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Citation for published version (APA):

Koning, H., Postma, D. S., Brunekreef, B., Duiverman, E. J., Smit, H. A., Thijs, C., Penders, J., Kerkhof, M., & Koppelman, G. H. (2012). Protocadherin-1 polymorphisms are associated with eczema in two Dutch birth cohorts. *Pediatric Allergy and Immunology*, *23*(3), 270-277. https://doi.org/10.1111/j.1399-3038.2011.01201.x

Document status and date:
Published: 01/05/2012

DOI:
10.1111/j.1399-3038.2011.01201.x

Document Version:
Publisher's PDF, also known as Version of record

Document license:
Taverne

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Pediatric Allergy and Immunology

ORIGINAL ARTICLE SKIN AND EYE DISEASE

Protocadherin-1 polymorphisms are associated with eczema in two Dutch birth cohorts

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To cite this article: Koning H, Postma DS, Brunekreef B, Duiverman EJ, Smit HA, Thijs C, Penders J, Kerkhof M, Koppelman GH. Protocadherin-1 polymorphisms are associated with eczema in two Dutch birth cohorts. *Pediatr Allergy Immunol* 2012: **23**: 270–277.

Keywords
Protocadherin-1; birth cohort; polymorphism; eczema; asthma.

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Accepted for publication 7 July 2011

DOI:10.1111/j.1399-3038.2011.01201.x

Abstract

Background: Eczema and asthma share a common genetic background and show linkage to chromosome 5q31-33. Protocadherin-1 (*PCDH1*) is located in this region and was identified as a susceptibility gene for bronchial hyper-responsiveness (BHR), a hallmark of asthma. *PCDH1* encodes an adhesion molecule, expressed in airway and skin epithelium. We determined whether *PCDH1* polymorphisms, previously associated with asthma or BHR, also associated with questionnaire and UK Working Party (UKWP) defined eczema.

Methods: Four *PCDH1* polymorphisms were genotyped in two Dutch birth cohorts, PIAMA (n = 967) and KOALA Birth Cohort Study (n = 1560). Association with eczema was determined by chi-square tests and generalized estimating equations (GEE).

Results: Insertion deletion IVS3-116 was associated with development of UKWP eczema in PIAMA [age 4, OR = 1.90 (1.14–3.18)] and borderline with questionnaire-reported eczema in PIAMA [GEE, OR = 1.33 (0.98–1.81)]. Furthermore, IVS3-116 was associated with questionnaire-reported eczema in KOALA [age 1, OR = 1.44 (1.00–2.07)]. Pooled analysis of questionnaire-reported eczema of both cohorts resulted in a significant association of IVS3-116 with eczema [OR = 1.26 (1.01–1.58)]. Rs3822357 (A-allele) associated with protection for eczema in PIAMA only [questionnaires, OR = 0.19 (0.06–0.63)].

Conclusion: *PCDH1* gene variant IVS3-116 associates with eczema in two independent birth cohorts. Combined with previous observations, this indicates a shared genetic susceptibility to BHR, asthma and eczema.

Abbreviations:
PCDH1, Protocadherin-1; BHR, Bronchial Hyper-responsiveness; PIAMA, Prevention and Incidence of Asthma and Mite Allergy birth cohort; KOALA, Dutch acronym for: Child, Parent, health, Focus on Lifestyle and Predisposition birth cohort; SNP, Single-Nucleotide Polymorphism; ISAAC, the International Study of Asthma and Allergies in Childhood; UKWP, UK Working Party criteria; FLG, Filaggrin; GEE, Generalized Estimating Equations; AD, Atopic Dermatitis; LD, Linkage Disequilibrium; OR, Odds Ratio; 95% CI, 95% Confidence Interval.

Atopic diseases such as eczema and asthma are increasingly prevalent in the western world (1). These complex diseases are caused by an interplay of genetic and environmental factors (2). Several prospective studies have shown that eczema pre-dates or co-exists with asthma (3, 4). Twin studies indicate that these atopic diseases share a common genetic background, but that also disease-specific genetic factors exist (5).

