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Association of Islet Cell Antibody and Human Leucocyte Antigen DQB1 Alleles (0201/0302) in siblings of type 1 diabetes mellitus

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Abstract

Type 1 diabetes is most often associated with auto antibodies (AAbs) against beta cell antigens and high levels of HLA mediated genetic susceptibility. The purpose of this study was to identify at risk siblings of type1 diabetic patients at an early stage by screening for HLA–DQB1 alleles, which carry the high-risk genotype (DQB1 *0201 / 0302) and its associations with measured islet cell antibody (ICA). Twenty-five siblings of subjects with type 1 diabetes aged 4 – 13 yr, median age (8.4 ± 1.02) were studied. They were screened for ICA as well as HLA-DQB1 (0201 and 0302) alleles.

Results of the study showed that among the 25 siblings, 20% tested positive for ICA. HLA-DQB1 was detected in 16% of siblings. Allele DQB1 0201 presented higher frequency (48%) than DQB1 0302 (36%). Association of DQB1 0201 and / or 0302 with ICA positive cases was detected in 20% of cases, while frequency of DQB1 0201 and / or 0302 in ICA negative cases was detected in 48% of them.

In conclusion: - Our results showed that among siblings of type 1 diabetes HLA-DQB1 alleles associated with highest genetic susceptibility i.e. DQB1 0201 and / or 0302 were detected with high significance. Also HLA-DQB1 0201 is more frequently associated with ICA.
Initial screening by high risk DQB1 *0201/0302 with subsequent autoantibody testing is shown to be useful in the assessment of diabetes risk among siblings of subjects with type 1 diabetes.

Introduction

Type 1 diabetes is most often associated with auto antibodies (AAbs) against "beta" cell antigens as AAbs to glutamic acid decarboxylase (GADA), insulin (IAA) as well as the heterogeneous islet cell cytoplasmic antibodies (ICA).

Although the complex associations and linkage of HLA antigens with type 1 diabetes are not yet fully understood, HLA–DQB1 and DQA1–DQB alleles provide the strongest genetic contribution to the disease (Robinson etal, 1989) accounting for approximately 50% of the genetic risk in type 1 diabetes (Todd and Farral 1996).

In Caucasian, DQB1 0302 and DQB10201 and their linked DR specificities DR4 and DR3 provide disease susceptibility (Michelson *et al*,1990), where's dominant protection is conveyed by DQB1 0602 linked to DR2 (Reijonen *et al*, 1991). The relationship between HLA markers and the occurrence of AAbs has been previously examined in recent onset patients of type 1 diabetes indicating positive correlation (Vande walle *et al*,1997) in recent years, several studies have been conducted to identify subjects at risk of developing type 1 diabetes by screening for ICA as well as by human leukocyte antigen (HLA) typing.

Aim of the work

To identify at risk siblings of type 1 diabetic patients at an early stage, by screening for HLA–DQB1 alleles which carry the high-risk genotype (DQB1 0201 and 0302) and its associations with measured islet cell antibody (ICA).

Subjects and methods

This study was carried out on twenty-five healthy siblings of type 1-diabetes. Fourteen females and 11 males. They aged 4-13 years, mean age was (8.4±1.02).

Samples of serum and blood were collected for immunological and HLA allele typing test, respectively. HbA1c was also measured.

Each family provided informed consent before samples were collected.

Methodology of HLA typing

Whole blood on EDTA was obtained from each subject. DNA was extracted from whole blood using DNA extraction kit (Manufactured by QIAGEN, USA) according to recommendation.

HLA DQB1 typing for both of the studied alleles was carried on using polymerase chain reaction (PCR) kit using sequence specific primaries multiplex PCR (all set SSP) manufactured by (DYNL BIOTECH Ltd. UK.). PCR cycling parameters were as followed: A denaturation step at 96 degrees centigrade (c°) for two min., 10 cycles of denaturation at 96 c° for 15 sec., annealing and extension at 96 c° for 60 sec. Twenty cycles of denaturation at 96 c°, for 10 sec. annealing at 61 c°. for 50 sec., and finally extension at 72 c°. for 30 sec. (Ilonen *et al*, 2000).

Immunological studies

ICA was determined by indirect immunofluorescence (IIF), (Reijonent *et al* 1994).

The ICA assay was controlled externally by an international proficiency test, (Immunology Diabetes Workshop, University of Florida USA, 1993).

