Network: Diseases Due to Environmental Factors

Project: Genetic Basis of Phenotype Expression in Atopy and Atopic Dermatitis

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Introduction

Atopy is defined as familial tendency to develop characteristic IgE-mediated allergic diseases on the basis of skin and mucuous membrane hyperreactivity (1). Epidemiologic studies showed a world-wide increase of the prevalence of atopic diseases (atopic eczema (AE), asthma, rhinoconjunctivitis) over the last decades (2). AE is one of the most common inflammatory, chronically relapsing skin diseases caused by combined influences of multiple genetic and environmental factors. It commonly represents the initial clinical manifestation of allergic disease and often precedes the onset of respiratory allergies. Family and twin studies clearly indicate that the genetic contribution is substantial (3). In recent years it has become clear that there are subpopulations of AE patients with regard to clinical parameters (e.g. age of onset, skin lesion morphology) and pathophysiology (extrinsic versus intrinsic AE) (4). In the context of genetics this is crucial. Therefore, in NGFN I large samples of trio families and single cases have been phenotyped on the basis of precise consensus criteria (5) that allow distinction of various subgroups of AE including intermediate phenotypes. Extensive phenotyping allowed unambiguous grouping of patients based on sensitization patterns and revealed a clear female preponderance for intrinsic AE, suggesting sex-related influence factors on disease expression. By detection of exclusively allergen-specific IgE against microbial components in a subgroup of patients with low serum IgE levels, a more precise differentiation between extrinsic and intrinsic type of disease was obtained. Further investigations showed that the surface expression of the high and low affinity IgE-receptor and of the interleukin-4-receptor alpha chain were significantly elevated in monocytes from patients with intrinsic AE. In addition, serum levels of IL-13 were significantly increased in these patients. Distinct AE subgroups could also be distinguished on the basis of reaction patterns towards climatic factors and exposure to allergens (6).

Genetics of atopy and atopic eczema is a research topic of both the Department of Dermatology and Allergy (Head: J. Ring) and the ZAUM Center of Allergy and Environment (Head: H. Behrendt), which is the umbrella organisation for different research groups active at the university campus Biederstein. The Department of Dermatology and Allergy TUM is one of the leading allergy centers in Germany with 76 in-patient facilities and a large outpatient clinic and has been established as one of eight national “Atopic Eczema Academies” (Neurodermitis-Akademie) in the National Consensus Program of the German Health Ministry in the development of schooling programs for eczema in children and adults. Also located at the Department of Dermatology and Allergy TUM is the Clinical Research Division of Molecular and Clinical Allergotoxicology (Head: M. Ollert), one of the five German national centers of excellence in allergy research funded by the German Ministry of Education and Science (BMBF). The ZAUM brings together different disciplines to study the influence of environmental factors upon development, elicitation and maintenance of allergic reactions. In the 6th framework of the European Union, both institutions have been established as “Centre of Excellence for Allergy” in the European Network for Centres of Excellence “Global Asthma and Allergy European Network GA^{2}LEN”.

Within our *Genotyping Platform* (S. Weidinger, T. Illig) identification of genes that may act specifically on a given phenotype or subphenotype is performed in a tandem approach using a population-based cohort of adults (KORA S3, 1407 adults), a case-control population for AE (KORA S4, 554 adults) and a affected offspring-parents-trio cohort (currently 250 families). Genotyping is carried out at the Genome Analysis Centre of the GSF-National Research Center for Environment and Health. Statistical analyses is performed by the Institute of Medical Statistics and Epidemiology (IMSE) of the Technische Universität Munich (S. Wagenpfeil).

Within our *Gene Validation Platform* (T. Jakob, M. Ollert) functional studies are carried out to evaluate the effects of polymorphisms on gene expression and protein function using sequencing and cloning experiments as well as expression profiling of lesional and and non-lesional tissue. In addition, murine models of AE are used to functionally validate observations obtained in the genotyping platform. For examinations on genetic control of epidermal intergrity, we use murine and human tissue culture skin models of fully reconstructed epidermis on a de-epidermized dermis (RE-DED-model) using the interfering RNA (siRNA) technology.

Both platforms closely cooperate with several partners within the NGFN Network. Material is continuously exchanged with projects NUW-S02T03 (Y. Lee, Berlin), NUW-S23T04 (S. Schreiber, Kiel) and NUW-S31T01 (M. Kabesch, Munich) as well as with the "German Mouse Clinic (GMC)", a worldwide unique phenotyping center within the systematic methodologic platform (SMP) "Mammalian models for inherited diseases in man" (7) located at the GSF Center for Environment and Health in München-Neuherberg (coordinated by M. Hrabé de Angelis). Joint core strategies for analysis on atopy and atopy-related phenotypes have been set up in close cooperation with project NUW-S31T01 (M. Kabesch, Munich).