The chromosomal region 5q31-33 is strongly linked to eczema (6, 7), asthma and bronchial hyper-responsiveness (BHR) (8–10). In this region, multiple atopy susceptibility

genes have been identified, such as *SPINK5* (eczema), *IL-13* (asthma, atopy) and *CD14* (atopy). One gene in this region, *Protocadherin-1* (*PCDH1*), has recently been discovered as a BHR susceptibility gene (11).

PCDH1 is thought to play a role in cell-cell adhesion (12, 13). PCDH1 mRNA and protein expression were identified in airway epithelium (11), whereas PCDH1 mRNA is expressed in skin keratinocytes (14, 15). A defect in epithelial barrier function may represent an underlying common mechanism in asthma, BHR and eczema (16).

We hypothesized that *PCDH1* gene variants may represent a common genetic background of eczema and asthma, owing to its proposed barrier function in epithelial cells. We therefore determined whether SNPs, that were shown previously to associate with asthma and BHR, are associated with eczema. To this aim, we investigated the association of *PCDH1* SNPs with eczema in two Dutch prospective birth cohorts.

Material and methods

Study populations

Two Dutch populations were investigated: (i) The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort and (ii) KOALA (Dutch acronym for: Child, Parent, health, Focus on Lifestyle and Predisposition) birth cohort. The local medical ethics committees approved these studies, and all participants provided written (parental) informed consent.

Ascertainment

Recruitment of subjects for PIAMA birth cohort

Study design of the PIAMA birth cohort has been described previously (17). In summary, recruitment took place during the first trimester of pregnancy with a validated screening questionnaire (18) and was conducted by 52 midwife practices in three different regions in the Netherlands: north (Groningen and surroundings), central (Bilthoven and Wageningen and surroundings) and southwest (Rotterdam and surroundings). Women reporting any of the following symptoms were defined as allergic (i.e. a history of asthma, current hay fever, current allergy to house dust mite or pets), and their children were defined as 'high-risk'. A total of 10,232 pregnant women completed the questionnaire and 2949 (29%) of them were 'allergic'. The participating children were born between May 1996 and December 1997 and were followed yearly up to 8 yr of age.

At baseline, the PIAMA study population consisted of 4146 children, 183 (5%) were lost to follow-up. The study therefore started with 3963 newborns: 1327 children from allergic mothers and a random sample of 663 children from non-allergic mothers were selected for medical examinations, from which 1808 children were eligible for a medical examination at age 4, and of whom 1288 children participated. DNA was collected at age 4 from 1037 children during medical examinations for atopic eczema. Children of non-Dutch mothers were excluded from the analysis. At the age of 1 yr, medical examination for eczema was performed only for the intervention group (n = 414). Intervention consisted of mite-impermeable mattress covers. Study population characteristics are described in Table 1.

Recruitment of subjects for the KOALA cohort

The design of the study has been described previously (19). Briefly, participants with diverse lifestyles (conventional and alternative) were recruited at 34 wk of gestation. Pregnant women with a conventional lifestyle (n = 2343) were recruited from an ongoing prospective cohort study on Pregnancy-related Pelvic Girdle pain in the Netherlands. Additionally, pregnant women with an alternative lifestyle (n = 491) were recruited through organic food shops, anthrophosophic doctors and midwifes, Steiner Schools and magazines. During the first 2 yr postpartum, and at the age of 6 yr, information on atopic outcomes and their determinants were collected for all members of the cohort by repeated questionnaires at ages 3, 7, 12, 24 months and 6 yr. The study was approved by the medical ethics committee of the Maastricht University. Written informed parental consent was obtained from all participants. All participants were asked for providing DNA via buccal swaps (n = 1560). Dutch children were medically examined for atopic eczema at the age of 2 yr only (n = 676). Characteristics of the study population are described in Table 1.

Clinical evaluation

Diagnosis of eczema

For the PIAMA cohort at the age of 1–8 yr, and for the KOALA cohort at 7, 12, 24 months and 6 yr, infants were defined as having developed eczema if their parents reported eczema based on ISAAC questions. The ISAAC questionnaire defines eczema as 'the presence of a history of itchy rash which was coming and going in the last 12 months, localized on flexural sites on folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes. Cases of only diaper rash, rash around the eyes and/or scalp scaling were excluded.

