

DIRECT
DIABETES RESEARCH ON PATIENT STRATIFICATION

Diabetes — a complex disease

Dr Ian Forgie, University of Dundee

The DIRECT project is not actually a single study but in fact a series of 7 different studies. Each study is designed either to examine how people develop type 2 diabetes, how the disease progresses in different individuals or why some people respond well to certain treatments while others do not. Ultimately our hope is to identify traits or "biomarkers" in individuals that can predict whether or not they will develop diabetes, how rapidly their disease may progress and what treatment is most likely to be of benefit to them. This is the goal of so called personalised or 'stratified' medicine whereby people are separated into different groups—with medical decisions and interventions being tailored to the individual patient based on their predicted response to, or risk of, disease.

Two of the DIRECT studies recruited over 3000 people attending 6 different specialist centres across Europe who had either been recently diagnosed with diabetes or were considered to be at high risk of developing the disease. The original plan was to monitor these individuals for up to 3 years to see how they fared and to examine them intensively every 18 months to discover if, and how, the disease had developed. At each visit the individual was given a full clinical examination, lots of samples were collected from them, and their blood sugar levels after consuming a liquid "meal" were examined. In addition, details of their diet were taken and their level of activity and exercise was assessed.

It soon became apparent that in most participants evidence of clear disease progression appeared more slowly than had originally been anticipated. For this reason the two studies were extended in order to be able to monitor participants for up to 4 years rather than just 3 years in order to improve the possibility of detecting clear evidence of developing disease.

There are several reasons why evidence of disease progression may have taken longer than expected. For example, our original estimates of how fast diabetes develops in the 'at risk' individuals we invited to join the study may have been mistaken. Alternatively we may not have given sufficient allowance to the impact of participants starting anti-diabetic drugs - or improving their diet or increasing their exercise because they knew they were taking part in a diabetes study.

It is clear from our preliminary findings, and those of previous studies, that Diabetes is a complex disease with many interrelated causes and factors to be considered. For example, the prime reason for high blood sugar levels in some individuals seems to be their high degree of obesity or unhealthy levels of one or more kinds of fat in their blood or how their fat is distributed. In other people, including those who are less obese, it appears the main reason is that they have stopped producing enough insulin (due a deterioration in the function of 'Beta cells' in their pancreas) whereas in yet others it seems there is enough insulin but its impact on their body is less than it once was (insulin resistance). In many people, perhaps most, it is a combination of these factors that leads to their illness. More information on these features is provided in a separate article in this newsletter that uses a 'tree-like' graph to illustrate the different groupings. By investigating the genes, proteins, metabolism and other characteristics of individuals within these groups we hope to be able to identify particular markers that may help doctors understand how best to treat or delay any evidence of diabetic disease.

DIRECT researchers gathered in Vienna in May 2018 to discuss the project's progress.

Getting in touch:

'The Direct Project'

@DIRECTdiabetes

http://www.direct-diabetes.org/

You probably have heard about the **General Data Protection Regulations (GDPR),** a new law that came into effect in Europe in May 2018.

Why does it matter for scientific research? The GDPR gives you more power over how your personal information is used. It applies to University and Hospital research, as well as to large companies. There are two things that are important to know:

1. You have a right to know how your information is used, as well as rights to object or to have your data deleted.
2. When your information is used for scientific research, your rights might not be applied if it would harm the research.

We hope that you are still happy for your information to be used for the DIRECT project. If you have any questions, please contact your project centre using the email address provided for each Centre on the DIRECT website:

https://www.direct-diabetes.org/information/#gdpr

A DIRECT participant's visit to the Diabetes Centre in The Netherlands

Lenka Groeneveld, PhD student takes us through the journey of a DIRECT participant at the Diabetes Center in Hoorn, The Netherlands.

Between August 2017 and November 2018 staff in the Diabetes Centre at Hoorn in The Netherlands welcomed participants enrolled in the DIRECT-1 (WP2.1) study for their third and final visit - around 48 months after their first visit. This is their experience...

In common with the previous two visits (at 0 and 18 months), all our participants came to the Center with great enthusiasm and curiosity regarding the scientific work being conducted.

Unfortunately for our participants the visit still involved the unpleasant task of drinking the infamous large glass of glucose solution, which had to be consumed within 5 minutes, something that they each bravely endured. After an Intravenous (IV) system had been inserted into their arm our participants then had to spend the next two hours in the waiting room. As soon as the alarm clock rang, they were back in our 'cloud room' for more blood collection.

Following a physical examination and collection of the last set of blood samples it was time for breakfast and coffee, to which all participants looked forward having endured a period of fasting prior to their visit!

We would very much like to thank all our participants for their time, openness and enthusiasm with which they have helped science move a step further forward and made our mornings in the 'cloud room' so enjoyable. No matter what others might say about our typically down-to-earth 'West-Frisian personalities', we hope that our little 'thank you' gifts of flowers blossom as passionately in your gardens as you have contributed to our research.

The DIRECT 'Participant Engagement Survey' results have been published!

