CLINICAL STUDY

Androgen receptor gene exon 1 CAG repeat polymorphism in Finnish patients with childhood-onset type 1 diabetes

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Abstract

Objective: Animal models suggest that androgen receptor gene polymorphisms might affect disease predisposition in human immune-mediated diabetes. The aim of this study was to investigate the effect of the human androgen receptor gene exon 1 CAG repeat polymorphisms on type 1 diabetes (T1D) susceptibility.

Design and methods: A combined strategy of case-control and family-based approaches was used. Affected sibling pair families ($n = 120$), nuclear families ($n = 645$) and cohorts of sporadic cases ($n = 208$) and controls ($n = 1381$) were genotyped for androgen receptor gene exon 1 CAG repeat polymorphism. An automated fluorescence-based DNA fragment-sizing method was used.

Results: The distribution of CAG repeat alleles did not differ significantly between patients and controls. However, short repeat alleles (7–14) were more prevalent among cases in girls compared with controls (8.77% vs 5.91%; $P = 0.03$). Long repeat alleles (19–28) were less frequent among HLA DR3-positive diseased boys than in DR3-positive control boys (32.6% vs 40.6%; $P = 0.011$). The differences were not significant after adjustment for multiple comparisons. Transmission of CAG repeat alleles was not different from expected in the total material. However, transmissions to girls deviated from the expected value significantly (extended transmission disequilibrium test (ETDT) 37.82; $P = 0.0016$). A decreased transmission of the alleles with 13, 20 and 26 repeats to girls was observed ($T\%0$, $P = 0.046$; $T\%25.5$, $P = 0.0003$, $T\%0$, $P = 0.025$).

Conclusion: The results do not support a common role for the androgen receptor gene exon 1 CAG repeat in T1D susceptibility; however, an effect of a disease variant in linkage disequilibrium could be detected.

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Introduction

Autoimmune disorders are, in general, more common in women than in men (1). This sex-specific difference has been implicated as reflecting the effect of sex steroids on immune responses, particularly the immune suppressive effects of androgens (2). Type 1 diabetes (T1D) makes an exception from the gender rule, as the disease tends to be more prevalent in men (the male:female ratio is 1.1:1.7) (3, 4).

The target organ response to androgens is determined mainly by the androgen receptor (AR) (OMIM No. 313700, Xq11-q12), the activation of which induces expression of a multitude of genes (5). The AR gene is more than 90 kilobases in length and contains eight exons (6). The large amino-terminal domain is encoded by exon 1, which includes a highly polymorphic CAG repeat that is translated into a poly-glutamine stretch

(Fig. 1). *In vitro*, the length of the CAG repeats correlates inversely with the transactivating function of the AR (7, 8). The exon 1 CAG repeat of the AR is associated with various disorders. A high number of CAG repeats (more than 40) considerably decreases receptor function and results in androgen insensitivity, oligospermic infertility and andropausal symptoms (9–12). In addition, long repeat alleles have been associated with Kennedy's disease, an X-linked spinal bulbar muscular atrophy (13). On the other hand, shorter repeats, although in the normal range (7–28), have been associated with increased androgen sensitivity which can lead to increased prostate cancer risk in males and anovulatory infertility in females (14, 15).

There are very limited data on the relationship of AR and human autoimmune diseases. It has been reported that a shorter exon 1 CAG repeat length is associated with rheumatoid arthritis in younger onset male

Figure 1 Gene structure and functional domains of the androgen receptor (Xq11-12).

patients (16). In addition, data from animal studies suggest that androgen action might affect diabetes susceptibility in humans. It has been shown that androgen treatment prevents diabetes in non-obese diabetic mice; moreover, testosterone increased the circulating insulin levels and induced islet hypertrophy in rat and mouse diabetes models (2, 17, 18). Mice transgenic to expanded CAG triplet repeats were prone to diabetes (19). The involvement of androgens in the pathogenesis of the beta-cell specific autoimmune process has not been confirmed in man. However, it is known that the age-specific T1D incidence curve has its highest peak during puberty, and after pubertal years an increase in the male to female incidence ratio can be seen (20, 21). These observations and the above mentioned experimental data suggest that androgen hormone action could be involved in the pathogenesis of T1D.

The aim of the study was to investigate the linkage and association of a functional polymorphism of the androgen receptor gene with T1D.

