

Genetic factors in contact allergy – review and future goals

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Summary

The genetics of contact allergy are still only partly understood, despite decades of research; this might be a consequence of inadequately defined phenotypes used in the past. A recommendation is to study an extreme phenotype, namely, polysensitization (sensitization to three or more unrelated allergens). Another approach to unravel the genetics of contact allergy is the study of candidate genes. In this review, we summarize studies on the associations between genetic variation (e.g. single-nucleotide polymorphisms) in certain candidate genes and contact allergy. Polymorphisms and mutations affecting the following proteins were studied: (i) filaggrin; (ii) *N*-acetyltransferase (NAT) 1 and 2; (iii) glutathione-*S*-transferase (GST) M and T; (iv) manganese superoxide dismutase; (v) angiotensin-converting enzyme (ACE); (vi) tumour necrosis factor (TNF); and (vii) interleukin-16 (IL-16). The polymorphisms of NAT1, NAT2, GSTM, GSTT, ACE, TNF and IL-16 were shown to be associated with an increased risk of contact allergy. In one of our studies, the increased risk conferred by the TNF and IL-16 polymorphisms was confined to polysensitized individuals. Other relevant candidate genes may be identified by studying diseases related to contact allergy in terms of clinical symptoms, a more general pathology (inflammation), and possibly an overlapping genetic background, such as irritant contact dermatitis.

Key words: allergic contact dermatitis; genetics; polymorphisms; delayed-type hypersensitivity.

Contact allergy, comprising allergic contact dermatitis as the clinical disease and underlying delayed-type sensitization as the latent condition, is triggered by chemicals of usually low molecular weight (contact allergens). Although some contact allergens are ubiquitous, only a minority of exposed individuals develop contact allergy. Several reasons may account for this observation: (i) exposure to allergens may differ (in terms of number, dose, and potency); and (ii) there may be different individual susceptibilities (1, 2). However, it would be

premature to attribute susceptibility to genetic traits only. Increased susceptibility to sensitization may be acquired, as in patients with leg dermatitis (1), and susceptibility to elicitation may be increased by a high induction dose of the allergen (3) or by co-factors such as irritation of the skin (1). Moreover, body homeostasis may temporarily be influenced by endocrinological or pharmacological factors causing increased or decreased susceptibility to contact allergy (Table 1). When studying the genetics of contact allergy, it is advisable, albeit difficult, to exclude these competing, possibly confounding, factors with an impact on susceptibility.

There are several approaches to investigate the genetic background of a disease: study of the inheritance of a disease in families, mapping of disease genes to specific locations on chromosomes, or analyses of the molecular

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Table 1. Inherent (acquired or naturally present) states probably influencing susceptibility to contact allergy

High induction dose of the allergen (3)
Irritant contact dermatitis (4)
'Status eczematicus' (1)
Leg dermatitis (5)
UV radiation (6)
Drugs (7, 8)
Climatic conditions (9)
Psychological stress (10)
Sex (11)
Age (5, 11)
Ethnicity (12)

mechanisms by which genes cause disease (13). Studies on the genetics of contact allergy fall roughly into three categories. The early studies comprised family studies and studies of immunogenetic markers in humans and animals. More recent studies have followed the 'candidate gene approach'. Genes are considered to be 'candidate genes' if their characteristics (e.g. a protein product) suggest that they may be responsible for a genetic disease, or at least partly involved in the development of a complex disease. Up to now, a vast number of different pathogenetic factors in contact allergy have been identified (14, 15), along with the genes controlling their expression. Therefore, they can be studied as 'candidate genes' in appropriately selected samples of patients with contact allergy. This approach is thus hypothesis-driven research, in contrast to genome-wide association studies, with an *a priori* 'agnostic' approach regarding the genes involved.

In this article, we review the existing evidence regarding the influence of genetic factors on contact allergy, at the same time challenging the traditional phenotype concept. In particular, we summarize studies of our group and others on associations between genetic variation [e.g. single-nucleotide polymorphisms (SNPs)] in certain candidate genes and contact allergy. Finally, we discuss and propose different approaches for future research.

Traditional Studies in the Genetics of Contact Allergy

In the past, different approaches were taken to study the question of 'contact allergy inheritance', in both humans and animals (Table 2). Studies up to 1985 were comprehensively reviewed by Menné and Holm (16), and recent studies on nickel allergy by Shram & Warshaw (17).

Experimental sensitization

Sulzberger and Rostenberg had noticed inter-individual differences in susceptibility to experimental sensitization

to p-nitroso-dimethylaniline (NDMA) and 2,4-dinitrochlorobenzene (DNCB) (18). Landsteiner et al. re-analysed these data, and concluded that the susceptibility to sensitization might depend on the nature of the allergen (2). Nevertheless, it was shown that individuals sensitized to one allergen are more easily sensitized to other allergens. However, the mechanism underlying differences in susceptibility remained unclear.

Family studies

The role of genetic factors became clearer through the study performed by Walker et al. (21). The authors studied experimental sensitization with NDMA and DNCB in 99 families with a total of 301 individuals. Interestingly, children were sensitized to the strong allergen DNCB independently of the success of sensitization in their parents, whereas in case of the weaker allergen NDMA, children were sensitized more often if their parents were also sensitized (Table 3). If both parents were sensitized to DNCB, 90.9% of the children were sensitized to DNCB, as compared with 52.9% when only one parent was sensitized. The authors concluded that a very potent allergen can be considered to 'overpower' genetic influences (21). In contrast, genetic factors seem to play a more prominent role in sensitization to weaker allergens (such as NDMA and nickel) (21, 24). This study was considered to be 'the most convincing human study' (26). Other, non-experimental, family studies (22, 23) were considered to be hampered by several limitations, such as differences in lifetime exposure for parents and offspring, and changing living conditions leading to a change of exposure patterns over time (16).

Twin studies

Clustering of diseases in families may result from either shared genetic influences or a shared family environment. With regard to nickel contact allergy, the results of twin studies are controversial (Table 2) (24–26). Menné and Holm demonstrated that nickel allergy could be influenced by genetic factors, as nickel sensitization was more common in monozygotic than in dizygotic twins (24), whereas Bryld and colleagues showed that nickel sensitization was mainly explained by environmental factors (Table 2) (26). These contradictory results may be explained by different intensities of exposure: in the Menné and Holms study, the main exposure came from suspenders, and in the study of Bryld et al., the main source of exposure may have been ear piercing (although not documented). Thus, the above notion that more potent allergens may overrule genetic influences could be extended to the impact of more intense exposure. The limitations of the study of Bryld et al. are:

Table 2. Summary of studies in the genetics of contact allergy

Authors	Study	Results	References
I. Experimental sensitization			
Sulzberger & Rostenberg (1939)	Sensitization to NDMA and DNCB in humans.	Inter-individual differences in susceptibility to sensitization; impact of pre-existing eczema.	(18)
Landsteiner et al. (1939)	Re-analysis of the above study.	Susceptibility to sensitization is chemical-specific. Individuals sensitized to one allergen are more easily sensitized to others.	(2)
Chase (1941)	Sensitization of guinea pigs with DNCB and poison ivy; identification of high and low responders. Controlled breeding of the two colonies.	The offspring of the high reactors also reacted intensely; the other group reacted poorly, although induction was even higher. Sensitivities (to organic compounds) not substance-specific.	(19)
Polak et al. (1968)	Sensitization of different inbred strains of guinea pigs with metal compounds.	One strain could be sensitized to potassium dichromate but not to mercury chloride; reverse sensitivity of the other strain. Sensitivities (to metal compounds) substance-specific.	(20)
II. Family studies			
Walker et al. (1967)	Experimental sensitization of parents and their children of 99 families with DNCB and NDMA.	Sensitization of children more frequent if parents were sensitized (only NDMA, not DNCB).	(21)
Forsbeck et al. (1971)	Relatives ($n = 404$) of patients with allergic contact dermatitis ($n = 94$) were patch tested with 23 standard allergens.	Positive reactions in female relatives more frequent than in controls (30%/18%).	(22)
Fleming et al. (1999)	Relatives ($n = 209$) of patients with nickel-allergic contact dermatitis ($n = 39$) were questioned about intolerance to nickel.	The risk ratio for first-degree relatives of nickel-positive patients was 2.83 (95% CI 2.45–3.27). Remark: confounders were not controlled for!	(23)
III. Twin studies			
Menné & Holm (1983)	Based on a questionnaire on a possible nickel allergy mailed to 1546 female twins from the Danish Twin register; 115 pairs were investigated (patch test in 75).	Difference in concordance rate for nickel allergy between monozygotic and dizygotic pairs. The heritability for nickel allergy was ~60%. For a medium-potency sensitizer (nickel), genetic factors may play a role (see Table 3).	(24)
Forsbeck et al. (1968)	101 twin pairs from the Swedish twin register: (a) patch tested with 23 standard allergens; (b) Sensitization with DNCB (see comment in Table 3).	(a) Insignificant concordance among the monozygotic pairs (b) No difference in sensitization between monozygotic and dizygotic pairs Conclusion: no evidence for genetic background.	(25)
Bryld et al. (2004)	A sample of female twins with hand eczema from the Danish twin register were patch tested with nickel ($n = 630$).	Patch test positive for nickel: $n = 146$. Only a small tendency for larger OR in monozygotic twins (OR 1.28; 95% CI 0.33–5.00). Nickel allergy mainly caused by environmental factors. Caveat: selection criterion!	(26)
IV. Studies of immunogenetic markers			
<i>Studies in animals</i>			
Ishii et al. (1990–1998)	Genetic control of metal sensitization in mice.	Various polymorphisms in the I-A region.	(27–30)
Asherson et al. (1990)	Sensitization and IFN- γ release in CBA (H-2k) and BALB/c (H-2d) mice.	The H-2d haplotype determines contact sensitivity and poor IFN- γ response to several antigens.	(31)
Okuda et al. (1980)	Genetic control of DNFB sensitization in mice.	Controlled by I-A region of H-2, and non-H-2 loci.	(32)
<i>Studies in humans (significant findings only)</i>		For further results, see references:	(16, 17, 33)
Walton et al. (1986)	Associations of nickel allergy with MHC loci.	HLA-B35.	(34)
Önder et al. (1995)	Associations of nickel allergy with MHC loci.	DQA1*061; DR15 decreased.	(35)
Silvennoinen-Kassinen et al. (1997)	Association with TAP genes, encoding the ABC (ATP-binding cassette) transporter associated with antigen processing (TAP).	RR for the alleles TAP2B increased, and RR for TAP2C decreased (both significantly).	(36)

CI, confidence interval; DNCB, 2,4-dinitrochlorobenzene; HLA, human leukocyte antigen; IFN- γ , interferon- γ ; MHC, major histocompatibility complex; OR, odds ratio; RR, relative risk; NDMA, p-nitroso-dimethylaniline.

Table 3. Difference in sensitization rates between children of sensitized and non-sensitized parents. 'The potent allergen DNCB is probably overpowering genetic influences'; Walker et al. (21)

Status of parents	Percentage of children sensitized	
	DNCB	NDMA
Sensitized	65	51
Not sensitized	52	29
	$p < 0.10$	$p < 0.01$

DNCB, 2,4-dinitrochlorobenzene; NDMA, p-nitroso-dimethylaniline.

(i) the use of a fairly heterogeneous phenotype, 'hand eczema', as a selection criterion; and (ii) a relatively small sample size per age group (37). Furthermore, lifestyle (living conditions) and, in particular, allergen exposure might be more similar in monozygotic than in dizygotic twins, leading to a systematic error in twin studies (38).

Studies of immunogenetic markers

Early studies used the human leukocyte antigen (HLA) encoded by the major histocompatibility complex (MHC) class I (HLA-A/HLA-B/HLA-C) and MHC class II (HLA-DP/HLA-DQ/HLA-DR) genes as serological immunogenetic markers. Up to now, >1200 class I and >336 class II alleles have been identified (39). MHC variations (HLA phenotype and/or MHC genotype) were found to be associated with disease susceptibility, mainly in autoimmune and infectious diseases (40). In contact allergy, the MHC (protein complex) on antigen-presenting cells (such as Langerhans cells) is thought to have a pivotal role in the induction process, insofar as the hapten is presented by a particular MHC molecule to naïve T-cells (14). In line with this model is the finding that the genetic control of delayed-type hypersensitivity reactions in mice (relying on the I-A subregion of the murine MHC) is antigen-specific. This was shown for nickel (27), mercury (28), chromium (29) gold (30), and organic haptens (32, 41).

Numerous studies on MHC class I and MHC class II have been performed in humans; most often with contact sensitization to nickel (16, 17, 33) and less often with sensitization to organic compounds (42–45). Most of the associations of MHC loci with contact allergy were not significant, with the exception of *HLA-B35* (34) and *DQA1*061* (35), which were increased, and *DR15*, which was decreased in nickel-allergic patients (35). In addition, polymorphisms of genes encoding the TAP transporter proteins located in the MHC class II region and of genes encoding complement factor B (a component of the C3 convertase enzyme activating the alternative pathway), particularly the subtype BF*FB, located in the MHC

class III region, were found to be associated with nickel allergy (36, 46).

Despite a large number of immunogenetic studies in contact allergy, the mechanistic rationale for studying the MHC in contact allergy, and the focus on mainly one single allergen (nickel), the lack of associations between contact allergy and the MHC complex (or specific HLA molecules) is striking. This may be explained by:

- the remarkably high heterogeneity of the MHC complex in humans (39),
- the non-specificity of nickel in terms of binding to nearly every HLA molecule, and even acting independently from HLA (47, 48).

