

The role of glutathione S-transferase and claudin-1 gene polymorphisms in contact sensitization: a cross-sectional study

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Summary

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Conflicts of interest

None declared.

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Background Contact sensitization is frequent in the general population and arises from excessive or repeated skin exposure to chemicals and metals. However, little is known about its genetic susceptibility.

Objectives To determine the role of polymorphisms of glutathione S-transferase (GST) genes and the claudin-1 gene (CLDN1) on the risk of contact sensitization, taking common filaggrin gene (FLG) mutations into account.

Methods In total, 3471 adult Danes from the general population were standard patch tested and filled out a questionnaire on their general health. They were genotyped for the following polymorphisms: GSTM1 and GSTT1 deletion, GSTP1 single nucleotide polymorphism (SNP) rs1695, four CLDN1 SNPs (rs893051, rs9290927, rs9290929 and rs17501010) and the FLG null mutations R501X and 2282del4.

Results In individuals without ear piercings, a higher prevalence of nickel sensitization was found in those with the minor allele of CLDN1 SNP rs9290927 ($P_{\text{trend}} = 0.013$). For CLDN1 rs17501010, contact sensitization to organic compounds was associated with the major allele ($P_{\text{trend}} = 0.031$). The risk pattern was also identified for self-reported nickel dermatitis ($P_{\text{trend}} = 0.011$). The fragrance sensitization prevalence differed in a pairwise comparison of the CLDN1 rs893051 SNP genotypes ($P = 0.022$), with the minor allele being associated with a higher prevalence. The associations were confirmed in logistic regression analyses.

Conclusions The CLDN1 polymorphisms rs9290927, rs893051 and rs17501010 were associated, respectively, with nickel contact sensitization in individuals without ear piercings, contact sensitization to fragrances, and with both organic compounds and nickel contact dermatitis. We could not find associations between GST gene polymorphisms and contact sensitization. FLG mutations did not affect the observed associations.

Contact sensitization to chemical substances and metals is frequent in the general population.¹ While the allergen dose per unit skin area is crucial for both induction and elicitation of dermatitis,^{2,3} only some individuals react when exposed to the same allergen dose, emphasizing the great interindividual differences regarding the threshold level.^{4,5} A recent comprehensive review article concluded that variations in the genes coding for N-acetyltransferases, glutathione S-transferases (GSTs), angiotensin-converting enzyme, tumour necrosis

factor, interleukin-16 and filaggrin (FLG) were associated with an elevated risk of contact sensitization or increased susceptibility to certain contact sensitizers.⁶ Also, it has been shown that loss-of-function mutations in FLG increase the risk of allergic nickel dermatitis^{7–9} and contact sensitization to nonmetals in individuals with episodes of dermatitis (article submitted for publication).

The outermost compartment of the epidermis, the stratum corneum, is a compact layer of flattened and rigid corneocytes

that are embedded in a lipid-rich matrix providing mechanical resistance against offending physical, chemical and microbial agents.¹⁰ Keratin filament alignment is facilitated by FLG proteins that are released from the keratohyalin granules at the junction between the stratum corneum and stratum granulosum.¹¹ FLG is formed by proteolytical cleavage of a precursor profilaggrin containing 10–12 FLG monomers.¹² FLG proteins are normally further degraded into their constituent amino acids, which help to maintain stratum corneum hydration (the so-called natural moisturizing factors).¹³ In the stratum granulosum, the keratinocytes are tightly bound by tight-junction proteins, which control the passage of molecules between the cells and prevent water loss.¹⁴ The components of tight junctions include claudin (CLDN)-1. While reduced expression of the CLDN-1 gene causes epidermal barrier dysfunction and increases the risk of atopic dermatitis – at least in individuals with darkly pigmented skin¹⁵ – no studies have yet investigated whether CLDN1 polymorphisms also increase the risk of contact sensitization.

GSTs are enzymes involved in cell protection by conversion of reactive molecules into less reactive products. They catalyse the conjugation of reactive electrophiles with reduced glutathione, hence their family name.¹⁶ There are seven cytosolic GST classes in humans: α , μ , π , σ , θ , ζ and ω , also designated A, M, P, S, T, Z and O, respectively.¹⁶ In the general population, complete deletion of the M1 and T1 members is frequently found.¹⁷ Gene polymorphism in a π -class GST, GSTP1, involves an amino acid substitution (Ile105 → Val105), resulting in decreased enzyme activity, at least with certain reactive groups.¹⁸ In relation to contact sensitization, the GSTT1-null genotype has been associated with chromium sensitization in Taiwanese construction workers,¹⁹ and the double deletion of GSTT1 and GSTM1 with thiomersal sensitization in Germans,²⁰ whereas GSTM1, GSTP1 and GSTT1 polymorphisms were not correlated with *p*-phenylenediamine sensitization.²¹

We investigated the association between multiple GST gene and CLDN1 polymorphisms, respectively, and contact sensitization in an adult general population, taking FLG mutation status into account. Contrary to clinical cohorts, environmental allergen exposure is suspected to be weaker overall in the general population, possibly allowing the detection of genetic susceptibility that would otherwise be overruled by high exposure to contact allergens.

