TYPE 1 DIABETES AND OTHER AUTOIMMUNE DISORDERS IN DOWN'S SYNDROME

Dr Julian Shield
Consultant Senior Lecturer, Department of Child Health, University of Bristol

There are a number of conditions with an autoimmune basis that present in people with Down's syndrome (DS). Thyroid disease is commonly screened for, but it is less well known that type 1 diabetes is more prevalent in people with DS. This is also true of some of the skin manifestations of autoimmunity, such as vitiligo, where lymphocytes attack the melanocytes in the skin resulting in pale patches, and alopecia areata (hair loss). There is some evidence that chronic active hepatitis is more common in people with DS, but more information is needed in this area. Although coeliac disease is a gastrointestinal intolerance to gluten, it is seen a lot in diabetes and thyroid disease and is associated with autoimmunity, and is therefore included here as an autoimmune manifestation of DS.

Thyroid disease

About 30% of people with DS will develop thyroid disease under the age of 25. In the general population it is said that about 10% of women will develop a thyroid problem at some time in their life, although it's much less common in men. So, there is a far greater preponderance of thyroid disease in DS than there is in the general population. Hypothyroidism is far more common than hyperthyroidism, although hyperthyroidism in itself is more common in DS than in the general population. Of those with thyroid disease, 50% will have developed it before the age of 8 years. There is a lot of evidence that thyroid autoimmunity causes the disease in those children over the age of 8; under age 8 there are lots of other causes and it is unusual to see autoantibodies.[1]

Congenital hypothyroidism

Congenital hypothyroidism, which is picked up by the heel prick test on day 7, is more common in children with DS. However, they often have normal thyroid scans, suggesting that something else is happening within the thyroid gland. There is a lot of

discussion about raised thyroid-stimulating hormone (TSH) levels in children with DS (sub-clinical hypothyroidism) where there are normal T4 levels but raised TSH levels. Over the short term this has little effect, because lots of the children will remain euthyroid for a long time and whether they are treated at that time is debatable. It has been suggested that TSH bioactivity may be abnormal in DS, such that more TSH is needed to make the thyroid gland work normally, but a recent study has shown that the TSH bioactivity is exactly the same as in the general population.[2] However, zinc was under a lot of scrutiny in the early 90s and there was an inference that zinc supplementation, at least in children with DS who were zinc deficient, would result in normalisation of TSH levels.[3]

Thyroid autoimmunity

Although only 30% of children will develop thyroid disease, about 40% of children with DS will have thyroid autoantibodies between the ages of 1 and 20 years.[4] This seems to be a recurring theme in DS – a lot more children manifest surrogate markers of autoimmunity than actually have the overt clinical disease. Interestingly, although human leukocyte antigen (HLA) is a major component of the body's immune system, HLA analysis in diabetes, thyroid disease and various other autoimmune diseases has shown no evidence that the HLA locus is associated with thyroid disease in the normal population. In people with DS, however, Nicholson and co-workers found that there was an association with the HLA locus. From this they inferred that there may be a permissive effect of chromosome 21, making the body more susceptible to these specific HLA haplotypes.[5]

Coeliac disease

A couple of studies have shown that IgA-positive antibodies – anti-gliadin antibodies – are present in about a quarter of the population with DS, but probably in less than 1% of the general population. In one study of 155 children, 21 patients with high levels of serological markers were biopsied and 7 had total villous atrophy.[6] This means that about 5% had coeliac disease, which is again more common than in the general population. In a study by Failla et al., 57 children with DS underwent serological tests. Those with positive findings went on to have a jejunal biopsy.[7]

Seven had positive biopsies, giving a prevalence of 12% for coeliac disease which is a very high level.

Diabetes

This is my own area of particular interest. We know that in the normal population approximately 2 out of 1000 children will develop type 1 diabetes in childhood; however, as far back as 1973 Jeremiah et al. described a prevalence of diabetes in children with DS of 20 out of 1000.[8] More recently there was a paper that came from the North of England suggesting a variable prevalence rate of between 1.4 and 10.6%, which is again far more common than in the general population.[9] Van Goor et al. looked at diabetes in The Netherlands and estimated a prevalence among children with DS of 1 in 300.[10] So diabetes is certainly more common in children with DS than expected from the general population.

In 1988, David Baum organised the British Paediatric Surveillance Unit (BPSU) Population Study in the UK of children who had developed diabetes and found that in about 7% the disorder developed before 2 years of age.[11] This subgroup is important because it is very difficult to treat. Young children can have very arbitrary eating and feeding patterns. People who look after children with diabetes like to give insulin at certain times and often find it difficult to adapt this to a young child's variable eating habits.