Summary

Contact allergy is apparently a complex disease, most likely caused by a combination of genetic effects and environmental influences. Several studies performed in humans and in animals suggest that genetic factors may play a role in contact allergy (Table 2). Beyond this general statement, however, no specific insights into the genetics of contact allergy have been gained.

In Search of the Appropriate Phenotype in Contact Allergy: Polysensitization

For many reasons, the genes involved in contact allergy have not yet been found. One of these may be the variety of phenotypes chosen. Most often, only sensitization to nickel or other metals was considered, on the implicit assumption that nickel allergy could be considered to be valid paradigm for contact allergy in general. This may be doubted (26); for instance, nickel may be recognized by T-cells directly (independently of MHC and peptide) (47, 48). Moreover, in contrast to many other (organic) allergens, which are often associated with other sensitizations, in allergen-association studies nickel remained mainly a solitary sensitizer (49), being associated only, if at all, with cobalt (50). In addition to the potentially confounding factors associated with altered susceptibility (Table 1), further factors have an impact on the manifestation of contact allergy (the observed phenotype): different doses, different potencies of the allergen and different susceptibilities of the individual all interact in causing different grades of sensitization [review: (1)]. On the other hand, as every human is equipped with the immunological tools to mount a delayed-type hypersensitivity reaction, such a reaction cannot reasonably be understood as a 'disease' or phenotype.

As such a complex situation is typical in the genetics of complex diseases, some researchers have advocated the

study of extreme phenotypes, where genetic influences are probably more pronounced and can be identified more easily (51). Therefore, we recommended focusing on patients with 'increased susceptibility to contact allergy', who could serve as the appropriate phenotype to be studied. However, how can 'increased susceptibility' be defined (1)?

The seminal work of a group from the UK 20 years ago contributed much to the concept of susceptibility (52). In a set of intriguing experiments using DNCB sensitization, they found, albeit in a rather small study group, that patients with pre-existing sensitization to three or more unrelated contact allergens were more easily (i.e. with lower doses) sensitized and exhibited stronger reactions upon re-challenge. Subsequently, a study was performed to determine whether there was an association between multiple sensitization and HLA molecules (43); however, no statistically significant association was found.

More recently, however, the concept of polysensitization has taken a new turn. In a number of analyses of the Information Network of Departments of Dermatology database on more than 100 000 patients, we found the following [review: (1)]:

- (1) The risk of being sensitized to an index allergen gradually increased with the number of additionally diagnosed allergens from the baseline series. This was shown for a group of quite heterogeneous substances, comprising neomycin, fragrance mix I, p-phenylenediamine (PPD), bufexamac, nickel, cobalt, and chromate.
- (2) The risk of having stronger (+++/+++ reactions in patch testing to an index allergen (e.g. fragrance mix) increased with the number of additional positive reactions to unrelated allergens.
- (3) The risk of being sensitized to a weak allergen such as paraben mix, as compared with sensitization to the stronger allergen methyl dibromoglutaronitrile, increased with the number of co-sensitizations.

These experimental and clinical observations in polysensitized patients, namely a higher risk of induction, a stronger reaction in elicitation, and additional sensitization to weak allergens, can be summarized under the concept of 'increased response'. However, 'increased response' in general may also be caused by other factors (Table 1). Nevertheless, it is plausible to consider an increased response as a sign of increased susceptibility. Hypothetically, the distribution of graded susceptibility as expressed by an increasing number of contact allergies (Fig. 1) could follow a distribution of quantitative traits that is often found in multifactorial disorders (13; p. 248).

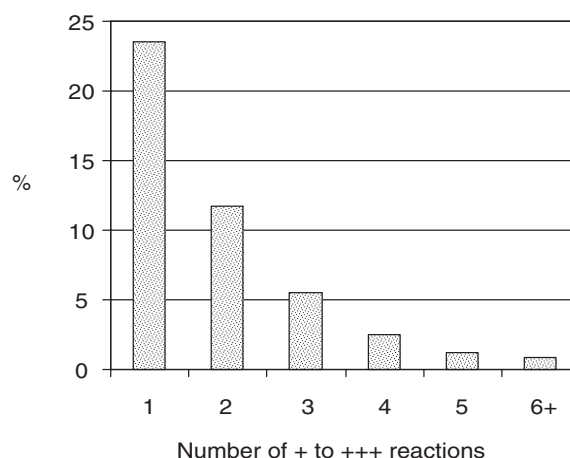


Fig. 1. Distribution of the number of positive reactions (+, ++ or +++) to allergens of the baseline series. 6+, six or more than six reactions. Total number of patients 58 268. Number of patients with no positive reaction to standard series allergens 31 865 (54.7%) (1).

One concern regarding the whole concept of polysensitization as a phenotype of increased susceptibility, comprehensively reviewed in (1), may be briefly discussed as follows: 'poly-exposure', and/or the interference of other factors (Table 1), may sometimes be a sufficient cause of polysensitization. However, while the influence of exogenous factors on sensitization is undisputed (Table 1), the influence of endogenous (inherent) factors should also be considered. The concept of polysensitization is aimed at elucidating the phenomenon of susceptibility. The following suggestions may be advanced:

- In early studies, individuals with a pre-existing sensitization were more easily sensitized to DNCB (18).
- Moss et al. considered 'poly-exposure' as a cause of polysensitization. However, polysensitized workers exposed to a multitude of occupational allergens were not shown to be more susceptible (52). Overall, polysensitization can possibly not be regarded as an unequivocal sign of increased susceptibility, but rather as a very probable one.
- Our finding that (i) stronger patch test reactions and (ii) sensitization to weak allergens were associated with polysensitization cannot be explained by poly-exposure.
- If poly-exposure was the only determinant of polysensitization, no genetic variation between monosensitized and polysensitized individuals would be expected. So far, it is our hypothesis that polysensitization is a phenotype that may have a genetic basis (see sections on TNF and

interleukin-16). Only future studies will be able to confirm the concept and the appropriateness of the research strategy, or otherwise.

- One indicator of cumulative exposure resulting in polysensitization is older age. Indeed, age was found to be a risk factor for polysensitization (53).
- Leg dermatitis was identified as an important confounding factor accompanying polysensitization (1). Therefore, our analyses were adjusted for these (and other) factors in logistic regression analyses (1).
- The prevalence of polysensitization remained stable over a 20-year period (54), despite probable changes in exposure patterns during this time and despite generally decreasing sensitization rates in the background population (55), indicating that polysensitization is a reliable characteristic.

Summary

Polysensitized individuals display different characteristics of increased susceptibility to contact allergy, and may represent a subgroup of patients in whom genetic risk factors are more prominent. Therefore, they are particularly suited for studies on the genetics of contact allergy.

Polymorphisms in Contact Allergy

The steadily increasing knowledge on the immune mechanism of contact allergy (14, 15) enabled the study of genetic variation in those parts of the genome whose products (e.g. enzymes or cytokines) were identified as important factors for the development of contact allergy (Table 4). The search for candidate genes in carefully selected populations characterized by a homogeneous phenotype is driven by hypotheses essentially based on the biochemical, physiological and immunological biology of the disease (56, 57). The concept behind this implies that many factors and several genes are probably involved, in accordance with the notion of contact allergy being a polygenic disease.

Following the scheme of immunological steps in contact allergy (Table 4), our group (58–62) and others (63–75) set about studying a number of potentially relevant polymorphisms in contact allergic patients. A polymorphism is a genetic variation located in specific DNA sequences found in >1% of the population. For instance, in the case of the well-known tumour necrosis factor (*TNF*)–308 G/A polymorphism, the 'G' (guanosine) at position –308 of the DNA sequence (minus indicating the promoter region) normally present in the *TNF* gene is replaced by 'A' (adenosine). This subtle change may have dramatic effects on the *TNF* protein.

Table 4. Functionally relevant steps in the pathogenesis of contact allergy possibly under the control of polymorphisms studied so far.

Function	Product of a polymorphic gene involved
<i>Factors influencing the availability of the allergen</i>	
Barrier structure and function	Filaggrin
Metabolism of the allergen (toxicification/detoxification)	N-Acetyltransferase I and II, Glutathione-S-transferase
<i>Factors interfering with inflammatory processes</i>	
Reactive oxygen species	Manganese superoxide dismutase
Metabolism of inflammatory (neuro)peptides	Angiotensin-converting enzyme
<i>Immunological factors (with impact on):</i>	
Antigen processing	TNF, IL-1 β , IL-1RA, IL-4, IL-6, IL-16
Migration and maturation of APCs	
Presentation of the allergen (MHC)	
Chemotaxis of lymphocytes	
T-cell subpopulations (activating or regulating)	

APC, antigen-presenting cell; IL-1 β , interleukin-1 β ; IL-1RA, interleukin-1 receptor antagonist; IL-4, interleukin-4; IL-6, interleukin-6; IL-16, interleukin-16; MHC, major histocompatibility complex; TNF, tumour necrosis factor.

The activity of a product is often not confined to one single function (step). *TNF*, for instance, influences barrier structure and function as well.

The results of the various studies are summarized in Table 5, and the relevant results are briefly discussed in the following sections.

Filaggrin

Filaggrin (filament-aggregating protein) is a key component of the stratum corneum [review: (78, 79)]. Multiple filaggrin peptides are cleaved from profilaggrin, which is encoded by the *FLG* gene on chromosome 1q21. Filaggrin aggregates keratin filaments, is a major component of the cornified envelope, and contributes – after degradation – to the pool of amino acids, metabolites and various ions known as 'natural moisturizing factor' (80). More than 20 loss-of-function mutations within the *FLG* gene have been reported (78). These *FLG* mutations are nonsense or frame shift mutations, each resulting in truncation of the profilaggrin molecule. Association of the *FLG* null genotype (in particular R501X and 2282del4) with atopic dermatitis and other allergic diseases has now been replicated in numerous studies (81).

Filaggrin deficiency may lead to skin barrier defects, as shown in atopic dermatitis patients with and without *FLG* mutations (80, 82). An increase in susceptibility to chronic irritant contact dermatitis was recently shown

Table 5. Studies on probably functionally relevant polymorphisms in contact allergic patients from our group* (columns I–IV, rows 2–4, 7, and 8), from more recent studies (columns I–IV, rows 1, 5, 6, and 9), and replication studies (column V).

I	II	III	IV	V
	Polymorphisms	Results	Ref.	Replicated
1	Filaggrin null mutations (combined genotypes for R501X and 2282del4)	Results inconclusive: (a) not associated with allergic contact dermatitis [†] (b) when (a) as compared with other controls: risk increased (c) not associated with allergic contact dermatitis [†] (d) associated with relevant sensitization to nickel only (e) only association in women with nickel dermatitis and without ear piercing	(72) [†] (73) [†] (74) [†] (75) (76)	
2	Genotype and phenotype of NAT2	Genotype and phenotype of 'rapid acetylators' increased	(58)	(65, 66)
3	NAT1 and NAT2 in patients allergic to 'para-compounds'	NAT2*4 allele (rapid acetylators) increased; NAT2*5b/2*6a (slow acetylators) decreased. Genetic linkage of NAT1*10 with NAT2*4	(59)	(65, 66) n.r. (67)
4	GSTM1 and GSTT1	Combined deletion (GSTT1/GSTM1) in patients allergic to organic mercury compounds as compared with controls and para group allergics	(60)	(68) [‡]
5	MnSOD	Valine (Val) to alanine (Ala) at amino acid – 9 (Val9→Ala) polymorphism. No difference between allergics [§] and controls	(71) [§]	
6	ACE (insertion/deletion polymorphism)	Insertion (I) or deletion (D) of 287 base pairs in intron 16/1 polymorphism (low ACE activity) increased	(70) [§]	
7	Cytokines: ILB – 511, ILB + 3953, ILRA, IL6 – 174, TNFA – 238, TNFA – 308	TNFA – 308 (G→A): increased (in polysensitized individuals) TNFA – 308 G/G and ILRA polymorphism (77) increased in Turkish patients (n = 50)	(61)	(68) [‡] (69) [§]
8	Cytokine: IL-16	IL16-295 (T→C) increased (in polysensitized individuals)	(62)	
9	Cytokine IL-4	No difference between chromate allergics and controls with regard to IL4-590 polymorphism	(68) [‡]	

ACE, angiotensin-converting enzyme; GST, glutathione-S-transferase; IL, interleukin; ILB, interleukin 1-beta; ILRA, interleukin 1-beta receptor antagonist; MnSOD, manganese superoxide dismutase; NAT, N-acetyltransferase; TNF, tumour necrosis factor.

* (a) University of Göttingen (Department of Occupational Medicine, Department of Dermatology) and (b) Department of Dermatology joining the Information Network of Departments of Dermatology.

[†]Sensitization not specified.

[‡]Allergic to chromate.

[§]Allergic to p-phenylenediamine (PPD).

n.r.: not replicated.

to be associated with *FLG* null mutations [odds ratio (OR) 1.91; 95% confidence interval (CI) 1.02–3.59] (74). This may also be relevant for contact allergy, because a compromised skin barrier will promote an inflammatory milieu and facilitate permeation of allergens, favouring an allergic response (83). More specifically, it was hypothesized that individuals with filaggrin deficiency may have a higher level of nickel absorption, because of the lack of nickel-chelating histidine-rich polypeptides of which filaggrin is composed (84).

However, the studies conducted so far have yielded conflicting results, and no clear association between contact allergy risk and *FLG* mutations has been

established (72, 74, 75). In our opinion, this may be attributable to a partly (74) or substantially (75) compromised selection of cases, or to an inadequate choice of controls (73).

