Trial to Reduce IDDM in the Genetically at Risk

TRIGR Newsletter

Autumn 2005
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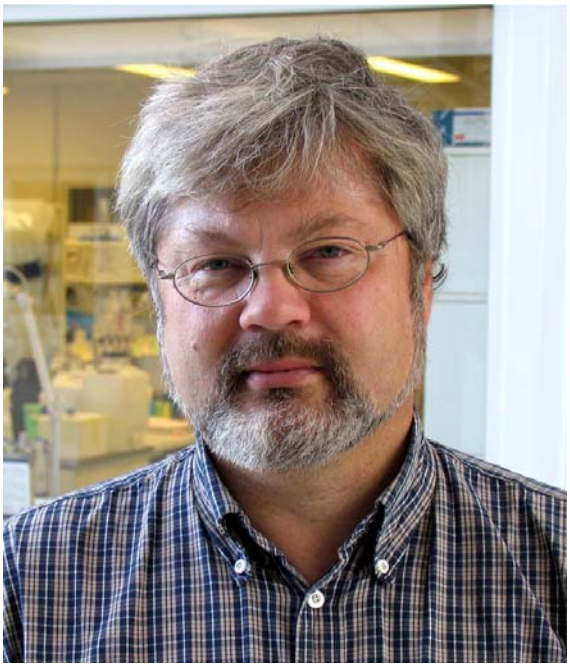
Editor: Matti Koski

On the genetics of diabetes in children



Professor Jorma Ilonen

Juvenile or type 1 diabetes is an example of a multifactorial disease where both the inherited genetic constitution and various environmental factors are contributing to the disease process. It has long been known that the susceptibility to the disease is inherited, however, it is also apparent that the rapid increase in the numbers of new cases can be explained only by changes in our environment. For example in Finland the proportion of children yearly diagnosed with type 1 diabetes has increased fivefold during the last 50 years.

It was shown already in the 1970's that the HLA gene region, known to be important in immune regulation was important in conferring genetic susceptibility to type 1 diabetes. Studies performed with twin and sibpairs demonstrated that about a half of the total genetic risk was defined by the HLA region. Later genome mapping studies using sibpairs have confirmed the crucial role of the HLA region but have also implied that there are several other genes contributing to the disease risk. The localisation and characterisation of these genes has been challenging. It has been observed, however, that polymorphisms within the insulin, CTLA-4, and most recently Lyp/PTPN22 genes affect the disease risk.

Genes most strongly affecting diabetes susceptibility were localised within the HLA class II region already in the late 70's. Although the gene region is now known in detail thanks to the development of molecular biology and tools for genetic research, no simple gene defining diabetes risk has been identified. Our view on HLA-defined risk determination is still surprisingly complex and imperfect. HLA-DQ molecules encoded by HLA-DQA1 and -DQB1 genes seem to be the most important single elements defining the disease risk. HLA-DQ and –DR gene loci are in very strong linkage and inherited together as haplotypes. HLA-DR3-DQ2 (HLA-DRB1*03-DQA1*05-DQB1*02) and HLA-DR4-DQ8 (DRB1*04-DQA1*03-DQB1*0302) haplotypes are strongly associated with disease risk. The former one is also a risk factor for several other autoimmune diseases such as celiac disease and systemic lupus erythematosus. On the other hand other haplotypes like HLA-DR2-DQ6 (HLA-DRB1*15-DQA1*0102-DQB1*0602) and HLA-DR5-DQ7 (HLA-DRB1*11/*12-DQA1*05-DQB1*0301) are protecting against diabetes. The genetic screening in the TRIGR project is based on typing for the presence of these haplotypes conferring susceptibility or protection. Genes inherited from both parents are important in the definition of disease risk, and their combined effect is dependent on the strength of both protecting and risk associated factors. The effect of strong protective factors is dominant implying that one protective haplotype is usually enough to prevent the disease despite the second haplotype conferring disease susceptibility. There are also interactions between risk factors and the risk is as a consequence greater if one has a combination of two different risk haplotypes compared to the presence of two similar haplotypes. In addition several other loci within the HLA region modify the risk defined by the class II region.