It occurred to me that, in clinical practice, I seemed to have seen quite a few children with DS and diabetes in this very young age group. I therefore placed an advertisement in the Down's Syndrome Association Newsletter asking parents to contact me if their child had developed diabetes. This then is a somewhat selected population, but 59 families responded and 22% of the children had developed diabetes in the first 2 years of life (Figure 1).[12] In the general population, very few children develop diabetes under 1 year of age, but in children with DS there is a massive increase, with about 15% developing diabetes in the first year of life. This small study then suggests that people with DS get diabetes earlier than the rest of the population. There are two possible reasons for this.

- *There may be a more aggressive autoimmune condition* – in the general population, whether diabetes develops at 2 or 15 years, auto-antibodies are likely to have developed before the age of 5 years and it is suspected that the autoimmune process occurs from birth, if not before. Therefore, people with more aggressive disease present very early whilst others have a gradual reduction of islet cell mass and smoulder along until they present clinically at about 15 years of age. The autoimmune process has been going on nearly all their lives, it's just a question of how quickly the islet cell mass is destroyed.
- *The islet cell population may be more prone to cell-mediated destruction* – it may be that the islet cells are weaker and more liable to be destroyed or they may be less in number in children with DS, and therefore any culling of islet cells by autoimmune destruction will result in an earlier manifestation.

Why is autoimmune disease more prevalent among people with DS?

There is abundant evidence of immune dysregulation in DS. It is known that the thymus is abnormal and that the morphology is different in children with DS. There are altered lymphocyte subpopulations in the peripheral blood and thymus, with evidence of T-cell activation and premature ageing, so the T-cell subsets are different from those in the general population. There's an increased susceptibility to infection, and the increase in some forms of malignancy may be related to the way the body monitors DNA mutations as well as the malignancy itself. Therefore, a lot of factors suggest that there is disordered immunity in DS, which may make people with DS more liable to autoimmune disease.

So how is the genetic susceptibility to autoimmunity conferred? There are two possibilities:

- The HLA alleles which confer genetic susceptibility to autoimmunity may be more prevalent in the DS population
- The three copies of chromosome 21 may cause disordered function of some genes which may be responsible for autoimmunity in general.

HLA genotypes

To investigate the first possibility we carried out a pilot study to see if some HLAs were more prevalent in children with DS. We collected DNA from three groups:

- parents and children who had been identified through the advertisement in the DSA Newsletter and who were open to further contact (30 individuals)
- an unselected group of children with DS in Gloucestershire (30 individuals)
- Professor Anna Kessling's (Imperial College, London) unselected cohort of children with DS and their parents (74 individuals).

From the DNA the HLA types were determined. The HLA region, which controls the immune system, is found on chromosome 6. It determines whether there is immune dysregulation or autoimmunity. In diabetes, the DR subregion (Figure 2) is of the most relevance – although there is now a lot of interest in DQ and DP – as those patients in the general population who develop diabetes are usually DR 3/4 (one DR 3 and one DR 4 from their parents). About 30% of the children with DS and diabetes had DR 3/4, but if you look at the general population with diabetes between 60 and 70% have DR 3/4. This suggests that there is a different genetic factor in people with DS that predicts whether or not they are going to get diabetes. Another interesting comparison was that there was an excess of DR 4 in the children with DS who didn't have diabetes. Obviously, a larger study is needed to confirm this finding, but it could be related not only to diabetes, but also to the higher prevalence of thyroid and coeliac diseases in people with DS.

Gene dose effects

There are several mechanisms whereby the presence of an extra chromosome 21 could influence autoimmunity. One of these is the possibility of disomic homozygosity. If two identical chromosomes are inherited from the parent of origin of the trisomy this may make you more liable to develop certain conditions. It has been putatively put forward as one of the causes of one of the types of leukaemia you see in childhood. To investigate this possibility we looked at the AIRE gene, the autoimmune regulator on chromosome 21 which causes autoimmune polyglandular syndrome type 1. We chose this because this syndrome has many features in common with DS. These include an increased incidence of candidiasis, thyroid disease, diabetes and vitiligo.

However we found no evidence for increased disomic homozygosity at the AIRE locus.

Another cause could be over-expression of one of the genes on chromosome 21 and if there are three genes they may all be active and therefore strongly expressed. This is true of superoxide dismutase (SOD) which has 150% of normal activity in people with DS – all three copies are active. If there is an autoimmune gene or genes over-expressed because the person has DS this may make them more liable to develop diabetes or other autoimmune conditions.