Nisha Shah, University of Oxford

In the last newsletter we reported the overall results of the participant engagement survey, which asked DIRECT participants in Denmark, Sweden, The Netherlands, and UK about their preferences for sharing data after the end of the DIRECT project. The results have now been published in the *Genetics in Medicine* journal and is titled "Sharing data for future research: Engaging participants' views about data governance beyond the original project". We would again like to thank the participants who completed the survey. We hope that this work will inform and inspire other scientific research studies to engage and involve their participants in the development of data sharing strategies in their own future research projects.

RESEARCH ROUND-UP

The DIRECT research programme is split up into a number of different studies (called work packages) all of which have been making good progress – here are some updates.

Diabetes progression in newly diagnosed patients

Dr Agata Wesolowska-Andersen, University of Oxford

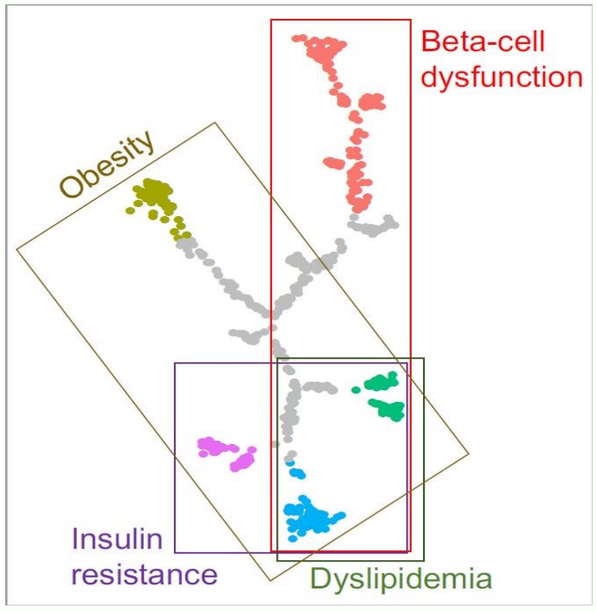
Type 2 diabetes is diagnosed based on measurements of high blood sugar levels. However this simple test does not capture the variability in patients' other characteristics, such as their weight, lifestyle, metabolism and genetics. One of the projects within the DIRECT study is investigating the differences in clinical characteristics of patients with newly-diagnosed type 2 diabetes to better understand the diversity among patients. We looked at various body measurements, including the body mass index (BMI) and waist-to-hip ratio, as well as the results of several clinical tests performed on the donated blood samples, including measures of cholesterol, triglycerides and liver enzymes. We also examined changes in glucose and insulin levels immediately after patients consumed a liquid 'meal' in order to evaluate how well the patients' beta cells, were producing the hormone insulin in their pancreas. In addition we measured each patients' sensitivity to insulin to check how well the cells in their body responded to it.

We used a special computer programme to visualise the similarities and differences between patients using a "tree-like" structure, with patients with similar characteristics grouped on the same branch of the "tree". See the diagram opposite. We focused on five smaller subgroups of patients at the tips of the tree branches, with the most clearly defined characteristics (the red, green, blue, purple and olive dots). The patient subgroups can broadly be defined by problems in four main areas: insulin production (beta-cell function), insulin resistance, obesity and unhealthy levels of one or more kinds of fat in your blood (LDL, HDL and triglycerides); this is called "dyslipidaemia". The different combinations of these underlying problems lead to disease of varying severity.

We identified two groups of patients who showed issues with only one of the above mentioned mechanisms: patients who are overweight but otherwise metabolically healthy (olive coloured dots), as well as patients who have a generally healthy lifestyle, but suffer from poor beta-cell function (red dots). Their condition generally doesn't get worse over time (at least within the 36 months duration of the DIRECT study), and majority of these patients did not require glucose-lowering drugs to manage their symptoms.

On the other hand, we found patients who had problems in all of the areas mentioned above (the green and blue coloured dots). Their type 2 diabetes tends to get worse more quickly than in patients with only a single affected disease mechanism. After 36 months of study over 70% of these patients required glucose-lowering drugs. One of the important findings of the study was that the fastest progressing subjects (blue and green dots) had abnormal lipid profiles (i.e. unusual levels of different fats in their blood) compared to otherwise similar overweight and insulin resistant individuals whose blood sugar levels remained more stable over time (shown as purple dots). This observation is being investigated further to see if this can help doctors identify at an early stage which patients may benefit from more prompt drug treatment.

We then investigated whether patients showed similar characteristics at 18 and 36 months after their initial visit. We observed that the majority of patients were classified into the same subgroups at the follow-up visits, as at the time of diagnosis, suggesting that some of the differences might be attributed to their genes (rather than for example the stage of their disease). Interest-



ingly, we found that the large group of patients with poor beta-cell function (shown as red dots) had a significantly higher number of changes in genes that have previously been linked to impaired insulin secretion, so supporting this possibility. Additionally, we looked at large numbers of molecular biomarkers measured in the patients' blood samples, including different circulating proteins and metabolites, and identified several markers associated with the different subgroups. We hope that these results will help us better understand the underlying mechanisms of how and why diabetes develops in different people, and may help us better identify patients likely to experience faster disease progression.

