Brief Genetics Report

Is Puberty an Accelerator of Type 1 Diabetes in *IL6-174CC* Females?

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The pubertal peak in onset of type 1 diabetes occurs earlier in girls than boys. We postulated that this sex difference might be mediated in part by estrogen or by genes regulated by estrogen, such as the interleukin-6 (*IL6*) gene. Previous studies concerning the role of an estrogen-sensitive single nucleotide polymorphism (SNP) in the *IL6* promoter in type 1 diabetes have proved contradictory. We therefore selected a large, genetically homogenous population-based cohort, analyzed by age at onset and sex, to test the hypothesis that the *IL6-174G>C* SNP affects age at onset of type 1 diabetes in females but not in males. We found that the *IL6-174CC* genotype was significantly less frequent in females diagnosed after than in those diagnosed before the age of 10 years (19 vs. 13%, $P = 0.016$). No genotype difference was observed in males stratified for age at onset. Among children diagnosed after age 10, the median age of onset was 11.9 years (intraquartile range 10.7–14.6) in 34 girls homozygous for *IL6-174C* compared with 13.2 years (11.6–15.4) in 229 girls with other genotypes and 13.5 years (12.0–15.6) in 339 males with any *IL6-174* genotype ($P = 0.012$). These data support the hypothesis that pubertal changes may contribute to accelerated onset of type 1 diabetes in genetically susceptible females. This phenomenon may be orchestrated by the action of estrogen on the *IL6* promoter. *Diabetes* 54:1245–1248, 2005

The peak age at onset of type 1 diabetes occurs between the ages of 8 and 14 years in females but not until ~2 years later in males (1). This has been attributed to earlier puberty in females, with associated endocrine and metabolic changes affecting insulin resistance. Since the hormonal changes of puberty differ between the sexes, genes regulated by sex hormones could play an important role in the different patterns of disease presentation. The gene for interleukin-6 (*IL6*) is a possible candidate as its promoter is regulated by 17-β-estradiol (E_2) (2).

IL6 is a pleiotropic cytokine expressed by a wide variety of cells: T- and B-cells, macrophages, dendritic cells, fibroblasts, and endothelial and pancreatic β-cells. A *G→C* polymorphism has been reported at position −174 of the *IL6* promoter affecting *IL6* transcription (3). A U.K. case-control study (4) found that the homozygous *IL6-174G/G* genotype was increased in patients with type 1 diabetes compared with healthy control subjects ($P < 0.002$), with a concomitant decrease in the *IL6-174C/C* genotype in the patients compared with control subjects (12.5 vs. 24.2%, respectively, $P < 0.004$) (4). In the 53 trios studied, the *IL6-174G* allele was transmitted in 29 of 53 (54.7%) informative meioses, and there was no association with age at onset. In contrast, in 206 Dutch simplex families where the proband was diagnosed under the age of 14 years, no association with the *IL6-174G>C* polymorphism was identified (5). A recent study of 253 Danish affected sibpair and simplex families (6) showed that the *IL6-174C* allele was strongly associated with type 1 diabetes in females but not in males and showed an age at onset effect in females. In this study, an increased transmission of 66% (95% CI 0.58–0.74) of the *IL6-174C* allele to females was observed.

The apparently discrepant results from these studies (4–6) may, however, be due to lack of power, to differences in selection criteria, or to differences between populations. The most rigorous test of association of a single nucleotide polymorphism (SNP) with a disease phenotype is through analysis of large homogeneous population-based cohorts (7). We therefore set out to test the hypothesis that there is a sex difference in the frequency of an estrogen-sensitive SNP in 1,130 individuals diagnosed with type 1 diabetes before the age of 21 years and participating in a population-based cohort from the U.K. (8). This study did not require control data, since it focused simply on whether there is a sex- and age-related difference in the frequency of a functional polymorphism in a genetically homogenous cohort with ages at diagnosis covering the onset of puberty.

Of the 1,130 individuals studied, 609 were male. Analysis of the frequency of the *IL6-174G>C* polymorphism demonstrated that the *IL6-174G>C* polymorphism was in

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BOX, Bart's Oxford; IL, interleukin; NF, nuclear factor; SNP, single nucleotide polymorphism.

TABLE 1
The frequency of the *IL6-174G>C* polymorphism in males and females overall and according to age at onset >10 and <10 years of age

	Females	Males	*P*
A: *-174IL6GC* frequency in males compared with females overall			
n	521	609	
G/G	183 (35)	198 (32.5)	
G/C	256 (49)	292 (48)	
C/C	82 (16)	119 (19.5)	0.27
B: *-174IL6GC* frequency in males compared with females diagnosed >10			
n	263	339	
G/G	95 (36)	113 (33)	
G/C	134 (51)	157 (46)	
C/C	34 (13)	69 (21)	0.016
C: *-174IL6GC* frequency in males compared with females diagnosed <10			
n	258	270	
G/G	88 (34)	85 (31)	
G/C	122 (47)	135 (50)	
C/C	48 (19)	50 (19)	0.78

Data are *n* (%) unless otherwise indicated.