In particular, in a larger population-based study on *FLG* mutations in Germany (KORA C) (75), data on contact sensitization were taken from a previous epidemiological study (*n* = 1141) (85). However, the frequency of sensitization to at least one allergen (40.0% versus 15%), to the fragrance mix (15.9% versus 1.6%) or to nickel (13.1% versus 5.9%) dramatically exceeded the frequencies found in other population-based studies (55, 86, 87), and, more surprisingly, even the figures reported

from clinical studies (54, 88). One reason for these highly divergent results could be selection bias (88). However, a certain proportion of 'false positives' among the nickel and fragrance mix positive 'cases' may have blurred possible associations. Indeed, neither sensitization to specific allergens (e.g. nickel or the fragrance mix) nor overall sensitization was significantly associated with the *FLG* mutations (75). However, when the diagnosis of nickel patch test positivity was strengthened by a positive history ('intolerance of fashion jewellery'), a significant association between contact allergy (to nickel) and filaggrin mutations was observed (OR 4.04; 95% CI 1.35–12.06) (75).

Finally, a recently published study on the *FLG* mutations in a random sample of 3335 adults from the general population in Denmark again found no associations with sensitization to nickel, sensitization to the fragrance mix, or overall sensitization (76). In contrast, an analysis in women who did not have ear piercings revealed a positive association between *FLG* null mutations and nickel sensitization (OR 3.7; 95% CI 0.73–18.96) and between *FLG* null mutations and nickel dermatitis (OR 6.75; 95% CI 1.17–38.91) (76).

In our view, these interesting observations support the notion that more intense allergen exposure, such as via piercing, overrules genetic influences (see above; chapter I 'Family studies' and 'Twin studies'). Alternatively, piercing may 'bypass' the nickel-chelating filaggrin protein (76), thus sensitizing individuals regardless of their *FLG* status.

N-acetyltransferase (NAT) 1 and 2

Acetylation is a major route of biotransformation for several therapeutic arylamine and hydrazine drugs (review: (89)). It plays an important role in the bioactivation as well as bioinactivation of numerous potential carcinogens. *N*-acetylation is generally regarded as a detoxifying reaction, whereas *N*-*O*-acetylation (the acetylation of the corresponding hydroxylamine) leads to highly reactive, toxic intermediates (review: (89, 90)). In humans, these acetylation reactions are catalysed by two closely related cytosolic enzymes, NAT1 (EC 2.3.1.5) and NAT2 (EC 2.3.1.5). Isoenzymes of NAT1 and NAT2 exhibit different enzyme activities, resulting in 'rapid' or 'slow' acetylation. In dermatology, the xenobiotic most studied with regard to *N*-acetylation is the contact allergen PPD. Consumers are exposed to PPD, for instance, through hair dyeing, together with couplers (e.g. 3-aminophenol) and immediately formed derivatives (91). PPD is metabolized in the skin to its mono-acetylated and di-acetylated derivatives by both NAT1 and NAT2,

and to oxidation products such as Bandrowski's base (an end-product of oxidation) (67, 92–95). However, it can be assumed that PPD is acetylated only partly, and its sensitizing properties are thus neutralized only partly (96, 97), the remainder still acting as a sensitizer (91, 98, 99).

The human *NAT1* and *NAT2* genes are located in close proximity on chromosome 8p22, and share 87% nucleotide sequence identity within their protein coding regions. At present, 26 alleles of *NAT1* and 59 of *NAT2* have been identified (<http://louisville.edu/medschool/pharmacology/NAT.html>; last accessed 10 February 2010). Both genes are polymorphic with regard to the 'slow' and 'rapid' acetylator phenotype. Epidemiological studies have provided some clues concerning the importance of variations in both *NAT1* and *NAT2* in altering the risk for a variety of disorders, most notably cancers (90, 100), but also non-malignant diseases (101) and, in particular, atopic diseases (102–105).

Several studies of NAT polymorphisms in contact allergy have been performed:

- (1) Early studies investigated the NAT phenotype in contact allergy. A 'slow acetylator' NAT1 phenotype was reported to be associated with contact allergy (63, 64, 106). As the studies (i) included patients from a patch test population as controls, (ii) used metabolism of a NAT2 substrate (caffeine), and (iii) were very small, no convincing conclusion can be drawn from the results with regard to the NAT1 phenotype in contact allergy patients (63, 64).
- (2) The first molecular-epidemiological study in contact allergy determined the NAT2 phenotype and genotype (58). Patients allergic to para-substituted aryl compounds such as PPD (other sensitization not excluded) ($n = 55$) and healthy controls ($n = 85$) were compared with regard to their capacity to metabolize caffeine (phenotype) and with regard to the NAT2 genotype. According to their phenotype and genotype, 48% and 51%, respectively, of contact allergic patients were classified as rapid acetylators, as compared with 24% and 31%, respectively, in the control group.
- (3) In a second study on NAT in a similarly characterized, but extended, group of patients ($n = 88$) and healthy controls ($n = 123$), we investigated polymorphisms in *NAT1* and *NAT2* (in total, 18 *NAT2* and 8 *NAT1*) (59). NAT2 rapid acetylators (carriers of at least one *NAT2*4* or *NAT2*12A* allele) were more common in the disease group (45%) than in the control group (30%) ($P = 0.029$). Furthermore, the slow acetylator phenotype associated with *NAT2*5b/2*6a* was significantly less

frequent in the disease group (14.8% versus 30.9%; OR 0.39; 95% CI 0.19–0.78). The high catalytic activity associated with NAT2*4 and the comparatively low activity associated with NAT2*5B were recently shown in cells transfected with either NAT2*4 or NAT2*5B (107). The haplotype NAT2*4/NAT1*10 (rapid acetylators) was increased in patients (27% versus 15%; OR 2.1; 95% CI 1.04–4.04). However, in this study, linkage disequilibrium between NAT2*4 and NAT1*10 was observed. This is a situation similar to confounding in epidemiology, with the consequence that, for example, in the subgroup of NAT1*10-negative individuals, the frequency of NAT2*4 carriers was similar among patients and controls. Hence, it would be misleading to attribute the increased risk exclusively either to the NAT1 or the NAT2 locus. Alternatively, both alleles may be linked to an unknown susceptibility factor.

- (4) These findings, namely an increased risk of contact allergy for the 'rapid' NAT2 polymorphism, were corroborated in studies from Turkey (66) and Iraq (65), although the results of the latter study are compromised by a small sample size and an unconventional, non-standardized patch test technique.
- (5) Finally, a recently published study on NAT1 and NAT2 genotypes in 147 PPD-sensitized individuals and 200 controls with no known history of sensitization to PPD or allergic contact dermatitis did not confirm the theory of an increased risk conferred by the rapid acetylator status (67). There was no significant difference between cases and controls with regard to rapid or slow acetylator genotypes of both NAT1 and NAT2. If anything, the rapid acetylator NAT1*10 allele seemed to be underrepresented in cases (OR 0.72; 95% CI 0.45–1.16), and this, if confirmed, would be in contrast to the above findings.

At first sight, the finding of a probably increased risk of sensitization to PPD in rapid acetylators seems paradoxical (64, 93). However, *N*-acetylation may be involved in the development of contact allergy through activation of aromatic amines or intermediates, for example, *N*-*O*-acetylation of the corresponding hydroxylamines (108). This would lead to higher levels of reactive metabolites, although, in the case of several aromatic hair dyes, evidence for *N*-hydroxylation is lacking (92, 109, 110). NAT enzymes may also play a role beyond xenobiotic metabolism, and may act on endogenous substrates (such as folic acid and derivatives, or other unknown substrates), or internal signalling

pathways (111, 112). Grant et al. pointed out the paradox that acetylated xenobiotics are less water-soluble than their parent compounds, which would make it unlikely that these enzymes evolved specifically in order to accelerate elimination of foreign chemicals (89).

Glutathione-S-transferases (GSTs) M1 and T1

Cytosolic/soluble GSTs constitute a superfamily of phase II enzymes. GSTs conjugate electrophilic substrates with the nucleophilic tripeptide glutathione (GSH). The cytosolic GSTs are subdivided into seven main classes, including the most studied families GST μ (mu, M) and θ (theta, T). Although some substrates are metabolized by several GSTs, others are specific for particular isoenzymes (reviews: (90, 113)). The observed substrate specificities (e.g. genotoxic aromatic epoxides, monohaloalkanes, dihaloalkanes, and aminophenols) were found in the context of specific toxicological research on carcinogenicity (113), and will probably not reflect the whole range of activities of these enzymes. GSH conjugation may result in toxification as well as detoxification. Additionally, like NATs, GSTs may act beyond xenobiotic metabolism, and may be involved in modulating internal signalling pathways (114).

The genes encoding for the proteins GSTM1 and GSTT1 are organized on chromosome 1p13.3 and on chromosome 22q11, respectively. Variations (null deletions resulting in null alleles, *GSTM1*0* and *GSTT1*0*) in both *GSTM1* and *GSTT1* were found to alter the risk for a variety of disorders, most notably cancers at different sites, but also other diseases; such as chronic cardiovascular disease, rheumatoid arthritis, and atopic asthma (113–115).

Studies of *GSTM1* and *GSTT1* polymorphisms in contact allergy include the following:

- (1) *GSTM1* and *GSTT1* may be involved in the inactivation of the organic mercury compound thiomersal and of its degradation products (e.g. ethyl mercury), as several organic mercury compounds were detoxified by GSH (116), as these compounds may interact with GSTs (117), and as GSH was shown to inhibit elicitation in patients allergic to thiomersal (118). Therefore, the *GSTM1* and *GSTT1* polymorphisms were studied in patients sensitized to mercury compounds (thiomersal, phenylmercury acetate, and ammonium mercury chloride, $n = 100$) (60). Healthy individuals ($n = 169$) and patients with contact allergy to para-substituted aryl compounds ($n = 114$) served as control groups. *GSTM1* deficiency was significantly more frequent in thiomersal-allergic persons, and *GSTT1*

deficiency more frequent in patients allergic to mercury compounds other than thiomersal. More importantly, the combined deletion (*GSTM1*0* and *GSTT1*0*) was significantly more frequent in the thiomersal group than in healthy controls (16/91 versus 11/169; $p = 0.0093$) and the para-compound group (16/91 versus 7/114, $p = 0.014$). This finding suggests a 'synergistic' effect of these enzyme deficiencies.

- (2) In a cohort study in cement workers ($n = 153$) conducted in Taiwan (68), those sensitized to chromate (cases, $n = 19$; 12.4%) were compared with non-sensitized individuals with regard to the *GSTM1* and *GSTT1* polymorphisms. Whereas the *GSTM1* deletion was equally distributed among cases (cement workers sensitized to chromate) and controls (cement workers without sensitization to chromate), there was a higher frequency of the *GSTT1* deficiency in chromate-sensitized individuals (3.2% versus 18.9%, RR 5.5; 95% CI 1.40–36.2).

Both studies point to a substrate-specific influence of *GST* polymorphisms. Although this influence is probably not restricted to mercury and chromate, a very general influence on pathogenetic steps in contact allergy seems not to be obvious. Generally, poor *GSTT1* and *GSTM1* activity may reduce the protection from reactive oxygen species (ROS) damage produced by reduction processes (113). There are metal allergens such as chromate, mercury, or nickel, but some organic compounds as well, such as PPD, which generate ROS, for example, hydroxyl radicals, singlet oxygen, and hydrogen peroxide (119–121), playing a role as 'danger signals' in the pathobiology of contact allergy (122–126).

More specifically, it was shown earlier that coupling of contact sensitizers such as methylchloroisothiazolinone to thiol groups is a key event for the activation of monocytes and monocyte-derived dendritic cells, as indicated by tyrosine phosphorylation (125), which can be blocked by thiol antioxidants such as α -tocopherol and pyrrolidine dithiocarbamate (126). Further mechanisms remain to be elucidated. Recent studies focused on defence systems against oxidative stress, the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway being regarded as the most important one (127). Nrf2 is a ubiquitous transcription factor. This protein regulates an enhancer sequence designated as antioxidant response element (ARE), which controls the expression of a number of antioxidant genes and their protein products, for example, enzymes (such as GSTs). Normally, Nrf2 activity is reduced to a basal level by the cytosolic protein Kelch-like-ECH-associating protein 1 (Keap1).

In response to oxidative stress, probably targeting thiol groups of distinct cysteines of Keap1, Nrf2 dissociates from Keap1, and activates ARE-mediated gene expression; the exact mechanisms of this are still disputed (127, 128). Regarding Keap1 with its highly reactive cysteines as a sensitive sensor protein recognizing electrophiles, the Nrf2–ARE signalling pathway has been used as an *in vitro* system to identify contact sensitizers (129). Fourteen of 15 strong sensitizers and 30 of 34 moderate sensitizers were correctly identified. However, the role of the Nrf2–ARE pathway in the pathogenesis of contact allergy is still unknown (129). Nevertheless, polymorphisms (SNPs) of *Nrf2*, *Keap1* and *AREs* were identified (130). The study of such regulatory polymorphisms, which result in variation in gene expression, appears to be important for discovering the mechanisms underlying complex diseases (131).

Manganese superoxide dismutase (MnSOD)

MnSOD (EC 1.15.1.1) is a mitochondrial protein that scavenges potentially toxic superoxide radicals by 'dismuting' superoxide (O_2^-) to O_2 plus H_2O_2 . The gene (*SOD2*) was mapped to chromosome 6q25.3. A peptide polymorphism in the target sequence of MnSOD, Val16 (flesh) Ala, is known to disrupt proper targeting of the enzyme from the cytosol to the mitochondrial matrix. This peptide polymorphism is related to a C/T SNP located at position 47 in exon 2 [reference sequence (rs)4880]. Several diseases have been suspected to be associated with this peptide polymorphisms, but there have been conflicting results [e.g. cancer (132), rheumatic diseases (133), or asthma (134)].

