glycan from bromelain) was assessed in 1,197 adults participating in a population-based study in Copenhagen, Denmark. Alcohol consumption and atopic symptoms (rhinitis, asthma and oral allergy syndrome) were assessed by questionnaire. Examinations included skin prick tests (SPTs), qualitative multiallergen IgE test (Phadiatop®), methacholine bronchial hyperreactivity, eosinophil cationic protein and alcohol dehydrogenase (ADH) gene polymorphisms. **Results:** Alcohol consumption

was significantly associated with CCD sensitization and this was particularly evident in SPT-negative individuals. The fast-metabolizing allele of the ADH1b polymorphism was significantly associated with CCD sensitization in alcohol drinkers. CCD sensitization was associated with atopic symptoms, but associations attenuated markedly when adjusting for SPT reactivity. **Conclusions:** Our results suggest that the positive association between alcohol consumption and CCD sensitization is universal and not specific to certain populations. The observed association between the ADH1b polymorphism and CCD sensitization may support that alcohol is causally related to the risk of CCD sensitization. The observed association between CCD sensitization and atopic phenotypes did not appear to be independent of SPT reactivity indicating limited significance of CCD sensitization per se.

Copyright © 2010 S. Karger AG, Basel

Introduction

N-linked glycans in plant and Hymenoptera (e.g. wasps, bees and other stinging insects) allergens may induce extensive IgE cross-reactivity [1–3]. Specific IgE to these glycans, which are commonly known as cross-reactive carbohydrate determinants (CCDs), was found to be

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2010 S. Karger AG, Basel
1018–2438/10/1531–0086\$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Allan Linneberg
Research Centre for Prevention and Health
Glostrup University Hospital, Building 84–85
Nordre Ringvej 57, DK-2600 Glostrup (Denmark)
Tel. +45 4323 3260, Fax +45 4323 3977, E-Mail alli@glo.regionh.dk

present in up to 63% of patients attending an allergy clinic due to hymenoptera venom allergy [4] and in up to 73% of subjects with allergy to 5 or more pollens [5]. In CCD-sensitized patients, in vitro IgE cross-reactivity may cause false-positive specific IgE determinations and therefore represents a diagnostic challenge [1–3, 6]. The clinical significance of CCD-IgE is a subject of debate. It is generally assumed that CCDs are of low biologic activity in vivo [2]. Thus, food challenge with CCD-containing allergens may not induce clinical symptoms in patients IgE sensitized to CCDs [7]. In contrast, in vitro biologic activity has been shown by positive basophil activation tests (reviewed in 2007 by Altmann [2]). The factors that increase the risk of CCD sensitization are not entirely known [2]. CCD sensitization has been found to be associated with alcohol consumption in studies in the regions of Spain and Portugal in the northwest of the Iberian peninsula, where wine is the preferred alcoholic beverage by far [8, 9]. It is not known whether the association between alcohol and CCD sensitization is present in other regions with different climatic and environmental conditions as well as different patterns of alcohol drinking.

The prevalence of IgE-mediated allergic diseases is high in countries that have adopted the so-called westernized, affluent, and urbanized lifestyle [10]. Alcohol consumption is a common lifestyle habit in such countries and might be of potential interest as a trigger or even risk factor for allergic disease. Alcohol (ethanol) is a strong immunological drug that suppresses Th1 responses, possibly favoring a net deviation of immune responses towards Th2. Furthermore, experimental animal studies [11, 12] and observational studies in humans [13, 14] strongly support the idea that alcohol consumption increases serum total IgE levels. In contrast, the relationship between alcohol consumption and specific IgE sensitization is less clear [15–17].

Ethanol is oxidized to acetaldehyde catalyzed by the class I alcohol dehydrogenase (ADH) isoenzymes (ADH1a, ADH1b, ADH1c) [18, 19]. There exist well-known functional polymorphisms of genes encoding ADH1b and ADH1c. Hence, both the ADH1b (rs1229984) A allele and the ADH1c (rs1693482) C allele are associated with faster metabolism of ethanol with subsequent higher peak levels of acetaldehyde following alcohol intake [20]. It has been proposed that acetaldehyde may be the substance responsible for alcohol-induced hypersensitivity reactions and possibly alcohol-induced Th2 skewing of the immune response [21]. Assuming that acetaldehyde is also the substance responsible for increased risk of CCD sensitization, one would expect that the fast

metabolizing alleles of the above-mentioned ADH1b and ADH1c polymorphisms would be associated with CCD sensitization in alcohol drinkers. Accordingly, we have recently found that the fast allele of the ADH1b (rs1229984) is associated with alcohol-induced hypersensitivity reactions in Caucasians [22].

The present study aimed to investigate the relationship of alcohol consumption with CCD sensitization in a Scandinavian general population that differed markedly from previous populations investigated with regard to many lifestyle, climatic and environmental factors. Furthermore, we investigated the potential association of CCD sensitization with different atopic symptoms and biomarkers as well as with ADH polymorphisms.