At home visits at the age of 1 and 4 yr for PIAMA and at 2 yr for KOALA, trained health nurses examined infants for atopic dermatitis according to the UK Working Party (UKWP) criteria (20–22) in a subset of the population. In PIAMA, home visits were performed at 1 yr for children of the intervention study and at 4 yr for a random sample of the cohort. In PIAMA, a mandatory modification was made to the UKWP criteria at 1 yr only, as the UKWP criteria 'early onset' and 'presence of other atopic diseases' are less relevant for children at 12 months of age (23).

In KOALA, home visits were performed in children whose parents gave permission to draw blood samples. Infants with a UKWP probability score (20) of atopic dermatitis (AD) >90% were regarded as infants with 'probable presence of atopic dermatitis' as was determined previously (24).

Diagnosis of asthma

In the PIAMA cohort we analysed 'doctor-diagnosed asthma', defined as asthma ever diagnosed by a doctor and

Table 1 Participant characteristics for PIAMA and KOALA study populations and total birth cohorts

Characteristics	PIAMA Study population	PIAMA Total cohort	KOALA Study population	KOALA Total cohort
Number	967	3963	1560	2834
Ethnicity (% Dutch origin)	100	94.2	100	95.8
Boys (%)	51.9	51.8	50.3	51.3
Education level mother (%)				
Low	18.3	23.5	8.3	10.7
Intermediate	42.7	41.5	37.3	39.4
High	39.0	35.0	54.4	49.9
Family history (%)				
Atopy mother	65.9	31.3	32.9	34.1
Asthma mother	16.3	7.9	8.9	9.3
Atopy father	31.7	30.8	36.6	35.0
Asthma father	7.3	7.7	10.1	10.0
Children at risk (%)*	75.6	51.5	55.8	56.5
Intervention (type)	Mattress covers	Mattress covers	No	No
No (%)	57.1	80.7	100	100
Placebo (%)	24.3	10.0	0	0
Active (%)	18.6	9.3	0	0
Environmental exposures				
Breast feeding (%)				
Never	14.6	17.9	14.6	19.8
<3 months	35.0	38.4	20.7	21.1
≥3 months	50.4	43.7	64.7	59.1
ETS at home first year (%)	22.9	27.8	11.5	15.4
Pet (dog and/or cat) first year (%)	39.6	43.2	39.8	41.9
Dog first year (%)	14.3	16.1	19.8	22.2
Cat first year (%)	29.1	32.8	24.3	23.8
Presence older siblings at birth (%)	48.8	50.5	58.3	55.7
Eczema manifestations				
ISAAC questionnaire				
1 yr (%)	18.5; n = 174	15.2; n = 559	18.0; n = 263	19.4; n = 484
2 yr (%)	20.1; n = 189	16.6; n = 616	15.4; n = 234	15.3; n = 389
3 yr (%)	22.3; n = 209	17.3; n = 632	N.a.	N.a.
4 yr (%)	21.5; n = 200	17.9; n = 631	N.a.	N.a.
5 yr (%)	17.9; n = 164	15.3; n = 515	N.a.	N.a.
6 yr (%)	18.0; n = 165	15.4; n = 527	13.6; n = 177	13.9; n = 275
7 yr (%)	16.9; n = 150	14.3; n = 479	N.a.	N.a.
8 yr (%)	18.3; n = 159	16.0; n = 515	N.a.	N.a.
UKWP criteria				
1 yr (%)	13.7; n = 56	14.3; n = 479	N.a.	N.a.
2 yr (%)	N.a.	N.a.	13.8; n = 93	13.4; n = 116
4 yr (%)	12.0; n = 115	12.2; n = 155	N.a.	N.a.
Total serum IgE				
1 yr (IU/ml)†	7.1 (2.0–17.0) n = 346	6.6 (2.0–17.0) n = 565	6.0 (2.6–12.4) n = 644	6.4 (2.7–13.3) n = 912
2 yr (IU/ml)†	N.a.	N.a.	12.0 (3.6–38.0) n = 658	12.2 (3.8–38.5) n = 829
4 yr (IU/ml)†	35.0 (12.0–95.0) n = 664	37.3 (13.0–107.3) n = 746	N.a.	N.a.
8 yr (IU/ml)†	65.0 (23.0–230) n = 703	62.8 (20.0–227.5) n = 1713	N.a.	N.a.
Asthma manifestations				
Asthma symptoms				
3 yr (%)	25.3; n = 245	22.9; n = 844	N.a.	N.a.
4 yr (%)	24.0; n = 230	18.8; n = 670	N.a.	N.a.
5 yr (%)	22.1; n = 211	17.6; n = 615	N.a.	N.a.