Patients and methods

Study population

Between 2006 and 2008, 3471 (43.8%) of 7931 randomly invited Danish adults (aged 18–69 years), born in Denmark and residing in the capital region of Copenhagen, completed a questionnaire and underwent a general health examination at Glostrup Hospital, Denmark. Hence, the population was mainly of white descent. The investigation was not restricted to skin health. More women than men, and more older than younger patients, participated (mean age 47.5 years).²² The

study was approved by the Ethics Committee of Copenhagen County (KA-20060011), and a written informed consent form was obtained from all of the participants.

Patch testing

In total, 3460 participants were patch tested with the standardized ready-to-apply TRUE Test® (Mekos Laboratories, Hillerød, Denmark) as previously described.²³ Readings were restricted to day 2 for practical reasons, and the reactions were read according to the recommendations from the International Contact Dermatitis Research Group.

GSTM1, GSTP1, GSTT1, FLG and CLDN1 genotyping

Blood samples were taken on the day of the health examination, and genomic DNA was purified from the leucocytes. Two multiplex real-time polymerase chain reaction (PCR)-based assays were used for the detection of GSTM1 and GSTT1 genomic DNA copy number, as previously described.^{24,25} The assays are based on amplification and quantification of the GSTM1 or GSTT1 sequence in relation to a reference gene, albumin, in a multiplex PCR using the StepOnePlus™ real-time PCR system (Applied Biosystems, Foster City, CA, U.S.A.). In total, 32 (0.9%) and 52 samples (1.5%) could not be assigned to the GSTM1 or GSTT1 genotypes, respectively, due to poor DNA quality.

The four CLDN1 single nucleotide polymorphisms (SNPs; rs893051, G/C; rs9290927, A/T; rs9290929, A/G; and rs17501010, G/T) were selected, as it has previously been shown that they are associated with atopic dermatitis and/or its severity, in a comprehensive association analysis including 27 CLDN1 SNPs in a North-American population.¹⁵ The intronic SNPs rs17501010 and rs9290927 were associated with a lower and higher risk of atopic dermatitis, respectively. The intronic rs893051 and rs9290929, situated in the promoter region, were both associated with greater disease severity.¹⁵ The CLDN1 SNPs and the GSTP1 SNP (rs1695, Ile105/Val105) were genotyped by the KBiosciences allele-specific PCR, KAS-Par (KBiosciences, Hoddesdon, U.K.). The genotyping success rate was above 97.4%, with a mismatch rate of 0.00% for the five SNPs, in a minimum of 362 duplicates per SNP.

Overall, 3346 participants (96.4% of the total) were FLG genotyped for the two most common mutations in the white population: R501X and 2282del. Regions covering these two mutations were amplified by PCR, and the PCR products were subsequently hybridized to microbeads carrying either mutation-specific or wild-type-specific probes. Detection was performed on a BioPlex 200 (BioRad, Hercules, CA, U.S.A.).²⁶

Questionnaire

The prevalence of dermatitis from nickel and cosmetic products, respectively, was assessed with the questions: 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or

earrings?' and 'Have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?'. Atopic dermatitis was defined by the U.K. Working Party's diagnostic criteria as one major plus at least two of four minor criteria.²⁷ The major criterion was an itchy skin condition; the minor criteria were (i) a history of involvement of the skin creases; (ii) a personal history of asthma or hay fever; (iii) a history of general dry skin within the last year; and (iv) onset before 2 years of age.²⁷

Statistical analyses

Contact sensitization was defined as at least one positive patch test reaction (+, ++ or +++). The genotype prevalence was compared for the following contact sensitization categories: 'overall' (at least one positive patch-test reaction), 'nickel sensitization', 'metal' (nickel, cobalt or chromium), 'fragrance sensitization' (fragrance mix I or Myroxylon pereirae) and 'organic compounds' (at least one contact sensitization excluding metal sensitization). In the case of nickel sensitization, association analyses were also performed for individuals without ear piercings, as nickel sensitization may have developed following skin piercing rather than following prolonged skin contact.²⁸ To test for dependency of GST and CLDN1 genotypes in contact sensitization, univariate analyses using the χ^2 -trend test (linear-by-linear association) were conducted. Additionally, P-values from pairwise comparisons were calculated. In the cases of GSTM1 and GSTT1, two deleted genes were denoted 'null/null', one deleted gene and one present were denoted 'functional/null', and 'functional/functional' referred to the presence of both gene copies being functional. In the case of GSTP1, 'functional/functional' referred to the presence of two genes coding for Ile at site 105 in the amino acid sequence; two genes coding for Val at site 105 were denoted 'null/null', and the heterozygote genotype was denoted 'functional/null'. To evaluate the combined effect of the GST variant genotypes and the FLG and CLDN1 genotypes, respectively, logistic regression analyses were performed. The abovementioned five contact sensitization categories were considered the dependent variables in five different regression analyses adjusted for sex (male or female), age (18–34, 35–49 or 50–69 years) and, in the case of nickel and metal sensitization, ear piercing (yes or no). The FLG genotype status was divided into wild type and null, where null denoted at least one non-functional copy. Associations were expressed as odds ratios with 95% confidence intervals. Hardy–Weinberg tests were performed using the Hardy–Weinberg calculator from the 'Online Encyclopedia for Genetic Epidemiology Studies'.²⁹ Statistical analyses were performed using SPSS v15.0 (SPSS Inc., Chicago, IL, U.S.A.) for Windows.

