635 Association of Toll-Like Receptor 6 (TLR6) Polymorphism Pro249Ser with Asthma Phenotypes

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RATIONALE: Toll-like receptor 6 (TLR 6) is an important receptor that mediates interaction with fungal components. Fungal allergens have been associated with asthma susceptibility and severity. The association of genetic polymorphisms in TLR6 with asthma phenotypes remains to be clarified although there are a few reports about it.

METHODS: The polymorphism Pro249Ser in TLR6 was genotyped in an asthmatic population recruited for a healthy home project, and examined for association with allergic sensitization to common allergens, asthma severity and emergency visit and related factors.

RESULTS: The T allele (Ser) in Pro249Ser of TL6 was not significantly associated with asthma severity or allergic sensitization to either all common allergens or to all common fungi in the population. T allele in Pro249Ser of TLR6 was associated with lower risk of emergency visit in asthmatic patients at close to threshold level ($p=0.06$). Asthma patients with T allele had about 5 fold less risk for emergency visit.

CONCLUSIONS: The polymorphism Pro249Ser of TLR6 tends to be associated with asthma emergency visits. The conclusion is being replicated in larger studies.

636 Allergy-causing Mite Identification based on PCR Amplification of their Ribosomal DNA

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RATIONALE: The aim of this study was to set up a system for the species identification of allergy-causing mites (order Astigmata), based on their rDNA sequence.

METHODS: DNA was extracted from different Astigmata species (purified bodies, complete cultures and fecal particles), and rDNA was amplified using Astigmata specific primers. PCR products were cloned and 10 clones from each species were randomly selected for sequencing in order to obtain data on intra- and inter-species variation. A Neighbour-Joining tree (1000 replicates) was obtained after the ClustalW alignment of all full-length sequences. PCR products were digested with *Rsa*I and *Hpa*II restriction enzymes to obtain Restriction Fragment Length Polymorphism (PCR-RFLP) profiles for each species.

RESULTS: rDNA comprising partial 18S and 28S, and full-length ITS1, 5.8S and ITS2 regions was amplified from the main allergy-causing mite species. Analysis of the PCR products showed the presence of a main band in each species. Complete direct sequencing was not possible due to the presence of insertions/deletions due to intra and/or inter-individual variability. Cloning and sequencing of PCR products demonstrated that sequence data can be used for the unambiguous identification of all species. PCR-RFLP profiles were unique for each species and could be used for species identification with no need of sequencing.

CONCLUSIONS: Once the correlation between morphological identification and rDNA sequence is established, molecular biology techniques can be routinely used for domestic mite species identification with no need of observation of adult stages.This system could be useful for quality control of mite extracts intended for clinical use.

637 Recombinant doubly-deglycosylated Der p 1 expression in Pichia pastoris

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RATIONALE: The major HDM group allergen Der p 1, from *Dermatophagoides pteronyssinus,* is one of the most potent of indoor allergens. Heterologous over-expression of mature Der p1 was previously attempted with limited success since N-glycosilation site N16 in proDer p 1 affected to allergen processing. In this study we establish an efficient system for the production of recombinant mature Der p 1.

METHODS: The previously de-glycosilated on site N52 proDer p 1-encoding DNA was mutated at the potential glycosilation site N16 in the pro-region and cloned in plasmid pPICZαA in phase with the α-factor signal sequence to transform *P. pastoris* cells. After induction, the presence of proDer p 1 in the extracellular medium was examined by SDS-PAGE and immunoblotting with polyclonal antibodies and IgE-reactive sera. Product was purified and dialysis in acidic buffer was required for processing.

RESULTS: ProDer p1 was secreted after methanol-induction into culture medium as a non-glycosilated protein of 34 kDa and purified with a final yield of 1.3 mg/l of culture. After purification and during processing by dialysis in acidic buffer, the prosequence was removed to give recombinant Der p 1 of 25 kDa with a yield of 0.265 mg/l of culture. In both cases, the recombinant allergens were recognized by specific IgG and IgE in immunoblotting or ELISA experiments.

CONCLUSIONS: This system of recombinant Der p 1 expression leads the way for the design of new diagnostics for HDM allergy, allergen engineering, structural and immunological studies and new IT-treatments.

638 Variability Introduced into Allergen Immunoassays during the Dust Extraction Process

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RATIONALE: Allergen exposure assessments are routinely based on reservoir dust samples. Specimens are sieved, weighed and extracted before their use in immunoassays. As house dust is not necessarily a homogeneous material, our aim was to determine the effect of dust weights used in extraction on the level of variability in immunoassays.

METHODS: Four bulk dust samples were used in this study. Dust weights of 2mg, 10mg, 25mg, 50mg and 100mg were weighed from each dust sample in triplicate by the same technician. Extracts were prepared of each aliquot at 50mg dust/ml. All 60 samples were analyzed for Der p 1, Der f 1, Fel d 1 and Can f 1 using MARIA. Variability between triplicates was determined using mean and CV%.

RESULTS: Variability was observed between triplicates of all dust weights for all measured allergens. Highest levels of variability were observed for the 2mg dust aliquots, which ranged between 7% and 160% CV between triplicate aliquots. Dust weights of 10-100mg showed reduced variability between triplicates of <30%CV with the exception of Can f 1.

CONCLUSIONS: This study indicates that there is potential for significant variability in allergen measurements that is introduced at the dust weighing stage. The level of reproducibility may differ between specimens, as matrix heterogeneity and allergen content vary. As dust weights of 2mg produced the least reproducible results, allergen analysis should not be attempted with less than 10mg of fine dust.