In view of the pathogenetic role of ROS in contact allergy (see above), the Val16→Ala polymorphism and a further polymorphism (Thr58→Ile) were studied in 157 patients with sensitization to PPD, who were compared with healthy controls ($n = 201$) (71). No heterozygous or homozygous carriers of the (Thr58→Ile) polymorphism were found. With regard to the Val16→Ala polymorphism, no significant difference in the distribution of allelic frequencies and genotypes between cases and controls was observed, although for subgroups defined by gender and age, there was a trend towards a overrepresentation of the CC carriers (Ala/Ala) (OR 1.3; 95% CI 0.8–2.1) (71). The authors concluded that the *SOD2* polymorphism studied has no strong impact on individual susceptibility to developing sensitization to PPD.

Angiotensin-converting enzyme (ACE)

ACE (kininase II, EC 3.4.15.1) catalyses the conversion of angiotensin I into angiotensin II, a potent vasoconstrictor,

and is involved in the inactivation of bradykinin, a potent vasodilator. In addition to these well-known substrates, ACE cleaves substance P, β -endorphins, and other peptides (135), which may modulate Langerhans cell and T-lymphocyte functions (136). In particular, it was shown in animal experiments that ACE modulated the inflammatory response to allergens, but not to irritants, by degrading bradykinin and substance P (7). The gene, *ACE*, is located on chromosome 17q.23.3. The presence or absence of a 287-bp *Alu* repeat element in this gene (a repeated DNA sequence that can be cut by the *Alu* restriction enzyme), that is, an 'insertion/deletion' (I/D) polymorphism, was found to be associated with the levels of circulating enzyme; namely, ACE levels were low for I/I homozygotes, and high for D/D homozygotes (137).

On the basis of this functional role of the polymorphism, it was hypothesized that individuals with the I/I genotype would be at greater risk, and those with the D/D genotype would be at lower risk, for developing contact allergy. The I/D polymorphism was studied in 90 patients with contact allergy (positive patch test reaction to PPD) and 160 controls (70). Carriers of the I allele (OR 1.6; 95% CI 1.1–2.4) as well as those with the I/I genotype (OR 2.0; 95% CI 1.1–3.7) were found significantly more often in the contact allergy group, and carriers of the 'protective' D allele were found less often in this study group (OR 0.6; 95% CI 0.4–0.9). These interesting results broaden our view of contact allergy beyond the chemical, immunological and inflammatory aspects to a neuro-endocrinological network, which is rarely considered in the pathogenesis of contact allergy (138, 139). Furthermore, they underline the importance of structural variants in the study of complex traits (140).

TNF

Following a change in nomenclature in 1998, TNF- α and TNF- β were renamed TNF and lymphotoxin- α . TNF is a pro-inflammatory cytokine, mainly produced by macrophages, that plays an essential role in various inflammatory conditions (141–143). Signals for the release of TNF can generally be characterized as 'danger signals' emitted from various sources, such as microbes, xenobiotics, immune complexes, or physical insults. In particular, both irritant and sensitizing compounds induce keratinocytes to release inflammatory cytokines such as TNF (144, 145).

The gene encoding TNF is located on chromosome 6p21.33, within the MHC class III complex (Fig. 2). Several gDNA variants or SNPs have been identified, for example, *TNF*-238 G→A, *TNF*-308 G→A, *TNF*-857 C→T, and *TNF*-1031 T→C). More than 90

case-control studies on the *TNF*-308 promoter SNP and disease have been performed so far (142, 146).

Studies of *TNF* polymorphisms in contact allergy include the following:

1. The first study investigating the relationship between polymorphisms of *TNF* and contact allergy found that the distribution of *TNF*-308 genotypes but not of *TNF*-238 genotypes was significantly different between cases with contact allergy and healthy controls, with carriers of the A allele being more frequent among polysensitized patients (61).
2. Individuals from Germany and The Netherlands sensitized to PPD ($n = 181$) and regional controls without a history of allergic contact dermatitis ($n = 161$), age-matched and gender-matched to cases, were selected for genotyping for the *TNF*-308 polymorphism (69). The frequency of the rare A allele was significantly higher in cases than in controls (22.1% versus 12.4%). A logistic regression analysis, using sex, age and *TNF* (A/A + A/G) versus GG as explanatory variables, confirmed the risk associated with the combined *TNF* (A/G + A/A) genotypes.
3. Patients with sensitization to various allergens of the standard series ($n = 50$) and healthy controls ($n = 100$) from Turkey were genotyped for *TNF* and interleukin-1 receptor antagonist (*IL-1RA*) (77). The *TNF*-308 G/G and *IL-1RA**1/2 polymorphisms were found to be significantly increased in patients with contact allergy. Ethnic differences may explain these divergent findings.
4. In a cross-sectional study in cement workers ($n = 153$) conducted in Taiwan (68), already quoted, those sensitized to chromate (cases, $n = 19$; 12.4%) were compared with non-sensitized individuals with regard to *TNF*-308 G/A and *IL-4*-590 C/T gene polymorphisms, respectively. The *TNF*-308 G/A genotype was found to be a significant risk factor for contact allergy to chromate [Relative Risk (RR) 3.9; 95% CI 1.14–13.2], whereas the distribution of genotypes of the *IL-4* polymorphism (C/T) did not differ between cases and controls. Assuming identical exposure (chromate-containing cement), the different outcomes are most likely attributable to different susceptibilities, which the genetic variation found (*TNF* and *GST*) may partly account for.

These largely consistent findings support the idea that the promoter polymorphism at position -308 of *TNF* might be a risk factor for acquiring contact allergy. In particular, in one study, the rare *TNF*-308 A allele was

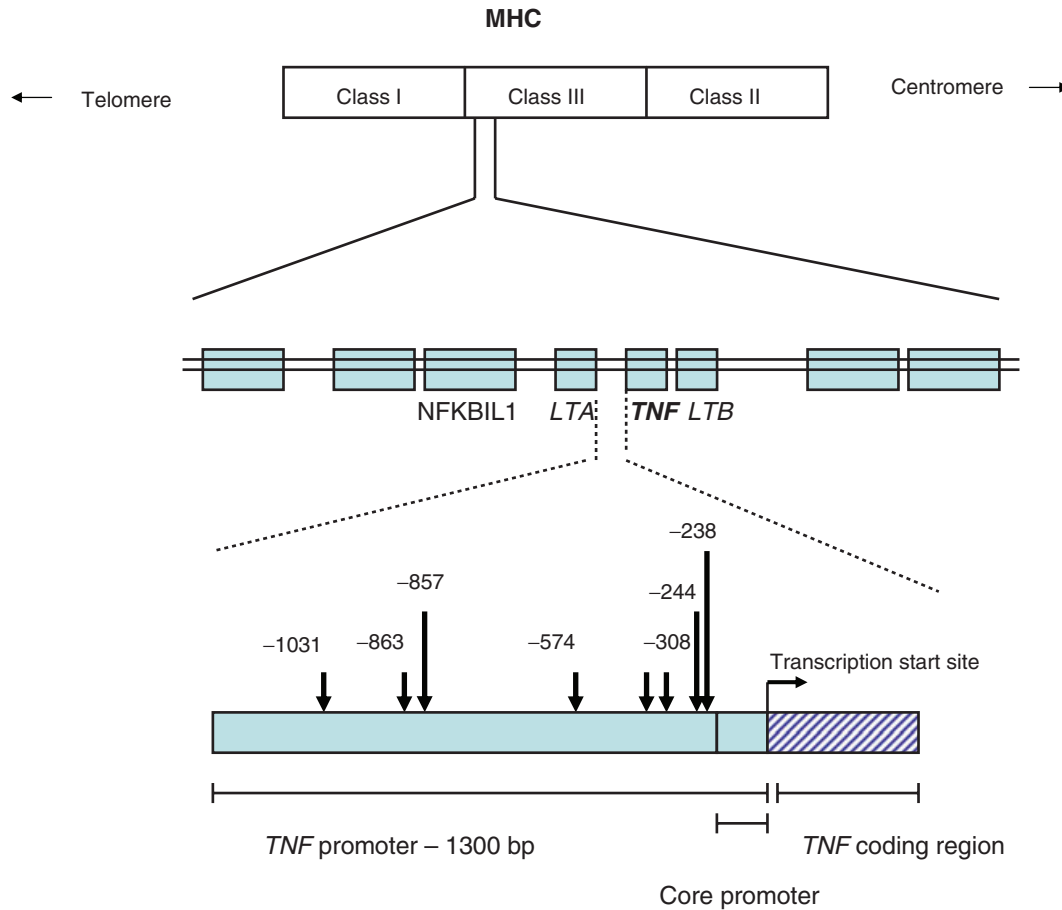


Fig. 2. Scheme depicting the major histocompatibility complex (MHC) on chromosome 6p21.31, the tumour necrosis factor gene (*TNF*) and flanking genes in the *TNF* region [lymphotoxin- α/β (*LTA/LTB*), NF-kappa-B inhibitor-like protein 1 (*NFKBIL1*)] and the *TNF*-promoter polymorphisms. Numbering is from +1 of the transcription start site [(146), modified].

more frequent among polysensitized patients. This finding supports the notion that polysensitization is probably the relevant phenotype. The finding of an increased granulomatous (delayed-type hypersensitivity) response to lepromin in carriers of the *TNF*-308 A allele may support the above results (147), although delayed-type hypersensitivity may not share all of the characteristics of contact allergy. As *TNF* is also involved in irritant contact dermatitis, and as the *TNF*-308 A polymorphism was shown to be associated with an increased risk of irritant contact dermatitis (148, 149), it is conceivable that this polymorphism might have an impact on contact allergy via unspecific trigger factors, as suggested by the 'danger model' (4).

Despite quite a large number of studies showing functional relevance, albeit 'highly context-specific', for example, in terms of an increase in transcriptional activity or production of *TNF*, Bayley et al. summarized the results of *in vivo* and *in vitro* functional studies,

and concluded that the -308 G/A polymorphism is probably not functional (146). The definite role of this polymorphism thus remains to be elucidated (150, 151).

Interleukin-16 (IL-16)

IL-16, originally described as a lymphocyte chemo-attractant, exerts a variety of pro-inflammatory effects [review: (152, 153)]. Its role could be to mediate directed locomotion of T-cells towards dendritic cells after these have captured antigen, and attract other dendritic cells to sites of antigenic challenge, resulting in a 10-fold higher accumulation of CD4⁺ T-cells (154). On the basis of several studies, an important role for IL-16 during delayed-type hypersensitivity reactions can be assumed (155–157). The gene encoding IL-16 (*IL16*) is located on chromosome 15q26.3. One polymorphism in the promoter region, a T/C SNP at position -295, was analysed with regard to, for example, Crohn's

disease, atopic dermatitis, and asthma, with conflicting results (158–160).

Up to now, only one study has investigated the *IL16*–295 polymorphism in contact allergy. It was found that the distribution of *IL16*–295 genotypes differed between patients with contact allergy and healthy controls (62). In particular, the *IL16*–295*C/C genotype was overrepresented among polysensitized individuals (7.0% versus 1.0% in the control group; OR 7.68; 95% CI 1.59–48.12). No association was found in patients sensitized only to para-arylic compounds ('monosensitized') as compared with controls, or in patients with atopic dermatitis, as compared with controls (62).

The fact that the homozygous combination of the rare allele *IL16*–295*C appeared to be more common among polysensitized patients with contact allergy adds, from the perspective of genetics, further support for the concept of polysensitization as a phenotype of increased risk. Both the observed association in contact allergy and in Crohn's disease (158), both thought to be driven by Th1 cytokines, and the lack of association in atopic dermatitis (159), dominated by Th2 cytokines, at least in acute lesions, are compatible with the hypothesis that this polymorphism may exert an enhancing (in Th1-driven diseases) or protective (in Th2-driven diseases) effect (161). Despite these interesting observations, the study needs to be replicated.

General remarks on the study of 'candidate gene' polymorphisms in contact allergy

In this review, we have presented studies supporting the view that manifestations of contact allergy may be influenced by distinct polymorphisms. The reason for investigating these polymorphisms was a pathogenetic hypothesis, that is, an established role of a factor in the immune mechanism underlying contact allergy. If a polymorphism is shown to be associated with a disease, then its functional relevance should be demonstrated. In this regard, the results could be regarded as plausible, although the functional role has not yet been proven experimentally.

Nevertheless, a number of limitations regarding the validity and the interpretation of the studies have to be addressed:

- *Choice of the phenotypes:* With the exception of our study group of patients, and the series ($n = 50$) from Turkey, which comprised a broader scale of sensitization, many studies were performed in individuals sensitized to PPD. This will probably achieve greater phenotype homogeneity, albeit at the expense of generalization. On the other

hand, as explained above, a study group defined by the mere presence of any one sensitization would be too heterogeneous with regard to susceptibility. Therefore, we suggested investigating genetic variation in a high-risk group (1), namely polysensitized patients, where genetic influences can probably be more readily identified (162, 163).

