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Screening for Diabetes is possible and should be based on Autoantibody and Genetic Profiles

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Abstract
Diabetes is a growing health problem with large health and economic implications. New technological advances give rise to the potential for effective national screening programmes. It has been known for some time that type 1 diabetes can be successfully predicted with a profile of autoantibodies. Recent developments in immunomodulatory therapies offer a suitable weapon. Type 2 diabetes is a common disease that is multifactorial but has several recently identified genetic polymorphisms that when combined contribute to a substantial risk of developing the condition. Lifestyle intervention in those with prediabetes is known to limit disease development and progression. This paper explores the above subjects and hypothesises that these developments suitably honed would be able to fulfil the criteria for an effective national screening programme.

Keywords: *Diabetes mellitus, screening programme, autoantibody profiling, case control studies, genetic profiling*

Introduction

For all of the advances in the treatment of diabetes, there has been relatively little work done on its prevention. In 2006, diabetes accounted for 10% of total NHS spending, and up to two thirds of this was for hospital treatments including diabetic complications. The number of people with diabetes is expected to rise by 15-25% over the next 20 years. In the UK, without further weight gain in the population, diabetes and its complications would still reach epidemic proportions within 10-20 years.[1]

Developing our understanding of the pathogenesis and natural history of diabetes has become an increasingly important focus of research. There are two hugely significant recent developments which have the potential to generate the basis of an accurate, viable screening programme which would help to identify individuals at risk and help to prevent the huge morbidity and mortality associated with this chronic, difficult disease.

The first advance is through the Wellcome Trust case control consortium which has identified 9 specific genes involved in the development of type 2 diabetes. The second is the large bulk of work done in diagnosing type 1 diabetes with a panel of immunoreagents and the development of specific vaccines designed to prevent autoimmune mediated pancreatic beta cell destruction.

When one considers the burden of diabetes on a public health scale there is the potential for the above advances, suitably honed, to have a large scale impact if applied to the robust model of a national screening programme.

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Early diagnosis of type 1 diabetes

Every year, thirty thousand people worldwide are diagnosed with type 1 diabetes mellitus (T1DM). It is a T-cell mediated autoimmune disease. An inflammatory cellular infiltrate consisting predominantly of CD8+ and CD4+ T lymphocytes and variable numbers of B lymphocytes, macrophages, dendritic cells and natural killer cells is present inside and around the pancreatic islets at and prior to diabetes onset, they are responsible for beta cell destruction.[2]

With regards to the immune trigger, autoantibodies can be very reproducibly detected in T1DM and are useful markers for diagnosis, pathogenesis and prediction. The best characterised autoantibodies are Glutamic acid decarboxylase (GAD65) and pancreatic islet cell antigens (ICA512, ICA12, IAA). They are all related to the secretory apparatus of beta cells. The identification and molecular cloning of islet antigens and autoantibodies has high concordance between laboratories and a WHO standard for worldwide comparison of antibody levels is now available.[3,4]

Most studies of islet autoantibody prevalence have been performed in relatives of patients with type 1 diabetes. Although a positive family history is a risk factor for type 1 diabetes, about 90% of new cases do not have an affected first degree relative.[5] Islet cell autoimmunity can precede the development of clinical type 1 diabetes by several years. Prospective studies in subjects with and without a family history of type 1 diabetes conclusively demonstrate that the risk for type 1 diabetes is strongly correlated with the number of positive antibodies. The 5-10 year risk for type 1 diabetes varies from 0-1% in individuals with only one positive antibody to 62-100% in subjects who are positive for 3 or more antibodies. Screening for multiple antibodies

is the most sensitive and specific strategy for identifying people at risk of diabetes.[6]

Natural history of type 1 diabetes

With regards to the natural history of type 1 diabetes, we need to understand its progression from latent to declared disease to demonstrate the significance of the autoantibody picture. Eisenbarth's model of type 1 diabetes characterises its evolving stages;[7]

1. Genetic susceptibility.
2. Triggering event for the initiation of beta cell autoimmunity.
3. Progressive loss of glucose stimulated insulin release.
4. Clinical manifestation of diabetes.

Several groups of researchers have initiated prospective studies from birth to determine which individuals are at risk by determining when islet autoantibodies first appear, which genetic and environmental factors influence their development and which characteristics of islet autoantibodies are most associated with progression to diabetes.

The German BABYDIAB study has demonstrated that children developing type 1 diabetes in early life (under 10 yrs) have first signs of islet autoimmunity very early in life, the majority by age 2.[8] These individuals have a 50% chance of developing type 1 diabetes before the onset of puberty. IAA's are usually the first to appear followed by GAD, IA-2 or IA2beta. Once islet autoantibodies appear they usually persist although significant fluctuations in antibody titre can be observed during the pre-diabetic phase. The BABYDIAB study has developed a marker to distinguish IAA relevant to type 1 diabetes from IAA which are not associated with disease progression or multiple autoantibodies.