Results

The prevalence of contact sensitization is shown in Table 1. Overall, 10.0% of subjects were contact sensitized to at least

Table 1 Allergens in the TRUE Test panel, and the prevalence of positive patch-test reactions in 3460 patch-tested adults from the general population

Allergen categories and single allergens	Positive reactions, % (n)
Metals	
Nickel sulphate	5.9 (204)
Cobalt dichloride	0.2 (8)
Potassium dichromate	0.1 (5)
Fragrances	
Fragrance mix I	1.6 (55)
Myroxylon pereirae	0.1 (3)
Other	
Wool alcohols	0.0 (0)
Neomycin sulphate	0.1 (2)
Caine mix	0.1 (2)
Colophony	0.6 (21)
Epoxy resin	0.5 (18)
Quinoline mix	0.1 (2)
Ethylenediamine dihydrochloride	0.2 (8)
p-tert-Butylphenol formaldehyde resin	0.1 (2)
Paraben mix	0.0 (0)
Carba mix	0.1 (4)
Black rubber mix	0.1 (4)
Cl+Me-isothiazolinone	0.2 (6)
Quaternium-15	0.2 (6)
Mercaptobenzothiazole	0.0 (0)
p-Phenylenediamine	0.1 (4)
Formaldehyde	0.2 (7)
Mercapto mix	0.0 (0)
Thiomersal	0.5 (18)
Thiuram mix	0.1 (3)
Total	10.0 (345)

one allergen (men 4.7% vs. women 14.2%, $P < 0.001$). The prevalence of atopic dermatitis was 10.1% (men 6.3% vs. women 13.2%, $P < 0.001$).

Glutathione S-transferases

The GSTM1, GSTT1 and GSTP1 genotype distributions did not deviate from the Hardy–Weinberg equilibrium ($P = 0.254$, $P = 0.920$ and $P = 0.671$, respectively). The outcome of patch testing is presented in Table 1. No associations with contact sensitization or contact sensitization subgroups ('metal', 'nickel', 'fragrance' or 'organic compound') were observed for GSTM1, GSTT1 or GSTP1 (Table 2). The prevalence of self-reported metal and cosmetic dermatitis was comparable between the GST variant genotypes. However, the prevalence of 'fragrance sensitization' differed between the GSTP1 genotypes ($P = 0.045$) (Table 2). As different GST variants can have overlapping substrate specificities, and even form functional heterodimers,¹⁶ we examined the combined effect of GST gene polymorphisms on contact sensitization using logistic regression analyses (Table 3). Besides traditional confounders such as age, sex and ear piercing, we also performed adjustment for the two most common FLG mutations and the

Table 2 Association between contact sensitization and the GSTT1, GSTM1 and GSTP1 genotypes

	Glutathione S-transferase genotype status			P (linear by linear in χ^2 -tests)
	Functional/functional, % (N/N _{tot})	Functional/null, % (N/N _{tot})	Null/null, % (N/N _{tot})	
At least one sensitization ^a				
GSTT1	9.0 (114/1269)	10.4 (163/1574)	10.6 (52/491)	0.213
GSTM1	9.6 (26/271)	9.8 (129/1320)	10.1 (178/1763)	0.727
GSTP1 ^b	10.6 (154/1455)	8.8 (130/1478)	9.7 (35/361)	0.247
Nickel sensitization ^a				
GSTT1	5.1 (65/1269)	6.2 (98/1574)	6.3 (31/491)	0.226
GSTM1	7.0 (19/271)	6.0 (79/1320)	5.7 (100/1763)	0.417
GSTP1 ^b	6.2 (90/1455)	5.4 (80/1478)	5.0 (18/361)	0.280
Nickel sensitization among individuals without ear piercing ^a				
GSTT1	1.0 (6/595)	0.8 (6/712)	2.4 (6/245)	0.169
GSTM1	0.7 (1/139)	1.3 (8/622)	1.1 (9/795)	0.881
GSTP1 ^b	0.7 (5/675)	1.2 (8/695)	2.5 (4/161)	0.081
Metal sensitization ^{a,c}				
GSTT1	5.4 (69/1269)	6.4 (101/1574)	6.7 (33/491)	0.231
GSTM1	7.0 (19/271)	6.1 (80/1320)	6.1 (108/1763)	0.729
GSTP1 ^b	6.5 (95/1455)	5.6 (83/1478)	5.3 (19/361)	0.242
At least one sensitization excluding metal ^{a,c}				
GSTT1	4.4 (56/1269)	4.3 (68/1574)	4.5 (22/491)	0.993
GSTM1	3.0 (8/271)	4.5 (59/1320)	4.5 (79/1763)	0.423
GSTP1 ^b	4.7 (69/1455)	3.7 (54/1478)	5.0 (18/361)	0.589
Fragrance sensitization ^{a,d}				
GSTT1	1.7 (21/1269)	1.4 (22/1574)	2.4 (12/491)	0.449
GSTM1	0.7 (2/271)	1.9 (25/1320)	1.6 (28/1763)	0.744
GSTP1 ^b	1.9 (27/1455)	1.1 (16/1478)	2.8 (10/361)	0.900 ^e
Nickel dermatitis ^f				
GSTT1	23.3 (294/1260)	25.0 (391/1561)	22.7 (111/489)	0.887
GSTM1	23.3 (63/270)	24.4 (320/1311)	24.0 (419/1749)	0.990
GSTP1 ^b	23.9 (347/1449)	23.7 (347/1465)	25.2 (90/357)	0.770
Nickel dermatitis among individuals without ear piercing ^f				
GSTT1	9.2 (55/595)	11.8 (84/711)	11.1 (27/244)	0.261
GSTM1	7.9 (11/139)	10.5 (65/620)	11.3 (90/795)	0.260
GSTP1 ^b	10.7 (72/674)	10.5 (73/696)	11.9 (19/159)	0.778
Dermatitis from a cosmetic product ^g				
GSTT1	46.3 (582/1256)	48.2 (750/1557)	48.4 (236/488)	0.514
GSTM1	47.0 (127/270)	47.5 (621/1306)	47.4 (826/1744)	0.980
GSTP1 ^b	48.5 (699/1441)	46.7 (683/1461)	48.2 (173/359)	0.787