The DNA locus of HLADQB1 genotype was typed using the amino acid sequencer of DNA (Sequence Specific Oligonucleotide) (SSO) probes 6.(Adojaan, *et al* 1996)

Statistical methods

Mean values, ranges and standard deviations were obtained for normally distributed values. Percentage of results were also calculated

Results: -

The results are shown in tables 1, 2, and 3

Table (1) mean value ± SD of age / year – FBS/mg/dl and HbA1c for all studied cases(25).

	Age / year	FBs/mg/dl	HbA1c
Range	4-13 years	65-101mg/dl	4.2-6.3
mean	(8.4 ± 1.02)	(84.8±5.07)	(6.26±1.58)

Table (2) percentage of –ve and +ve HLADQB1 0201, 0302 and ICA in all studied cases.

Parameters	-ve	+ve
HLADQB1 0201	52%	48%
HLA-DQB1 0302	64%	36%
HLADQB1 0201 and 0302	84%	16%
ICA	80%	20%

Table (3) Frequency of Association of HLADQB1 0201 and 0302 with positive and negative ICA.

	HLADQB1 0201	HLADQB1 0302	Total	Percentage
ICA (-ve)	7 (28%)	5 (20%)	12	48%
ICA (+ve)	3 (12%)	2 (8%)	5	20%

Discussion:

Type 1 diabetes is an autoimmune disease caused by destruction of pancreatic insulin secreting B-Cells, a process that may occur sub clinically years before the onset of clinical symptoms. The risk of sibling is 15 fold higher than is the population prevalence, but the etiology of the disorder is complex and probably involves multiple genetic and environ-mental factors (Charles *et al*, 1998).

Among the general population, the presence of islet autoantibodies carries increased risk (Schatz *et al*, 1993). Thus it would appear that islet autoantibodies are universal markers of the destruction of insulin producing islet cells that ultimately results in type 1 diabetes.

In the present study, ICA were used as primary screening tool for beta – cell autoimmunity in siblings with increased genetic risk of type 1 diabetes. This decision was based on the observation that ICA were more sensitive and specific than GADA or IAA, with more than 84% of children with newly diagnosed type 1 diabetes testing positive (Savila et al,1998 and Kimpim etal, 2002).

In the current study, ICA were detected in 20% of studied cases. Kulmala et al,(2001) reported that the prevalence of ICA had been observed to be 3-4% among Finnish school children, while Krischer et al (2003) found 82% positive cases of ICA. Further follow up and analysis of actual progression to diabetes may revert some of our negative ICA cases to positive one. Bonifacio *et al*, (1999) had shown that ICA titer was directly related to risk of type 1 diabetes. Also for trails in which screening is necessary to identify a population with sufficient diabetes risk, ICA would augment the population identified by GADA and ICA 512 AA (or vise versa).

Type 1 diabetes has strong class II HLA association, with linkage to the DQA and B genes and is influenced by the DRB genes. The risk presented by the DR4 allele is primarily attributable to an association in a haplotype with HLA-DQB1 *0302. Susceptibility associated with HLA-DR_3 may be determined directly by HLA-DQB1 *0201 (Cavan etal, 1992).

In the current study, high risk HLA-DQB1 0201 allele represented higher frequency in siblings of type 1 diabetes (48%) than HLA-DQB1. 0302 allele (36%). Analysis for both HLA-DQB1allele was found in 16% of cases. These results are in agreement with Hagopain (1995) and Bonifacio et al (1995).

High proportion of this genetic marker indicate that those siblings carry the susceptibility for developing type 1 and that their predicted risk of diabetes is greater with more than one high risk allele.

In a study, investigating genetic and hum-oral markers for prediction type 1 diabetes in siblings has reported that among the genetic markers DQB1 *0201/0302 was associated with the highest positive predictive value of 22% (Schlosser et al, 2002).

In our study, the frequency of HLA-DQB1 *0201 and / or 0302 in ICA negative cases was detected in 48% of cases. This is in accordance with results of Schlosser et al (2002), who found 46% association.

On the other hand HLA-DQB1 *0201 and / or 0302 was detected in 20% of our ICA positive cases. Kimpini et al, (2002)

found the proportion of children who tested positive for ICA was significantly higher among those with the high risk genotype (5.1%).

Schlosser *et al* (2002) found HLA heterozygostiy to occur in 4.5% with single AAb.

ICA was associated with 0201 in 8% of cases and was associated with both alleles in 12% of cases. Subjects with positive AAbs revealed an increased frequency of diabetes when associated with HLA-DQB1 alleles 0201 or 0302 (P=0.001 and 0.006) respectively (Kimpim etal, 2002).

