1. FINAL PUBLISHABLE SUMMARY REPORT

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Asthma is the most common chronic inflammatory disease affecting up to 30 million children and adults in Europe and many more around the world. Due to the complexity of the disease many of the underlying mechanisms are still not well understood. Genetic predisposition as well as environmental factors influence asthma development. Recently, systematic approaches to identify genetic susceptibility factors by means of Genome Wide Association Study were successful. Within the GABRIEL Consortium a novel and major susceptibility locus specifically for childhood asthma was identified on chromosome 17q21, which has now been independently replicated in a number of studies in different ethnicities. *ORDML3*, a gene within this locus, seems to contribute significantly to the observed association with asthma. ORMDL3 is a member of a highly conserved protein family (ORMDL1-3), members of which seem to be expressed in the endoplasmic reticulum as transmembrane proteins. However, insight in the biological function of these proteins is limited and even less is known on their influence on asthma development.

This project aimed to increase our basic understanding of the function of the *ORMDL* gene family *in vivo,* and *in vitro* and to subsequently characterize their role in asthma pathogenesis. The first project phase at the Outgoing host institute, the National Jewish Health Hospital in Denver (USA), focused on studying ORMDL in two mouse strains (C57BL/6, and Balb/c) which are both frequently used in asthma models. While Balb/c mice are relatively hyperresponsive in allergy models, C57BL/6 mice display low airway responsiveness after allergen exposure and differences in the immune response of both strains exist. We observed ubiquitous expression of all three members of the *ORMDL* gene family in all analyzed tissues (brain, gut, heart, kidney, liver, lung, skeletal muscle, spleen, and thymus) and primary cells (isolated from lymph nodes and blood) with comparable baseline expression levels in both mouse strains. When we applied a well established animal model of allergic inflammation and airway responsiveness expression levels of *ORMDL* genes were exclusively altered in asthma-related tissues (lung, spleen, and thymus). Also, the timing of *ORMDL* expression after allergen exposure seems to be critical in this model. We conclude from these experiments that there is evidence that *ORMDL* genes play a functional role in asthma pathogenesis.

During the second phase of the project we initially compared general gene expression patterns of the *ORMDL* family members in different human and mouse tissues including brain, gut, heart, liver, lung, lymph nodes, and spleen. Furthermore, immunocytochemistry was performed in different immunologically relevant human cell lines including B- and T-cells, lung epithelium cells, primary fibroblasts, and cells not directly related to asthma development to determine if ORMDL proteins were detectable and in which cell compartments they were localized. By Westernblot analyses using a specific antibody against all ORMDLs we confirmed the presence of this protein family only in the membranic and the fraction of the endoplasmic reticulum. When analyzing *ORMDL3* gene expression in human primary cells a significant increase of *ORMDL3* expression in cord blood mononuclear cells was detectable following stimulation (innate and adaptive) indicating that *ORMDL3* may be relevant for immune regulation already early in life.

In addition, we have for the first time delineated pathways which may be regulated by the ORMDL protein family as novel protein-protein-interaction partners in living human cells were identified using the BRET (Bioluminescence Resonance Energy Transfer) technology. In total, interactions of 160 proteins against each member of the ORMDL family were tested with proteins representing different cell compartments including cytoplasmic, extracellular,

endoplasmic reticulum, mitochondrial, cell membranic, plasma membranic, peroxisomal, and nuclear proteins. Positive interactions for ORMDL1, ORMDL2, and ORMDL3 were observed for 55 proteins including protein of the endoplasmic reticulum, membranic, plasma membranic, mitochondrial and peroxisomal compartment. Our data indicated that ORMDLs most likely possess similar biological functions due to the fact that properties of interactions between all three members of the ORMDL family and their respective binding partners were overlapping. Intriguingly, a number of positive ORMDL binding partners comprised proteins of immunologically relevant pathways likely involved in asthma development. Hence, we tested the hypothesis if levels of ORMDL affect further downstream effects. Indeed, in human cells we observed a direct influence on the expression of a proinflammatory chemokine *in vitro* after the down-regulation of endogenous *ORMDL1*, *ORMDL2*, and *ORMDL3* through small interfering siRNAs (siRNA).

The knowledge gained from mice experiments in the first phase of this project in combination with the results from these human studies provided a better insight into functional properties of the ORMDL family *per se*. This dual approach improves our understanding of causal mechanisms leading to asthma susceptibility. Hence, this new data may in the future offer the potential to develop novel preventive and therapeutic strategies.

Prof. Dr. med Michael Kabesch
Medical School Hannover
Department of Pediatric Pneumology, Allergy and Neonatology
kabesch.michael@mh-hannover.de
www.asthmagene.de