^aDefined as positive patch-test readings (+, ++, +++) according to the International Contact Dermatitis Research Group criteria. ^bFor GSTP1, functional/functional refers to the Ile105/Ile105 genotype, functional/null refers to the Ile105/Val105 genotype and null/null refers to the Val105/Val105 genotype. ^cMetals included in the TRUE Test are nickel, cobalt and chromium. ^dFragrances included in the TRUE Test are fragrance mix I and Myroxylon pereirae. ^eIn pairwise χ^2 -test P = 0.045. ^fAn affirmative answer to the question 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' ^gAn affirmative answer to the question 'Have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?'

four CLDN1 SNPs to evaluate the significance of GST function in subjects having an inherited skin barrier abnormality. No associations between the GST gene polymorphisms and contact sensitization were found in any of the models (data not shown).

Claudin 1

The genotype distributions of the CLDN1 SNPs rs893051, rs9290927, rs9290929 and rs17501010 were in Hardy–Weinberg

equilibrium (P = 0.699, P = 0.752, P = 1.00 and P = 0.075, respectively). The four SNPs were analysed for association with contact sensitization (Table 4). In individuals without ear piercings, we found a higher prevalence of nickel sensitization associated with the minor allele compared with the major allele of SNP rs9290927 (P_{trend} = 0.013). For rs17501010, a significant trend towards a decreasing prevalence of contact sensitization to organic compounds was observed (P_{trend} = 0.031). The rs17501010 SNP was also significantly associated with self-reported nickel dermatitis, with the highest prevalence identi-

Table 3 Logistic regression analyses on contact sensitization in the general population adjusted for GSTT1, GSTM1, GSTP1 and FLG genotypes and other known risk factors

	Adjusted odds ratio (95% confidence interval)				
	At least one sensitization ^a (n = 3197)	Nickel sensitization ^a (n = 3197)	Metal sensitization ^{a,b} (n = 3197)	At least one sensitization other than metal ^{a,b} (n = 3227)	Fragrance sensitization ^{a,c} (n = 3227)
Age					
18–35	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
36–49	1.06 (0.78–1.44)	1.17 (0.80–1.71)	1.16 (0.79–1.68)	0.99 (0.63–1.56)	0.90 (0.45–1.78)
50–69	0.72 (0.50–1.03)	0.49 (0.30–0.81)*	0.58 (0.36–0.94)*	0.93 (0.57–1.52)	0.61 (0.28–1.36)
Sex					
Male	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Female	2.79 (1.95–3.99)**	7.21 (3.64–14.3)**	7.88 (4.00–15.6)**	1.30 (0.92–1.84)	1.30 (0.74–2.28)
Ear piercing					
No	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	—	—
Yes	1.41 (1.01–1.98)*	2.93 (1.66–5.19)**	2.75 (1.60–4.72)**	—	—
GSTT1 ^d					
Functional/functional	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Functional/null	1.05 (0.81–1.37)	1.12 (0.80–1.58)	1.08 (0.77–1.51)	0.91 (0.63–1.32)	0.79 (0.43–1.45)
Null/null	1.19 (0.83–1.70)	1.28 (0.81–2.04)	1.29 (0.82–2.03)	0.97 (0.58–1.63)	1.38 (0.66–2.88)
GSTM1 ^e					
Functional/functional	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Functional/null	0.94 (0.59–1.51)	0.71 (0.41–1.24)	0.72 (0.41–1.26)	1.59 (0.72–3.53)	4.78 (0.64–35.5)
Null/null	0.95 (0.60–1.50)	0.64 (0.37–1.10)	0.70 (0.41–1.20)	1.65 (0.75–3.63)	4.08 (0.55–30.2)
GSTP1 ^f					
Ile/Ile	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Ile/Val	0.82 (0.64–1.06)	0.86 (0.62–1.19)	0.84 (0.61–1.16)	0.77 (0.54–1.11)	0.59 (0.32–1.10)
Val/Val	0.90 (0.60–1.33)	0.76 (0.44–1.30)	0.76 (0.45–1.28)	1.10 (0.62–1.79)	1.50 (0.72–3.13)