Table 1 (Continued)

Characteristics	PIAMA Study population	PIAMA Total cohort	KOALA Study population	KOALA Total cohort
6 yr (%)	18.3; n = 174	14.6; n = 507	11.2; n = 146	11.6; n = 228
7 yr (%)	16.6; n = 151	12.3; n = 416	N.a.	N.a.
8 yr (%)	17.2; n = 158	13.0; n = 429	N.a.	N.a.
Doctor-diagnosed asthma				
1 yr (%)	7.2; n = 69	6.1; n = 224	N.a.	N.a.
2 yr (%)	5.6; n = 54	4.8; n = 177	N.a.	N.a.
3 yr (%)	5.1; n = 49	4.1; n = 152	N.a.	N.a.
4 yr (%)	5.2; n = 50	4.1; n = 145	N.a.	N.a.
5 yr (%)	5.1; n = 48	4.0; n = 138	N.a.	N.a.
6 yr (%)	5.8; n = 55	3.9; n = 134	N.a.	N.a.
7 yr (%)	4.2; n = 38	2.8; n = 98	N.a.	N.a.
8 yr (%)	5.1; n = 45	3.6; n = 116	N.a.	N.a.
Bronchial hyper-responsiveness				
8 yr (%)	44.9; n = 293	42.9; n = 402	N.a.	N.a.

n, number of samples available; N.a., not available. Definitions of characteristics were described previously (27). *Defined as: children who have at least one parent with asthma or atopy. †Geometric mean (interquartile range).

asthma present in the last 12 months. Second, we used a definition of asthma based on symptoms, defined as at least one attack of wheeze or dyspnoea and/or the prescription of inhaled corticosteroids in the last 12 months (25). In PIAMA, bronchial hyper-responsiveness at 8 yr was defined as a fall of ≥20% in FEV1 after inhalation of a maximum of 0.62 mg methacholine bromide (26). In the KOALA cohort, asthma at the age of 6 yr was defined by asthma symptoms: at least one attack of wheezing or dyspnoea, or regular use of inhaled asthma medications in the last year.

Genotyping

Subjects from the PIAMA and KOALA cohorts were genotyped for three *PCDH1* single-nucleotide polymorphisms (SNPs) (rs3797054, rs3822357 and rs14359) and one insertion deletion (IVS3-116, Fig. 1a), in amplified DNA by competitive allele-specific PCR using KASPar™ genotyping chemistry, performed under contract by K-Biosciences, as described previously (27). These polymorphisms were selected based on previous associations of *PCDH1* with BHR and asthma (11).

Linkage disequilibrium (LD) was calculated using Haploview v4.1, by determining D′ and r^2 values (http://www.broad.mit.edu/mpg/haploview). Genotyping was performed blinded of the clinical status and considered successful when < 5% of the genotypes were missing. Furthermore, all SNPs were tested for deviations from Hardy–Weinberg equilibrium, using chi-square tests ($p > 0.05$).

Statistical analysis

Eczema was investigated for association with *PCDH1* gene variants using chi-square tests, comparing genotype frequencies between affected and non-affected individuals. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated from logistic regression analysis. To detect a possible influence of asthma on the associations of *PCDH1* with eczema, analyses were repeated adjusted for asthma.

Generalized estimating equations (GEE) were used to assess associations between *PCDH1* gene variants and the presence of eczema in the first 8 yr of life. GEE is a longitudinal statistical technique that takes correlations between repeated measurements in the same individual into account. As eight observations on atopic dermatitis were made on the same subject, we performed a GEE analyses to analyse the relationships of *PCDH1* SNPs with eczema simultaneously. A 7-dependent working correlation structure fitted the data best. This assumes that the correlations t measurements apart are equal, the correlations t + 1 measurements apart are equal and so on for t = 1 to t = 7.

