CHeartED

News Bulletin
March 2011

What causes cardiovascular malformations?
Can cardiovascular malformations be prevented?

To find an answer to these questions, CHeartED focuses on the interaction between genes and environment in the development of heart defects.

Cardiovascular malformations (CVM) are the commonest birth defect. In the European Union, 51,000 infants are born with CVM each year. Two million people are affected and CVM is the commonest cause of childhood death. Despite intensive research, the cause of 80% of CVM remains elusive. The CHeartED project is based on the idea that interactions between genetic and environmental factors play an important role in the development of CVM.

In this News bulletin professor Heather Cordell of research line 1c and professor Wout Lamers of research line 2a give an update on their part of the project.

Genome wide association studies in Tetralogy of Fallot

Professor Heather Cordell heads the statistical genetics group in the Institute of Human Genetics of Newcastle University. She leads work package 1c which is part of the Genetic Epidemiology line.

Cordell: We study genetic factors as well as the interaction between genotypes and the environment. We hope to identify novel factors which can be modulated in the population or in high-risk families. Folate is such an example: sufficient intake of folate during pregnancy, considerably reduces the incidence of neural tube defects.

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Gene- Environment Interactions in Heart Development

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COOPERATION

Professor
Heather Cordell

Our approach is to look genome wide. Our genome wide association studies (GWAS) will help us to identify novel genetic factors. We hope to identify both associations with cardiac developmental genes and associations with genes in pathways directly affected by the environment. The results will give an indication of the total contribution of common genetic variation to CVM. Having this knowledge could help to reduce the incidence of CVM.

An important decision in our study design was whether to pool all cardiac malformations or to select a single one. Pooling samples could reduce the power to detect associations. We therefore decided to focus on tetralogy of Fallot (TOF).

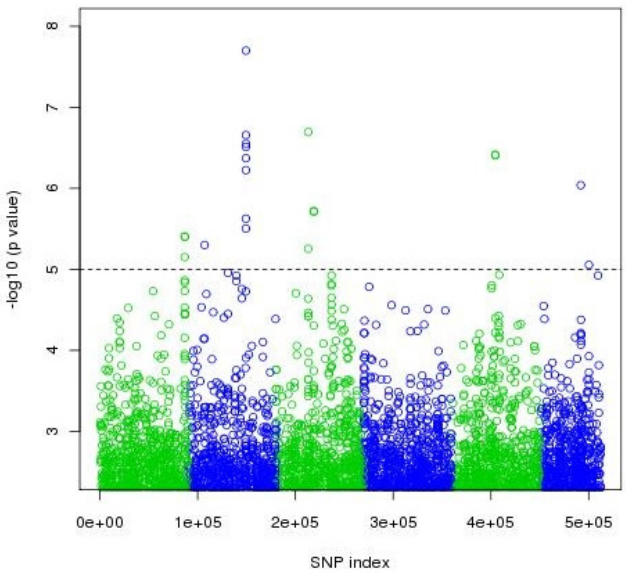
Within the Genetic Epidemiology line we work closely together: work package 1a gathers and prepares the samples, work package 1b performs the high resolution genotyping and in work package 1c we perform statistical analysis on the data in order to detect genetic associations with CVM.

We have genotyped 979 TOF cases, together with 754 of their parents. We used a chip – the Illumina Huan660W-Quad v1 DNA Analysis BeadChip – that contains nearly 660,000 single nucleotide polymorphism (SNP) markers that are evenly spaced throughout the genome. This chip can also detect copy number variants (CNVs) that may be major contributors in the aetiology of malformations. The chip provided us with genotype data for each individual. We did extensive quality control checks in order to retain only reliable samples and SNPs.

This resulted in a final set of 843 TOF cases for analysis, of which 320 had both parents genotyped. We analysed these 320 child-parent trios using the transmission disequilibrium test (TDT), a test that reveals alleles that are significantly over-transmitted from heterozygous parents to affected children.

To find significant differences in frequency, we also compared the 843 TOF cases with 5163 controls. These population based controls were available from the Wellcome Trust Case-Control Consortium. Each test was performed at a subset of about 500,000 SNPs. These SNPs had passed stringent quality checks.

A Manhattan plot of the results from the case/control analysis reveals several genomic regions showing suggestive significance. We will follow up these regions, together with the regions identified from the TDT analysis, by replication genotyping. We will use an independent sample of cases and controls. This will reveal which regions are genuinely associated with disease and thus worthy of more detailed bioinformatic and functional investigation.



GWAS Manhattan plot showing the strongest associations that have the smallest P-values, *i.e.* the greatest negative logarithms of the P-values.