*P < 0.05; **P < 0.001; ref., reference value. ^a Defined as positive patch-test readings (+, ++, +++) according to the International Contact Dermatitis Research Group criteria. ^b Metals included in the TRUE Test are nickel, cobalt and chromium. ^c Fragrances included in the TRUE Test are fragrance mix I and Myroxylon pereirae. ^d GSTT1 genotype for the deletion allele. ^e GSTM1 genotype for the deletion allele. ^f GSTP1 genotype for the Ile105/Val105 single nucleotide polymorphism.

fied among individuals who were homozygous for the major allele ($P_{\text{trend}} = 0.011$). The fragrance sensitization prevalence differed significantly when a pairwise comparison of the rs893051 SNP genotypes ($P = 0.022$) was performed, with the minor allele being associated with a higher prevalence of fragrance sensitization. No associations were found for the SNP rs9290929. The four SNPs and their associations with contact sensitization and contact dermatitis were also evaluated in logistic regression analyses that were adjusted for age, sex, ear piercing and atopic dermatitis. The associations between rs9290927 and nickel sensitization in individuals without ear piercing, rs893051 and fragrance sensitization, and rs17501010 and nickel dermatitis were all confirmed in the logistic regression analyses (Table 5). The association between rs17501010 and contact sensitization to the organic substances of the TRUE Test was only borderline significant (Table 5). Additional adjustment for FLG mutation status did not alter the outcomes for rs17501010 and rs893051. However, in individuals without ear piercings, the association between the rs9290927 minor allele and nickel sensitization became insignificant even though the increased risk pattern was still found (data not shown). None of the four CLDN1 SNPs was associated with atopic

dermatitis in this cohort. We had insufficient study power to perform interaction analyses on FLG and CLDN1, but crude risk analyses showed no indications of additive effects.

Discussion

Skin exposure to contact allergens is practically impossible to avoid due to their frequent use in consumer and occupational products. Identification of the critical determinants of interindividual variation of sensitization and elicitation threshold levels may ultimately improve prevention, as genetically susceptible individuals may be instructed to take particular precautions. GST gene polymorphisms have previously been associated with contact sensitization and other inflammatory disorders,^{16,19,20} whereas CLDN1 polymorphisms have been associated with atopic dermatitis in a case-control study.¹⁵ We investigated the association with contact sensitization using data from a general adult population study. Moreover, common FLG mutations, which have been associated with nickel sensitization, were taken into account.

Some CLDN1 SNPs were significantly associated with nickel sensitization and dermatitis, as well as contact sensitization to

Table 4 Association between contact sensitization and the claudin-1 gene (CLDN1) single nucleotide polymorphism (SNP) genotype

	CLDN1 SNP genotype status			
	A/A, % (N/N _{tot})	A/a, % (N/N _{tot})	a/a, % (N/N _{tot})	P (linear by linear in χ^2 -tests)
At least one contact sensitization ^a				
rs893051	9.6 (110/1142)	10.2 (164/1609)	9.5 (55/580)	0.966
rs9290927	10.1 (250/2485)	9.5 (75/791)	11 (7/66)	0.750
rs9290929	9.9 (96/967)	9.9 (164/1656)	9.5 (67/708)	0.768
rs17501010	10.1 (280/2781)	9.2 (50/545)	0 (0/17)	0.289
Nickel sensitization ^a				
rs893051	6.2 (71/1142)	5.8 (94/1609)	5.7 (33/580)	0.630
rs9290927	5.7 (141/2485)	6.6 (52/791)	6 (4/66)	0.401
rs9290929	5.6 (54/967)	5.9 (98/1656)	5.9 (42/708)	0.746
rs17501010	5.8 (161/2781)	6.6 (36/545)	0 (0/17)	0.724
Nickel sensitization among individuals without ear piercing ^a				
rs893051	1.3 (7/546)	1.4 (10/732)	0.4 (1/268)	0.352
rs9290927	0.9 (10/1149)	1.6 (6/375)	8 (2/26)	0.013*
rs9290929	1.1 (5/450)	0.9 (7/756)	1.7 (6/346)	0.463
rs17501010	1.2 (16/1301)	0.8 (2/246)	0 (0/6)	0.539
Metal sensitization ^{a,b}				
rs893051	6.7 (76/1142)	6.0 (97/1609)	5.9 (34/580)	0.468
rs9290927	6.0 (150/2485)	6.6 (52/791)	6 (4/66)	0.648
rs9290929	5.7 (55/967)	6.3 (104/1656)	6.2 (44/708)	0.623
rs17501010	6.0 (167/2781)	7.2 (39/545)	0 (0/17)	0.542
At least one sensitization excluding metal ^{a,b}				
rs893051	3.9 (44/1142)	4.7 (75/1609)	4.0 (23/580)	0.717
rs9290927	4.5 (112/2485)	3.7 (29/791)	6 (4/66)	0.597
rs9290929	4.8 (46/967)	4.2 (70/1656)	4.0 (28/708)	0.411
rs17501010	4.6 (129/2781)	2.8 (15/545)	0 (0/17)	0.031*
Fragrance sensitization ^{a,c}				
rs893051	0.9 (10/1142)	2.2 (36/1609)	1.6 (9/580)	0.107 ^d
rs9290927	1.6 (40/2485)	1.6 (13/791)	2 (1/66)	0.981
rs9290929	2.0 (19/967)	1.8 (30/1656)	0.8 (6/708)	0.092
rs17501010	1.8 (50/2781)	0.9 (5/545)	0 (0/17)	0.116
Nickel dermatitis ^e				
rs893051	23.5 (267/1134)	24.0 (383/1595)	25.4 (147/579)	0.426
rs9290927	23.6 (583/2470)	25.3 (198/784)	29 (19/65)	0.192
rs9290929	24.7 (237/958)	23.7 (390/1648)	23.5 (165/703)	0.526
rs17501010	24.9 (688/2762)	19.6 (106/542)	24 (4/17)	0.011*
Nickel dermatitis among individuals without ear piercing ^e				
rs893051	10.5 (57/545)	10.5 (77/730)	10.8 (29/269)	0.894
rs9290927	10.4 (119/1149)	11.2 (42/374)	16 (4/25)	0.410
rs9290929	12.1 (54/448)	9.4 (71/756)	11.6 (40/346)	0.717
rs17501010	10.9 (141/1299)	8.5 (21/246)	0 (0/6)	0.197
Dermatitis from a cosmetic product ^f				
rs893051	46.9 (532/1134)	48.0 (762/1589)	48.2 (277/575)	0.574
rs9290927	47.1 (1158/2458)	48.4 (380/785)	59 (39/66)	0.136
rs9290929	47.0 (448/953)	47.4 (778/1642)	47.4 (334/704)	0.853
rs17501010	47.2 (1300/2754)	49.2 (266/541)	41 (7/17)	0.540