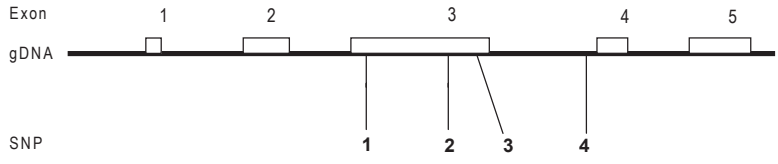
We present the overall estimates based on yearly measurements. We repeated GEE analysis adjusted for parental atopy, sex, breastfeeding, intervention and study population, and inspected whether these covariates alter the regression coefficient ≥10%. In addition, analyses were repeated adjusted for asthma. We previously investigated and reported the validity of pooling data from these two highly comparable birth cohorts (27). For all statistical analysis, we report associations obtained by the best-fitting genetic model.

Results

Genotyping

All tested SNPs were located in or nearby exon 3 of *PCDH1*. All SNPs were in Hardy–Weinberg equilibrium ($p > 0.05$). The allele frequencies were similar in the PIAMA and KOALA cohorts (Fig. 1a). Measures of LD were comparable between study populations. Strong LD was observed for rs3822357, rs3797054, rs14359 and IVS3-116, as D′ approaches the value of 1. LD expressed as r^2 was remarkably lower (0.0–0.17), owing to differences in allele frequencies between SNPs (Fig. 1b).

(a)



SNP	region	name	AC094107.3	rs-number	Allele 1	Allele 2	Frequency (allele 2) in cohorts	
							PIAMA	KOALA
1	exon 3	Ala514Thr	77474	rs3822357	G	A	0.08	0.07
2	exon 3	Ala750Ala	76764	rs3797054	T	C	0.36	0.36
3	exon 3	IVS3 +164	75751	rs14359	G	C	0.23	0.23
4	intron 3	IVS3 -116	70270	(submitted)	TTC Ins	TTC Del	0.06	0.07

gDNA = genomic DNA, SNP = Single Nucleotide Polymorphism, IVS = intervening sequence. Nucleotide sequence of IVS3_116 is as follows: gaatgtgctgtgctggggcctgggtctctt (-/ctt) ggttgctgttgaagcctgagtaggatggtg.

(b)

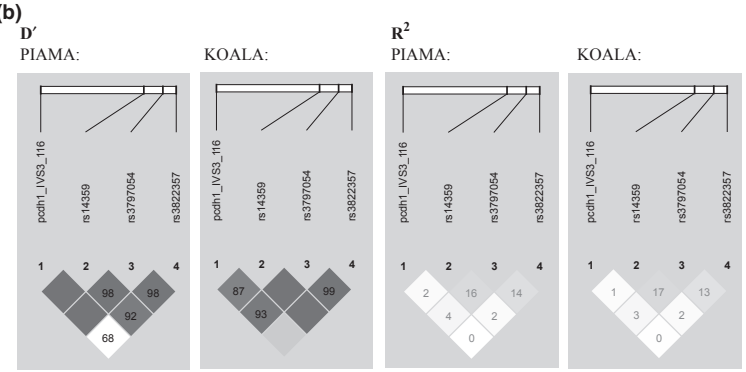


Figure 1 Protocadherin-1: Gene, mRNA expression, SNP position and LD-pattern. *PCDH1* SNPs are numbered 1–4 and corresponding minor allele frequencies are calculated per population (a). Linkage disequilibrium plots are displayed for D′ and R-square values both for PIAMA and KOALA. D′ is represented by gray colour, meaning D′ is >0.9. $r^2 = 0$ is white and $r^2 = 1$ is represented in black (b).

Association with eczema in PIAMA and KOALA cohorts

In the PIAMA cohort (see Table 1 for characteristics), the del IVS3-116 variant was significantly associated with UKWP-defined eczema at the age of 4 yr, using the best-fitting dominant model [OR = 1.90 (95% CI: 1.14–3.18)], and a tendency in a similar direction was observed at 1 yr of age [OR = 1.74 (0.81–3.72)] for this eczema definition. Furthermore, IVS3-116 was border-line associated with increased risk of eczema, defined by questionnaire, in the PIAMA cohort [GEE analysis of 0–8 yr; dominant model: OR = 1.33 (0.98–1.81)] (Table 2).

