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

<table>
<thead>
<tr>
<th>State</th>
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<tbody>
<tr>
<td>High induction dose of the allergen (3)</td>
</tr>
<tr>
<td>Irritant contact dermatitis (4)</td>
</tr>
<tr>
<td>‘Status eczematicus’ (1)</td>
</tr>
<tr>
<td>Leg dermatitis (5)</td>
</tr>
<tr>
<td>UV radiation (6)</td>
</tr>
<tr>
<td>Drugs (7, 8)</td>
</tr>
<tr>
<td>Climatic conditions (9)</td>
</tr>
<tr>
<td>Psychological stress (10)</td>
</tr>
<tr>
<td>Sex (11)</td>
</tr>
<tr>
<td>Age (5, 11)</td>
</tr>
<tr>
<td>Ethnicity (12)</td>
</tr>
</tbody>
</table>

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. reanalysed 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

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Experimental sensitization</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sulzberger &amp; Rostenberg (1939)</td>
<td>Sensitization to NDMA and DNCB in humans.</td>
<td>Inter-individual differences in susceptibility to sensitization; impact of pre-existing eczema.</td>
<td>(18)</td>
</tr>
<tr>
<td>Landsteiner et al. (1939)</td>
<td>Re-analysis of the above study.</td>
<td>Susceptibility to sensitization is chemical-specific. Individuals sensitized to one allergen are more easily sensitized to others.</td>
<td>(2)</td>
</tr>
<tr>
<td>Chase (1941)</td>
<td>Sensitization of guinea pigs with DNCB and poison ivy; identification of high and low responders. Controlled breeding of the two colonies.</td>
<td>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.</td>
<td>(19)</td>
</tr>
<tr>
<td>Polak et al. (1968)</td>
<td>Sensitization of different inbred strains of guinea pigs with metal compounds.</td>
<td>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.</td>
<td>(20)</td>
</tr>
<tr>
<td><strong>II. Family studies</strong></td>
<td></td>
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<tr>
<td>Walker et al. (1967)</td>
<td>Experimental sensitization of parents and their children of 99 families with DNCB and NDMA.</td>
<td>Sensitization of children more frequent if parents were sensitized (only NDMA, not DNCB).</td>
<td>(21)</td>
</tr>
<tr>
<td>Forsbeck et al. (1971)</td>
<td>Relatives (n = 404) of patients with allergic contact dermatitis (n = 94) were patch tested with 23 standard allergens.</td>
<td>Positive reactions in female relatives more frequent than in controls (30%/18%).</td>
<td>(22)</td>
</tr>
<tr>
<td>Fleming et al. (1999)</td>
<td>Relatives (n = 209) of patients with nickel-allergic contact dermatitis (n = 39) were questioned about intolerance to nickel.</td>
<td>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!</td>
<td>(23)</td>
</tr>
<tr>
<td><strong>III. Twin studies</strong></td>
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<tr>
<td>Mennè &amp; Holm (1983)</td>
<td>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).</td>
<td>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).</td>
<td>(24)</td>
</tr>
<tr>
<td>Forsbeck et al. (1968)</td>
<td>101 twin pairs from the Swedish twin register: (a) patch tested with 23 standard allergens; (b) sensitization with DNCB (see comment in Table 3).</td>
<td>(a) Insignificant concordance among the monozygotic pairs (b) No difference in sensitization between monozygotic and dizygotic pairs. Conclusion: no evidence for genetic background.</td>
<td>(25)</td>
</tr>
<tr>
<td>Bryld et al. (2004)</td>
<td>A sample of female twins with hand eczema from the Danish twin register were patch tested with nickel (n = 630).</td>
<td>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!</td>
<td>(26)</td>
</tr>
<tr>
<td><strong>IV. Studies of immunogenetic markers</strong></td>
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<tr>
<td>Asherson et al. (1990)</td>
<td>Sensitization and IFN-γ release in CBA (H-2k) and BALB/c (H-2d) mice.</td>
<td>The H-2d haplotype determines contact sensitivity and poor IFN-γ response to several antigens. (31)</td>
<td></td>
</tr>
<tr>
<td><strong>Studies in humans (significant findings only)</strong></td>
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<tr>
<td>Walton et al. (1986)</td>
<td>Associations of nickel allergy with MHC loci.</td>
<td>HLA-B35. (34)</td>
<td></td>
</tr>
<tr>
<td>Ønder et al. (1995)</td>
<td>Associations of nickel allergy with MHC loci.</td>
<td>DQA1 *061; DR15 decreased. (35)</td>
<td></td>
</tr>
<tr>
<td>Silvennoinen-Kassinen et al. (1997)</td>
<td>Association with TAP genes, encoding the ABC (ATP-binding cassette) transporter associated with antigen processing (TAP).</td>
<td>RR for the alleles TAP2B increased, and RR for TAP2C decreased (both significantly). (36)</td>
<td></td>
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</table>

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; NMDA, p-nitroso-dimethylaniline.
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 (+++ or ++++) 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 methyldibromo glutaronitrile, 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).