- *Identification of the phenotypes:* Even if there is an unequivocal definition of the phenotype to be investigated, the study may fail to identify the phenotype correctly. For instance, the lack of association between *FLG* mutations and contact allergy (75) may be attributable to the 'diluent' effect of very probably false-positive cases from an 'historical' study (85). The same problem arises if the control group includes cases bearing the target phenotype (72, 73).
- *Control for allergen exposure:* With few exceptions (68, 76), allergen exposure was not considered or controlled for. Wang et al. investigated polymorphisms in cement workers, sensitized or not to chromate (68). Thyssen et al. detected *FLG* mutations significantly more often only in women with nickel dermatitis without ear piercing (76). Possible consequences of not considering exposure would be: (a) individuals with high susceptibility but without allergen exposure (and thus not sensitized) may thus be allocated to the control group. (b) exposure may be 'high' in terms of frequency, dose, and potency, and a possible genetic influence could thus be 'overruled' and not detected.
- *Interaction of polymorphisms:* As cytokines, for example, TNF/interleukin- 1β (*IL-1 β*) (164) or TNF/interferon- γ (165), and enzymes, for example, cytochrome P450/GST (15), interact *in vivo*, polymorphisms may act synergistically; for example, the combination of *CYP1A1* with *GSTM1*0* may increase the risk of cancer (113, 166), or the combination of *NAT2* and *TNF* polymorphisms may increase the susceptibility for psoriasis (167). Although synergism between *NAT1* and *NAT2* and between *GSTM1* and *GSTT1* polymorphisms has been demonstrated (see above), no analyses addressing these potential interactions, and in particular interactions between cytokine and enzyme polymorphisms, have hitherto been performed.
- *The problem of linkage:* Often, the question remains unresolved of whether a disease-associated polymorphism is itself functionally important or is acting only as a marker for a co-inherited, perhaps yet unidentified, polymorphism. For example: the starting point of most studies was the hypothesis

that genetic variants of the *TNF* locus are likely to be involved in the disease, because *TNF* is clearly involved in pathogenesis. This is, in fact, the rationale of the candidate gene approach. However, this locus may be linked to other candidate SNPs within or outside the *TNF* gene (e.g. *LTA*, encoding lymphotoxin- α) or to unknown susceptibility markers, which may extend over a large region of the MHC, including many genes (168). Thus, it may be a complex haplotype (e.g. HLA-A1, HLA-B8, or HLA-DR3), and not the lone SNP, that impacts on disease susceptibility (169). This possibility demonstrates the need to study frequencies of rather long-range haplotypes containing *TNF*-*308 together with other susceptibility markers (Fig. 2).

- *Basic requirements:* In view of the poor reproducibility of the majority of studies – only 6 of 600 associations could consistently be replicated – Bayley et al. refer to some basic requirements proposed by the editors of *Nature Genetics*: large sample size, small *p*-values, associations that ‘make biological sense’, and alleles that have a relevant physiological function. An initial study should be accompanied or followed by an independent replication study (146).

The studies of polymorphisms in contact allergy reviewed all have small sample sizes, and the *p*-values are rather poor. All studies are essentially underpowered, or false-positive results cannot be excluded.

Nevertheless, most studies tentatively indicated a functional role of the polymorphisms, notwithstanding the limitations outlined above. It is noteworthy that three polymorphisms first identified by the Göttingen group as being associated with an increased risk of contact allergy, namely *TNF*, *NAT* and *GST* polymorphisms, were replicated at least once, albeit with slightly different study designs.

Taken together, the results presented here may support the view that contact allergy should be regarded primarily as an entirely exogenous (‘environmental’) disease caused by a contact allergen. However, a closer look, together with varying exposure conditions, indicates that genetic factors might become important. The interplay of many (environmental and heritable) factors should be regarded as a challenge, and is shared by all complex diseases, such as diabetes, coronary heart disease, and cancer. Future research may help to determine ‘heritability’ or the effect of a disease variant on the ‘population attributable fraction’ (for example, *NAT2* and *GSTM1* polymorphisms explained 31% of bladder cancers) (170). Only then it might be appropriate to reflect on ‘gene–environment interactions’ or the balance (or rather imbalance)

between environmental and heritable aetiology of contact allergy (170).

Perspectives – Different Approaches to Detect the Relevant Genes

The many possible approaches to examine the genes that underlie common diseases and quantitative traits fall broadly into two categories: (i) candidate gene studies; and (ii) genome-wide association studies, as summarized in (171). The studies outlined above belong to the former category.

Gene expression analysis

On the basis of the reasonable assumption that a pathobiological link between a manifest disease, and the anatomical site, together with its microscopic, molecular and functional elements (cells and biochemical mediators) exists, multiple intra-individual comparisons of the genes expressed in normal and specific (diseased) tissues may help to elucidate the role of genes, and hopefully their specific responsibility for the disease. With the advent of DNA and RNA microarrays, an *a priori* ‘agnostic’ approach with regard to the genes involved became possible (172). Thus, microarrays were used to study gene expression in diseases, for example, atopic dermatitis (173).

In contact allergy, gene expression microarrays were used in animals and humans, providing some new evidence on the role of different cytokines, chemokines, proteases, and transcription factors (174, 175). More specifically, the microarray technique was used to study the well-known changes in phenotype and function of cultured dendritic cells after contact allergen exposure at a transcriptional level (176). Although approximately 2880 genes were differentially regulated, subsequent, functionally guided, selection resulted in a group of 10 genes that were strongly associated with allergen exposure (177, 178).

Genome-wide association studies

In addition to the above applications, gDNA microarrays have been used in the unbiased analysis of inter-individual genome variation, the most important polymorphisms being expressed as single base-pair changes (SNPs), copy number variants (CNVs), or deletions/insertions (indels) (140, 163, 172, 179). The International HapMap Project has created a genome-wide database of patterns of human genetic variation (180, 181). However, genome-wide association studies on contact allergy have not yet been performed.

The term 'whole-genome microarray' might be misunderstood: not every one of the estimated 11 million SNPs has been identified, but preferentially so-called tagSNPs (several hundred thousand, depending on the array), which are strongly linked with other SNPs. With this technique, knowledge regarding over 80% of SNPs present with a frequency of at least 5% across the whole genome has been obtained (140). On the other hand, disease-related variations, for example, indels (see the section on ACE), or CNVs, which are thought to play an important role in immunological diseases (179), may evade detection, because they are not covered by the platform used. Furthermore, the 5% detection limit may be too high to detect rare variants (140, 182), such as the *FLG* mutations (see the section on filaggrin), that were not 'tagged' by conventional tagSNPs (78).

Further limitations put into perspective the usefulness of genome-wide association studies. The benefits, misconceptions and limitations of genome-wide association studies in general terms are summarized in (140, 163). Most of the susceptibility risk for complex traits were found to be in the range 1.1 (heterozygote genotypes) to 1.6 (homozygote genotype). Only a small fraction of the genetic variability (at most 10%) has so far been explained by the many loci identified in complex traits. For instance, the > 30 associated markers in Crohn's disease account for less than 10% of the cumulative genetic variance. It was estimated that genome-wide association studies studies with 60 000–100 000 individuals are needed to capture no more than 10–15% of the genetic variance (140).

Therefore, the question may arise of whether such broad approaches are the only ones capable of yielding relevant results within the foreseeable future.

Where do we go from now in the genetics of contact allergy?

Genes underlying common (complex) diseases, such as contact allergy, are likely to be multiple, each with a relatively small effect, and presumably have to act in combination, together with environmental influences, to lead to clinical disease. However, some diseases are not simply present or absent, but can be viewed as quantitative traits expressed on a measured continuum. Only if a normal trait variation exceeds a defined threshold can it be considered as disease (e.g. hypertension). Similarly, contact allergy was considered here as a quantitative trait, and it was suggested that the genetics should be studied in individuals where this trait is expressed at the extreme tail of the distribution of sensitization, namely as polysensitization (Fig. 1).

Back to candidate genes. Contact allergy seems to be in a better position than a number of common diseases for which the aetiology and pathobiology are not fully understood, and for which genome-wide association studies are performed in the hope of obtaining, via the genes, further insights into the mechanisms of the disease. Although not every aspect of contact allergy is known, there is considerable knowledge about the functionally important steps. It may thus be a fruitful alternative to reverse the approach: not from gene to function, but again, from established functions to disease-associated genes. The 'filaggrin story' may exemplify the efficiency of such an approach (78). In general, by testing only a limited number of distinct hypotheses, some limitations hampering statistical testing (insufficient power and low prevalence of rare variants) could be overcome (163). Possible areas of interest are summarized in Table 6.

Table 6. Areas where polymorphisms could be candidates for study, on the basis of their role in pathogenesis in contact allergy

- Factors influencing the availability of the allergen
 - Barrier structure and function (e.g. role of filaggrin),
 - Metabolism of the allergen (e.g. NAT, GST, cytochrome P450 enzymes)
 - Cellular membrane trafficking (e.g. ABC transporters)
- (Inflammatory) co-stimulatory factors
 - From the cutaneous innate immune system (e.g. inflammasome and TLRs)
 - From the cutaneous (anti)-inflammatory repertoire (e.g. generation and processing of ROS, activity of NOS, neuronal inflammation)
- Basic cellular signalling involved in both, unspecific (inflammatory) and specific (immunological) mechanisms, for example, class II nuclear receptors (e.g. Ret X, vitamin D, and PPAR), transcription factors (e.g. NF- κ B and STAT4), and different protein kinases (MAP), and immuno-specific co-stimulation and signalling (e.g. CTLA4)
- Immunological factors, for example, cytokines and receptors (e.g. IL-12, IL-23, IL-6, and IL-17), chemokines and ligands (CCR7 and CCL27), intracellular adhesion molecules (ICAM), or enzymes (e.g. MMP and caspase), with impacts on:
 - antigen processing
 - migration and maturation of APCs
 - Presentation of the allergen (MHC/TCR structure and function)
 - T-cell subpopulations and their activating or regulating role (e.g. NKT, CD8, Th17, and Treg)

ABC, ATP-binding cassette; APC, antigen-presenting cell; CTLA4, cytotoxic T-lymphocyte-associated protein; GST, glutathione-S-transferase; IL, interleukin; ICAM, intercellular adhesion molecule; MAP, mitogen-activated protein kinase; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; NAT, N-acetyltransferase; NF- κ B, nuclear factor 'kappa-light-chain enhancer' of activated B-cells; NKT, natural killer T-cell; NOS, nitric oxide synthetase; PPAR, peroxisome proliferator-activating receptor; Ret X, retinoid receptor X; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; TLR, toll-like receptor; Treg, T-regulatory cell; ROS, reactive oxygen species.

Broadening the focus on pathology. There have been several instances in which one genomic interval has been associated with two or more diseases, which may share clinical symptoms, pathogenesis, and genetics, as in the case of ichthyosis vulgaris and atopic dermatitis (79), or which are, at first sight, not suspected to be related at all, such as metabolic and autoimmune diseases. For example, the glucokinase regulatory protein (encoded by the *GCKR* gene) is involved in regulating triglyceride levels and is associated with Crohn's disease and psoriasis (140). The autoimmune diseases have been suspected to share a common immune-mediated aetiology. Nowadays, evidence of a common genetic underpinning has accumulated (40, 140, 183, 184).

Likewise, contact allergy was found to be associated with other diseases (phenotypes). The association with irritant contact dermatitis is undisputed (see above). Leg (stasis) dermatitis is acknowledged as an important risk factor for sensitization to contact allergens (5). Additionally, epidemiological studies from Denmark found that contact sensitization to unrelated (not airborne) allergens (e.g. nickel) was associated with an increased risk of respiratory symptoms after exposure to various airborne chemicals (laser printers, drying paint, car exhausts, and newspapers) (185). Interestingly, the risk increased with the number of positive patch test reactions (polysensitization) (185). These diseases may share certain polymorphisms, of which some are already known: those affecting filaggrin (74, 75, 186), TNF (143, 148, 187–189), IL-16 (161), and GSTs (115, 190). Such a broadened view could include, for instance, the study of those loci in contact allergy that have been found in

non-atopic asthma, another inflammatory disease (191). One may speculate about a more general trait associated with inflammatory diseases (192), instead of, or in addition to, disease-specific polymorphisms. The attempts to group autoimmune diseases according to shared pathogenesis and shared genetics (183, 193) may go beyond the narrow area of 'immunological diseases' to inflammation-related diseases in a more general sense, as the association between asthma and inflammatory bowel disease (192) or between psoriasis and cardiovascular diseases might suggest (194, 195). Such findings have prompted some to suggest that the textbooks of medicine need to be rewritten to account for the interconnectivity of the molecular bases underlying distinct diseases (140).

A future research programme for the genetics of contact allergy. Therefore, the future programme in contact allergy could comprise, in addition to distinct contact allergy-related mechanisms (Table 6), the search for shared pathogenesis and shared genetics of

- (1) immune (delayed-type hypersensitivity)-related or
- (2) inflammation-related or
- (3) inflammatory skin-related diseases or states.

The impact of considering and studying contact allergy as a disease partly influenced by genetic factors would be: (i) to better understand its mechanisms; (ii) to be able to protect susceptible individuals; and (iii) to add a further quantitative element (susceptibility) to the well-known determinants of contact allergy, namely dose and potency of the allergen.