Subjects and methods

Subjects

Two sets of families with affected children were analysed in this study. The first family set included 120 affected sibling pair families with two or more affected children. The second set comprised 645 nuclear families with one child affected by T1D and healthy parents. In addition, a cohort of sporadic cases was also analysed ($n = 208$). Most of the patients and families were collected from four university centres in Finland (Turku, Tampere, Oulu and Helsinki). All cases presented with classical T1D were diagnosed according to the World Health Organisation's criteria before the age of 15 years. Consecutively recruited healthy newborns from the same geographical regions were used as controls ($n = 1381$; 891 boys, 490 girls). The local Ethics Committees had approved the study

and informed consent was obtained from the participating subjects and/or from their parents.

Methods

DNA was extracted from peripheral EDTA-blood using a salting out method (22). HLA DQA1-DQB1 genotypes were identified using a PCR-based lanthanide-labelled oligonucleotide hybridisation and time-resolved fluorometry detection method (23, 24). The androgen receptor (AR) gene (Xq11-12) exon 1 CAG repeat polymorphism was analysed using PCR where one primer was labelled with a fluorescent dye. After initial denaturation for 1 min at 96 °C, samples were subjected to 30 cycles of amplification, consisting of a 20 s denaturation phase at 96 °C, a 1 min annealing phase at 62 °C and a 1 min extension phase at 72 °C. The forward primer was: 5'AGA ATC TGT TCC AGA GCG TGC 3', and the reverse primer was: 5'GAC TGC GGC TGT GAA GGT TGA 3' labelled with HEX dye. The AR gene exon 1 CAG repeat alleles were identified by fluorescence-based automated DNA fragment sizing according to the manufacturer's instructions (MegaBace 1000, Amersham Biosciences, CA, USA).

Statistics

Transmission disequilibrium test (TDT) and linkage analysis were carried out using TDTPhase (developed by Frank Dudbridge, 2000), MapMaker/Sibs (<ftp://ftp-genome.wi.mit.edu/distribution/software/sibs>) and Genehunter Z.O. (<http://galton.uchicago.edu/>) public softwares.

Chi-square statistics or Fisher's exact test were used for comparisons of the frequencies of CAG repeat polymorphisms in the groups studied. Independent samples *t*-test was used for comparison of lengths of repeat alleles between various groups (SPSS 11.0). A *P* value of 0.05 or less was considered significant. Correction for multiple comparisons was applied where appropriate.

Results

Case-control series

The number of exon 1 CAG repeats identified in cases and controls were between 7 and 28, which corresponds to the normal range (25). The distribution of CAG repeat alleles did not differ significantly between patients (1315 alleles) and the control group (1871 alleles) (Table 1). Similarly, no difference was found when boys were analysed separately. Interestingly, the short repeat alleles (7–14) were more prevalent in girls with diabetes compared with control girls (8.77% vs 5.91%; $P = 0.03$, odds ratio (OR) 1.53, 95% confidence interval (CI) 1.03–2.26); however the difference disappeared when correction

Table 1 Distribution of androgen receptor gene exon 1 CAG repeat alleles in children with type 1 diabetes and controls.

Allele	Diabetes (<i>n</i> = 1315)		Controls (<i>n</i> = 1871)	
	No.	%	No.	%
7	1	0.08	1	0.05
8	0	0	0	0
9	1	0.08	7	0.37
10	3	0.23	2	0.11
11	5	0.38	6	0.32
12	9	0.68	13	0.69
13	8	0.61	5	0.27
14	84	6.39	95	5.08
15	91	6.92	123	6.57
16	135	10.27	190	10.15
17	217	16.50	327	17.48
18	185	14.07	243	12.99
19	162	12.32	235	12.56
20	88	6.69	133	7.11
21	159	12.09	224	11.97
22	76	5.78	141	7.54
23	39	2.97	61	3.26
24	28	2.13	23	1.23
25	14	1.06	31	1.66
26	5	0.38	9	0.48
27	1	0.08	2	0.11
28	4	0.3	0	0

for multiple comparisons was applied. Cases were stratified according to age at diagnosis (0–4.9, 5–9.9 and 10–15 years) and groups were compared with each other and also with controls. No differences were detected (data not shown).