Rs3822357 (A-allele) was significantly associated with decreased risk of eczema, defined by questionnaire [GEE of 0–8 yr, co-dominant model OR = 0.19 (0.06–0.63)] in the PIAMA study. Numbers were too low to accurately determine association with UKWP criteria.

In the KOALA birth cohort (see Table 1 for characteristics), we also observed an association of IVS3-116 with increased risk of eczema. IVS3-116 del was significantly associated with ISAAC questionnaire–defined eczema at 1 yr [OR = 1.44 (1.00–2.07), dominant model], but not at later age and not with UKWP criteria (Table 2). The protective effect of rs3822357 (A-allele) was not identified in KOALA (Table 2). Other *PCDH1* SNPs were not significantly associated, neither with ISAAC questionnaire– nor UKWP-defined eczema, both in PIAMA and KOALA cohorts (data not shown).

Importantly, GEE analysis on pooled ISAAC questionnaire data of PIAMA and KOALA cohorts resulted in a significant association of IVS3-116 with eczema [OR = 1.26 (1.01–1.58), dominant model] (Table 2 and Fig. 2), but not of rs3822357 [OR = 0.68 (0.23–2.03)]. For IVS3-116, associations with eczema were specifically identified at age 1 and 3 yr [OR = 1.38 (1.03–1.84) and OR = 1.59 (1.04–2.43)], and border-line at age 7 yr [OR = 1.60 (0.98–2.60), Fig. 2]. Adjustment for potential confounders (paternal atopy, sex, breastfeeding, intervention or study population) did not change any of the earlier mentioned associations.

When we constructed haplotypes using the selected SNPs, the significant associated deletion variant IVS3-116 was only in a haplotype with wild type rs14359, rs3797054 and rs3822357 alleles, with similar allele frequencies as IVS3-116 alone (PIAMA 0.063; KOALA 0.070). Therefore, the haplotype analysis did not result in different associations.

As *PCDH1* was previously associated with BHR and asthma, we investigated whether asthma status, at the same age as when the association of *PCDH1* with eczema was determined, had influence on the association of *PCDH1* with eczema. For the association of UKWP eczema at 4 yr, we observed no confounding relationship of asthma phenotypes on the association of *PCDH1* with eczema. In addition, correction for doctor-diagnosed asthma had no influence on the association analysed by GEE between *PCDH1* IVS3-116 and questionnaire-defined eczema [OR = 1.33 (0.98–1.81), p = 0.07]. Correction for asthma symptoms even showed a

Table 2 Associations of *PCDH1* SNPs with eczema in PIAMA and KOALA cohorts

Cohort	Eczema definition	Age (yr)	SNP	No. total subjects	Risk allele	Outcome [OR (95%CI)]	p-value
PIAMA	Questionnaire (GEE Analysis)	0–8	IVS3-116	961	TTC Del	(3) 1.33 (0.98–1.81)	0.070
	Questionnaire (GEE Analysis)	0–8	rs3822357	961	A	(1) 1.16 (0.84–1.61)	0.36
						(2) 0.19 (0.06–0.63)	0.0068
	UKWP	1	IVS3-116	392	TTC Del	(3) 1.74 (0.81–3.72)	NS
		4	IVS3-116	929	TTC Del	(3) 1.90 (1.14–3.18)	0.013
KOALA	Questionnaire	1	IVS3-116	1427	TTC Del	(3) 1.44 (1.00–2.07)	0.032
		2	IVS3-116	1483	TTC Del	(3) 0.91 (0.60–1.39)	NS
		6	IVS3-116	1283	TTC Del	(3) 1.10 (0.70–1.72)	NS
	Questionnaire	1	rs3822357	1438	A	(1) 0.75 (0.49–1.15)	NS
						(2) 0.48 (0.06–3.84)	NS
		2	rs3822357	1496	A	(1) 0.90 (0.59–1.39)	NS
						(2) 2.32 (0.60–9.05)	NS
		6	rs3822357	1290	A	(1) 0.63 (0.37–1.09)	NS
						(2) 1.72 (0.35–8.33)	NS
	UKWP	2	IVS3-116	652	TTC Del	(3) 1.00 (0.52–1.92)	NS
			rs3822357	658	A	(1) 0.72 (0.33–1.55)	NS
						(2) 6.21 (0.86–44.73)	NS
Pooled	Questionnaire (GEE Analysis)	0–8	IVS3-116	2486	TTC Del	(3) 1.26 (1.01–1.58)	0.042
		0–8	rs3822357	2486	A	(1) 1.03 (0.81–1.31)	NS
						(2) 0.68 (0.23–2.03)	NS