Methods

Participants

This study was based on the Copenhagen Allergy Study, which was a population-based study carried out between October 1997 and November 1998. The aim of the study was to assess the prevalence and risk factors of allergic diseases. The study design and general characteristics of the study population have been reported in more detail elsewhere [23–25]. In brief, random samples of 15- to 69-year-old individuals, born in Denmark and with Danish citizenship, living in 11 municipalities in the southwestern part of Copenhagen (the capital of Denmark), were drawn from the Civil Registration System and invited to a health examination. A total of 1,216 persons (62% of the invited) were examined. The invitation procedure was a 2-stage design in order to enrich the study population with persons reporting allergic respiratory symptoms. The first stage included a screening questionnaire survey of allergic respiratory symptoms and the second stage included a health examination, a self-administered questionnaire and allergy tests. In addition, the health examinations included measurement of skin prick test (SPT) reactivity and serum-specific IgE positivity against common inhalant allergens, and bronchial hyper-reactivity (BHR) as described below. The Copenhagen Allergy Study was approved by the local ethics committee, and informed written consent was obtained from all participants.

Specific IgE Measurements

The enzyme-enhanced fluorescence ImmunoCAP system in a Uni-CAP® 250 analyzer (Phadia, Uppsala, Sweden) was used for specific IgE measurements. We measured IgE to MUXF (Ro214, Phadia), the N-glycan epitope from bromelain, as a marker of CCD reactivity. MUXF, like all known N-glycans, has a backbone with two N-acetylglucosamines and a mannose, and the additional mannose (M), xylose (X), and core α (1,3) fucose (F) substitutions. The lower detection threshold for the method used is 0.01 kU/l. According to the manufacturer's instructions, any sIgE level ≥ 0.1 kU/l may be considered positive. Thus, CCD sensitization was defined as sIgE levels at ≥ 0.1 kU/l. Aeroallergen IgE reactivity was estimated by the commercial multiallergen UniCAP-Phadiatop® test (Phadia), a solid-phase immunoassay for specific

IgE using a balanced mixture of relevant inhalant allergens. This test provides a qualitative result, either positive or negative depending on the presence of IgE against allergens present in the mixture.

Serum Eosinophil Cationic Protein

UniCAP ECP (Pharmacia Diagnostics AB, Uppsala, Sweden) was used to measure serum eosinophil cationic protein (ECP) according to the manufacturer's instructions. Values of ECP were transformed into a dichotomous variable coded positive for values higher than 20 µg/l and otherwise negative.

Skin Prick Test

The Soluprick SQ® system (ALK-Abelló, Denmark) was used for the skin prick test (SPT) as previously described [24]. The panel of allergens comprised birch pollen (*B. verucosa*), grass pollen (*P. pratense*), mugwort pollen (*A. vulgaris*), animal danders (horse, cat and dog), 2 house dust mites (*D. pteronyssinus* and *D. farinae*), and 2 moulds (*C. herbarium* and *A. alternata*). A mean wheal diameter ≥ 3 mm was considered positive. SPT positivity was defined as a positive SPT to at least 1 of the above 10 allergens. Furthermore, an ordinal variable was defined as 11 levels of SPT positivity (0–10 positive SPTs).

Methacholine Bronchial Hyperreactivity

Methacholine BHR was measured with a modification of the method of Yan et al. [26], as described in detail elsewhere [27]. A maximal cumulative dose of 1,200 µg methacholine was inhaled beginning with 9.39 µg and ending at a dose of 600 µg with each inhalation representing a doubling of the cumulative dose. Lung function was measured 1 min after each dose and the test was stopped if the forced expiratory volume in the first second (FEV₁) was 20% below the initial level (following saline inhalation). The provocative dose of methacholine required to cause a 20% fall in FEV₁ (PD₂₀) was calculated, and BHR was defined by PD₂₀. In the present study, the test for BHR was considered positive if PD₂₀ was below the maximal cumulated dose of 1,200 µg [27].

Genotyping of ADH Polymorphisms

Participants were genotyped for the ADH1b (rs1229984) and for ADH1c (rs1693482 and rs698) polymorphisms using the Taqman allelic discrimination assay (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Reactions were performed on the Taqman 7900HT. Genotype distributions did not deviate significantly from the Hardy-Weinberg equilibrium ($p > 0.05$).

Symptoms of Atopic Diseases

Having symptoms of allergic rhinitis was defined as a confirmative answer to at least 1 of the questions 'Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing when (a) near grass, trees, or flowers, (b) following exposure to furry animals, e.g. horse, dog, cat, rabbit, guinea pig, or hamster, or (c) following exposure to dust from cleaning or from making a bed?'

Having symptoms of asthma was defined as a confirmative answer to at least 1 of the questions 'Within the last 12 months have you experienced shortness of breath or trouble breathing following exposure to (a) grass, trees, or flowers, (b) furry animals, e.g. horse, dog, cat, rabbit, guinea pig, or hamster, or (c) to dust when cleaning rooms or making beds, or when in bed?'