References

- 1 Schnuch A, Brasch J, Uter W. Polysensitization and increased susceptibility in contact allergy: a review. *Allergy* 2008; **63**: 156–167.
- 2 Landsteiner K, Rostenberg A, Sulzberger M B. Individual differences in susceptibility to eczematous sensitization with simple chemical substances. *J Invest Dermatol* 1939; **2**: 25–29.
- 3 Hostynek J J, Maibach H I. Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis? *Food Chem Toxicol* 2004; **42**: 1859–1865.
- 4 Smith H R, Basketter D A, McFadden J P. Irritant dermatitis, irritancy and its role in allergic contact dermatitis. *Clin Exp Dermatol* 2002; **27**: 138–146.
- 5 Uter W, Geier J, Pfahlerberg A, Effendy I. The spectrum of contact allergy in elderly patients with and without lower leg dermatitis. *Dermatology* 2002; **204**: 266–272.
- 6 Cruz PD Jr. Effects of UV light on the immune system: answers to five basic questions. *Am J Contact Dermatitis* 1996; **7**: 47–52.
- 7 Scholzen T E, Stander S, Riemann H, Brzoska T, Luger T A. Modulation of cutaneous inflammation by angiotensin-converting enzyme. *J Immunol* 2003; **170**: 3866–3873.
- 8 Elliott J C, Picker M J, Nelson C J, Carrigan K A, Lysle D T. Sex differences in opioid-induced enhancement of contact hypersensitivity. *J Invest Dermatol* 2003; **121**: 1053–1059.
- 9 Uter W, Hegewald J, Kranke B, Schnuch A, Gefeller O, Pfahlerberg A. The impact of meteorological conditions on patch test results with 12 standard series allergens (fragrances, biocides, topical ingredients). *Br J Dermatol* 2008; **158**: 734–739.
- 10 Saint-Mezard P, Chavagnac C, Bosset S, Ionescu M, Peyron E, Kaiserlian D, Nicolas J F, Berard F. Psychological stress exerts an adjuvant effect on skin dendritic cell functions in vivo. *J Immunol* 2003; **171**: 4073–4080.
- 11 Kwangsuksith C, Maibach H I. Effect of age and sex on the induction and elicitation of allergic contact dermatitis. *Contact Dermatitis* 1995; **33**: 289–298.
- 12 Dickel H, Taylor J S, Evey P, Merk H F. Comparison of patch test results with a standard series among white and black racial groups. *Am J Contact Dermatitis* 2001; **12**: 77–82.

- 13 Jorde LB, Carey JC, Bamshad MJ, White RL. *Medical Genetics*, 3rd edition: St Louis, Mosby, 2006.
- 14 Rustemeyer Th, van-Hoogstraten IMW, von-Blomberg BME, Scheper RJ. Mechanisms in allergic contact dermatitis. In: *Contact Dermatitis*, 4th edition, Frosch PJ, Menné T, Lepoittevin J-P (eds): Berlin, Springer, 2006: pp. 11–43.
- 15 Smith CK, Hotchkiss SH, A M. Allergic contact dermatitis. Chemical and Metabolic Mechanisms: London, Taylor & Francis, 2001.
- 16 Menné T, Holm N V. Genetic susceptibility in human allergic contact sensitization. *Semin Dermatol* 1986; **5**: 301–306.
- 17 Shram SE, Warshaw EM. Genetics of nickel allergic contact dermatitis. *Dermatitis* 2007; **18**: 125–133.
- 18 Sulzberger MB, Rostenberg A. Acquired specific supersensitivity (allergy) to simple chemicals. IV. A method of experimental sensitization; and demonstration of increased susceptibility in individuals with eczematous dermatitis of contact type. *J Immunol* 1939; **36**: 17–27.
- 19 Chase MW. Inheritance in guinea pigs of the susceptibility to skin sensitization with simple chemical compounds. *J Exp Med* 1941; **73**: 711–726.
- 20 Polak L, Barnes M, Turk JL. The genetic control of contact sensitization to inorganic metal compounds in guinea pigs. *Immunology* 1968; **14**: 707–711.
- 21 Walker FB, Smith PD, Maibach HI. Genetic factors in human allergic contact dermatitis. *Int Arch Allergy Appl Immunol* 1967; **32**: 453–462.
- 22 Forsbeck M, Skog E, Ytterborn KH. Allergic diseases among relatives of patients with allergic contact dermatitis. *Acta Derm Venereol (Stockh)* 1971; **51**: 123–128.
- 23 Fleming CJ, Burden AD, Forsyth A. The genetics of allergic contact hypersensitivity to nickel. *Contact Dermatitis* 1999; **41**: 251–253.
- 24 Menné T, Holm N V. Nickel allergy in a female twin population. *Int J Dermatol* 1983; **22**: 22–28.
- 25 Forsbeck M, Skog E, Ytterborn KH. Delayed type of allergy and atopic disease among twins. *Acta Derm Venereol (Stockh)* 1968; **48**: 192–197.
- 26 Bryld LE, Hindsberger C, Kyvik KO, Agner T, Menné T. Genetic factors in nickel allergy evaluated in a population-based female twin sample. *J Invest Dermatol* 2004; **123**: 1025–1029.
- 27 Ishii N, Ishii H, Ono H, Horiuchi Y, Nakajima H, Aoki I. Genetic control of nickel sulfate delayed-type hypersensitivity. *J Invest Dermatol* 1990; **94**: 673–676.
- 28 Ishii N, Ishii H, Nakajima H, Aoki I, Shimoda N. Contact sensitivity to mercuric chloride is associated with I-A region in mice. *J Dermatol Sci* 1991; **2**: 268–273.
- 29 Ishii N, Takahashi K, Kawaguchi H, Nakajima H, Tanaka S, Aoki I. Genetic control of delayed-type hypersensitivity to chromium chloride. *Int Arch Allergy Immunol* 1993; **100**: 333–337.
- 30 Ishii N, Takahashi K, Ishiwa M, Sugita Y, Nakajima H. Genetic control of delayed-type hypersensitivity to gold antigens in mice. *J Dermatol Sci* 1998; **16**: 104–110.
- 31 Asherson GL, Dieli F, Gautam Y, Siew LK, Zembla M. Major histocompatibility complex regulation of the class of the immune response: the H-2d haplotype determines poor interferon-gamma response to several antigens. *Eur J Immunol* 1990; **20**: 1305–1310.
- 32 Okuda K, Ishii N, Ikezawa Z, Tani K, Ishigatsubo Y. Genetic control of contact hypersensitivity. I. I-A subregion as well as non-H-2 loci codes for the gene of 2,4-dinitro-1-fluorobenzene antigen. *Eur J Immunol* 1980; **10**: 969–971.
- 33 Emtestam L, Zetterquist H, Olerup O. HLA-DR, -DQ and -DP alleles in nickel, chromium, and/or cobalt-sensitive individuals: genomic analysis based on restriction fragment length polymorphisms. *J Invest Dermatol* 1993; **100**: 271–274.
- 34 Walton S, Keczes K, Learoyd P A, Rajah S M. HLA-A, -B and -DR antigens in nickel sensitive females. *Clin Exp Dermatol* 1986; **11**: 636–640.
- 35 Önder M, Aksakal B, Gulekon A, Makki S, Gurer M A, Barut A. HLA DR, DQA, DQB and DP antigens in patients allergic to nickel. *Contact Dermatitis* 1995; **33**: 434–435.
- 36 Silvennoinen-Kassinen S, Ikäheimo I, Tiilikainen A. TAP1 and TAP2 genes in nickel allergy. *Int Arch Allergy Immunol* 1997; **114**: 94–96.
- 37 Bataille V. Genetic factors in nickel allergy. *J Invest Dermatol* 2004; **123**.
- 38 Bataille V. The role of twin studies in the genetics of skin diseases. *Clin Exp Dermatol* 1999; **24**: 286–290.
- 39 Holdsworth R, Hurley CK, Marsh SGE, Lau M, Noreen HJ, Kempenich JH, Setterholm M, Maier M. The HLA dictionary 2008: a summary of HLA-A, -B, -C, DRB1/3/4/5, and DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens* 2009; **73**: 95–170.
- 40 Fernando MM, Stevens CR, Walsh EC, Dejager P L, Goyette P, Plenge RM, Vyse TJ, Rioux JD. Defining the role of the MHC in autoimmunity: a review and pooled analysis. *PLoS Genet* 2008; **25**: e100024. doi:10.1371/journal.pgen.000024.
- 41 Kato H, Hayashi M, Fukumori Y, Kaneko H. MHC restriction in contact hypersensitivity to dicyclohexylcarbodiimide. *Food Chem Toxicol* 2002; **40**: 1713–1718.
- 42 Valsecchi R, Bontempelli M, Vicari O, Scudeller G, Cainelli T. HLA antigens and contact sensitivity to para group. *Ital Gen Rev Dermatol* 1981; **18**: 99–103.
- 43 White SI, Friedmann P S, Stratton A. HLA antigens and Langerhans cell density in contact dermatitis. *Br J Dermatol* 1986; **115**: 447–452.
- 44 Hegyi E, Busova B, Niks M. HLA-A and -B antigens in IPPD-sensitive persons. *Contact Dermatitis* 1990; **22**: 228–229.
- 45 Wilkinson SM, Morrey K, Hollowood K, Heagerty AH, English JS. HLA-A, -B and -DR antigens in hydrocortisone contact hypersensitivity. *Contact Dermatitis* 1993; **28**: 295–297.
- 46 Orecchia G, Perfetti L, Finco O, Dondi E, Cuccia M. Polymorphisms of HLA class III genes in allergic contact dermatitis. *Dermatology* 1992; **184**: 254–259.
- 47 Moulon C, Wild D, Dormoy A, Weltzien HU. MHC-dependent and -independent activation of human nickel-specific CD8+ cytotoxic T cells from allergic donors. *J Invest Dermatol* 1998; **111**: 360–366.
- 48 Gernerding K, Moulon C, Karp DR, VanBergen J, Koning F, Wild D, Pflugfelder U, Weltzien HU. A new type of metal recognition by human T cells: contact residues for peptide-independent bridging of T cell receptor and major histocompatibility complex by nickel. *J Exp Med* 2003; **197**: 1345–1353.
- 49 Brasch J, Uter W, Geier J, Schnuch A. Associated positive patch test reactions to standard contact allergens. *Am J Contact Dermatitis* 2001; **12**: 197–202.
- 50 Hegewald J, Uter W, Pfahlerberg A, Geier J, Schnuch A. A multifactorial analysis of concurrent patch-test reactions to nickel, cobalt, and chromate. *Allergy* 2004; **60**: 372–378.
- 51 Tsao H, Florez JC. Introduction to genetic association studies. *J Invest Dermatol* 2007; **127**: 2283–2287.

- 52 Moss C, Friedmann P S, Shuster S, Simpson J M. Susceptibility and amplification of sensitivity in contact dermatitis. *Clin Exp Immunol* 1985; **61**: 232–241.
- 53 Carlsen B C, Menné T, Johansen J D. Associations between baseline allergens and polysensitization. *Contact Dermatitis* 2008; **59**: 96–102.
- 54 Carlsen B C, Menné T, Johansen J D. 20 Years of standard patch testing in an eczema population with focus on patients with multiple contact allergies. *Contact Dermatitis* 2007; **57**: 76–83.
- 55 Thyssen J P, Linneberg A, Menné T, Nielsen N H, Johansen J D. Contact allergy to allergens of the TRUE-test (panels 1 and 2) has decreased modestly in the general population. *Br J Dermatol* 2009; **161**: 1124–1129.
- 56 Tabor H K, Risch N J, Myers R M. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 2002; **3**: 391–397.
- 57 Lohmueller K E, Pearce C L, Pike M, Lander E S, Hirschhorn J N. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003; **33**: 177–182.
- 58 Schnuch A, Westphal G A, Müller M M et al. Genotype and phenotype of N-acetyltransferase 2 (NAT2) polymorphism in patients with contact allergy. *Contact Dermatitis* 1998; **38**: 209–211.
- 59 Westphal G A, Reich K, Schulz T G, Neumann Ch, Hallier E, Schnuch A. N-Acetyltransferases 1 and 2 polymorphisms in para-substituted arylamine-induced contact allergy. *Br J Dermatol* 2000; **142**: 1121–1127.
- 60 Westphal G-A, Schnuch A, Schulz T-G 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**: 384–388.
- 61 Westphal G.-A, Schnuch A, Moessner R, Koenig I R, Kränke B, Hallier E, Ziegler A, Reich K. Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermatitis* 2003; **48**: 93–98.
- 62 Reich K, Westphal G, König I R, Mössner R, Krüger U, Ziegler A, Neumann Ch, Schnuch A. Association of allergic contact dermatitis with a promoter polymorphism in the IL16 gene. *J Allergy Clin Immunol* 2003; **112**: 1191–1194.
- 63 Kawakubo Y, Nakamori M, Schoepf E, Ohkido M. Acetylator phenotyping in patients with p-phenylenediamine (PPD) allergy. *Skin Pharmacol* 1995; **8**: 273.
- 64 Kawakubo Y, Nakamori M, Schöpf E, Ohkido M. Acetylator phenotype in patients with p-phenylenediamine allergy. *Dermatology* 1997; **195**: 43–45.
- 65 Najim R A, Al-waiz M, Al-Razuqi R A. Acetylator phenotype in Iraqi patients with allergic contact dermatitis. *Ann Saudi Med* 2005; **25**: 473–476.
- 66 Nacak M, Erbagci Z, Aynacioglu A S. Human arylamine N-acetyltransferase 2 polymorphism and susceptibility to allergic contact dermatitis. *Int J Dermatol* 2006; **45**: 323–326.
- 67 Blömeke B, Brans R, Coenraads P-J et al. Para-phenylenediamine and allergic sensitization: risk modification by N-acetyltransferase 1 and 2 genotypes. *Br J Dermatol* 2009; **161**: 1130–1135.
- 68 Wang B J, Shiao J S, Chen C J, Lee Y C, Guo Y L. Tumour necrotizing factor-alpha promoter and GST-T1 genotype predict skin allergy to chromate in cement workers in Taiwan. *Contact Dermatitis* 2007; **57**: 309–315.
- 69 Blömeke B, Brans R, Dickel H, Bruckner T, Erdmann S, Heesen M, Merk H F, Coenraads P J. Association between TNFA-308 G/A polymorphism and sensitization to para-phenylenediamine: a case-control study. *Allergy* 2009; **64**: 279–283.
- 70 Nacak M, Erbagci Z, Buyukafsar K, Yurtsever A S, Tiftik R N. Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with allergic contact dermatitis. *Basic Clin Pharmacol Toxicol* 2007; **101**: 101–103.
- 71 Brans R, Dickel H, Bruckner T, Coenraads P-J, Heesen M, Merk H F, Blomeke B. MnSOD polymorphisms in sensitized patients with delayed-type hypersensitivity reactions to the chemical allergen para-phenylene diamine: a case-control study. *Toxicology* 2005; **212**: 148–154.
- 72 Lerbaek A, Bisgaard H, Agner T, Ohm-Kyvik K, Palmer C N, Menné T. Filaggrin null alleles are not associated with hand eczema or contact allergy. *Br J Dermatol* 2007; **157**: 1199–1204.
- 73 Brown S J, Cordell H J. Are filaggrin mutations associated with hand eczema or contact allergy? – we do not know. *Br J Dermatol* 2008; **158**: 1383–1384.
- 74 de Jongh CM, Khrenova L, Verberk MM, Calkoen F, van Dijk FJ, Voss H, John SM, Kezic S. Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. *Br J Dermatol* 2008; **159**: 621–627.
- 75 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**: 1430–1435.
- 76 Thyssen J P, Johansen J D, 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**: 1278–1285.
- 77 Ertam I, Itirli G, Onay H, Alper S, Ozkinay F. Interleukin-1 receptor antagonist and tumour necrosis factor-alpha gene polymorphisms in Turkish patients with allergic contact dermatitis. *Contact Dermatitis* 2009; **61**: 86–90.
- 78 Brown S J, McLean W H. Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009; **129**: 543–552.
- 79 Sandilands A, Smith F J, Irvine A D, McLean W H. Filaggrin's fuller figure: a glimpse into the genetic architecture of atopic dermatitis. *J Invest Dermatol* 2007; **127**: 1282–1284.
- 80 Kezic S, Kemperman P M, Koster E S et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol* 2008; **128**: 2117–2119.
- 81 van den Oord R A H M, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: systematic review and meta-analysis. *Br Med J* 2009; **339**: b2433. doi:10.1136/bmj.b2433.
- 82 Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean W H, Shimizu H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. *J Invest Dermatol* 2009; **129**: 632–639.
- 83 Schnuch A, Uter W, Reich K. Allergic contact dermatitis and atopic eczema. In: *Handbook of Atopic Eczema*, Chapter 17, 2nd edition, Ring J Przybilla B Ruzicka T (eds); Berlin, Heidelberg, New York, Springer, 2005; pp. 176–199.
- 84 Thyssen J P, Carlsen B C, Menné T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008; **19**: 303–307.
- 85 Schäfer T, Bohler E, Ruhdorfer S, Weigl L, Wessner D, Filipiak B, Wichmann H E, Ring J. Epidemiology of contact