There are numerous loci (areas of chromosomes) associated with insulin-dependent diabetes in childhood. Interestingly, none of them have so far been mapped to chromosome 21; therefore, in the general population there is no inference that chromosome 21 has an effect on the genesis of type 1 diabetes.

Chromosome 21

Chromosome 21 has between 500 and 800 genes. Possibly less than 20 of these are responsible for the major phenotypic features of DS and are found on the distal segment (22.3 is the critical region) of the long arm (q). However, the genes for autoimmunity could be somewhere else and there are certain conditions related to DS where the genes are actually outside this critical region, which probably explains the phenotypic variation among children with DS. Therefore, although the critical region is important for the phenotype, all the other associated conditions may well lie outside this critical region.

Potential autoimmune genes

The following genes are all mapped to chromosome 21 and may be a cause of autoimmune disease:

- *AIRE*

  AIRE causes autoimmune polyglandular syndrome type 1 and even though disomic homozygosity does not occur, AIRE can't be ruled out entirely as it might be being expressed in excess because of the three copies of chromosome 21

- *Amyloid precursor protein (APP)*

  Most people with DS manifest neuropathological features of Alzheimer's disease

much younger than the general population because of beta amyloid deposition, increased cell death due to apoptosis and possibly increased reactive oxygen free radicals. The amyloid protein is linked to increased expression of APP and SOD-1 in people with DS. So, as well as being responsible for the Alzheimer changes, it is possible that this overexpression may confer a propensity to develop other major problems, such as tissue destruction and increased tissue inflammation. This would lead to an increased risk of organ damage with lymphocytic infiltration.

- *Superoxide dismutase*

  Superoxide dismutase converts superoxide (O_2^-) to oxygen and peroxide, rendering it non-dangerous. In normal diabetes you see a reduced amount of superoxide dismutase and, because there is more superoxide about, more tissue damage is caused and therefore more islet cells are destroyed. With 150% activity of SOD in people with DS you might expect a protective effect. However more hydrogen peroxide is produced than can be eliminated, and the system is saturated causing hydroxyl ions to be formed (Fenton's reaction) which are as damaging as the superoxide. This may be one of the reasons why there's more organ damage in DS.

- *Ligand of ICOS (LICOS)*

  LICOS is part of a co-stimulatory mechanism by which antigen-presenting cells interact with T cells (where ICOS is found) to produce proliferation and cytokine production, and therefore more tissue damage and inflammation (Figure 3). Furthermore, what appears to happen is that these are only expressed on CD4-positive and CD8-positive cells that are actively proliferating and thereby involved in tissue damage and cytokine production. It might be that over-expressing LICOS leads to increased lymphocyte damage and cytokine production, thereby making organ damage more severe.

Future studies

The Department of Child Health at Bristol University has developed a research protocol to identify the cause of autoimmunity in DS. Information from 200 children with DS and diabetes will be compared with information from 200 children with only

DS and with a control group. The information collected will be the autoantibody status for:

- IAA (insulin)
- GAD (glutamic acid decarboxylase)
- IA-2 (protein tyrosine phosphatase)

Although these autoantibodies have little to do with tissue destruction *per se*, diabetes is caused by cell-mediated lymphocytic infiltration and destruction of islet cells, and these autoantibodies are very good markers of diabetes. The study will also be looking at TPO (thyroid peroxidase) and tissue transglutaminase – markers for thyroid and coeliac disease respectively, but it is primarily focussing on diabetes as this has a known origin and is a well-defined subgroup. These populations will be compared and then some genetic analysis will be performed using the published map of chromosome 21 (from the human genome sequencing projects) and single nucleotide polymorphisms (SNPs). SNPs are small variations on the chromosome that can be labelled and followed through a family. By doing linkage studies (looking at SNPs that tend to be inherited together) it may be possible to determine loci (places on a chromosome) for genes involved in the excess of autoimmune disease in DS.

Conclusions

Autoimmunity is very prevalent in people with DS and it's a very under-researched area with very little having been written about it over the last 20–25 years. This means that there is very little to tell parents except that all these conditions appear to be more common and that having three copies of chromosome 21 obviously has a permissive effect.

A point for discussion concerns screening for coeliac disease. It is putatively associated with small bowel lymphomas in later life, and from personal experience I know that a lot of children with diabetes and DS have subclinical coeliac disease which can be determined by a biopsy. These children can be put on a gluten-free diet and suddenly feel a lot better, as well as preventing possible long-term problems. One concern is that a quarter of the DS population has basic anti-gliadin antibodies, which implies that a lot of biopsies will be performed on children who are normal.