To establish the genetic screening protocol used in the TRIGR project we analysed in Finnish pilot studies the frequencies of the most common risk and protection associated HLA haplotypes in affected members of multiplex case families as well

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as in infants with a first-degree relative with type 1 diabetes. Table 1 shows the major genotypes and their odds ratios and positive predictive values. The three genotypes selected for the screening protocol cover about 86% of those who eventually will develop type 1 diabetes, whereas among infants in affected families less than half have one or two of these genotypes. Although the risk among those selected for the study is considerable higher, one has to notice that the disease risk is not absent in those who are excluded from the study. In fact, it is still clearly higher than in the unselected general population. The probability to develop the disease is also variable among those participating in the study. The figures in Table 1 are based on the Finnish population but we know that they are of the same magnitude in all countries involved in the TRIGR study, although the relative proportions of the two risk haplotypes vary. There is a gradient of an increasing frequency of DR3-DQ2 southwards in Europe and there is also evidence that the associated risk, which is relatively low in the North, may be higher in the South.

Our understanding of the mechanisms by which the genes associated with disease risk are exerting their effect is still unclear. HLA molecules play a central role in the mounting of any immune responses, since they present peptides digested from proteins to T-lymphocytes, which recognise these as their antigenic epitopes. The allelic polymorphism in HLA molecules is reflected in the shape of the peptide binding groove affecting which specific peptides can be bound to each HLA allele. The combination of HLA molecules inherited by a subject is accordingly defining which type of antigenic epitopes he or she is able to recognise. It has been suggested that only molecules associated with increased risk for diabetes risk are able to bind certain critical epitopes in autoantigens, which are targets of the autoimmune response against pancreatic beta cells. It is also possible that autoantigen peptides bound by certain risk molecules are similar to peptides digested from microbes and recognised during infections. It has been shown, that the same T-cell can recognise peptides derived from both an autoantigen and a virus or bacterium. This type of molecular mimicry is actually quite common but is alone probably not enough for triggering an autoimmune disease.

Table1. *Frequencies of screened genotypes among diabetes cases in multiplex families and newborns with a diabetic first-degree relative.*

	Diabetes cases N=289		Newborn Infants N=475	
Genotype	N	%	N	%
(DR3)*-DQ2 /DR4-DQ8	99	34,3	52	10,9
DR4-DQ8/x**	120	41,5	104	21,9
DR3-DQ2/x	29	10,0	73	15,4
Other genotypes	41	14,2	246	51,8

**The presence of DR3 allele is not confirmed in this genotype. Part of cases similar to the following genotype (DR4-DQ8/x)*
***Other haplotype not any of typed protection associated haplotypes*

HLA-typing laboratory personnel, Turku

TRIGR in Australia

Australia has 3 study centres all based in the state of New South Wales. The coordinating centre for TRIGR in Australia is at the Children's Hospital at Westmead. There is one other study centre in the Sydney metropolitan area at Randwick with the third study centre in Newcastle which is 150 kilometres north of Sydney.

Recruitment commenced in October 2003 with registered participants living within driving distance of one of the study centres. Advertising for the study saw us receiving enquiries from country areas and interstate so we are now recruiting across Australia. We certainly have some area to cover as Australia has 7.7 million square kilometres which

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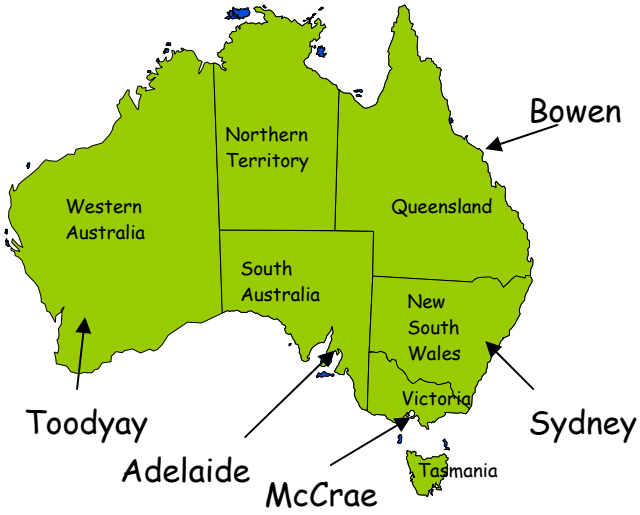
makes us the 6th largest nation after Russia, Canada, China, the USA and Brazil but with only 20 million in population.



So far 157 TRIGR babies have been born throughout Australia and of those 60 are eligible for follow-up. Of these, 22 families live in the Sydney area (which has the largest population in Australia) and are therefore being followed up at either Westmead or Randwick. The study centre at Newcastle has 13 eligible babies that they are following up. That leaves 25 babies being followed up by local practitioners with the support of the nurse coordinator from the Children's Hospital at Westmead.