- allergy in adults. *Allergy* 2001; **56**: 1192–1196.
- 86 Schnuch A, Uter W, Geier J, Gefeller O. Epidemiology of contact allergy: an estimation of morbidity employing the clinical epidemiology and drug utilisation research (CE-DUR) approach. *Contact Dermatitis* 2002; **47**: 32–39.
- 87 Thyssen J P, Uter W, Schnuch A, Linneberg A, Johansen J D. 10-year prevalence of contact allergy in the general population in Denmark estimated through the CE-DUR method. *Contact Dermatitis* 2007; **57**: 265–272.
- 88 Uter W, Ludwig A, Balda B-R, A, Schnuch, Pfahlberg A, Schäfer T, Wichmann H-E, Ring J. The prevalence of contact allergy differed between population-based and clinic-based data. *J Clin Epidemiol* 2004; **57**: 627–632.
- 89 Grant D M, Goodfellow G H, Sugamori K S, Durette K. Pharmacogenetics of the human arylamine N-acetyltransferases. *Pharmacology* 2000; **61**: 204–211.
- 90 Thier R, Bruning T, Roos P H, Rihs H P, Golka K, Ko Y, Bolt H M. Markers of genetic susceptibility in human environmental hygiene and toxicology: the role of selected CYP, NAT and GST genes. *Int J Hyg Environ Health* 2003; **206**: 149–171.
- 91 Rastogi S C, Sosted H, Johansen J D, Menne T, Bossi R. Unconsumed precursors and couplers after formation of oxidative hair dyes. *Contact Dermatitis* 2006; **55**: 95–100.
- 92 Nohynek G J, Duche D, Garrigues A, Meunier P A, Toutain H, Leclaire J. Under the skin: biotransformation of para-aminophenol and para-phenylenediamine in reconstructed human epidermis and human hepatocytes. *Toxicol Lett* 2005; **158**: 196–212.
- 93 Kawakubo Y, Merk H F, Masaoudi T A, Sieben S, Blomeke B. N-Acetylation of paraphenylenediamine in human skin and keratinocytes. *J Pharmacol Exp Ther* 2000; **292**: 150–155.
- 94 Krasteva M, Nicolas J-F, Chabeau G, Garrigue J-L, Bour H, Thivolet J, Schmitt D. Dissociation of allergenic and immunogenic functions in contact sensitivity to para-phenylenediamine. *Int Arch Allergy Immunol* 1993; **102**: 200–204.
- 95 White J M, Kullavanijaya P, Duangdeeden I, Zazzeroni R, Gilmour N J, Basketter D A, McFadden J P. p-phenylenediamine allergy: the role of Bandrowski's base. *Clin Exp Allergy* 2006; **36**: 1289–1293.
- 96 Goebel C, Hewitt N J, Kunze G, Wenker M, Hein D W, Beck H, Skare J. Skin metabolism of aminophenols: human keratinocytes as a suitable in vitro model to qualitatively predict the dermal transformation of 4-amino-2-hydroxytoluene in vivo. *Toxicol Appl Pharmacol* 2009; **235**: 114–123.
- 97 Aeby P, Sieber T, Beck H, Gerberick G F, Goebel C. Skin sensitization to p-phenylenediamine: the diverging roles of oxidation and N-acetylation for dendritic cell activation and the immune response. *J Invest Dermatol* 2009; **129**: 99–109.
- 98 Sieben S, Kawakubo Y, Al Masaoudi T, Merk H F, Blomeke B. Delayed-type hypersensitivity reaction to paraphenylenediamine is mediated by 2 different pathways of antigen recognition by specific alphabeta human T-cell clones. *J Allergy Clin Immunol* 2002; **109**: 1005–1011.
- 99 Coulter E M, Jenkinson C, Wu Y et al. Activation of T-cells from allergic patients and volunteers by p-phenylenediamine and Bandrowski's base. *J Invest Dermatol* 2008; **128**: 897–905.
- 100 Agundez J A. Polymorphisms of human N-acetyltransferases and cancer risk. *Curr Drug Metab* 2008; **9**: 520–531.
- 101 Ladero J M. Influence of polymorphic N-acetyltransferases on non-malignant spontaneous disorders and on response to drugs. *Curr Drug Metab* 2008; **9**: 532–537.
- 102 Orzechowska-Juzwenko K, Milejski P, Patkowski J, Nittner-Marszalska M, Malolepszy J. Acetylator phenotype in patients with allergic diseases and its clinical significance. *Int J Clin Pharmacol Ther Toxicol* 1990; **28**: 420–425.
- 103 Zielinska E, Niewiarowski W, Bodalski J, Stanczyk A, Bolanowski W, Rebowski G. Arylamine N-acetyltransferase (NAT2) gene mutations in children with allergic diseases. *Clin Pharmacol Ther* 1997; **62**: 635–642.
- 104 Gawronska-Szklarz B, Luszawska-Kutrzeba T, Czaja-Bulsa G, Kurzawski G. Relationship between acetylation polymorphism and risk of atopic diseases. *Clin Pharmacol Ther* 1999; **65**: 562–569.
- 105 Brocvielle H, Muret P, Goydadin A-C, Boone P, Broly F, Kantelip J-P, Humbert P. N-Acetyltransferase 2 acetylation polymorphism: prevalence of slow acetylators does not differ between atopic dermatitis patients and healthy subjects. *Skin Pharmacol Appl Skin Physiol* 2003; **16**: 386–392.
- 106 Olszewska Z, Jablkowska-Gajdzinska J, Majewska E. Acetylation phenotype in patients with eczema. *Przeg Dermatol* 1984; **LXXI**: 213–214.
- 107 Bendaly J, Doll M A, Millner L M, Metry K J, Smith N B, Pierce W M, Hein D W. Differences between human slow N-acetyltransferase 2 alleles in levels of 4-aminobiphenyl-induced DNA adducts and mutations. *Mutat Res* 2009; **671**: 13–19.
- 108 Cheung Y L, Snelling J, Mohammed N N D, Gray T J B, Ioannides C. Interaction with aromatic hydrocarbon receptor, CYP1A induction, and mutagenicity of a series of diaminotoluenes: implications for their carcinogenicity. *Toxicol Appl Pharmacol* 1996; **139**: 203–211.
- 109 Stanley L A, Skare J A, Doyle E, Powrie R, D'Angelo D, Elcombe C R. Lack of evidence for metabolism of p-phenylenediamine by human hepatic cytochrome p450 enzymes. *Toxicology* 2005; **210**: 147–157.
- 110 Skare J A, Hewitt N J, Doyle E, Powrie R, Elcombe C. Metabolite screening of aromatic amine hair dyes using in vitro hepatic models. *Xenobiotica* 2009; **39**: 811–825.
- 111 Butcher N J, Ilett K F, Minchin R F. Inactivation of human arylamine N-acetyltransferase 1 by the hydroxylamine of p-aminobenzoic acid. *Biochem Pharmacol* 2000; **60**: 1829–1836.
- 112 Gillam E M J. The dark side of a 'detoxification' mechanism. *Trends Pharmacol Sci* 2001; **22**: 11.
- 113 Bolt H M, Thier R. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. *Curr Drug Metab* 2006; **7**: 613–628.
- 114 Hayes J D, Flanagan J U, Jowsey I R. Glutathione transferases. *Annu Rev Pharmacol Toxicol* 2005; **45**: 51–88.
- 115 Holla L I, Stejskalova A, Vasku A. Polymorphisms of the GSTM1 and GSTT1 genes in patients with allergic diseases in the Czech population. *Allergy* 2006; **61**: 265–267.
- 116 Bohets H H, van Thienen M N, VanderBiest I, VanLandeghem G F, D'Haese P C, Nouwen E J, DeBroe M E, Dierickx P J. Cytotoxicity of mercury compounds in LLC-PK1, MDCK and human proximal tubular cells. *Kidney Int* 1995; **47**: 395–403.