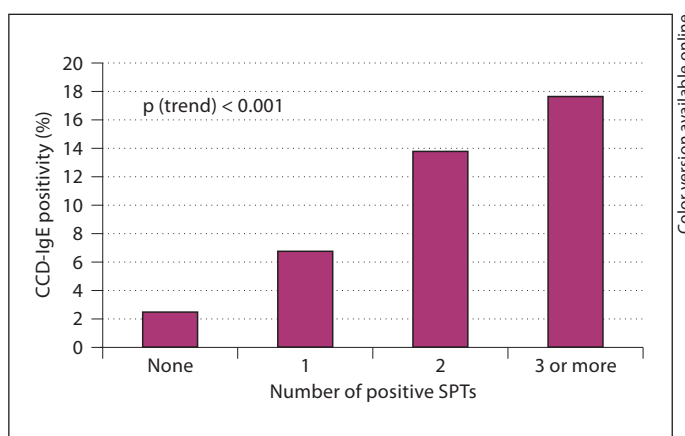


Fig. 1. Prevalence of a positive (≥ 0.1 kU/l) test for serum-specific IgE antibodies to CCDs, as reflected by specific IgE to MUXF (Ro214, Phadia) according to SPT reactivity to inhalant allergens, as reflected by the number of positive SPTs.

Having symptoms of oral allergy syndrome was defined as a confirmative answer to the question 'Have you ever had itching in the mouth following intake of any food or beverage item?'

Alcohol Intake and Smoking

The questions used were as follows: 'During the last 12 months, how many of the following have you been drinking on average per week? Number of beers per week? Number of glasses of wine per week? Number of glasses/units of spirits per week?'. A test of reliability of self-reported alcohol intake using these questions was previously conducted in our background population and it showed consistency with self-reported alcohol intake and the alcohol intake information obtained by a diet interview, just as reporting bias between types of alcohol beverages seemed to cause only minor problems [28]. The average total amount of alcoholic beverages consumed per week during the preceding 12 months was calculated. One beer, 1 glass of wine, or 1 glass of spirit was defined as 1 unit or standard drink (1.5 cl or 12 g of pure ethanol). The total average amount (units) consumed per week was calculated and categorized into 4 groups. Total alcohol intake as assessed by this method has previously been found to correlate with levels of gamma glutamyl transferase [15]. A beverage preference was defined as a specific type of alcohol if the intake of this type exceeded 50% of their total alcohol intake. If no type of alcohol exceeded 50%, alcohol preference was defined as 'mixed'. Smoking status was defined by 3 categories: never smokers, ex-smokers and daily smokers (≥ 1 g tobacco/day).

Statistics

Statistics were performed using the statistical program SAS, version 9.2 (SAS Institute, Cary, N.C., USA). All p values reported are 2-tailed and statistical significance was defined as $p < 0.05$.

Associations of CCD positivity with alcohol intake (4 groups), ADH polymorphisms, BHR, atopic symptoms (rhinitis, asthma and oral allergy syndrome), sex and smoking status (3 groups) were examined by frequency tables as well as by multivariate lo-

Table 1. Characteristics of the study population

	All, n (%) (n = 1,216)	Female, n (%) (n = 649)
Age, years		
15–30.5	361 (29.7)	207 (31.2)
30.5–46	467 (38.4)	258 (38.9)
46.5–61.5	262 (21.6)	149 (22.5)
61.5–77	126 (10.4)	49 (7.4)
CCD sensitization ^a	(n = 1,197) 77 (6.4)	(n = 649) 24 (3.7)
SPT reactivity	(n = 1,213)	(n = 661)
Positive	472 (38.9)	213 (32.2)
Smoking status	(n = 1,204)	(n = 657)
Never	547 (45.4)	315 (48.0)
Former	214 (17.8)	102 (15.5)
Current	443 (36.8)	240 (36.5)
Total alcohol consumption	(n = 1,202)	(n = 656)
Non-drinkers	223 (18.6)	181 (27.6)
1–7 drinks/week	534 (44.4)	306 (46.7)
7–14 drinks/week	258 (21.5)	121 (18.5)
≥14 drinks/week	187 (15.6)	48 (7.3)
Alcohol preference ^b	(n = 1,202)	(n = 656)
Non-drinkers	223 (18.6)	181 (27.6)
Beer	318 (26.5)	86 (13.1)
Wine	383 (31.9)	258 (39.3)
Spirits	32 (2.7)	19 (2.9)
Mixed	246 (20.5)	112 (17.1)

^a Positive (≥0.1 kU/l) determination of specific IgE to MUXF (Ro214, Phadia).

^b Beer, wine, spirits: more than 50% of total alcohol intake.

gistic regression analyses. The following confounders (explanatory variables) were considered in the regression models: sex, age, alcohol intake, smoking status and SPT positivity as noted in the tables. *p* values of likelihood ratio tests were used to test for statistical significance in all logistic regression analyses. For analyses of frequency differences between groups, the χ^2 test or Fisher's exact test were used, whereas for analyses of linearity the Cochran-Armitage trend test was used. Differences in the levels of IgE to CCD were tested by the Wilcoxon Signed Rank test.