Results/Project Status

STAT6: Several studies have shown linkage of chromosome 12q 13-24 with atopy-related phenotypes. Among the candidate genes in this region is *STAT6* (“signal transducer and activator of transcription”), which is essential for Th2-cell differentiation, recruitment and effector function. We evaluated 6 previously identified polymorphisms of *STAT6* for evidence of associations with serum IgE levels and atopic diseases within our population-based cross-sectional cohort of 1407 German adults (KORA S3). Genotyping was performed using MALDI-TOF MS. A significant association with total serum IgE levels ($p = 0.015$) was observed for one polymorphism in intron 2 (rs324011) where two transcription factor binding sites for NFκB are found close to each other suggesting a regulatory function of this region for the transcription of *STAT6*. In addition, a *STAT6* risk haplotype for elevated IgE levels showing odds ratios of 1.7 ($p=0.015$) for IgE cut-off 100 kU/l, and 1.54 ($p = 0.032$), 1.6 ($p = 0.025$), 2.54 ($p = 0.007$) for IgE percentiles 50%, 66%, 90%, was detected. The increased risk of this haplotype was strongly confirmed by linear haplotype trend regression on log-transformed IgE values ($p = 0.007$). Analyses further revealed a risk haplotype for specific sensitization and a risk haplotype for asthma. Thus, it could be demonstrated that *STAT6* polymorphisms play a role in IgE expression and thereby are a factor in expression of the allergic or "atopic" state (8). The findings of this study have been highlighted by the Faculty of 1000 Biology (http://www.f1000biology.com).

IL-18: Human IL-18 is a potent proinflammatory cytokine that plays a role in atopic diseases by enhancing the IL-4 and IL-13 production and stimulating the synthesis of immunoglobulin E (IgE). The gene for human IL-18 is located

on chromosome 11q22.2-22.3, a region that has been linked to atopy-related traits. *IL18* polymorphisms have been reported to be associated with specific sensitisation and allergic rhinoconjunctivitis. To evaluate associations with AE, we genotyped SNPs in the regulatory region of the *IL18* gene in 225 AE patients and 175 healthy controls. Analysis revealed significant associations of SNPs 113G and 127T in Exon 1, and -137C and -133G in the promoter regions with AE. On the functional level active IL-18 in the sera of AE patients was enhanced at disease exacerbation. Furtheron IL-18 in the supernatant of PBMC of AE patients stimulated with *S.aureus* enterotoxin B was significantly higher than in controls. Our data suggest that SNPs in the regulatory region of the *IL18* gene might be involved in the development of AE by contributing to a dysregulation of the IL-18 production (9).

Chymase: Mast cell chymase is a proinflammatory serine protease abundantly expressed by dermal mast cells. Expression of chymase is decreased in non-leasional skin of AE patients and further decreased in leasional skin suggesting a role of chymase in suppressing skin inflammation. Association analyses between the promoter polymorphism rs1800875 in the chymase gene (*CMA1*) and atopy-related traits have yielded inconsistent results. Therefore we sequenced the *CMA1* locus in 24 unrelated healthy individuals with serum IgE levels < 50% percentile and 24 individuals with AE and serum IgE levels > 90% percentile. 7 *CMA1* SNPs were evaluated for associations with atopic phenotypes within our large population-based cohorts of German adults (n=1875). Polymorphism rs1800875 showed significant association with AE. No associations between any other SNP and atopic traits could be detected. These results confirm previous observations of a significant association between the *CMA1* promoter polymorphism rs1800875 and AE, but not with other atopic phenotypes, and support the hypothesis that *CMA1* serves as candidate gene for AE (10).

NOD1 and NOD2: Bacterial cell wall muramyl peptides can signal via recently discovered intracellular pathogen-recognition receptors of the innate immune system, NOD1 (CARD4) and NOD2 (CARD15). Interestingly, for polymorphisms of both *NOD1* and *NOD2* associations with susceptibility to Crohn's disease, a predominantly Th1-mediated condition, have recently been shown (11, 12). In a tandem approach using our large population-based cohort of German adults (n=1417), a case-control population for AE (n=454), and our cohort of parent-offspring trios for AE (189 trios), we could show that genetic variants within *NOD1* (13) as well as *NOD2* (14) are also important determinants of atopy susceptibility. In the case of *NOD1* it is noteworthy that one polymorphism which showed replicated association with atopic traits is located in intron 9, where two putative transcription factor binding sites for Pax1 are found, and alters Pax1 binding probability. These results strengthen the hypothesis that inflammatory barrier diseases (eg inflammatory bowel disease, asthma, AE, psoriasis) share disease genes and regulatory mechanisms in the interaction between susceptibility and trigger factors and underline the importance of variations in innate immunity genes for both Th1- and Th2-mediated immunoregulatory disorders.