*P < 0.05. A, major allele; a, minor allele. Actual SNP bases – rs893051: major allele G, minor allele C; rs9290927: major allele A, minor allele T; rs9290929: major allele A, minor allele G; rs17501010: major allele G, minor allele T. ^aDefined as positive patch-test readings (+, ++, +++) according to the International Contact Dermatitis Research Group criteria. ^bMetals included in the TRUE Test are nickel, cobalt and chromium. ^cFragrances included in the TRUE Test are fragrance mix I and Myroxylon pereirae. ^dIn pairwise χ^2 -test P = 0.022. ^eAn affirmative answer to the question 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' ^fAn affirmative answer to the question 'Have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?'

organic compounds and fragrances (Tables 4 and 5). However, none of the CLDN1 SNPs increased the overall risk of contact sensitization. We suspect that the study lacked sufficient statistical

power to detect small effects; for example, the association between CLDN1 SNP rs893051 and fragrance sensitization was stronger in heterozygous than homozygous individuals. Also,

Table 5 Logistic regression analyses of contact sensitization in the general population involving statistically significant claudin-1 gene single nucleotide polymorphism associations from Table 4

	Adjusted odds ratio (95% confidence interval)			
	Nickel sensitization in individuals without ear piercings ^a (n = 1550)	At least one sensitization excluding metals ^{a,b} (n = 3343)	Fragrance sensitization ^{a,c} (n = 3331)	Nickel dermatitis ^d (n = 3202)
Age				
18–35	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
36–49	3.17 (0.40–25.0)	1.03 (0.65–1.62)	0.90 (0.45–1.78)	1.40 (1.11–1.77)*
50–69	1.43 (0.17–12.1)	0.95 (0.58–1.54)	0.72 (0.33–1.55)	0.84 (0.64–1.10)
Sex				
Male	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Female	4.81 (1.85–12.5)**	1.31 (0.93–1.85)	1.27 (0.73–2.20)	4.23 (3.27–5.47)**
Ear piercing				
No				1.00 (ref.)
Yes				1.96 (1.54–2.50)**
Atopic dermatitis ^e				
No				1.00 (ref.)
Yes				1.84 (1.42–2.39)**
rs9290927				
A/A	1.00 (ref.)			
A/T	1.63 (0.58–4.55)			
T/T	7.29 (1.44–36.8)*			
rs893051				
G/G			1.00 (ref.)	
G/C			2.56 (1.27–5.19)*	
C/C			1.77 (0.71–4.38)	
rs17501010				
G/G		1.00 (ref.)		1.00 (ref.)
G/T		0.59 (0.34–1.01) ^f		0.72 (0.56–0.92)*
T/T		0 (—)		0.62 (0.16–2.39)

*P < 0.05; **P < 0.001; ref., reference value. ^aDefined as positive patch-test readings (+, ++, +++) according to the International Contact Dermatitis Research Group criteria. ^bMetals included in the TRUE Test are nickel, cobalt and chromium. ^cFragrances included in the TRUE Test are fragrance mix I and Myroxylon pereirae. ^dAn affirmative answer to the question 'Have you ever had eczema under the tightener of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' ^eDefined by the U.K. Working Party's diagnostic criteria for atopic dermatitis (one major and at least two of four minor criteria). ^fP = 0.053.

we could not confirm the association between atopic dermatitis and CLDN1 polymorphisms, which could be explained by the use of a general population cohort where morbidity is generally limited. Although our findings need to be replicated in other populations to rule out random error, they could indicate that tight junctions may indeed be involved in susceptibility to contact sensitization and allergic contact dermatitis. The results were not corrected for multiple testing (e.g. Bonferroni) as an *a priori* hypothesis was defined. We acknowledge that the probability of making type I errors is higher without correction, but on the contrary the type II error rate is not exaggerated.