Results

Determinants of CCD Sensitization

The characteristics of the study population are shown in table 1. The overall prevalence of CCD sensitization in the study population was 6.4 % (77/1,197, 95% confidence limits 5.0–7.8%). The prevalence of CCD sensitization was higher in males than in females (53/548, 9.7% vs. 24/649, 3.7%, *p* < 0.001). CCD sensitization was not sig-

Table 2. The prevalence of CCD sensitization according to alcohol intake

Alcohol consumption (drinks per week)	CCD sensitization ^a	
	% (n/N)	OR (95% CI) ^b
All participants		
Non-drinkers	6.4 (14/219)	2.00 (0.96–4.18)
1–7 drinks	4.6 (24/524)	1 (reference)
7–14 drinks	7.4 (19/258)	1.60 (0.83–3.08)
≥14 drinks	11.0 (20/182)	2.03 (1.01–4.06)
<i>p</i> trend	0.02	0.37
SPT-negative participants		
Non-drinkers	0.7 (1/138)	0.64 (0.07–5.92)
1–7 drinks	1.2 (4/328)	1 (reference)
7–14 drinks	4.0 (6/151)	2.67 (0.73–9.84)
≥14 drinks	7.0 (7/100)	3.75 (0.98–14.28)
<i>p</i> trend	<0.001	0.02
SPT-positive participants		
Non-drinkers	16.3 (13/80)	2.21 (1.00–4.90)
1–7 drinks	10.2 (20/196)	1 (reference)
7–14 drinks	12.2 (13/107)	1.16 (0.55–2.47)
≥14 drinks	16.1 (13/81)	1.45 (0.65–3.23)
<i>p</i> trend	0.78	0.70

^a Positive (≥0.1 kU/l) determination of specific IgE to MUXF (Ro214, Phadia).

^b Odds ratio (95% CI) obtained by logistic regression adjusting for sex, age, smoking, and SPT reactivity_{0–10} (the latter only in the model with all participants).

nificantly associated with age (data not shown). The prevalence of CCD sensitization was higher in SPT-positive (reactivity to at least 1 of 10 SPTs) individuals than in SPT-negative individuals (59/464, 12.7% vs. 18/717, 2.5%, *p* < 0.001) and increased markedly with increasing number of positive SPTs (fig. 1).

Alcohol consumption was significantly associated with the prevalence of CCD sensitization. Furthermore, the risk of CCD sensitization tended to increase with increasing consumption of alcoholic drinks, although a clear dose-response relationship was mainly seen in SPT-negative persons (table 2). These associations remained essentially similar after adjustment for several potential confounders (table 2). The mean levels of CCD-IgE were generally low and they differed significantly (*p* = 0.031, Wilcoxon Signed Rank test) across the 4 groups of alcohol consumption. Thus, the mean levels were 0.029, 0.028, 0.086 and 0.089 kU/l among the categories 'non-drinkers', '1–7 drinks per week', '7–14 drinks per week', and '≥14 drinks per week', respectively. The association of CCD sensitization with alcohol consumption was inde-

Table 3. The prevalence and risk of CCD sensitization (IgE to MUXF, Ro214, Phadia) according to genetic polymorphisms of alcohol dehydrogenase (ADH)

Genotype	Effect on ethanol metabolism	Prevalence of CCD sensitization (IgE ≥0.1 kU/l)			
		All participants		Alcohol drinkers	
		% (n/N)	OR (95% CI) ^b	% (n/N)	OR (95% CI) ^b
ADH1b rs1229984					
GG	Slow	6 (71/1158)	1 (reference)	6 (58/934)	1 (reference)
AG	Intermediate	21 (4/19)	3.20 (0.94–10.89)	31 (4/13)	4.71 (1.29–17.11)
AA	Fast	– ^c	–	– ^c	–
p value ^a		0.03		0.01	
ADH1c rs1693482					
TT	Slow	7.4 (16/216)	1 (reference)	7.5 (13/173)	1 (reference)
CT	Intermediate	7.0 (42/598)	0.92 (0.49–1.7)	7.3 (35/478)	0.93 (0.47–1.84)
CC	Fast	5.0 (19/383)	0.66 (0.32 –1.34)	4.8 (15/298)	0.63 (0.29–1.39)
p value ^a		0.36	0.22	0.31	0.22

^a p values determined by χ^2 or Fisher's exact test (frequency analyses) or trend test for linearity in logistic regression.

^b Odds ratio (95% CI) obtained by logistic regression adjusted for sex, age, alcohol (continuous), smoking (never/former/current), and SPT reactivity (positive/negative).

^c There were no persons homozygous for the A allele.

pendent of the type of alcoholic beverage. Thus, wine, beer and spirit consumers ran an equal risk of CCD sensitization (data not shown). Increasing alcohol consumption tended to be associated with increasing prevalence of SPT reactivity and Phadiatop positivity, but this was not statistically significant (data not shown).

In alcohol drinkers, subjects carrying the A-allele of the ADH1b polymorphism were significantly more likely to be CCD-IgE positive (table 3). The two ADH1c polymorphisms were in 100% concordance (complete linkage disequilibrium) with one another and therefore results for only one of them (rs1693482) are shown. The ADH1c polymorphisms were not significantly associated with CCD sensitization (table 3).