Hardy-Weinberg equilibrium ($P = 0.78$), and no overall significant difference ($P = 0.27$) according to sex was observed in the overall *IL6-174G>C* genotype distributions (Table 1*A*). When males and females diagnosed over the age of 10 years were analyzed, there was, however, a difference in the frequency of the *IL6-174G>C* polymorphism between the two groups (genotype-wise $P = 0.02$ and allele-wise $P = 0.05$) (Table 1*B*). This difference was not detected in boys and girls diagnosed under the age of 10 years (Table 1*C*). The frequency of the *IL6-174G>C* polymorphism in girls diagnosed after the age of 10 was significantly lower compared with girls diagnosed earlier and compared with all males regardless of age (13 vs. 19% $P = 0.016$).

The effect of genotype and sex on the cumulative distribution function of age of onset is shown in Fig. 1. This figure indicates that the frequency of all genotypes is remarkably similar until the age of 9 years; after this age, girls homozygous for *IL6-174CC* show a left shift or acceleration in the age at diagnosis. Among children diagnosed after age 10, the median age of onset was 11.9 years (intraquartile range 10.7–14.6) in 34 girls homozygous for *IL6-174C* compared with 13.2 years (11.6–15.4) in 229 girls with other genotypes and 13.5 years (12.0–15.6) in 339 males with any *IL6-174* genotype ($P = 0.012$).

HLA class II DRB1-DQA1-DQB1 data were available on 1,115 of 1,130 (98.6%) subjects studied for the *IL6-174G>C* polymorphism, and no association between the presence of HLA class II haplotypes and *IL6* genotype was identified.

This study, designed to examine the role of an estrogen-sensitive polymorphism in the *IL6* promoter in a population-based cohort with type 1 diabetes, showed that the frequency of the *IL6-174CC* genotype decreases with increasing age of onset in females but not males with type 1 diabetes. Twice as many females as males homozygous for the *IL6-174C* allele develop diabetes between the ages of 8.5 and 11.5 years. These data are in agreement with a recent Danish study demonstrating association between the *IL6-174C* allele and type 1 diabetes in females but not males in a Danish population (6) but are contrary to other studies of the *IL6* gene in type 1 diabetes. It is worth noting that in the two studies (4,5) that did not find an association between *IL6-174G>C* and type 1 diabetes, the age at diagnosis was defined as <14 years, with a mean age

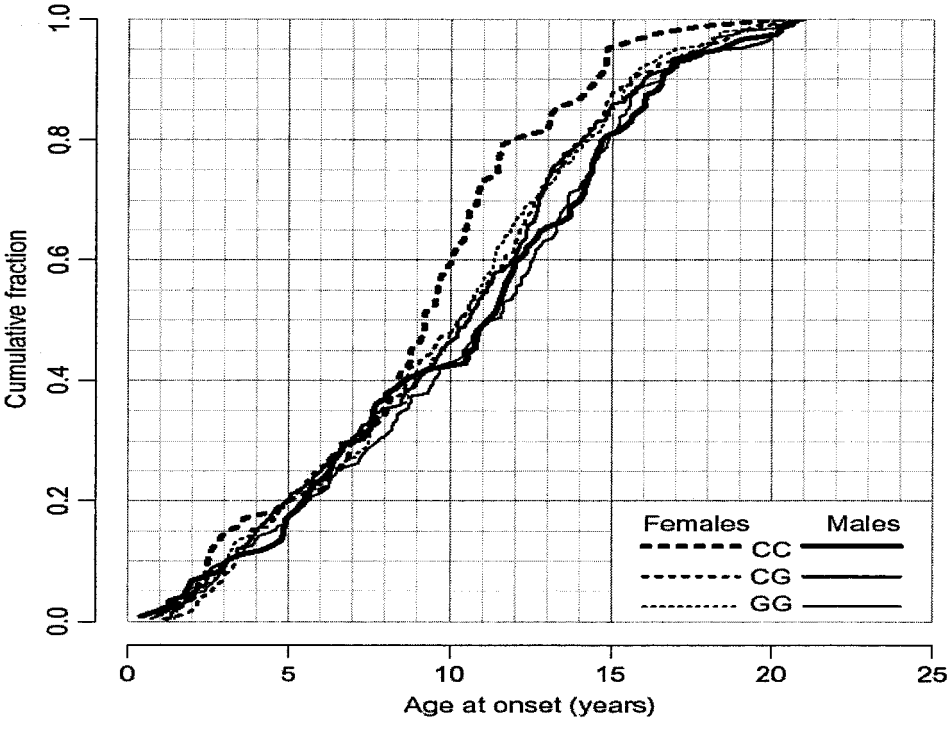

FIG. 1. The cumulative age of onset in 1,130 individuals with type 1 diabetes stratified by each *IL6-174G>C* genotype and sex.

of onset of 4 years in one study (5), and in the other (4), the data were not analyzed according to sex. It is possible that this type of sex effect, which is visible during adolescence, might also have been missed by whole-genome screens that usually focus on those diagnosed early in life (9,10).