- 117 Almar M M, Dierickx P J. In vitro interaction of mercury, copper (II) and cadmium with human glutathione transferase pi. *Res Commun Chem Pathol Pharmacol* 1990; **69**: 99–102.
- 118 Santucci B, Cannistraci C, Cristaudo A, Camera E, Picardo M. Thimerosal positivities: the role of SH groups and divalent ions. *Contact Dermatitis* 1998; **39**: 123–126.
- 119 Picardo M, Zompetta C, Marchese C, de Luca C, Faggioni A, Schmidt R J, Santucci B. Paraphenylenediamine, a contact allergen, induces oxidative stress and ICAM-1 expression in human keratinocytes. *Br J Dermatol* 1992; **126**: 450–455.
- 120 Valko M, Morris H, Cronin M T. Metals, toxicity and oxidative stress. *Curr Med Chem* 2005; **12**: 1161–1208.
- 121 Kaur S, Zilmer M, Eisen M, Rehema A, Kullisaar T, Vihalemm T, Zilmer K. Nickel sulphate and epoxy resin: differences in iron status and glutathione redox ratio at the time of patch testing. *Arch Dermatol Res* 2004; **295**: 517–520.
- 122 Rutault K, Alderman C, Chain B M, Katz D R. Reactive oxygen species activate human peripheral blood dendritic cells. *Free Radic Biol Med* 1999; **26**: 232–238.
- 123 Young C N, Koepke J I, Terlecky L J, Borkin M S, Boyd S L, Terlecky S R. Reactive oxygen species in tumor necrosis factor- α -activated primary human keratinocytes: implications for psoriasis and inflammatory skin disease. *J Invest Dermatol* 2008; **128**: 2606–2614.
- 124 Mizuashi M, Ohtani T, Nakagawa S, Aiba S. Redox imbalance induced by contact sensitizers triggers the maturation of dendritic cells. *J Invest Dermatol* 2005; **124**: 579–586.
- 125 Becker D, Valk E, Zahn S, Brand P, Knop J. Coupling of contact sensitizers to thiol groups is a key event for the activation of monocytes and monocyte-derived dendritic cells. *J Invest Dermatol* 2003; **120**: 233–238.
- 126 Bruchhausen S, Zahn S, Valk E, Knop J, Becker D. Thiol antioxidants block the activation of antigen-presenting cells by contact sensitizers. *J Invest Dermatol* 2003; **121**: 1039–1044.
- 127 Kaspar J W, Nitire S K, Jaiswal A K. Nrf2:1Nrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med* 2009; **47**: 1304–1309.
- 128 Nguyen T, Pickett C B. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* 2009; **284**: 13291–13295.
- 129 Natsch A, Emter R. Skin sensitizers induce antioxidant response element dependent genes: application to the in vitro testing of the sensitization potential of chemicals. *Toxicol Sci* 2008; **102**: 110–119.
- 130 Wang X, Tomso D J, Chorley B N, Cho H Y, Cheung V G, Kleeberger S R, Bell D A. Identification of polymorphic antioxidant response elements in the human genome. *Hum Mol Genet* 2007; **16**: 1188–1200.
- 131 Knight J C. Regulatory polymorphisms underlying complex disease traits. *J Mol Med* 2005; **83**: 97–109.
- 132 Bag A, Bag N. Target sequence polymorphism of human manganese superoxide dismutase gene and its association with cancer risk: a review. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 3298–3305.
- 133 Yen J H, Chen C J, Tsai W C, Lin C H, Ou T T, Hu C J, Liu H W. Manganese superoxide dismutase and cytochrome P450 1A1 genes polymorphisms in rheumatoid arthritis in Taiwan. *Hum Immunol* 2003; **64**: 366–373.
- 134 Mak J C, Leung H C, Ho S P, Ko F W, Cheung A H, Ip M S, Chan-Yeung M M. Polymorphisms in manganese superoxide dismutase and catalase genes: functional study in Hong Kong Chinese asthma patients. *Clin Exp Allergy* 2006; **36**: 440–447.
- 135 Scholzen T E, König S, Fastrich M, Bohm M, Luger T A. Terminating the stress: peripheral peptidolysis of proopiomelanocortin-derived regulatory hormones by the dermal microvascular endothelial cell extracellular peptidases neprilysin and angiotensin-converting enzyme. *Endocrinology* 2007; **148**: 2793–2805.
- 136 Scholzen T, Armstrong C A, Bunnett N W, Luger T A, Olerud J E, Ansel J C. Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. *Exp Dermatol* 1998; **7**: 81–96.
- 137 Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; **86**: 1343–1346.
- 138 Masek K, Slansky J, Petrovicky P, Hadden J W. Neuroendocrine immune interactions in health and disease. *Int Immunopharmacol* 2003; **3**: 1235–1246.
- 139 Hendrix S. Neuroimmune communication in skin: far from peripheral. *J Invest Dermatol* 2008; **128**: 260–261.
- 140 Frazer K A, Murray S S, Schork N J, Topol E J. Human genetic variation and its contribution to complex traits. *Nat Rev Genet* 2009; **10**: 241–251.
- 141 Clark I A. How TNF was recognized as a key mechanism of disease. *Cytokine Growth Factor Rev* 2007; **18**: 335–343.
- 142 Bradley J R. TNF-mediated inflammatory disease. *J Pathol* 2008; **214**: 149–160.
- 143 Tracey D, Klareskog L, Sasso E H, Salfeld J G, Tak P P. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008; **117**: 244–279.
- 144 Corsini E, Galli C L. Cytokines and irritant contact dermatitis. *Toxicol Lett* 1998; 102–103.
- 145 Lisby S, Jongeneel V, Hauser C. Irritants and allergens induce TNF α gene expression in keratinocytes. *Arch Dermatol Res* 1994; **286**: 161–162.
- 146 Bayley J P, Ottenhoff T H, Verweij C L. Is there a future for TNF promoter polymorphisms? *Genes Immun* 2004; **5**: 315–329.
- 147 Moraes M O, Duppre N C, Suffys P N, Santos A R, Almeida A S, Nery J A C, Sampaio E P, Sarno E N. Tumor necrosis factor- α promoter polymorphism TNF2 is associated with a stronger delayed-type hypersensitivity reaction in the skin of borderline tuberculoid leprosy patients. *Immunogenetics* 2001; **53**: 45–47.
- 148 Allen M H, Wakelin S H, Holloway D, Lisby S, Baadsgaard O, Barker J N W N, McFadden J P. Association of TNF α gene polymorphism at position – 308 with susceptibility to irritant contact dermatitis. *Immunogenetics* 2000; **51**: 201–205.
- 149 de Jongh C M, John S M, Bruynzeel D P et al. Cytokine gene polymorphisms and susceptibility to chronic irritant contact dermatitis. *Contact Dermatitis* 2008; **58**: 269–277.
- 150 Taudorf S, Krabbe K S, Berg R M, Moller K, Pedersen B K, Bruunsgaard H. Common studied polymorphisms do not affect plasma cytokine levels upon endotoxin exposure in humans. *Clin Exp Immunol* 2008; **152**: 147–152.
- 151 Kroeger K M, Steer J H, Joyce D A, Abraham L J. Effects of stimulus and cell type on the expression of the – 308 tumour necrosis factor promoter polymorphism. *Cytokine* 2000; **12**: 110–119.
- 152 Glass W G, Sarisky R T, Vecchio A M. Not-so-sweet sixteen: the role of IL-16 in infectious and immune-mediated inflammatory diseases. *J Interferon Cytokine Res* 2006; **26**: 511–520.
- 153 Cruikshank W W, Kornfeld H, Center D M. Interleukin-16. *J Leukoc Biol* 2000; **67**: 757–766.
- 154 Laberge S, Ghaffar O, Boguniewicz M, Center D M, Leung D Y, Hamid Q. Association of increased CD4+ T-cell infiltration with increased IL-16 gene

- expression in atopic dermatitis. *J Allergy Clin Immunol* 1998; **102**: 645–650.
- 155 Yoshimoto T, Wang C R, Yoneto T, Matsuzawa A, Cruikshank W W, Nariuchi H. Role of IL-16 in delayed-type hypersensitivity reaction. *Blood* 2000; **95**: 2869–2874.
- 156 Masuda K, Katoh N, Soga F, Kishimoto S. The role of interleukin-16 in murine contact hypersensitivity. *Clin Exp Immunol* 2005; **140**: 213–219.
- 157 Stoitzner P, Ratzinger G, Koch F et al. Interleukin-16 supports the migration of Langerhans cells, partly in a CD4-independent way. *J Invest Dermatol* 2001; **116**: 641–649.
- 158 Glas J, Torok H P, Unterhuber H, Radl-mayr M, Folwaczny C. The –295T-to-C promoter polymorphism of the IL-16 gene is associated with Crohn's disease. *Clin Immunol* 2003; **106**: 197–200.
- 159 Reich K, Westphal G, König I R et al. Cytokine gene polymorphisms in atopic dermatitis. *Br J Dermatol* 2003; **148**: 1237–1241.
- 160 Akesson L S, Duffy D L, Phelps S C, Thompson P J, Kedda M A. A polymorphism in the promoter region of the human interleukin-16 gene is not associated with asthma or atopy in an Australian population. *Clin Exp Allergy* 2005; **35**: 327–331.
- 161 Burkart K M, Barton S J, Holloway J W et al. Association of asthma with a functional promoter polymorphism in the IL16 gene. *J Allergy Clin Immunol* 2006; **117**: 86–91.
- 162 Hayes J D, Strange R C. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* 2000; **61**: 154–166.
- 163 Hardy J, Singleton A. Genomewide association studies and human disease. *N Engl J Med* 2009; **360**: 1759–1768.
- 164 Cumberbatch M, Dearman R J, Kimber I. Langerhans cells require signals from both tumour necrosis factor-alpha and interleukin-1 beta for migration. *Immunology* 1997; **92**: 388–395.
- 165 Bartee E, Mohamed M R, McFadden G. Tumor necrosis factor and interferon: cytokines in harmony. *Curr Opin Microbiol* 2008; **11**: 378–383.
- 166 Lira M G, Provezza L, Malerba G et al. Glutathione S-transferase and CYP1A1 gene polymorphisms and non-melanoma skin cancer risk in Italian transplanted patients. *Exp Dermatol* 2006; **15**: 958–965.
- 167 Reich K, Westphal G, Schulz Th, Müller M, Zipprich S, Fuchs Th, Hallier E, Neumann Ch. Evidence for a synergistic effect of polymorphisms of N-acetyltransferase 2 and TNF-alpha promoter as susceptibility factors for psoriasis in males. *J Invest Dermatol* 1999; **113**: 214–220.
- 168 Stenzel A, Lu T, Koch W A, Hampe J, Guenther S M, De LaVega F M, Krawczak M, Schreiber S. Patterns of linkage disequilibrium in the MHC region on human chromosome 6p. *Hum Genet* 2004; **114**: 377–385.
- 169 Lio D, Candore G, Romano G C et al. Modification of cytokine patterns in subjects bearing the HLA-B8,DR3 phenotype: implications for autoimmunity. *Cytokines Cell Mol Ther* 1997; **3**: 217–224.
- 170 Hemminki K, Lorenzo Bermejo J, Forsti A. The balance between heritable and environmental aetiology of human disease. *Nat Rev Genet* 2006; **7**: 958–965.
- 171 Hirschhorn J N, Daly M J. Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* 2005; **6**: 95–108.
- 172 Gresham D, Dunham M J, Botstein D. Comparing whole genomes using DNA microarrays. *Nat Rev Genet* 2008; **9**: 291–302.
- 173 Sugiura H, Ebise H, Tazawa T, Tanaka K, Sugiura Y, Uehara M, Kikuchi K, Kimura T. Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. *Br J Dermatol* 2005; **152**: 146–149.
- 174 Hartmann B, Staedtler F, Hartmann N, Meingassner J, Firat H. Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. *Inflamm Res* 2006; **55**: 322–334.
- 175 Pedersen M B, Skov L, Menne T, Johansen J D, Olsen J. Gene expression time course in the human skin during elicitation of allergic contact dermatitis. *J Invest Dermatol* 2007; **127**: 2585–2595.
- 176 Ryan C A, Gildea L A, Hulette B C, Dearman R J, Kimber I, Gerberick G F. Gene expression changes in peripheral blood-derived dendritic cells following exposure to a contact allergen. *Toxicol Lett* 2004; **150**: 301–316.
- 177 Gildea L A, Ryan C A, Foertsch L M, Kennedy J M, Dearman R J, Kimber I, Gerberick G F. Identification of gene expression changes induced by chemical allergens in dendritic cells: opportunities for skin sensitization testing. *J Invest Dermatol* 2006; **126**: 1813–1822.
- 178 Ryan C A, Kimber I, Basketter D A, Pallardy M, Gildea L A, Gerberick G F. Dendritic cells and skin sensitization: biological roles and uses in hazard identification. *Toxicol Appl Pharmacol* 2007; **221**: 384–394.
- 179 Schaschl H, Aitman T J, Vyse T J. Copy number variation in the human genome and its implication in autoimmunity. *Clin Exp Immunol* 2009; **156**: 12–16.
- 180 Manolio T A, Brooks L D, Collins F S. A HapMap harvest of insights into the genetics of common disease. *J Clin Invest* 2008; **118**: 1590–1605.
- 181 Hindorf L A, Jankins H A, Mehta J P, Manolio T A. A Catalog of Published Genome-Wide Association Studies Available at: www.genome.gov/26525384 (accessed 12 February 2010).
- 182 Goldstein D B. Common genetic variation and human traits. *N Engl J Med* 2009; **360**: 1696–1698.
- 183 Zhernakova A, van Diemen C C, Wijmenga C. Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 2009; **10**: 43–55.
- 184 Hyatt G, Melamed R, Park R et al. Gene expression microarrays: glimpses of the immunological genome. *Nat Immunol* 2006; **7**: 686–691.
- 185 Elberling J, Linneberg A, Mosbech H et al. Airborne chemicals cause respiratory symptoms in individuals with contact allergy. *Contact Dermatitis* 2005; **52**: 65–72.
- 186 Palmer C N, Ismail T, Lee S P et al. Filaggrin null mutations are associated with increased asthma severity in children and young adults. *J Allergy Clin Immunol* 2007; **120**: 64–68.
- 187 Wallace H J, Vandongen Y K, Stacey M C. Tumor necrosis factor-alpha gene polymorphism associated with increased susceptibility to venous leg ulceration. *J Invest Dermatol* 2006; **126**: 921–925.
- 188 Gao J, Shan G, Sun B, Thompson P J, Gao X. Association between polymorphism of tumour necrosis factor alpha-308 gene promoter and asthma: a meta-analysis. *Thorax* 2006; **61**: 466–471.
- 189 Li-Kam-Wa T C, Mansur A H, Britton J et al. Association between –308 tumour necrosis factor promoter polymorphism and bronchial hyperactivity in asthma. *Clin Exp Allergy* 1999; **29**: 1204–1208.
- 190 Kamada F, Mashimo Y, Inoue H et al. The GSTP1 gene is a susceptibility gene for childhood asthma and the GSTM1 gene is a modifier of the GSTP1 gene. *Int Arch Allergy Immunol* 2007; **144**: 275–286.
- 191 Moffatt M F, Kabesch M, Liang L et al. Genetic variants regulating ORMDL3 expression contribute

- to the risk of childhood asthma.
Nature 2007; **448**: 470–473.
- 192 Björner L. Time for a paradigm shift in asthma treatment: from relieving bronchospasm to controlling systemic inflammation. *J Allergy Clin Immunol* 2007; **120**: 1269–1275.
- 193 Thorisson G A, Muilu J, Brookes A J. Genotype-phenotype databases: challenges and solutions for the post-genomic era. *Nat Rev Genet* 2009; **10**: 9–18.
- 194 Prodanovich S, Shelling M L, Federman D G, Kirsner R S. Cytokine milieu in psoriasis and cardiovascular disease may explain the epidemiological findings relating these 2 diseases. *Arch Dermatol* 2008; **144**: 1518–1519.
- 195 Federman D G, Shelling M, Prodanovich S, Gunderson C G, Kirsner R S. Psoriasis: an opportunity to identify cardiovascular risk. *Br J Dermatol* 2009; **160**: 1–7.