Network: Obesity and Related Disorders

Project: Potential Polymorphisms of the VLDL Receptor and other Obesity Candidate Genes in Patients with and without Coronary Artery Disease

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Introduction

Coronary heart disease is the leading cause of death in the western population. Data from the Framingham Heart Study has consistently shown that lipid and lipoprotein disturbances are related to increasing degrees of obesity and are accompanied by greater rates of CAD. It also has been shown that polymorphisms/mutations in a number of genes modulating intra/extrahepatic lipid metabolism, contribute to increased triglyceride levels in obese subjects with CAD. One new candidate gene that is poorly studied for its potential in causing obesity and CAD is the very low density lipoprotein (VLDLR) receptor (Fig. 1). The VLDLR is a member of the LDL receptor (LDLR) super- family[1], but unlike the LDLR it only binds ApoE containing lipoproteins. (Chylomicrons, VLDL and IDL) and its expression is limited to fatty acid active tissues (adipose, heart, muscle). Studies with VLDL receptor knockout mice under high fat diet showed impaired fatty acids uptake and cellular triglyceride storage in adipose tissue and a protection from the development of obesity[2]. These data strongly implicate an important role of the VLDL receptor in the development of obesity. Within the NGFN2 we will investigate for the first time the role of the VLDLR and its modulating cofactors (apolipoproteins, lipolytic enzymes) on obesity and CAD. The study population that we will use for this purpose, comes from The „Marburger Präventions-Allianz“, which is a multimodular concept for the prevention of CAD and started 1998 in the Dept. of Cardiology at the University of Marburg. Since this time from each patient who underwent a coronary angiographic examination in our clinic and who is willing to participate, all crucial clinical information reaching from lipid profile, medication, BMI, blood pressure and coronary angiograms are sampled in a database. Furthermore DNA for molecular genetic examinations is available in an anonymous database and used for the screening of gene mutations that contribute to atherosclerosis development (eg. defects in ApoB/E and the LDL receptor). In combination with the clinical data, results from genetic examinations generate a well defined cardiovascular risk profile of the patients. In the last years a number of novel genetic defects were identified on the basis of the „Marburger Präventions-Allianz“- database. These included a very frequent german ApoB-100 mutation (ApoB-H3543Y)[3] and a novel mutation in the cytochrome P450 epoxygenase (CYP2J2) gene[4]. Furthermore combinations of various SNP´s such as the Apo A5 S19W polymorphism together with the ApoE2/2 genotype and the combination of two polymorphisms within the osteoprotegerin gene promoter were identified, and appeared to be more frequent in subjects with hyperlipidemia or CAD respectively [5,6]. The findings of the last years showed that the „Marburger-Präventions-Allianz is an attractive tool when screening for novel genetic variations and their relevance for coronary artery disease (CAD). Within NGFN2 our major research goal is the identification of novel alleles/genotypes relevant for obesity and CAD in a number of candidate genes encoding apolipoproteins, as well as in genes encoding proteins known to regulate intra/extrahepatic lipid metabolism. The genotypes in these candidate genes may explain lipid and lipoprotein disturbances relevant for obesity and atherosclerosis.

Structure of the VLDL receptor: A member of the LDLR super family

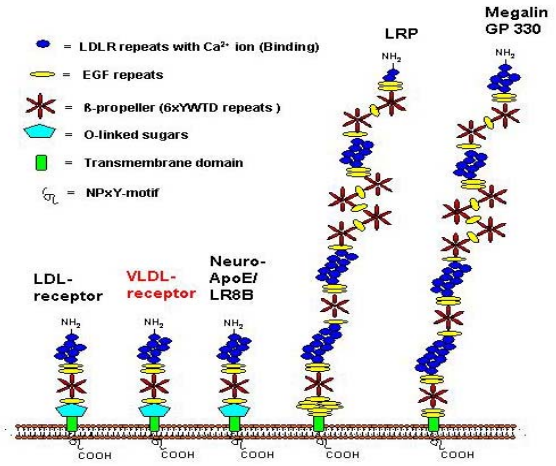

Fig 1: Modular domain architecture of the VLDLR. The VLDLR is a member of the LDLR super family. The only difference to the LDLR is an additional ligand binding repeat. Unlike the LDLR, expression of the VLDLR is limited to fatty acid active tissues (adipocytes and muscle).