Finally, it is important to recognise that studying rare diseases may help the understanding of more common diseases in the general population. DS and autoimmunity is itself relatively rare, and to encourage the financing of these sorts of studies they must also be relevant to the general population. Autoimmunity is extremely common in the general population. Studies, such as the one at Bristol University, will not only benefit our understanding of autoimmunity in DS, but may also help us to understand more about the condition in the general population.

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Question session

Andrew Tandy (Taunton, Somerset) I've not seen any of the children that I look after who have diabetes, so I've got a picture in mind where they present as the thin, polydypsic, polyuric child who doesn't have DS. I'm wondering whether – because their body mass index is different – they have so-called obesity **–** and added to their learning difficulties this gives them a different behaviour. Do you think this so-called obesity and the behavioural differences lead to a lack of usual signs? Particularly, if you've got a slow insidious concept of diabetes, maybe we're not picking up cases – and if that was the case, should we be screening for diabetes in DS children?

Julian Shield I think there's quite a significant increase in type 2 diabetes in the general population and I think children with DS do tend to become relatively

obese – certainly in adolescence from my observation. We do have a few patients who have been referred to us for obesity and glycosuria but I think they actually have type 2 diabetes. In many respects, children with DS and diabetes seem to present far more acutely – a lot of them present at a very young age and we must remember that diabetes is not that common a condition – 2 per 1000 is not a huge proportion. There aren't that many children with DS in the country – so even having a 10-fold or 20-fold increase in risk, it's still not a huge amount, 1 in 100 or maybe 1 in 200, so I don't think diagnoses are being missed.

It's very interesting that children with DS have an increased propensity to develop diabetes and once you've considered thyroid disease, coeliac disease, vitiligo and everything else, I think you can argue that a sub-population (maybe a third) of children with DS have a very aggressive autoimmune type phenotype. It's probably more than just diabetes and they are a very interesting group; what makes them different to the other ones?

Monica Pinto and Dr Miguel Palha (Portugal) In our population with DS in Portugal, it's quite different – probably because of a different genetic basis. We have about 500 children and not one has diabetes. We have seven with alopecia, three with vitiligo and only one probable (currently being investigated) case of coeliac disease. There might be something with a genetic basis that gives our children some sort of protection against diabetes.

Julian Shield Portugal has quite a Mediterranean climate doesn't it? As you know the incidence of diabetes in Mediterranean climates such as Italy and Spain is probably a fifth to a sixth of what it is for example in Scotland, where it is about 25 per 100,000 per year and climbing. Except for Sardinia, which is obviously very hot, but has a closed population, the incidence of diabetes in Mediterranean populations is much lower and there's undoubtedly an environmental influence as well as a genetic one.

Monica Pinto One surprising thing for us is coeliac disorder, which in our general population is increasing compared with other populations. However, in our population with DS it's practically absent.

Julian Shield Really!

Monica Pinto We have one clinical case – from the Islands – which is being investigated, but we checked anti-endomysial and anti-gliadin antibodies in 120 children and they were all negative – we did not have a single clinic case of coeliac disease.

Dr Julian Shield In Italy they seem to have a lot of coeliac disease, so perhaps the Portuguese population is different in many respects.

Patricia Jackson I think it perhaps illustrates that there is a need for a lot of research. We've done a similar study locally (South East Scotland) and we identified only one child with coeliac disease, and yet we have a high rate of the disease in the general population. I think it illustrates the need to get information together to look at the real problems.

Figure 1: Variations in age of onset of insulin dependent diabetes mellitus (IDDM) between children with Down's syndrome and the 1988 British Paediatric Surveillance Unit Survey[12]