Definition of models: (1) = Heterozygous minor allele compared to homozygous wild type, (2) = Homozygous minor allele compared to homozygous wild type, (3) = Dominant model (heterozygous and homozygous minor alleles compared to homozygous wild type). NS, not significant; GEE, generalized estimating equations.

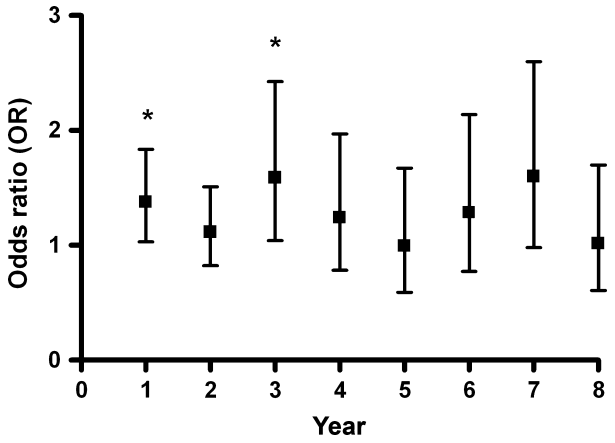


Figure 2 Association of IVS3-116 with eczema in pooled PIAMA and KOALA cohorts. Results of generalized estimating equations are shown for IVS3-116. Odds ratios and 95% confidence intervals are calculated per year for pooled PIAMA and KOALA cohorts, using a dominant model (*$p < 0.05$).

small increase in odds ratio [OR = 1.38 (0.99–1.92), p = 0.058]. Finally, in children with eczema, there was no significant association of *PCDH1* with BHR or asthma at later age (data not shown).

Discussion

This study showed significant association between the *PCDH1* insertion deletion polymorphism IVS3-116 and increased risk of eczema, defined by objective measurements for eczema, the UK Working Party criteria. Moreover, in a pooled analysis of 2486 children of these birth cohorts, IVS3-116 was significantly associated with questionnaire-defined eczema at age 0–8.

We observed association between the *PCDH1* polymorphism IVS3-116 and eczema in two independent Dutch birth cohorts. Importantly, the annual ISAAC core questions provided similar prevalence rates of eczema in these cohorts. We excluded children from non-Dutch origin and investigated a homogeneous Dutch population. Our studies were hypothesis driven, and we therefore did not correct for multiple testing but rather determined association of *PCDH1* polymorphisms in a second cohort. Furthermore, the same allele (del IVS3-116) was previously found to be associated with asthma and BHR (11), providing evidence that these diseases share a genetic background.

We detected several differences in the pattern of the association of *PCDH1* with eczema that related to an age-specific effect, and the association of *PCDH1* with eczema defined through UKWP criteria. For KOALA, data on eczema are available in the first 2 yr, and IVS3-116 associated with questionnaire eczema at age 1, but not at 2 or 6 yr, in children not selected for maternal allergy. This may suggest that *PCDH1* is associated with an early onset of eczema. However, when analysing PIAMA questionnaire data longitudinally with GEE, no change in risk was observed in time. In addition, when pooling the questionnaire data of both cohorts, a significant association was identified during the whole period in this larger sample size (Fig. 2). Additionally,

the association of IVS3-116 with eczema was not modified by paternal allergy.

The association of UKWP eczema with IVS3-116 was identified at the age of 4 yr in a subset of the PIAMA cohort, while no association existed in KOALA at age 2 using this definition. One explanation for the difference in association of IVS3-116 with UKWP eczema can relate to the slightly different usage of UKWP criteria. As in KOALA at the age of 2 yr no data are available on asthma in children, the UKWP criterion 'History of asthma or hay fever' was not applied, while in PIAMA for the eczema assessment at the age of 4 yr this criterion was included. This could have led to an enrichment of children in the PIAMA cohort with asthma or hay fever, and thereby influence the association. However, we detected no confounding relationship of asthma on the association of *PCDH1* with eczema. The detected differences in the associations may relate to the onset or severity of eczema, or cohort-specific gene–gene or gene–environment interactions.