Recently it has been shown that activity and binding of ApoE-containing lipoprotein-particles to the VLDL receptor is modulated in a complex together with Lipoprotein lipase (LPL) (Fig. 2). Several possible functions of the VLDL receptor have been reported in atherosclerosis, obesity/insulin resistance and cardiac fatty acid metabolism. Studies with VLDL receptor knockout mice (VLDLR -/-) under high fat diet showed an impaired uptake of fatty acids by adipose tissue and a protection from obesity in these animals

Mechanism of triglyceride delivery to tissues by the VLDL receptor:

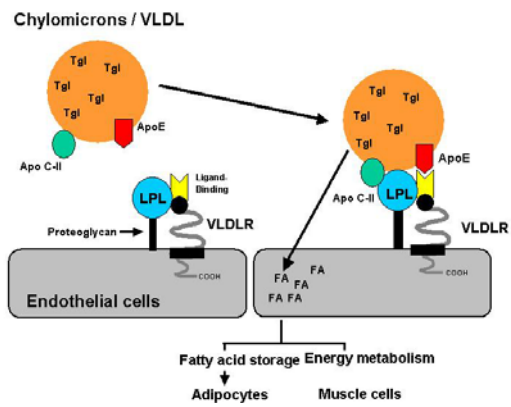

Fig 2: Mechanism of triglyceride hydrolysis and fatty acid delivery to tissues by the VLDLR. In this model triglycerides are hydrolysed from VLDL and Chylimicrons upon binding of ApoE to the VLDLR and subsequent action of proteoglycan bound lipoprotein lipase (LPL) on endothelial cells. In this process Apo C-II serves as an activator of LPL.

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Project Status

As a new project within the NGFN2 we started to evaluate the role of VLDLR polymorphisms/mutations on obesity and CAD in the study population utilizing Denaturing Gradient Gel Electrophoresis (DGGE) techniques. In a first approach we designed primer pairs that covered the complete VLDL receptor gene and its promoter sequence for the DGGE screening procedures. In a method evaluation study we constructed a series of in vitro generated control mutations (Transitions/transversions and deletion/insertions) at different positions within the VLDLR gene to test the reliability of our DGGE system. Our data with this mutated control fragments showed that all constructed mutations were detected by the system and that the DGGE- mutation screening system is a reliable mutation detection system for the subsequent screening studies of our selected candidate genes. Based on this findings we continued with our screening ofubjects from the database of the „Marburger Präventions-Allianz“ (which at present now consists of more than 5000 subject). So far only two known non-synonymous SNPs have been identified within the VLDR gene. To assess whether there are putative VLDLR mutations that are associated with CAD and obesity, we first screened the complete VLDLR gene (19 exons and promoter) in 129 preselected subjects (BMI < 25; triglycerides >150 mg/dl) from the collection of the „Marburger-Präventions-Allianz“. DGGE based screening detected a total of 6 mutations, 4 of which were new (Fig. 3.) and located within functional conserved domains of the VLDLR. In addition screening of 135 subjects from a control group (BMI < 25; triglycerides <150 mg/dl) revealed that three of the novel mutations were not detectable in this group. This data strongly point out that there are more non-synonymous mutations within the VLDLR gene than previously detected and published within the Ensembl database. In additional experiments, genotyping of 6 SNPs within the VLDLR gene in all identified mutation carriers revealed that all had an individual VLDLR genotype.

DGGE-screening of the VLDLR gene

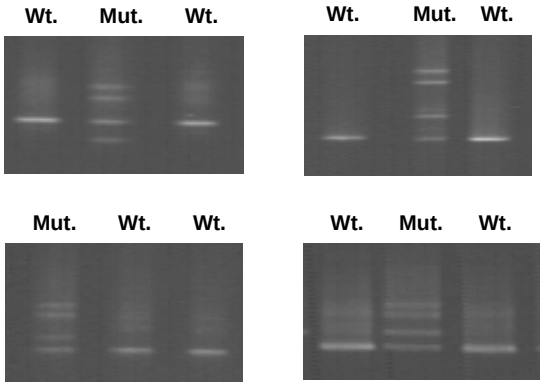

***Fig 3**: Detection of novel mutations in the VLDLR gene by DGGE. The gels show wildtype (Wt) and carriers of heterozygous mutations (Mut) from the study population of the „Marburger Präventions-Allianz“ that lie within functional domains of the VLDLR.*