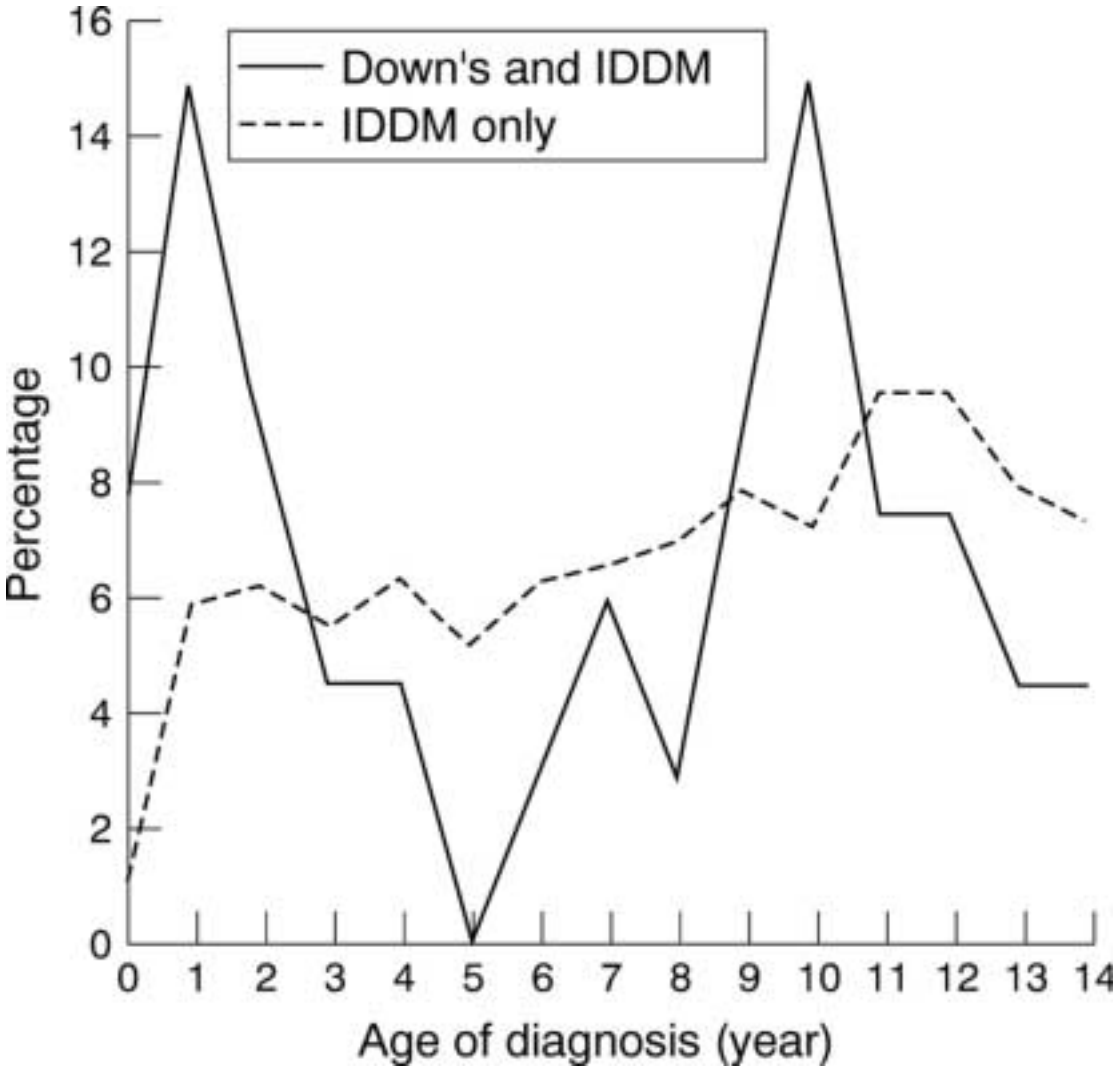

Figure 2: Chromosome 6

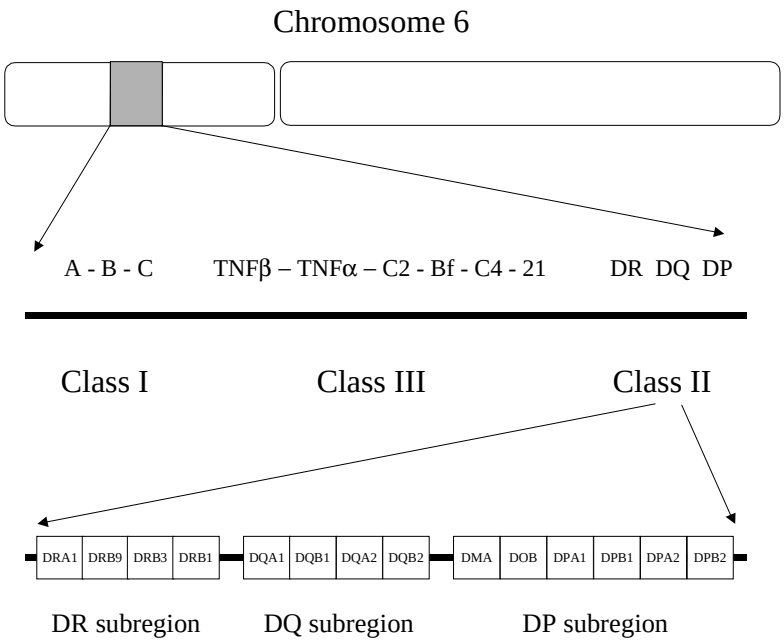

Figure 3: TcR MHC Peptide Interactions