PROJECT PERIODIC REPORT

Grant Agreement number: 603091

Project acronym: TRANSCARD

Project title: Translating disease to cardiovascular health

Funding Scheme: Collaborative project

Date of latest version of Annex I against which the assessment will be made: 03/11/2014

Periodic report: **1st** ☐ **2nd X** **3rd** ☐ **4th** ☐

Period covered: **from** 01/03/2015 **to** 31/08/2016

Name, title and organisation of the scientific representative of the project's coordinator:
Dr. Jan Albert Kuivenhoven, Academisch Ziekenhuis Groningen

Tel: +31 503632687

Fax:

E-mail: j.a.kuivenhoven@umcg.nl

Project website address: www.transcard-research.org

Publishable summary

TransCard is a collaborative effort between 2 industrial and 4 academic partners in 4 European countries, i.e. Switzerland (UZH and Nebion; lead small medium enterprise), Germany (PolyQuant; second small medium enterprise), Denmark (REGIONH) and The Netherlands (UMCG, AMC) from which the work is coordinated.

IN DEPTH STUDIES OF INTIALLY CHOSEN CANDIDATE GENES

After three years, the TransCard consortium is extending studies into the most promising candidate genes that have been selected at the start of the project.

One of these genes is **COMMD1** as it was just discovered that this protein is required for normal trafficking of the low-density lipoprotein (LDLR) to the membrane of hepatocytes, which in turn controls the levels of circulating low-density lipoprotein (LDL) cholesterol. Additional investigations have been published in 2016 which showed that COMMD1 acts in concert with members of the CCC complex and others. The function of these protein complexes in endosomal cargo sorting (such as LDLR) is evolutionary conserved and is shown to control plasma cholesterol concentration in mice, dogs and humans. Further genetic epidemiological studies into the relation between COMMD1, lipid traits, and clinical endpoints of ischemic disease by REGIONH were unfortunately hampered by the lack of powerful genetic tools but alternative approaches to study the genetics of this family of proteins and its binding partners are being explored. Ongoing studies indicate that we have multiple tools to study the endosomal sorting of lipoprotein receptors which control plasma lipoprotein levels. These studies are supported by targeted proteomic analyses which were made possible through PolyQuant and the UMCG that provides the mass spectroscopy expertise and machinery. The ongoing basic research studies are anticipated to increase our understanding of cholesterol and lipid homeostasis at the cellular and systemic level.

LRP1 was a second gene that was picked up for study at the start of TransCard. It concerns a long-established gene which is thus far almost exclusively studied in mice and in cell culture. Through a collaborative effort between AMC, UMCG, we show that functional mutations in LRP1 in humans are likely causally related to low high HDL cholesterol. While LRP1, a member of the LDLR protein family, is mostly known for its function in the clearance of remnant (triglyceride-rich) lipoproteins, these investigations show that one of the other ligands of LRP1 affects the expression of major players in HDL metabolism, i.e. ABCA1 and SRB1). REGIONH has corroborated the finding that LRP1 is associated with HDL cholesterol and also shows an association with triglycerides in a large genetic epidemiological study.

Early on, TransCard's lead small medium enterprise, NEBION, identified **Gene 1** (in collaboration with UMCG) as an interesting novel lead gene through in silico analyses. Soon after our discovery, **Gene 1** was also identified through genome wide association studies to be associated with total cholesterol levels. Studying whole body knock-out mice by UMCG shows interesting lipid and other phenotypes while REGIONH showed that rare variation at the respective gene locus is associated with the major protein component of LDL. In view of the promising data thus far we will continue our studies in both humans and mice.

STAP1 was identified by the AMC as a novel candidate gene for familial hypercholesterolemia in several families. Interestingly, this gene is expected to exert a function in cells of the immune system (B cells). If we can prove that there exists a causal link between loss of STAP1 and hypercholesterolemia, this would be a breakthrough. In the AMC, blood products of affected individuals and unaffected family members are currently studied. Genetic studies of STAP1 by REGIONH were hampered by lack of powerful genetic tools. The UMCG has generated Stap1 knock-out mice but the first results are negative. We are currently challenging these mice with an inflammatory diet as well as a hepatic knock down of the LDLR.

NOVEL CANDIDATE GENES

In 2016, TransCard has also started studies into the role of two novel candidate **Genes 2 and 3**. Gene 2 was identified by NEBION through additional mRNA co-expression analyses while gene 3 was identified through literature studies. For both genes, the UMCG is generating whole body knock-out mice. This has been successful for gene 3, while for gene 2, we have not yet obtained homozygous knock-out mice. Preliminary studies show that complete loss of gene 3 is associated with increased cholesterol levels. We are currently investigating whether gene 3 products are affected in a rare human disorder that is studied within the UMCG.

REGIONH furthermore identified (in collaboration with UMCG) **Gene 4** as candidate gene for further study.

Through studies in families, the AMC identified **Gene 5** as a gene involved in accelerated development of atherosclerosis. Preliminary findings suggest a role of its gene product in vessel integrity. In addition, AMC has collected precious materials of patients with various dyslipidemias and premature atherosclerosis which will be studied at the gene, mRNA, protein and metabolome levels.

In a drug screen for genes that control the transport of lipoprotein across in cells by UZH, **Genes 6 and 7** were identified and validated as players in this important phenomenon in atherogenesis. Studies in mice are being considered. Finally, we look forward to the result of a massive whole genome RNAi based screening effort for genes that are involved in the uptake of HDL and LDL in a hepatoma cell line by the same partner. This screening will undoubtedly reveal new insights that will become important to TransCard.

TECHNICAL ADVANCES

The new projects will need quick validation steps in view of only two years till the end of the project. We have therefore requested for prolongation of TransCard by six months.

NEBION has improved their power to identify new genes after curating additional publicly available mRNA datasets (incuding RNAseq) and is continuously developing tools to prioritize genes for further study.

Our studies will also benefit from other technical improvements in TransCard. The use of CRISPR/Cas9 to quickly generate whole body knockout mouse models has recently been extended by the possibility to quickly study the impact of liver specific ablation of novel candidate genes. The latter will be important in that it renders outcome in a 3 month time period. Further support comes from targeted proteomics studies, enabled by UMCG and PolyQuant, that will provide rapid insight into changes in multiple proteins involved in cholesterol and beyond. In addition, REGIONH will have expanded their repertoire of variants in candidate genes through further sequencing efforts. This will help to better select and prioritize genes of potential interest for large scale studies in humans.

CONCLUSION AND OUTLOOK

TransCard is well underway with studies in multiple candidate genes with proven or yet anticipated roles in the regulation of plasma lipids and lipoproteins. Most of our work is directed to get better insight into the molecular aetiology of genotype-phenotype relations. It is too early to speculate whether this knowledge can be directly used to develop pharmaceutical intervention strategies but of all genes studied thus far, **Gene 1** is likely most targetable of all.