Vandewalle *et al*, (1997) in their study on recent onset diabetic patients as well as their siblings found positive correlation between the occurrence of diabetes associated AAbs and high risk HLA markers. These results might be of importance for population screening strategies based on detection of islet autoantibodies in associations with high risk HLA genotypes. Furthermore they indicate significant increased risk for subsequent development of type 1 diabetes.

In conclusion: - our results showed that among siblings of type 1 diabetes HLA-DQB1 alleles associated with highest genetic susceptibility i.e. DQB1 0201 and / or 0302 were seen with high significance. Also HLA-DQB1 0201 is more frequently associated with ICA. Initial screening by high risk DQB1 *0201/0302 with subsequent autoantibody testing is shown to be useful in the assessment of diabetic risk among siblings of subjects with type 1 diabetes.

References

1. **Adojaan B, Knip M, Vahasalo P, *et al* (1996).** Relation between incidence of childhood IDDM and frequency of ICA positivity in un affected children in the general population. Diabetes Care; 19:1451-1452.
2. **Bonitacio E , Bmgly PJ, Shallock M, Dean BM, *et al* (1990)** Quantity of islet cell antibodies and prediction of insulin dependent diabetes .Lancet 335; 147-149
3. **Cavan DA, Bain SC, Barnett AH:** Genetics of type 1 (Insulin-Dependent) diabetes mellitus med. Genet. 29:441-446.
4. **Charles FV, Pnina V,Sunanda B, Fei Bao., *et al* (1998)** Evidence For Oligogenic Inheritance of type 1 diabetes in a large Bedouin Arab Family. J. Clin. Investigation 102 (8) 1569-1575
5. **Culmala P , Rahko J ,Savola K, Salo P, *et al.* .(2001)** (Beta) cell autoimmunity, genetic susceptibility and progretion to type1diabetes in unaf-fected school children. Diabetes Care24: 171-173
6. **Green baum C J, Sears KL, Kahn SE,Palmer JP. .(1999)** Relationship of B-cell Function and autoantiboies to progression and non progression of subclinical type 1 diabetes Diabetes 48:170-175
7. **Hagopain W,Sanjeevi C,Kockum I *et al* (1995)** Glutamate decarboxylase, insulin and islet cell antibodies and HLA typing to detect diabetes in a general population based study of Swedish children. J Clin Inv; 95;001-7
8. **Ilonen J, Reijonen H, Green A, Reunanen A ,*et al* (2000)** Childhood diabetes in Finland study group. Geographical differences with Finland in the frequency of HLA- DQ genotypes associated with type 1 diabetes susceptibly. Eur. J. Immunogenet; 27: 225-230.
9. **Kimpim KI, Kulmala P, Savoca K, Kupila A, *et al*. .(2002)** Natural history of beta cell autoimmunity in young children with increased genetic susceptibility to type1 diabetes recruited from the general population.J. Clin.Endo.Metab. 87(10) 4572-79
10. **Krischer JP, Cuthbertson DD, YU, liping I, *et al*. (2003).** The diabetes prevention trial type1 study group. Screening Strategeis for the. Identification of Multiple Antibody-Positive Relation of individuals with Type1 Diabetes. J. clin. Endo. Metab. 88(1): 103-108
11. **Michelson B ,Wassmuth R, Ludvlgsson J, Lrnmark A, *et al*.(1990).** HLA hterozygosity in insulin dependent diabetes is most frequent at DQ locus. Scan. J. Immunology 31:405-413
12. **Pugliese A, Eisenbarth GS. (1996).** Human type1 diabetes mellitus: genetic susceptibility and Resistance inisenbarth

GS, Lafercy KJ eds. Type1diabetes: molecular, cellular and clinical immunology. Oxford University Press; 134-152.