Clinical Significance of CCD Sensitization

CCD sensitization was significantly associated with self-reported atopic symptoms in crude analyses (table 4). However, atopic symptoms seemed to be more strongly associated with SPT reactivity than with CCD sensitization. Thus, for each additional positive SPT (0 to 10) the risk of atopic symptoms increased significantly (table 4). When including both CCD sensitization and SPT reactivity in the multivariate regression models, the associations of CCD sensitization with symptoms were substantially attenuated and became statistically non-significant

(table 4), while the association of SPT reactivity remained strong. BHR and ECP were not significantly associated with CCD sensitization (table 4).

The prevalence of CCD sensitization was markedly higher among Phadiatop-positive than among Phadiatop-negative individuals (72/471, 15.3% vs. 5/726, 0.7%, $p < 0.001$). There was a strong concordance between results of the multiallergen specific IgE test (Phadiatop) and SPT reactivity (table 5). There were only 48 individuals (4.1%) with discordant negative Phadiatop results (negative Phadiatop test with positive SPT) and 52 individuals (4.3%) with discordant positive Phadiatop result (positive Phadiatop test with negative SPT). Individuals with positivity to both Phadiatop test and SPT showed the highest prevalence of atopic symptoms. Individuals with discordant positive Phadiatop results showed the highest prevalence of CCD sensitization (table 5).

Discussion

In this Scandinavian general population, we found a positive association between alcohol consumption and the prevalence of CCD sensitization. This is in line with findings of 2 studies from the northwestern part of the Iberian peninsula (parts of Spain [9] and Portugal [8]).

Table 4. The prevalence of different atopic manifestations according to CCD sensitization status

	Prevalence % (n/N)	Crude OR (95% CI)	Adjusted OR ^b (95% CI)
Symptoms of rhinitis			
CCD-IgE-negative	44.7 (500/1,119)	1 (reference)	1 (reference)
CCD-IgE-positive ^a	74.0 (57/77)	3.53 (2.09–5.95)	1.93 (1.03–3.58)
Number of positive SPTs ^c	–	2.24 (1.97–2.54)	2.32 (2.03–2.65)
Symptoms of asthma			
CCD-IgE-negative	15.4 (172/1,120)	1 (reference)	1 (reference)
CCD-IgE-positive ^a	35.1 (27/77)	2.98 (1.81–4.89)	1.62 (0.91–2.89)
Number of positive SPTs ^c	–	1.59 (1.46–1.73)	1.62 (1.48–1.78)
Symptoms of OAS			
CCD-IgE-negative	15.2 (170/1,119)	1 (reference)	1 (reference)
CCD-IgE-positive ^a	31.2 (24/77)	2.53 (1.52–4.21)	1.73 (0.95–3.17)
Number of positive SPTs ^c	–	1.46 (1.34–1.58)	1.56 (1.42–1.72)
Methacholine BHR			
CCD-IgE-negative	13.0 (142/1,089)	1 (reference)	1 (reference)
CCD-IgE-positive ^a	18.6 (13/70)	1.52 (0.81–2.85)	0.89 (0.44–1.83)
Number of positive SPTs ^c	–	1.35 (1.24–1.47)	1.57 (1.41–1.74)
Eosinophilic cationic protein (levels ≥ 20 μ g/l)			
CCD-IgE-negative	15.8 (183/1,088)	1 (reference)	1 (reference)
CCD-IgE-positive ^a	24.7 (18/73)	1.62 (0.93–2.82)	1.30 (0.72–2.35)
Number of positive SPTs ^c	–	1.13 (1.04–1.23)	1.15 (1.05–1.26)

OAS = Oral allergy syndrome.

^a CCD sensitization is defined as a positive test (cut-off of ≥ 0.1 kU/l) for specific IgE to MUXF (Ro214, Phadia).

^b Adjusted by logistic regression for sex, age, alcohol and smoking as well as for the variables in the table.

^c Introduced as a continuous variable, i.e., the odds ratio reflects the increase in risk for each additional positive SPT (on a scale from 0 to 10).

Thus, the association between alcohol and CCD sensitization seems to be universal and independent of cultural (e.g. drinking patterns), environmental, and climatic differences between Northern and Southern Europe. For example, the preference for wine drinking is likely to be lower in the present population as compared to, for example, the Spanish/Portuguese studies [8, 9]. However, the prevalence of IgE sensitization and the levels of IgE-CCD in this urban Scandinavian population were lower than those reported in a mostly rural Spanish population [8]. This finding may suggest that other factors than alcohol determine the risk of CCD sensitization.