CAG repeat allele frequencies were also compared in HLA DR3-positive cases (*n* = 562) and controls (*n* = 365) and no differences were found either when the whole group or various age at diagnosis groups were compared.

However, when DR3-positive boys with T1D (*n* = 420) were compared with DR3-positive control boys (*n* = 229) the frequency of long repeat alleles (19–28) was reduced in the cases (32.6% vs 40.6%; *P* = 0.011, OR 0.66, 95% CI 0.47–0.91). The corrected *P* value was not significant. There was no difference between cases and controls when DR3-positive girls were analysed.

When the length of repeat alleles were compared between cases and controls using independent samples *t*-test, no difference was found. Similar comparisons were carried out on groups stratified according to sex, age at onset and HLA genotype, and no differences were seen.

TDT results

Transmission analysis including the 20 alleles identified in the 765 families was not statistically significant (global *P* value of 0.06 (extended transmission disequilibrium test (ETDT) 29.3)). However, a decreased transmission of the allele with 20 repeats to cases

was observed (T42, NT 72, T% 36.8; *P* = 0.005). No significant distortion from expected was seen when transmissions to boys were analysed. In contrast, the transmission of CAG repeat alleles to girls differed from the expected value significantly (ETDT 37.82; *P* = 0.0016). A profoundly decreased transmission of the allele with 20 repeats to girls was observed (T14, NT41, T%25.5; *P* = 0.0003). In addition, transmissions of alleles with 13 and 26 repeats to affected girls were also decreased (T0, NT4; *P* = 0.046; and T0, NT 5, *P* = 0.025 respectively). The ETDT finding in girls is mostly explained by the transmission distortion of these three alleles.

Linkage

Genetic linkage was analysed in sibling pair families using Genehunter 2.0 program, and a LOD score of 1.43 was obtained for the AR exon 1 CAG repeat polymorphism.

Discussion

In this study we analysed the association and linkage of the androgen receptor gene exon 1 CAG repeat polymorphism with type 1 diabetes in the Finnish population. A combined strategy of case-control and family-based approaches was used. The case-control comparison indicated that the short repeat alleles (7–14) were more prevalent in girls and the long repeat alleles (19–28) were less common in DR3-positive male patients than in controls, although the differences did not remain significant after correction for multiple comparisons. We conclude that the results do not support a common role for this polymorphism in the genetic predisposition to type 1 diabetes.

Interestingly, ETDT showed a profoundly decreased transmission of the repeat allele 20 in girls. It might indicate that it is not the studied AR polymorphism itself but a putative disease variant in linkage disequilibrium with this particular allele which may confer T1D susceptibility. The existence of such a diabetes susceptibility locus on chromosome X has been suggested, and the AR gene region was also identified in the first T1D genome scan with a maximum LOD score of 1.2 (3, 26). We found a similar evidence of linkage in this study. Since a more pronounced disease predisposing effect for the chromosome X T1D locus was indicated in HLA DR3-positive individuals, we analysed transmission of the AR alleles in the HLA DR3-positive family set. No distortion from the expected value could be identified.

We observed no difference in the length of AR repeat alleles between different age at onset and gender groups, which might indicate that androgen action does not contribute to the pubertal peak in disease incidence.

There is very limited data in the literature on the relationship of AR gene polymorphism and autoimmune diseases. Kawasaki and co-workers (16) found that young-onset male patients with rheumatoid arthritis had significantly shorter CAG repeat lengths than controls (21.8 vs 23.2) and suggested that increased androgen sensitivity could affect disease development. Similarly, shorter exon 1 CAG repeats were found to be more prevalent in patients with ankylosing spondylitis (22 vs 23) in the study of Mori *et al.* (27). Accordingly, both studies described a shift of AR alleles towards the short repeat range; however the biological significance of the findings is doubtful, since the difference in the allele length between the groups was about one repeat only. We analysed the distribution of the CAG repeat alleles and also compared the length of the alleles in both sexes, in different age at onset groups and also in the HLA DR3 subgroup. However, no differences were found.

In summary, our findings indicate that the AR exon 1 CAG polymorphism does not contribute to T1D susceptibility in Finnish patients. The ETDT results might indicate an effect of a disease variant in linkage disequilibrium and justify further T1D gene mapping efforts in this chromosome region.

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