It is also acknowledged that the investigated contact allergens have different chemical properties, being either water or lipid soluble and also having different valency, factors that may influence the capacity to penetrate the epidermis and induce contact sensitization. Hence, the stratum corneum works as an air–liquid barrier where keratinocytes are embedded in lipids to prevent water loss. The lipid-rich stratum corneum may also theoretically facilitate the penetration of

especially lipid-soluble contact allergens, and hamper the penetration of water-soluble ones. However, barrier disruption caused by exposure to irritants will affect the quantity of lipids and therefore, at least in theory, increase the capacity of water-soluble allergens to also penetrate the stratum corneum and reach the tight junctions. Thus, the risk of allergen penetration is determined not only by dose and exposure time, but also by the chemical properties of the contact allergens in question, as well as the presence of irritants. In a recent review article, the stratum corneum, tight junctions and dendritic cells were referred to as 'the three musketeers' of the epidermal barrier due to their crucial role in skin homeostasis.¹⁰ The tight-junction barriers exist covertly under the stratum corneum and divide two adjacent compartments to maintain different solute concentrations.¹⁰ As the tight-junction barrier may be regarded as a second line of defence, CLDN1 mutations could have an impact on the susceptibility to develop contact sensitization and dermatitis. Even though CLDN-1 is just one of several tight-junction proteins, it is a

key component of the epidermal protein structures. Absence of CLDN-1 is implicated in neonatal sclerosing cholangitis associated with ichthyosis, and it has been suggested that hypomorphic CLDN1 mutations are responsible for related mild phenotypes.³⁰ Provided that an inherited impairment of the stratum corneum, e.g. due to FLG mutations, causes increased allergen penetration of 'the first line of defence', one would also expect increased effects of a concomitant leaky 'second line of defence' due to genetic variation in the tight junctions. However, we could not confirm an increased effect of CLDN1 polymorphisms when FLG mutations were present. It must be underscored that the numbers in the subanalyses were often small and that the results therefore should be interpreted with caution.

Some previous studies have suggested an association between GST gene polymorphisms and contact sensitization to certain chemicals;^{19,20} however, a recent meta-analysis by Pot *et al.* concluded that polymorphisms of GST genes are not major contributors to contact sensitization, and that they are disease modifying rather than causal.²¹ When we examined one GST gene at a time (GSTT1, GSTM1 and GSTP1), no association between the functional gene copy number and contact sensitization was identified. Moreover, we could not find evidence for an association in mutually adjusted regression analyses. Taking FLG or CLDN1 polymorphisms into account did not change the outcome, even though a compromised skin barrier would theoretically increase the effect of GST enzyme presence or absence. The seven classes of human cytosolic GSTs comprise 16 different isoforms, which gives 16 different functional homodimers.¹⁶ It has also been shown that some GSTs are capable of forming functional heterodimers,³¹ and if one counts in the numerous gene polymorphisms giving rise to an even broader repertoire of functional dimers, the potential detoxification spectre could be enormous. Considering differential regulation of GST gene variant expression, and the tissue specificity and overlapping metabolic function of this huge enzyme network, it may be that analyses on single variant polymorphisms are too simplified to interpret at disease level.

We are aware that the contact sensitization categories that were constructed for this study grouped structurally different chemical compounds, although GST variants display a degree of substrate preference. However, as GST enzyme activities have not been evaluated for all chemical substances, and because GST variants have been shown to have overlapping substrate specificities, we chose a clinical approach. GST variant polymorphisms might have an impact on sensitization rates for specific allergens such as thiomersal or chromate, as previously reported.^{19,20} Such specific allergen associations would have been missed in our study due to low prevalence rates. Similarly, the penetration efficiency of single allergens may vary with CLDN1 SNP genotype status, depending on which route, inter- or intracellular, is the primary. Nevertheless, general population studies are typically unbiased, offering reliable estimates. Another limitation of the study includes the fact that patch-test readings were performed on day 2 only,

which may introduce both false-negative and false-positive readings, depending on the allergen in question.^{32–34}

In summary, the CLDN1 SNP polymorphisms rs9290927, rs893051 and rs17501010 may be associated, respectively, with nickel contact sensitization in individuals without ear piercings, contact sensitization to fragrances, and with both organic compounds and nickel contact dermatitis. We could not find associations between GST gene polymorphisms and contact sensitization in a general population for GSTT1, GSTM1 and GSTP1 variants in combination or alone.

What's already known about this topic?

- Polymorphisms in the gene encoding the tight-junction protein claudin (CLDN)-1 were recently associated with atopic dermatitis.
- Polymorphisms in the glutathione S-transferase (GST) genes have been associated with contact sensitization, but their role remains unclear.

What does this study add?

- CLDN1 polymorphisms may affect the propensity to develop contact sensitization to common haptens.
- Polymorphisms in the GST genes do not seem to be of importance in contact sensitization when assessed in a general population.