Although not strongly replicated, we do observe significant association in two independent cohorts. The significant associations were identified using 115 and 263 eczema subjects respectively. To investigate replication, we suggest analysing IVS3-116 in a larger sample size.

We did identify an association of the coding SNP rs3822357 (Ala514Thr) with eczema in PIAMA. Again, the same risk allele was observed for BHR in a family study from the UK and a birth cohort from the US (11). This finding was not significantly replicated in KOALA. This difference in association could not be explained by the difference in study design, as correction for potential confounders (paternal atopy, sex or breastfeeding) did not alter the association of rs3822357 with eczema in PIAMA. Given the low allele frequency of the risk allele, power was low to investigate replication, and we therefore suggest that larger studies have to analyse this SNP to increase power to detect association.

Three *PCDH1* polymorphisms (rs3797054, IVS3-116 and rs3822357) have been previously reported to associate with BHR and asthma in Dutch, UK and US populations (11), and we now report that one of these polymorphisms (IVS3-116) to be associated with eczema. The functional effects of these gene variants are not known. Together with the published evidence on the adhesion function of PCDH1 (13) and its expression in skin keratinocytes (14), this points towards a role of this gene affecting epithelial integrity in BHR and asthma, a mechanism that also has been proposed for eczema (16, 28).

It is of interest to compare our results with data on filaggrin (*FLG*), a replicated gene for eczema, resulting in loss of epithelial integrity in the skin. Previously, we genotyped the PIAMA cohort for three *FLG* SNPs, and confirmed the relative high prevalence of the *FLG* null mutations in the north of Europe (29), which clearly differs for Mediterranean populations, were the frequency of these SNPs was low or not detected (30). These results point towards genetic heterogeneity of this disease. For *FLG*, we and others have observed an association with early age at onset of eczema (31–34). In addition, it has been reported that persons with eczema carrying *FLG* mutations are at increased risk to develop asthma. Albeit not that strong as for *FLG,* we do observe significant associations of relative rare *PCDH1* gene variants with eczema. We were not able to determine *FLG* and *PCDH1* gene–gene interactions owing to low numbers of children carriers of both *FLG* and *PCDH1* deletions. Interestingly, for *PCDH1* we did not observe a progression towards asthma at age 6–8. As the initial genetic association of *PCDH1* gene variants with asthma was identified in an older age group, longer follow up may be needed to assess progression from eczema to asthma.

In conclusion, our study provides evidence for the association of *PCDH1* polymorphism IVS3-116 with eczema. Previously, IVS3-116 was shown to be associated with BHR. Our data show that *PCDH1* may have a pleiotropic effect on BHR and eczema in different populations.

Acknowledgments

We would like to thank all participants from the PIAMA and KOALA birth cohorts. In addition, we thank Judith Vonk for critically reading the manuscript. Henk Koning was supported by the European Commission as part of GABRIEL (A multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) contract number 018996 under the Integrated Program LSH-2004-1.2.5-1 and a grant from the University Medical Center Groningen. Funding PIAMA birth cohort: The Netherlands Organisation for Health Research and Development (ZonMW 2100.0090); the Netherlands Organisation for Scientific Research; the Netherlands Asthma Fund (EBO 3.2.03.48); the Netherlands Ministry of Spatial Planning, Housing, and the Environment; and the Netherlands Ministry of Health, Welfare and Sport. The KOALA birth cohort study was co-financed by: Netherlands Asthma Foundation, Netherlands Organisation for Health Research and Development (ZonMw), Royal Friesland Foods, Triodos Foundation, Foundation for the Advancement of Heilpedagogie, Phoenix Foundation, Raphaël Foundation, Iona Foundation and Spinoza Award (2000, Prof. D.S. Postma). Genetic studies were supported by ZonMw, The Netherlands Organisation for Health Research and Development, grant number 912-03-031 and 91656091 (ZON MW VENI grant to Dr Koppelman).

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