The cross-sectional nature of the present and previous studies makes it difficult to draw any firm conclusions about whether alcohol and CCD sensitization are causally related. The biological mechanisms underlying alcohol-induced CCD sensitization are not clear. Individuals carrying the A-allele of the ADH1b polymorphism are faster metabolizers of ethanol compared to those who are homozygous for the wild-type allele (the G-allele) [29,

30]. Fast metabolizers experience higher peak levels of acetaldehyde following alcohol drinking. Interestingly, we found that among alcohol drinkers fast-metabolizers of alcohol (carriers of the A-allele) were significantly more likely to be CCD-IgE positive. This finding lends support to the hypothesis that acetaldehyde – and not ethanol – through a histamine releasing effect is the substance responsible for the immunological effects of alcohol consumption, as previously proposed [21]. Interestingly, acetaldehyde is responsible for the abnormal protein glycosylation that is commonly observed in alcohol abusers [31, 32]. The ADH1b polymorphism has a much stronger effect on the rate of conversion of alcohol to acetaldehyde than the ADH1c polymorphism [29, 30], which may explain why we only found an association between CCD sensitization and the ADH1b, and not the ADH1c, polymorphism. Furthermore, the low frequency of the A allele of the ADH1b polymorphism and the fact that none of the participants was found to be homozygote for this allele made it impossible to investigate a potential

Table 5. The prevalence of CCD sensitization and atopic manifestations stratified by SPT reactivity to inhalant allergens and qualitative multiallergen test for specific IgE to a panel of inhalant allergens (Phadiatop)

	SPT-negative and Phadiatop-negative	SPT-positive and Phadiatop-negative	SPT-negative and Phadiatop-positive	SPT-positive and Phadiatop-positive	p value ^b
CCD sensitization ^a , % (n/N)	0.74 (5/676)	0 (0/48)	25.0 (13/52)	14.1 (59/419)	<0.001
Symptoms of rhinitis, % (n/N)	27.4 (185/675)	39.6 (19/48)	42.3 (22/52)	79.1 (332/420)	<0.001
Symptoms of asthma, % (n/N)	8.4 (57/676)	12.5 (6/48)	19.2 (10/52)	30.2 (127/420)	<0.001
Symptoms of OAS, % (n/N)	8.3 (56/676)	20.8 (10/48)	13.5 (7/52)	28.9 (121/419)	<0.001
Metacholine BHR, % (n/N)	10.5 (69/655)	12.8 (6/47)	8.2 (4/49)	18.7 (76/406)	0.004

OAS = Oral allergy syndrome.

^a CCD sensitization is defined as a positive test (cut-off of ≥ 0.1 kU/l) for specific IgE to MUXF (Ro214, Phadia).

^b p value determined by χ^2 test or Fisher's exact test.

dose-response effect. Other potential biological mechanisms should be considered. For example, it is well-known that alcohol increases the permeability of the gastro-intestinal tract thereby facilitating the introduction of food proteins including glycoproteins to the immune system. Furthermore, alcohol may influence gastric acid levels, which in turn influences degradation and allergenicity of food proteins [33]. Interestingly, experimental studies have shown that alcohol-induced gastritis decreases oral tolerance [34].

In vitro CCD sensitization is generally considered as being of limited clinical importance, as reviewed by Altmann [2]. In our study, the significant associations between atopic symptoms and CCD sensitization in crude analyses were markedly attenuated when the models were adjusted for SPT reactivity to inhalant allergens. In contrast, SPT reactivity in itself was strongly associated with symptoms of atopic disease, and these associations were not affected by adjustment for CCD sensitization. Thus, for each additional positive SPT the relative risk of e.g. rhinitis increased 2-fold (table 4). This may suggest that CCD sensitization per se does not confer any major risk of atopic symptoms and that any such risk seems to be mediated through the effects of SPT reactivity, which is closely related to CCD sensitization, as also noted by Mari [5]. It may therefore be speculated that CCD sensitization is mainly a marker of IgE reactivity.

The validity of the questions used to obtain data on atopic symptoms should be considered. The questions employed for assessment of allergic rhinitis and asthma symptoms have been validated against aeroallergen sensitization in previous studies [35] and the validity was found to be reasonably comparable to the validity of similar questions in other general population settings [36–

38]. Including additional data on, for example, seasonality and conjunctivitis yielded essentially similar results with regard to the association between CCD sensitization and atopic symptoms.

The apparent ability of alcohol consumption to cause CCD sensitization may have important implications for clinical practice. Thus, it is well-known that sensitization to CCDs may influence in vitro determinations of serum-specific IgE to some allergens. This has been shown for determinations of IgE to plant allergens (pollens, natural rubber latex or peanuts) [8, 39–41] and to Hymenoptera venom allergens [8, 39], whereas tests for house dust mite seems to be less susceptible to the presence of CCDs [40]. Multiallergen IgE tests may be particularly susceptible to CCD interference [1, 42]. Consistently, in the group of subjects with a positive multiallergen IgE test but without SPT reactivity to inhalant allergens we observed the highest frequency of CCD sensitization, and numerically the levels of CCD-IgE were higher among these subjects than in the other groups (table 5). It is thus possible that the multiallergen IgE test may have been falsely positive due to CCD sensitization in at least some of the cases. Our results may suggest that cross-reactivity between CCDs and some types of inhalant allergens may cause false-positive in vitro allergy tests, particularly in persons with a high alcohol intake.