References

- 1 Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population – prevalence and main findings. *Contact Dermatitis* 2007; **57**:287–99.
- 2 Rees JL, Friedmann PS, Matthews JN. The influence of area of application on sensitization by dinitrochlorobenzene. *Br J Dermatol* 1990; **122**:29–31.
- 3 Fischer LA, Menné T, Johansen JD. Dose per unit area – a study of elicitation of nickel allergy. *Contact Dermatitis* 2007; **56**:255–61.
- 4 Fischer LA, Voelund A, Andersen KE *et al.* The dose–response relationship between the patch test and ROAT and the potential use for regulatory purposes. *Contact Dermatitis* 2009; **61**:201–8.
- 5 Johansen JD, Frosch PJ, Menné T. Allergic contact dermatitis in humans: experimental and quantitative aspects. In: *Contact Dermatitis* (Johansen JD, Frosch PJ, Lepoittevin JP, eds), 5th edn. Berlin: Springer-Verlag, 2011; 241–9.
- 6 Schnuch A, Westphal G, Mössner R *et al.* Genetic factors in contact allergy. *Contact Dermatitis* 2011; **64**:2–23.
- 7 Ross-Hansen K, Menné T, Johansen JD *et al.* Nickel reactivity and filaggrin null mutations – evaluation of the filaggrin bypass theory in a general population. *Contact Dermatitis* 2011; **64**:24–31.
- 8 Thyssen JP, Johansen JD, Linneberg A *et al.* The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. *Br J Dermatol* 2010; **162**:1278–85.
- 9 Novak N, Baurecht H, Schäfer T *et al.* Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008; **128**:1430–5.

- 10 Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *J Clin Invest* 2012; **122**:440–7.
- 11 Gruber R, Elias PM, Crumrine D *et al.* Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *Am J Pathol* 2011; **178**:2252–63.
- 12 Irvine AD, McLean WHI, Leung DYM. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011; **365**:1315–27.
- 13 Kezic S, Kammeyer A, Calkoen F *et al.* Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. *Br J Dermatol* 2009; **161**:1098–104.
- 14 Brandner JM, Haftek M, Niessen CM. Adherens junctions, desmosomes and tight junctions in epidermal barrier function. *Open Dermatol J* 2010; **4**:14–20.
- 15 de Benedetto A, Rafaels NM, McGirt LY *et al.* Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011; **127**:773–86.
- 16 Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005; **45**:51–88.
- 17 Bolt HM, Thier R. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. *Curr Drug Metab* 2006; **7**:613–28.
- 18 Sundberg K, Johansson A-S, Stenberg G *et al.* Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis* 1998; **19**:433–6.
- 19 Wang B-J, Shiao J-S, Chen C-J *et al.* Tumour necrotizing factor- α promoter and GST-T1 genotype predict skin allergy to chromate in cement workers in Taiwan. *Contact Dermatitis* 2007; **57**:309–15.
- 20 Westphal GA, Schnuch A, Schulz TG *et al.* Homozygous gene deletions of the glutathione S-transferases M1 and T1 are associated with thimerosal sensitization. *Int Arch Occup Environ Health* 2000; **73**:384–8.
- 21 Pot LM, Alizadeh BZ, Ahrenberg D *et al.* No major role for glutathione S-transferase gene polymorphisms in sensitization to para-phenylenediamine and other xenobiotics: a study of association and meta-analysis. *Br J Dermatol* 2011; **164**:890–2.
- 22 Thyssen JP, Linneberg A, Menné T *et al.* The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol* 2009; **161**:95–101.
- 23 Thyssen JP, Linneberg A, Menné T *et al.* Contact allergy to allergens of the TRUE-test (panels 1 and 2) has decreased modestly in the general population. *Br J Dermatol* 2009; **161**:1124–9.
- 24 Brasch-Andersen C, Christiansen L, Tan Q *et al.* Possible gene dosage effect of glutathione-S-transferases on atopic asthma: using real-time PCR for quantification of GSTM1 and GSTT1 gene copy numbers. *Hum Mutat* 2004; **24**:208–14.
- 25 Hersoug L-G, Brasch-Andersen C, Husemoen LLN *et al.* The relationship of glutathione-S-transferase copy number variation and indoor air pollution to symptoms and markers of respiratory disease. *Clin Respir J* 2012; **6**:175–85.
- 26 Meldgaard M, Szecsi PB, Carlsen BC *et al.* A novel multiplex analysis of filaggrin polymorphisms: a universally applicable method for genotyping. *Clin Chim Acta* 2012; **413**:1488–92.
- 27 Williams HC, Burney PGJ, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994; **131**:406–16.
- 28 Thyssen JP, Carlsen BC, Menné T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008; **19**:303–7.
- 29 Rodriguez S, Gaunt TR, Day INM. Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; **169**:505–14.
- 30 Hadj-Rabia S, Baala L, Vabres P *et al.* Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. *Gastroenterology* 2004; **127**:1386–90.
- 31 Prova C. Glutathione transferases in the genomics era: new insights and perspectives. *Biomol Eng* 2006; **23**:149–69.
- 32 Thyssen JP, Jensen CS, Johansen JD, Menné T. Results from additional nickel patch test readings in a sample of schoolgirls from the general population. *Contact Dermatitis* 2008; **59**:317–8.
- 33 Jonker MJ, Bruynzeel DP. The outcome of an additional patch-test reading on days 6 or 7. *Contact Dermatitis* 2000; **42**:330–5.
- 34 Uter WJC, Geier J, Schnuch A. Good clinical practice in patch testing: readings beyond day 2 are necessary: a confirmatory analysis. Members of the Information Network of Departments of Dermatology. *Am J Contact Dermat* 1996; **7**:231–7.