Predicting the risk for development of type 1 diabetes has so far been important for identification of individuals that might profit from inclusion in pre-emptive interventional trials aiming to prevent the onset of disease. In the Joslin/Denver study it was found that the risk of developing type 1 diabetes by five years after the first detection of islet antibodies is 44% if two and 100% if three islet antibodies are present.[9] Several other studies have confirmed this observation. Using various combinations of these islet antibody characteristics it was possible to stratify diabetes risk from less than 10% to around 90% within 5 years. We can also see that there is a hierarchy in the diabetes risk associated with the different islet antibodies and also the age at which they first appear. Progression to diabetes is significantly faster in those who have multiple islet antibodies in the first year of life, compared with 2 or 5 year olds. Similarly, in the Florida, Washington and Karlsburg studies, 27-50% of subjects with multiple islet antibodies developed type 1 diabetes within 3-8 years of follow up.[10,11] Overall we now understand a lot more about the aetiology and development of type 1 diabetes and the importance of antibody analysis has become apparent with regards to this. Outside of research studies, screening may be requested from individuals at increased risk for type 1 diabetes who want to know their own or their children's risk status. In these circumstances screening can be offered, but the psychological impact of testing should always be considered. However up until recently there were no effective interventions to alter the apparent course of disease. There have now been several studies to evaluate therapies to prevent or delay the onset of disease, the avoidance of severe illness (ketoacidosis) and hospitalisation.

Preventing type 1 diabetes onset

With regards to applying these theoretical considerations to practical treatments there have been several different approaches already; Polyclonal anti-T cell therapy; chemokine targeting drugs; CCL4; non-activating humanised monoclonal antibody against CD3 – hOKT3gamma1(Ala-Ala); combined azathioprine and thymostimulin and the 'vaccine' Diapep277.[12-17]

Research trials on these new applications have mostly been performed in individuals with early or newly diagnosed diabetes. They work on the basis of immunosuppression and have varying degrees of success when scored on such variables as; time taken to commencement of insulin therapy, periods of remission, preservation of beta cell mass etc. Recent studies have provided proof of principle that short term treatment with anti-T-cell antibodies are able to preserve the residual pancreatic beta cell function for at least 18 months.[12,18] The resultant stabilising effect on metabolic control is expected to delay or limit chronic complications in these patients.

A diabetes vaccine

DiaPep277 is a peptide derived from Heat Shock Protein 60 (HSP60) which is normally found in islet cells and is a known target self antigen. In the context of immunity, peptides act as chemical markers on the surface of cells, they influence how T-cells identify enemy invaders such as viruses. By manipulating these markers the goal of its use is to modulate the autoimmune responses underlying type 1 diabetes. It is believed to work through induction of a shift from T-helper-1 to T-helper-2 cytokines produced by the autoimmune T cells.[19]

There has been much work done in the past couple of years in mice and newly diagnosed human patients with type 1 diabetes.[20] Researchers have sought to discover whether administration of DiaPep277 is safe and the consequent effects on endogenous insulin secretion, metabolic control and exogenous control. Early results are promising and show a significant preservation of insulin secretion up to 18 months from the time of diagnosis.[17,21] However, it has to be said that in these particular groups of patients being studied, the individuals have already reached the final stage in the development of clinically revealed disease, that is; loss of insulin production through destruction of beta cells.[22] That particular war has already been lost, (until perhaps islet cell transplants become a practical reality). Where we really

Table 1: UK National Screening Committee Criteria applied to our hypothetical models of screening for Type 1 & 2 diabetes.

	Type 1 Diabetes	Type 2 Diabetes
Screening criteria		
1. The Condition		
Condition is an important health problem	Yes	Yes
Natural history is understood	Yes	Yes
Identifiable disease marker and latent period	Yes – autoantibody profile	Yes – genetic polymorphisms
2. The Test		
Simple, safe, precise and validated screening test	Yes – serum blood test, early in life	Yes – serum blood test, at any point in life
Distribution of test values should be known	Yes	Yes
Test should be acceptable	Yes	Yes
Agreed policy on the further diagnostic investigation of individuals with a positive test result and the choices available to those individuals	Not yet	Lifestyle modification
3. The Treatment		
There should be an effective intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.	Combination immunomodulatory therapies.	Lifestyle interventions at an appropriately early point

need to intervene is back at stage 1 in the natural history of diabetes, before any damage is done. As the studies in the previous section have shown, it is now possible for a sensible risk stratification to take place on the basis of autoantibody profiling, not just in relatives, but in all individuals.

To date, only three phase II/III clinical trials have demonstrated safety and efficacy: anti-CD3 antibody, DiaPep277, and GAD65 (in patients with latent autoimmune diabetes in adults). Unfortunately, a significant number of patients did not respond positively and remained insulin-dependent after completion of therapy.[20,21] Several reasons account for this. Firstly, the severity of the disease as well as the auto-aggressive T cell repertoire vary from patient to patient leading to a broad range of therapeutic efficacies, and secondly at the time of the treatment the number of remaining beta-cells will directly impact the level of insulin production post-treatment. The question is how do we enhance efficacy of future immuno-interventions in patients with T1DM. Many have suggested that combination therapies might be the best approach.[23]

Type 1 Screening Programme

In the context of developing a screening programme for type 1 diabetes we propose the following hypothetical method to pilot and trial its effectiveness.