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.

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

APC, antigen-presenting cell; IL-1β, interleukin-1β; 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).

<table>
<thead>
<tr>
<th>I</th>
<th>Polymorphisms</th>
<th>Results</th>
<th>Ref.</th>
<th>Replicated</th>
</tr>
</thead>
</table>
| 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)† |  |
| 2 | Genotype and phenotype of NAT2 | Genotype and phenotype of ‘rapid acetylators’ increased | (58) | (65, 66) |
| 3 | \textit{NAT}1 and \textit{NAT}2 in patients allergic to ‘para-compounds’ | \textit{NAT}2*4 allele (rapid acetylators) increased;  
\textit{NAT}2*5b/2*6a (slow acetylators) decreased. Genetic linkage of \textit{NAT}1*10 with \textit{NAT}2*4 | (59) | (65, 66) n.r. (67) |
| 4 | \textit{GSTM1} and \textit{GSTT1} | Combined deletion (\textit{GSTT1/GSTM1}) in patients allergic to organic mercury compounds as compared with controls and para group allergies | (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/I 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; II, 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 p-phenylenediamine (PPD).

n.r.: not replicated.

to be associated with \textit{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 \textit{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 \textit{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*T2A 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 allergy seems not to be obvious. 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).

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 (\( \text{O}_2^- \)) to \( \text{O}_2 \) plus \( \text{H}_2\text{O}_2 \). 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 an 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 17q23.3. 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
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 $\text{IL16} - 295$ polymorphism in contact allergy. It was found that the distribution of $\text{IL16} - 295$ genotypes differed between patients with contact allergy and healthy controls (62). In particular, the $\text{IL16} - 295^C/C$ 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-aryllic 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 $\text{IL16}-295^C$ 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 $\text{FLG}$ 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 $\text{FLG}$ 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, $\text{TNF/interleukin-1\beta}$ (IL-1\beta) (164) or $\text{TNF/interferon-\gamma}$ (165), and enzymes, for example, cytochrome P450/GST (15), interact in vivo. polymorphisms may act synergistically; for example, the combination of $\text{CYP1A1}$ with $\text{GSTM1}*$0 may increase the risk of cancer (113, 166), or the combination of $\text{NAT2}$ and $\text{TNF}$ polymorphisms may increase the susceptibility for psoriasis (167). Although synergism between $\text{NAT1}$ and $\text{NAT2}$ and between $\text{GSTM1}$ and $\text{GSTT1}$ 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 lymphotxin-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 *apriori* ‘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 (homozygote 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>
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<tr>
<th>Table 6. Areas where polymorphisms could be candidates for study, on the basis of their role in pathogenesis in contact allergy</th>
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<tr>
<td><strong>Factors influencing the availability of the allergen</strong></td>
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<tr>
<td>– Barrier structure and function (e.g. role of filaggrin),</td>
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<tr>
<td>– Metabolism of the allergen (e.g. NAT, GST, cytochrome P450 enzymes)</td>
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<td>– Cellular membrane trafficking (e.g. ABC transporters)</td>
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<td><strong>Inflammatory co-stimulatory factors</strong></td>
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<td>– From the cutaneous innate immune system (e.g. inflammasome and TLRs)</td>
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<tr>
<td>– From the cutaneous (anti)-inflammatory repertoire (e.g. generation and processing of ROS, activity of NOS, neuronal inflammation)</td>
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<tr>
<td><strong>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-κB and STAT4), and different protein kinases (MAP), and immuno-specific co-stimulation and signalling (e.g. CTLA4)</strong></td>
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<tr>
<td><strong>Immunological factors, for example, cytokines and receptors</strong></td>
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<tr>
<td>– IL-12, IL-23, IL-16, and IL-17, chemokines and ligands (CCR7 and CCL27), intracellular adhesion molecules (ICAM), or enzymes (e.g. MMP and caspase), with impacts on:</td>
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<tr>
<td>– Antigen processing</td>
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<td>– Migration and maturation of APCs</td>
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<td>– Presentation of the allergen (MHC/TCR structure and function)</td>
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<tr>
<td>T-cell subpopulations and their activating or regulating role</td>
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<tr>
<td>(e.g. NKT, CD8, Th17, and Treg)</td>
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</table>

ABC, ATP-binding cassette; APC, antigen-presenting cell; CTLA4, cytotoxic T-lymphocyte-associated protein; GST, glutathione-Stransferase; IL, interleukin; ICAM, intercellular adhesion molecule; MAP, mitogen-activated protein kinase; MHC, major histocompatibility complex; MMP, matrix metalloprotease; NAT, N-acetyltransferase; NF-κB, 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 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

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18 Sulzberger M B, Rostenberg A. Acquired hypersensitivity to nickel. Chemical and Metabolic Mechanisms in allergic contact dermatitis. In: Contact Dermatitis, pp. 11–43.


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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
190 Moffatt M F, Kabesch M, Liang L et al. Genetic variants regulating ORMDL3 expression contribute...