Diabetes during pregnancy causes congenital malformations

Diabetes during pregnancy of the mother significantly increases the risk of congenital malformations in her children. In research line 2a, professor Wout Lamers of the Amsterdam Medical Center in the Netherlands, works with his team on a mouse model to study changes in gene expression caused by hyperglycemia. Lamers: Diabetes during pregnancy can affect any developing organ system of the embryo, but defects of the neural tube and heart are among the most common.

In this project, we will establish the gene-expression profile of embryos of diabetic mice. We look in the period that the embryos are sensitive to the teratogenic effect of a high glucose level in de mother. We can make our mice diabetic with a drug called streptozotocin. In these mice the glucose level increases and we can lower it by treating the mice with insulin. After two weeks of insulin treatment, the mice are mated. During pregnancy the glucose level increases again. We sacrifice the mice at 7.5-10.5 days of pregnancy and isolate the embryos.

The diet is critical. If we feed the mice a diet which is low in fat and high in antioxidants (*e.g.* vitamin A and E), only 1 embryo out of 192 showed an unclosed neural tube on day 10.5 (0.5%). However, if we feed the mice a diet which is high in fat and low in antioxidants, we observe malformations in 13% of the embryos.

Jing Zhao
PhD student

This rate of malformations is identical to that published in literature. The malformed embryos had a neural tube defect, an enlarged pericardial cavity, myocardial apoptosis in the atrium, and/or were retarded in development.

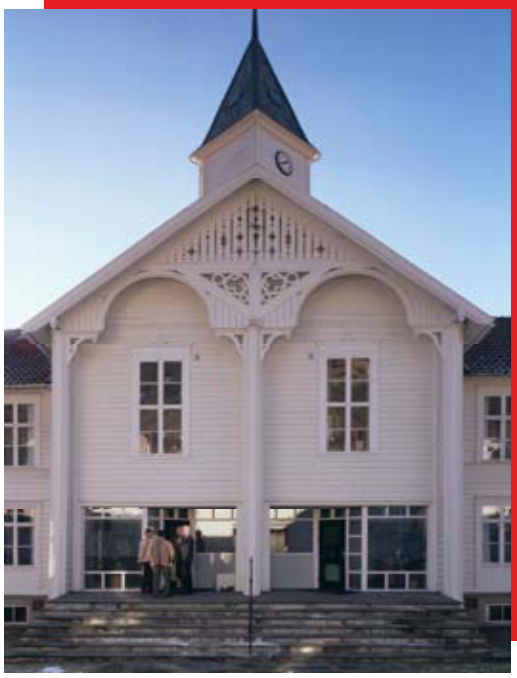
We will now determine changes in gene expression due to exposure to hyperglycemia in embryos of 8.5 and 9.5 days, *i.e.* during the 2 days following the presumably sensitive period at day 7.5. We have collected 116 embryos that were exposed to maternal hyperglycemia and 66 control embryos. 23% of the former group showed growth retardation. Because male embryos are more sensitive than female, we have selected the embryos having the male sex-determining gene Sry. For our studies we use embryos expressing low levels of *Pax3* and *Glut4* mRNA, markers that were previously shown to be associated with diabetic pregnancy.

To establish the prevalence of affected genes we will separate mRNAs and microRNAs of the embryos and analyze them by deep sequencing.

New Training, Mobility and Dissemination Manager

Nanna Claij has taken over the dissemination related issues of CHeartED from her colleague Hubert the Leeuw. Nanna: “Since January 2008 I am employed at the Netherlands Heart Foundation. As research manager I keep in contact with the scientists that receive funding of the Heart Foundation. I am looking forward to extend my tasks with dissemination activities for CHeartED.”

2nd Annual Meeting in Bergen, Norway May 18-19, 2011



The meeting will start around 14.00 h on May 18 and will end around 15.00 h on May 19, 2011.

The meeting will be held at Kalfarveien 31 in the “Bjerkedalsrommet”.

Rooms for CHeartED are reserved at the Grand Terminus Hotel, which is quite close to Kalfarveien 31. You may book them at the Hotel webpage: http://www.ght.no/en/about/

If you take the Airport bus you may ask the driver to stop outside the Bus terminal, which is close to the hotel. Link to time table: http://vitaminw.no/kunde/flybussen/FilVedlegg/Flybussrute_010211.pdf .

General remarks for CHeartED consortium

Website

• We ask you to check the information on the website. If you have any adjustments, please notify the dissemination manager.

• Visit the open-access CHDwiki via the CHeartED website. The Wiki contains lots of information about genetic and environmental knowledge on heart development. We invite you to take a look at the website and actively help to enlarge the database.

Dissemination

• To keep all consortium members informed, we ask the beneficiaries to announce CHeartED related meetings, courses, workshops etc. on the website.

• To share publications related to CHeartED with all consortium members, please upload all publications on the website.

To upload events or publications yourself, you have to login first. You may also ask the dissemination manager.

Do not forget to mention EU support in all publications, courses, workshops etc. as follows:

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