1. A blood test for all individuals prior to the age of 2 years (this could theoretically be performed post-partum with the heel-prick test) screening for islet cell antibodies.
2. If an individual is deemed to be at high risk of developing diabetes on the basis of their autoantibody profile, then they should have appropriate (parental) counselling and consideration of a trial of the above immunomodulatory therapies.
3. Follow up would consist of monitoring pancreatic function, blood glucose levels, insulin and C-peptide secretion.
4. Early detection should lead to delay and / or avoidance of diabetes and its complications. This would lead to better outcomes than later or standard treatment.

We suggest that by picking up those individuals with latent, non-declared disease we have the opportunity to have a significant impact on avoidance of developing autoimmune pancreatic destruction. We know that combination immunomodulatory therapies have an effect in those who are newly diagnosed, what would truly be a breakthrough is the application of that intervention to those who are deemed to be at high risk on the basis of their islet antibodies but may not develop the disease for several years. See table 1 for a summary of how this fits into the criteria for a national screening programme.

Type 2 Diabetes

In comparison, type 2 diabetes is a much larger problem; however there may well be a simple approach to pre-diagnosis and avoidance that would also fit nicely into a screening programme. Type 2 diabetes is multi-factorial disease culminating in insulin resistance and deranged glycaemic control. It is said that 'genes load the gun and environment pulls the trigger' and this is especially true for diabetes in our age of the obesity epidemic.

The Welcome Trust Case Control Consortium (WTCCC) is the largest ever study of the genetic polymorphisms in common diseases. Using data generated by the WTCCC, type 2 diabetes research groups recently reported the discovery of several novel genes involved in the development of type 2 diabetes and obesity.[32] One of these genes (FTO) influences risk of diabetes through an effect on weight and risk of obesity. The other three regions contain genes (CDKAL1, CDKN2A and IGF2BP2) that have a direct effect on diabetes risk. In total there are 9 major genes identified which relate to the pancreatic islet cells, the liver, gut and brain which are involved in the polygenic

development of type 2 diabetes. The findings provide insight into the genetic architecture of type 2 diabetes, emphasizing the contribution of multiple variants of modest effect. Not every individual who possesses these genes will go on to have diabetes because they will all have different lifestyle risk factors. However, given the rising tide of obesity in the western world, it is worthwhile considering targeted pre-emptive intervention in those most at risk.

Type 2 Screening Programme Hypothesis

There have been several studies exploring ways of reducing the risk of developing type 2 diabetes and many of these are in individuals with 'pre-diabetes' or impaired glucose tolerance. An example is the Finish Diabetes Prevention study which used tailored lifestyle interventions aimed at improving dietary habits and increasing physical activity in such patients and this brought about a significant reduction, sustained for several years, in the development of diabetes.[33,34] This has been repeated in several further studies and has been shown to be even better than using oral hypoglycaemic agents such as pioglitazone.[35-38] It is also an economically effective strategy.

If we look at the NICE guidelines (national institute of clinical excellence) which attempt to measure the cost utility of any intervention by calculating the cost per quality of life year gained (QALY), then this type of treatment falls well below the £20,000 per year cut off which is used to denote worthwhile interventions that will bring about significant reductions in mortality and morbidity.[39] It is well proven in those with impaired glucose tolerance.[35,36]

Therefore, if we take the next logical step and not only apply those patients who are traditionally believed to be at risk of type 2 diabetes, the obese, those with a family history and those with impaired glucose tolerance to these type of lifestyle treatments but also those with a recognised high genetic risk, then we can hope to generate a significant, financially viable approach to avoiding type 2 diabetes and formulating an appropriate screening programme to implement this. See table 1.

Conclusions

The above offers a rough outline of the directions we should take in developing novel screening programmes for type 1 and 2 diabetes. Certainly there are issues that need to be clarified and ironed out but the general framework is present.

With regards to type 1 diabetes, we know that autoimmune mechanisms trigger the destruction of pancreatic beta cells and if we can identify those at a risk suitably early on then the opportunities exist for targeting the immune response and T-cell activity. Research so far has only been performed in those who have 'declared' disease. We really need to approach the high risk sub-clinical group and autoantibodies provide the best route for doing this.

Type 2 diabetes is hugely important. It is a common polygenic disorder but has recognised environmental influences. Awareness of one's genotype is an important step in modifying one's risk factors accordingly. If patients understand that they are at high risk of developing diabetes in the future with its inherent complications then we can seek to stem the non-inevitable tide of its development. Diabetes is a spectrum from normality to impaired glucose tolerance to clinically revealed disease and again any preventative measures work best when they are introduced early. Studies have shown that lifestyle modification is cost effective and it works. Essentially if we can relieve the burden on the health economy caused by the expansion of type 2 diabetes then there will be significant population benefits. The information and knowledge is all there, it is just a question of how we implement it.

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