13. **Reijonen H, Ilonen J, Knip M, Kerblom HK, .(1991)** HLADQB1 Alleles and agsence of Asp-57 suscept factors of IDDM in finland. Diabetes. 40:1640-44
14. **Robinson , DM, Holbeck S, Plammei J, Nepom GT. (1989).** HLADQ3.2 identifies subtypes of Dr4 Haplotype permissive for IDDM. Genet. Epidemiolology 6:149-154
15. **Savola K, Bonifacio E, Sabbah E, Kuolmala P, Salo P *et al*. .(1998).** The childhood diabetes in Finland study group .IA-2 antibodies –a sensitive marker of IDDM with clinical conception childhood adolecance. Diabetologia 41:424-79
16. **Schatz D, Krischer J Horne G, Riley W,Spillar R, *et al*. .(1994).** Islet cell antibodies predict insulin-dependent diabetes in United state school age children as powerfullyas in unaffected relatives.J.Clin. Invest.;93:2403-2407
17. **Schlosser, Michael, trebelow, Martina, Wassmuth. *et al* (2002).** The Karlsburg Type 1 diabetes Risk Study of a Normal School Children Population: Association of (beta) –cell auto antibodies and Human Leucocytic antigen-DQB1 alleles in Antibody-Positive Individuals . J. Clin.End. Metab . 87(5) 2254 – 61 ,
18. **Todd JA, and Farralm: (1996).** Panning for gold: genome wide scanning for link age in type1 diabetes. Hum. Mol.Genet. 5:1443-1448
19. **Vandewalle CL, Farlorni A, Lernmark,, Goubert P ,*et al*. .(1997).** Association of GAD65-and LA-2 autoantibodies with genetic risk markers in new-onset IDDM patientsand their siblings. The Belgiam Diabetes Registng. Diabetes care 20:1547-1552

مصاحبة الاجسام المضادة لخلايا جزر لانجرهانز مع الجزيء الجينى DQB1 0302 , 0201 في اشقاء مرضى السكر من النوع الاول

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السكر من النوع الأول هو اكثر الانواع مصحوبة بظهور الاجسام المضادة ضد خلايا البيتا (خلايا بيتا فى جزر لانجرهانز) وايضا مستويات عالية من الجزئيات الجينية القابلة لظهور المرض

الهدف من هذا البحث هو تحديد مدى قابلية تعرض اشقاء مرضى السكر من النوع الاول لهذا المرض وهذا عن طريق قياس الاجسام المضادة لخلايا بيتا وايضا عن طريق التتبع الجينى لهذا المرض ومدى مصاحبته للأجسام المضادة لخلايا بيتا وهذا عن طريق تحليل الجزيء الجينى فى الحمض الامينى فى الكروموسوم المسئول عن هذا المرض وقد تم اجراء هذا البحث على 25 فردا من اشقاء مرضى السكر من النوع الاول يتراوح اعمارهم ما بين 4 الي 13 سنة بمتوسط عمرى من 8.4±1.02 وقد تم قياس الاجسام المضادة لخلايا بيتا في جميع الافراد وايضا تم تتبع التحليل الجينى للحمض الامينى في الكروموسوم المسئول عن ظهور هذا المرض (0302) ,(0201) HLA-DQB

وكانت النتائج الاتى

20% من الحالات كانت اجابية للاجسام المضادة لخلايا بيتا

16% من الحالات كانت اجابية للتحليل الجينى HLA-DQB1

48% من الحالات كانت اجابية للتحليل الجزئى الجينى HLA-DQB1 0201

بينما كانت 36 % فقط اجابية للتحليل الجزئى الجينى HLA-DQB10 302

وكانت هذه النتائج ذات دلالة احصائية قوية لمصاحبة الاجسام المضادة لخلايا البيتا مع التحليل الجينى 0302, 0201 التي تزيد من احتمالات ظهور اعراضة وبمحاولة ايجاد العلاقة بين مصاحبة الاجسام المضادة لخلايا بيتا مع كلا من DQB1 0 302 , DQB1 0 201 وجد ان الاجسام المضادة لخلايا بيتا اكثر مصاحبة للجزيء الجينى DQB1 0201 فقط اكثر من 0302 والخلاصة من هذه النتائج وجد انه بخصوص اشقاء مرضى السكر من النوع الاول وجد عندهم قابلية وراثية عالية لظهور مرض السكر وذلك في حالة وجود الجزئ الجينى DQB10201 مع الاجسام المضادة لخلايا بيتا واقل منها احتمالا في حالات ايجابية DQB1 0302 مع الاجسام المضادة لخلايا بيتا ويزيد احتمالات ظهور المرض فى حالة ايجابية الجزئيين الجينيين 0302, 0201 مصاحبين للأجسام المضادة لخلايا بيتا ويكاد ينعدم احتمال الاصابة بالمرض فى حالة سلبية الجزئ الجينى 0201, 0302 ومن هذا يمكننا التنبؤء المبكر لحدوث مرض السكر من النوع الأول في أشقاء مرضى السكر من هذا النوع الاول وذلك قبل ظهور المرض وذلك يمكن ان يفيد فى محاولة تاجيل ظهور المرض او التدخل المبكر للعلاج لمنع المضاعفات .