Outlook

Our preliminary screening data of the complete VLDLR gene with only a minor preselected subset of the study population from the „Marburger Präventions-Allianz“, so far detected 4 novel mutations that are all located within functionally important regions of the VLDLR, thus leading to impaired VLDLR function and maybe a protection from the development of obesity in the identified subjects. In addition we conclude from our preliminary data that all of this mutations cluster within a particular region of the VLDLR gene in our study population, which restricts the screening of the complete gene to a subset of a few exons in a high-throughput screening approach with the remaining study population. At present we study the role of the detected mutations on impaired VLDLR function in various in vitro assays (eg. like with defective LDL receptors in familial hypercholesterolemia), these include transfection experiments with the mutated receptors to monitor the uptake of labelled lipoproteins and the cellular location of defective receptors Fig. 4. These data will provide information about the underlying mechanism that might protect from obesity and subsequently may used for a targeted drug therapy against the VLDLR in obesity.

Lipoprotein uptake and detection of normal and defective lipoprotein receptors in cells:

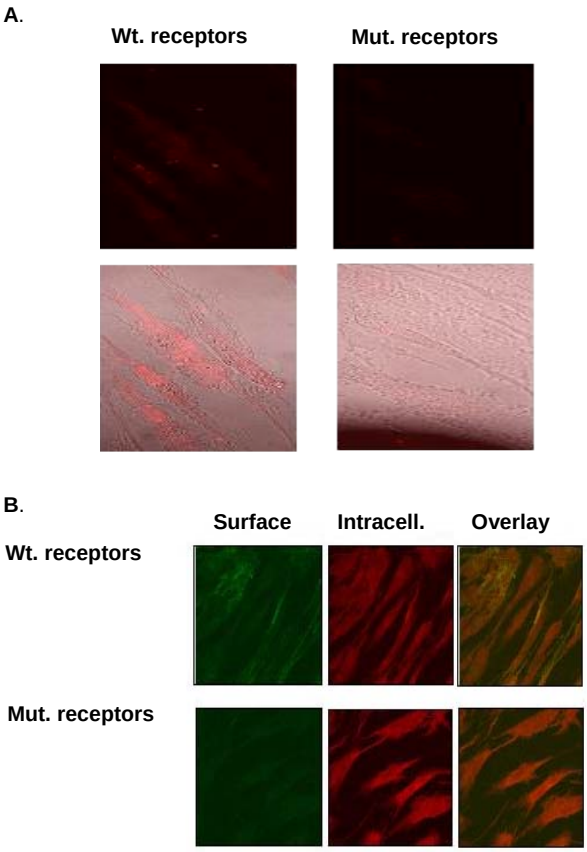

***Fig 4**: **A.** Cellular uptake of fluorescence labelled lipoproteins by cells with normal or defective lipoprotein receptors. **B.** Detection and cellular location of defective lipoprotein receptors in cells by immunofluorescence experiments. The images show wildtype and FH-fibroblasts with a transport defective LDLR. Similar experiments will be used to characterize the novel detected VLDLR mutations.*

*Lit.: **1.** Sakai J et al. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. J.Biol Chem.1994;269:2173-82. **2**. Goudriaan JR et al. Protection from obesity in mice lacking the VLDL receptor. Arterioscler Thromb Vasc Biol.21:1488-93. **3.** Soufi M et al. Description of a new but frequent mutation of ApoB-100- apoB His3543Tyr. Atherosclerosis. 2004; 174:11-16. **4.** Spiecker M et al. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. Circulation. 2004;110 :2132-6. **5.** Schaefer JR et al. Hyperlipidemia in patients with apolipoprotein E 2/2 Phenotype: Apolipoprotein A5 S19W mutation as a cofactor. Clin Chem. 2004; 50:2214. **6.** Soufi M et al. Osteoprotegerin gene polymorphisms in men with coronary artery disease. J Clin Endocrinol Metab. 2004;89:3764-8.*

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