Conclusions

In this Scandinavian general population study, we could confirm previous observations in Spanish and Portuguese populations in that increasing alcohol consumption is associated with increasing prevalence of CCD

sensitization. Hence, this association seems to be independent of regional drinking patterns, climate, and environmental factors. These findings deserve attention due to that fact that CCD sensitization may interfere with in vitro IgE determinations due to cross-reactivity. The finding that the risk of CCD sensitization was significantly higher in persons with a genetically determined faster metabolism of ethanol may support the idea that alcohol is causally related to risk of CCD sensitization and that acetaldehyde (the metabolite of ethanol) plays a role in alcohol-induced CCD sensitization. The association between CCD sensitization and atopic phenotypes did not appear to be clearly independent of SPT reactivity, indicating that CCD sensitization may be of limited clinical significance in itself.

Acknowledgments

The Copenhagen Allergy Study was supported by grants from the Danish Medical Research Council, the Danish Health Insurance Fund, the Danish Ministry of Health (the National Health Fund for Research and Development), the Danish Medical Research Council and the Danish Ministry of Health (Research Centre for Environmental Health: Environmental Health Research Programme 1997), and ALK – Abelló A/S, Denmark. The present project was supported by a grant from Trygfonden for Health (Lyngby, Denmark), the Spanish ISCIII (project No. 071173) and the Danish Agency for Science Technology and Innovation (grant No. 2101-06-0065).

References

- 1 Malandain H: IgE-reactive carbohydrate epitopes: classification, cross-reactivity, and clinical impact. *Eur Ann Allergy Clin Immunol* 2005;37:122–128.
- 2 Altmann F: The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007; 142:99–115.
- 3 van Ree R: Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. *Int Arch Allergy Immunol* 2002;129:189–197.
- 4 Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Muller C, Enk A: In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220–1229.
- 5 Mari A: IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002; 129:286–295.
- 6 Guilloux L, Morisset M, Codreanu F, Parisot L, Moneret-Vautrin DA: Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: roles of peanut and cross-reactive carbohydrate determinants specific IgE. *Int Arch Allergy Immunol* 2009;149:91–97.
- 7 Mari A, Ooievaar-de Heer P, Scala E, Giani M, Pirrotta L, Zuidmeer L, et al: Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. *Allergy* 2008; 63:891–896.
- 8 Coutinho V, Vidal C, Garrido M, Gude F, Lojo S, Linneberg A, et al: Interference of cross-reactive carbohydrates in the determination of specific IgE in alcohol drinkers and strategies to minimize it: the example of latex. *Ann Allergy Asthma Immunol* 2008; 101:394–401.
- 9 Gonzalez-Quintela A, Garrido M, Gude F, Campos J, Linneberg A, Lojo S, et al: Sensitization to cross-reactive carbohydrate determinants in relation to alcohol consumption. *Clin Exp Allergy* 2008;38:152–160.
- 10 Linneberg A: Are we getting enough allergens? *Int Arch Allergy Immunol* 2008;147: 93–100.
- 11 Heinz R, Waltenbaugh C: Ethanol consumption modifies dendritic cell antigen presentation in mice. *Alcohol Clin Exp Res* 2007;31: 1759–1771.
- 12 Linneberg A, Roursgaard M, Hersoug LG, Larsen ST: Effects of alcohol consumption on the allergen-specific immune response in mice. *Alcohol Clin Exp Res* 2008;32:553–556.
- 13 Linneberg A, Petersen J, Nielsen NH, Madsen F, Frolund L, Dirksen A, et al: The relationship of alcohol consumption to total immunoglobulin E and the development of immunoglobulin E sensitization: the Copenhagen Allergy Study. *Clin Exp Allergy* 2003;33:192–198.
- 14 Gonzalez-Quintela A, Gude F, Boquete O, Rey J, Meijide LM, Suarez F, et al: Association of alcohol consumption with total serum immunoglobulin E levels and allergic sensitization in an adult population-based survey. *Clin Exp Allergy* 2003;33:199–205.
- 15 Linneberg A, Hertzum I, Husemoen LL, Johansen N, Jorgensen T: Association between alcohol consumption and aeroallergen sensitization in Danish adults. *Clin Exp Allergy* 2006;36:714–721.
- 16 Assing K, Bødtger U, Linneberg A, Malling HJ, Poulsen LK: Association between alcohol consumption and skin prick test reactivity to aeroallergens. *Ann Allergy Asthma Immunol* 2007;98:70–74.
- 17 Linneberg A, Friedrich N, Husemoen LL, Thuesen B, Gonzalez-Quintela A, Vidal C, et al: Incidence and remission of specific IgE aeroallergen sensitization from age of 40 to 60 years, and association with alcohol consumption. *Int Arch Allergy Immunol* 2009; 151:142–148.
- 18 Gemma S, Vichi S, Testai E: Individual susceptibility and alcohol effects: biochemical and genetic aspects. *Ann Ist Super Sanita* 2006;42:8–16.
- 19 Crabb DW, Matsumoto M, Chang D, You M: Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. *Proc Nutr Soc* 2004;63:49–63.
- 20 Eriksson CJ, Fukunaga T, Sarkola T, Chen WJ, Chen CC, Ju JM, et al: Functional relevance of human adh polymorphism. *Alcohol Clin Exp Res* 2001;25:157S–163S.
- 21 Linneberg A, Berg ND, Gonzalez-Quintela A, Vidal C, Elberling J: Prevalence of self-reported hypersensitivity symptoms following intake of alcoholic drinks. *Clin Exp Allergy* 2008;38:145–151.
- 22 Linneberg A, Gonzalez-Quintela A, Vidal C, Jørgensen T, Fenger M, Hansen T, Pedersen O, Husemoen LL: Genetic determinants of both ethanol and acetaldehyde metabolism influence alcohol hypersensitivity and drinking behaviour among Scandinavians. *Clin Exp Allergy* 2010;40:123–130.
- 23 Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart: the Copenhagen Allergy Study. *J Allergy Clin Immunol* 2000;106: 247–252.