Outlook

Together with partners and based on previous findings, associations of polymorphisms in various "innate immunity genes" with atopic traits are subject of ongoing investigations. Special emphasis will be put on investigations on the role of Toll-like receptors in the pathogenesis of AE (15). Subsequent analyses of gene-gene- and gene-environment-interaction are planned. We further aim at testing the effects of variations found in the coding regions of examined genes on the functionality of the resulting proteins both in murine models and in reconstituted human or murine skin equivalents.

In a joint project with the Wellcome Trust Center of Hunman Genetics the epidermal differentiation complex (EDC), which is located on chromosome 1q21, a region that has been linked to both atopic eczema and psoriasis, will be evaluated. EDC genes are expressed late during maturation of epidermal cells and are therefore positional candidate genes for linkage to this locus. Together with international partners whole genome association studies are currently being planned in order to identify new candidate genes for AE.

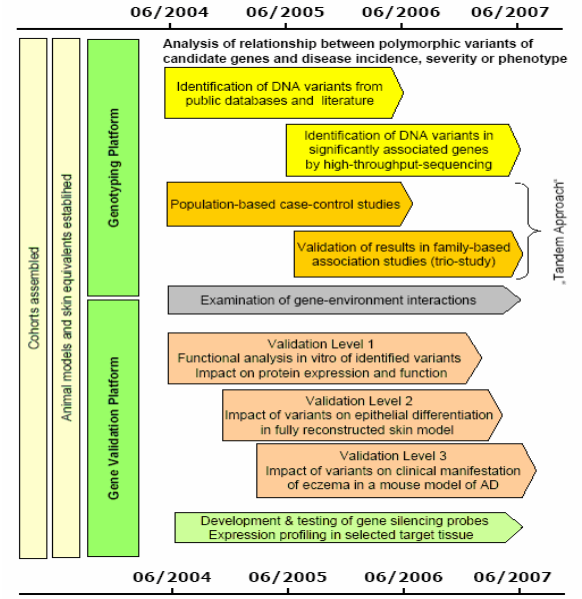

Fig 1: *Genetic basis of pathophysiological and dermatological phenotype expression in atopy and atopic eczema (Project NUW-S31T05). Timelines.*

Lit.: **1.** *Leung DYM, Bieber T. Atopic dermatitis. Lancet. 2003;361:151-160.* **2.** *Ring J, Kramer U, Darsow U, Behrendt H. Why are allergies increasing? Curr Opin Immunol* 2001;13:701-708. **3.** *Cookson WO, Moffat MF. The genetics of atopic dermatitis. Curr Opin Allergy Clin Immunol 2002;2:383-387.* **4.** *Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. J Allergy Clin Immunol 2003;112:252-262.* **5.** *Weidinger S, Lee Y. Standard operational procedures for phenotyping atopic dermatitis. NGFN 2003.* **6.** *Kramer U, Weidinger S, Darsow U, Mohrenschlager M, Ring J, Behrendt H. Seasonality in symptom severity influenced by temperature or grass pollen: results of a panel study in children with eczema. J Invest Dermatol 2005;124:514-523.* **7.** *Gailus-Durner V et al. Introducing the German Mouse Clinic: Open access platform for standardized phenotyping. Nat Methods 2005; 2: 403-404.* **8.** *Weidinger S et al. Association of a STAT 6 haplotype with elevated serum IgE levels in a population-based cohort of caucasian adults. J Med Gen 2004;41:658-663.* **9.** *Novak N, Kruse S, Potreck J, Maintz L, Jenneck C, Weidinger S et al. Single nucleotide polymorphisms of the IL18 gene are associated with atopic eczema. J Allergy Clin Immunol 2005;115:828-833.* **10.** *Weidinger S et al. Association study of mast cell chymase polymorphisms with atopy. Allery 2005; in press.* **11.** *Ogura Y et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603–606.* **12.** *McGovern DP et al. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease.Hum Mol Genet 2005;14:1245-12450.* **13.** *Weidinger S et al. Association of NOD1 polymorphisms with atopic eczema and related phenotypes. J Allergy Clin Immunol 2005;116:177-184.* **14.** *Weidinger S et al. Association of CARD15 polymorphisms with atopy-related traits in a population-based cohort of Caucasian adults. Clin Exp Allergy 2005; 35:866–872* **15.** *Köllisch G et al. Various members of the Toll-like receptor family contribute to the innate immune response of human epidermal keratinocytes. Immunology 2005; 114: 531-541.*