Predicting glucose deterioration in Finland

Dr Tarja Kokkola at the University of Eastern Finland writes about the DIRECT research in Finland.

The University of Eastern Finland (UEF) in Kuopio has been an important part of the DIRECT project. The principal investigator at the UEF/Laboratory of Internal Medicine is Professor Markku Laakso, who is one of the leading scientists on the genetics of type 2 diabetes.

The DIRECT participants in eastern Finland are all middle-aged or elderly men selected from a group of 10,197 men previously enrolled in the so-called METSIM (Metabolic Syndrome in Men) study that ran between the years 2005-2010. More than 1300 of these men, who were considered to have a high risk of developing type 2 diabetes, have participated in the DIRECT study in Kuopio. Most of the men have been followed closely for 4 years to see if they have any detectable difference or characteristic (a so called "biomarker") that will help us to predict which of the men show deterioration in their blood glucose balance or the development of diabetes. Furthermore, various kinds of blood samples from other Centres in Europe participating in the DIRECT biomarker study were analysed at Kuopio. In particular, UEF has been responsible for glucose and insulin analyses in the DIRECT study.

The project has employed more than 10 people at the UEF. The project has been running smoothly due to the earlier involvement of the staff of the Laboratory of Internal Medicine in METSIM and several other longitudinal cohort studies. Of the UEF participants the last had their examination visits on the 25th of September 2018.

The information collected from participants in Kuopio include glucose tolerance and other factors from blood samples, different measures of size and fat content of the body, blood pressure, physical activity, diet and voluntary urine and toenail samples. Extensive statistical analyses will be used to identify factors predicting deterioration in the blood glucose balance. This data will help identify new medical strategies focused on early detection, prevention and treatment of type 2 diabetes.

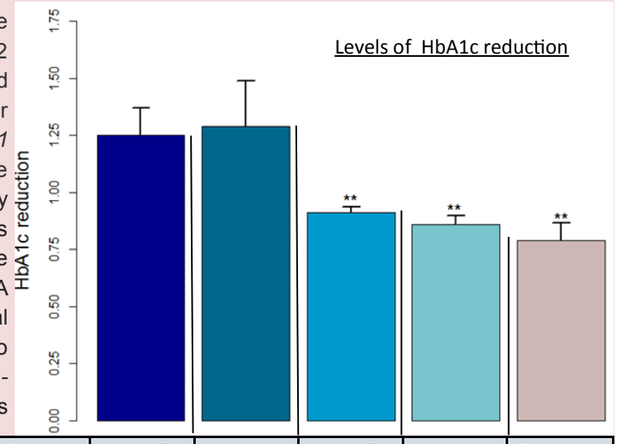
Your genes may explain how well you respond to anti-diabetic drugs

Dr Adem Dawed, University of Dundee, explains his work on DIRECT genetic data.

GLP-1 receptor agonists (GLP-1RA) are injectable anti-diabetic drugs that improve blood sugar control by mimicking a hormone called GLP-1 that is secreted from the gut. This hormone is released when you eat and stimulates increased insulin secretion by the pancreas. GLP-1RA drugs such as: Exenatide (Byetta, Bydureon), Liraglutide (Victoza, Saxenda) and others also show other beneficial effects such as weight reduction, lower blood pressure and better blood fat levels. It has been known for some time that GLP-1RA works better in some people than others but the reasons for this have not been understood.

As part of the DIRECT research programme, we studied the genetics of glycaemic response in 4,563 patients with Type 2 Diabetes who were treated with a GLP-1RA drug. We found changes in the DNA code (so called 'genetic variants') of particular genes, alters how well GLP-1RA drugs work. *GLP-1R* and *ARRB1* are proteins found on the insulin producing cells (beta cells) of the pancreas and are involved in the control of blood sugar level by enhancing insulin secretion. Around 5% of the population has been found to have one or more copies of the altered code for the *ARRB1* gene.. They show a much better response to GLP-1RA drug treatment, whereas in the rest of the population with a normal or altered *GLP-1R* genetic code only a slightly better response to GLP-1RA treatment is observed (as measured by HbA1c levels - an indicator of long-term blood glucose control). The difference is equivalent to receiving an extra 0.6mg of Liraglutide or 10µg of Exenetide.

This study sheds important light by identifying distinct subgroups of diabetic patients that respond well to GLP-1RA-based medicines, so implying doctors in the future may need to check your genes before prescribing these drugs. Thus this is a step towards personalised medicine in type 2 diabetes.



GLP-1R-Gly168Ser	Normal	Variant	Normal	Variant	Two Variants
ARRβ1	Variant	Variant	Normal	Normal	Normal
% of People with this combination	4%	1%	58%	30%	7%
Average fall in HbA1c (%)	1.25	1.29	0.91	0.86	0.79