To have these families involved in TRIGR has meant the involvement of many hospitals and co-operation of medical staff throughout Australia. We have families living as far as Bowen, QLD 2142 kilometres from Sydney, Toodyay, WA 3967 kilometres from Sydney, Adelaide, SA 1395 kilometres from Sydney & McCrae, Vic 929 kilometres from Sydney.

Hospital kits (containing letters of explanation for hospital staff, TRIGR labels, blood tubes to collect cord blood for HLA testing, courier consignment note and packaging to send bloods to Sydney as well as study formula if the mother is unable to fully breastfeed) are given to the families before the baby is born. The kit provides the hospital staff with everything that is needed for the baby to participate.

Many enquiries have been made to the study centre by parents who have heard about TRIGR through their endocrinologists, obstetricians, diabetes educators, nursing staff or paediatricians. Enquiries have also been received from families who have been in contact with other TRIGR participants or who have read about the study in articles that have been published in diabetes magazines, woman's magazines and various websites.

With the help of enthusiastic TRIGR staff and the cooperation of many families we will continue to recruit and have many more Australians involved in TRIGR.

TRIGR staff meeting in Newcastle August 2005

Glenda Fraser, Nurse Coordinator, TRIGR Australia

Family McCallum

After hearing about the TRIGR study during my final weeks of pregnancy, my husband (Andrew) and I decided that not only would our baby benefit from participating, but it was also a positive way to be involved in type 1 diabetes research.

Glenda Fraser and Ros Bongiorno (the Children's Hospital at Westmead) were terrific support for us, particularly because we lived 10 hours west of the nearest TRIGR centre. They phoned regularly and were always available to answer any questions or concerns a nervous first mother often has. Oliver would not breastfeed and therefore started using

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the study formula, which was always sent promptly when ever we called.

With Oliver's mother, grandmother and uncle all type 1 diabetics, being involved in this study has given us a little more peace of mind knowing that Oliver has annual checkups and that perhaps we may detect any early onset in him. (This peace of mind is what lets me put the poor little thing through the blood tests).

Andrew and I feel privileged to be involved in this study and we hope that in some small way, we may contribute to prevention of type 1 diabetes in generations to come.

Jane, Andrew and Oliver McCallum (Goondiwindi, Qld, Australia)
6th October 2005

Flavor test: Study Formula

The Study Formula used in the TRIGR study is either a normal cow's milk based infant formula or a special hydrolysed formula, where the proteins have been broken into smaller pieces. Some hydrolysed formula has been mixed into the cow's milk based formula, so that the different formulas cannot be distinguished based on smell or taste. The hydrolysed proteins give the formula a smell and taste that is unpleasant to most adults. Hydrolysed infant formula is being widely used for treating allergies in infants.

Research has shown that hydrolysed formula is readily accepted by young infants. It seems that their perception of flavor differs from adults, so that they do not find the taste of hydrolysed formula unpleasant. The flavor perception of infants appears to develop, so that after the age of about 4 months the taste of hydrolysed formula is not so easily accepted anymore. If the infant has got used to hydrolysed formula before 4 months of age, he/she usually easily accepts this taste also later.

To get an older infant to accept the taste of hydrolysed formula may need several times of exposure – this is true with any new food that is introduced to a child. To make the introduction easier, the formula can be mixed with a food that the baby already likes.

To get an idea of the smell and taste of the Study Formula, a group of TRIGR researches arranged a Study Formula tasting. We were amazed that the taste of the Study Formula could not be distinguished from foods with a strong taste. The test winner was infant mango puré!

These were the foods that were tested, about 1 scoop of Study Formula powder was added to 1 dl of food:

Infant mango puré – the taste of Study Formula could not be distinguished – TEST WINNER
Infant plum puré – the taste of Study Formula could not be distinguished
Ready-made infant cereal with lingonberries – the taste of Study Formula could not be distinguished
Infant potato and carrot puré – the taste of Study Formula was distinguishable, but not unpleasant
Oatmeal cereal – the Study Formula could be clearly distinguished, but it was more acceptable than pure Study Formula liquid

Professor Hans Åkerblom liked the taste of mango puré with Study Formula.

Study Coordinators Stina Bodén from Sweden, Glenda Fraser from Australia and Heli Suomalainen from Finland tasting baby foods with Study Formula.

Sonja Bärlund, Nutrition Fellow, TRIGR Helsinki