- 24 Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T: Smoking and the development of allergic sensitization to aeroallergens in adults: a prospective population-based study. *The Copenhagen Allergy Study. Allergy* 2001;56:328–332.
- 25 Thuesen BH, Husemoen LLN, Fenger M, Linneberg A: Lack of association between the MTHFR (C677T) polymorphism and atopic disease. *Clin Resp J* 2009;3:102–108.
- 26 Yan K, Salome C, Woolcock AJ: Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760–765.
- 27 Elberling J, Linneberg A, Dirksen A, Johansen JD, Frølund L, Madsen F, et al: Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity. *Clin Exp Allergy* 2005;35:75–81.
- 28 Gronbaek M, Heitmann BL: Validity of self-reported intakes of wine, beer and spirits in population studies. *Eur J Clin Nutr* 1996;50:487–490.
- 29 Lee SL, Chau GY, Yao CT, Wu CW, Yin SJ: Functional assessment of human alcohol dehydrogenase family in ethanol metabolism: significance of first-pass metabolism. *Alcohol Clin Exp Res* 2006;30:1132–1142.
- 30 Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A, Gronbaek M: Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes. *Pharmacogenomics J* 2008;8:220–227.
- 31 Xin Y, Lasker JM, Lieber CS: Serum carbohydrate-deficient transferrin: mechanism of increase after chronic alcohol intake. *Hepatology* 1995;22:1462–1468.
- 32 Liinamaa MJ, Hannuksela ML, Ramet ME, Savolainen MJ: Defective glycosylation of cholesteryl ester transfer protein in plasma from alcohol abusers. *Alcohol* 2006;41:18–23.
- 33 Untermayr E, Jensen-Jarolim E: The role of protein digestibility and antacids on food allergy outcomes. *J Allergy Clin Immunol* 2008;121:1301–1308.
- 34 Andrade MC, Menezes JS, Cassali GD, Martins-Filho OA, Cara DC, Faria AM: Alcohol-induced gastritis prevents oral tolerance induction in mice. *Clin Exp Immunol* 2006;146:312–322.
- 35 Linneberg A, Nielsen NH, Madsen F, Frølund L, Dirksen A, Jørgensen T: Secular trends of allergic asthma in Danish adults. *The Copenhagen Allergy Study. Respir Med* 2001;95:258–264.
- 36 Bergmann RL, Forster J, Schulz J, Bergmann KE, Bauer CP, Wahn U: Atopic family history: validation of instruments in a multicenter cohort study. *Pediatr Allergy Immunol* 1993;4:130–135.
- 37 Lakwijk N, Van Strien RT, Doekes G, Brunekreef B, Gerritsen J: Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. *Clin Exp Allergy* 1998;28:454–458.
- 38 Braun-Fahrlander C, Wuthrich B, Gassner M, Grize L, Sennhauser FH, Varonier HS, et al: Validation of a rhinitis symptom questionnaire (ISAAC core questions) in a population of Swiss school children visiting the school health services. SCARPOL-team. Swiss Study on Childhood Allergy and Respiratory Symptom with Respect to Air Pollution and Climate. *International Study of Asthma and Allergies in Childhood. Pediatr Allergy Immunol* 1997;8:75–82.
- 39 Mahler V, Gutgesell C, Valenta R, Fuchs T: Natural rubber latex and hymenoptera venoms share immunoglobulin E-epitopes accounting for cross-reactive carbohydrate determinants. *Clin Exp Allergy* 2006;36:1446–1456.
- 40 Malandain H, Giroux F, Cano Y: The influence of carbohydrate structures present in common allergen sources on specific IgE results. *Eur Ann Allergy Clin Immunol* 2007;39:216–220.
- 41 Vidal C, Vizcaino L, az-Peromingo JA, Garrido M, Gomez-Rial J, Linneberg A, et al: Immunoglobulin-E reactivity to a glycosylated food allergen (peanuts) due to interference with cross-reactive carbohydrate determinants in heavy drinkers. *Alcohol Clin Exp Res* 2009;33:1322–1328.
- 42 Gonzalez-Quintela A, Garrido M, Gude F, Campos J, Lojo S, Linneberg A, et al: Discordant positive results of multiallergen immunoglobulin E tests in relation to cross-reactive carbohydrate determinants and alcohol consumption. *J Invest Allergol Clin Immunol* 2009;19:70–71.