Both the Danish and UK-BOX (Bart's Oxford) populations showed that females homozygous for *IL6-174CC* had a younger age of onset than females with an *IL6-174G* allele and all males studied. There were, however, interesting differences in the results obtained from both studies. When the Danish data were corrected for age at diagnosis <21 years, the median age of onset in *IL6-174CC* females was 6.4 years compared with 9.2 years in the BOX cohort. Furthermore, the cumulative incidence of type 1 diabetes, stratified according to age at onset and genotype, is linear in the U.K. population until the age of 8.5–9.5 years (Fig. 1), after which *IL6-174CC* females appear to have a left shift in age of onset of disease. In the Danish population, *IL6-174CC* females have an earlier age of onset throughout life (6).

We postulate that differences in the two populations tested may be responsible for the stronger effect of the *IL6(-174)CC* genotype in the Danish study. The cohort of 253 families comprised 150 affected sibpair families and 103 parent/offspring families collected retrospectively through adult and pediatric clinics. The families participating in the Bart's Oxford population-based study were identified prospectively with 95% case ascertainment (8); only 10% are affected sibpair families. Affected sibpair and affected parent/offspring families may be enriched for diabetes susceptibility genes, resulting in a greater effect in these families. We also considered that Denmark may be a higher-risk population than the U.K., but recent epidemiological data indicate that the incidence of type 1 diabetes is similar in both populations (11).

The mechanism by which the *IL6-174CC* genotype increases risk of type 1 diabetes in females but not in males is unclear. One possible explanation of the U.K. cumulative incidence stratified according to age at onset and genotype (Fig. 1) is that estrogen, switched on from ~9 years of age onwards (12) in females, has increased risk in *IL6-174CC* females, resulting in earlier age of onset. An effect of oral contraceptives in young women may be hidden within the 15- to 20-year-old group, as it has been reported that in some women, use of oral contraceptives affects *IL6* production (13).

Previous reporter studies have confirmed that estrogen plays a role in *IL6* regulation, although some of these data remain controversial (2,3,6). The first 225 bp of the promoter is sufficient for synergistic activation of *IL6* by the transcription factors nuclear factor (NF)-IL6 and NF-κB, and estrogen (E_2) receptors can interact directly with the NF-IL6/NF-κB complex, inhibiting their ability to bind to the *IL6* promoter and activate gene transcription (14). The *IL6-174GC* SNP lies adjacent to the multiple response binding site for NF-IL6, but its effect has proven contentious. An initial study using reporter assays showed that the *IL6-174 G* allele was more active than the *IL6-174C* allele in stimulated HeLa cells (3). A later study presented data indicating that the *IL6-174C* allele may be more active than the G allele in HeLa cells, but these differences did not reach statistical significance. The study emphasized that genetic regulation of *IL6* is controlled by several promoter variants and is cell specific (15).

The effect of the *IL6-174G>C* polymorphism has recently been analyzed in Ishikawa cells expressing the human estrogen receptor to elucidate the role of estrogen on the two different alleles of the *IL6-174G>C* polymorphism (6). The two alleles responded differently to E_2 pretreatment; when stimulated in the absence of estrogen, the *IL6-174C* allele was ~70% more active than the *IL6-174G* allele, and in the presence of estrogen, the activity of the *IL6-174G* allele was increased so that both alleles have equal function. These results do not offer an explanation of our genetic data, but it is possible, indeed likely, that the regulation of *IL6* in lymphocytes and islet β-cells differs from its regulation in the cell types previously studied. Further studies of the genetic regulation of *IL6* will be required to explain the mechanism underlying the increased risk to *IL6-174CC* females at puberty demonstrated in this study.

In conclusion, we have used a rigorous strategy to test the hypothesis that there is a sex difference in the frequency of an estrogen-sensitive SNP in individuals with type 1 diabetes. Our data demonstrate a role for the *IL6-174CC* genotype in the susceptibility of adolescent females to type 1 diabetes and provide preliminary evidence for a role for an estrogen-sensitive gene in determining the observed sex difference in type 1 diabetes susceptibility at puberty. On the basis of these data, we propose that haplotypes of *IL6* in combination with alleles of other genes regulated by estrogen may provide the key to the sex differences observed in type 1 diabetes.

RESEARCH DESIGN AND METHODS

DNA was collected from the probands of families participating in the BOX Study of Childhood Diabetes. This is a prospective, population-based family study that has, since 1985, recruited >95% of the families of children who have developed type 1 diabetes before age 21 years in the former Oxford Health Authority Region, U.K. (8). By March 2002, 1,746 families had been recruited into the study, with 89% under regular follow-up. The study population is 95% white European, and the remainder originate mainly from the Indian subcontinent. The classification of type 1 diabetes was based on assignment by the referring clinician and was made on the basis of World Health Organization criteria (16) and a clinical requirement for insulin treatment from diagnosis. Patients with secondary diabetes, known genetic subtypes including MODY (maturity-onset diabetes of the young), or clinical type 2 diabetes were not included in the study. The study was approved by the research ethics committees in all participating centers.

Genetic analysis. DNA extraction and HLA class II genotyping were performed as previously described (17). The *IL6-174G>C* SNP was analyzed in 1,130 individuals (609 male) with type 1 diabetes using PCR-RFLP (restriction fragment–length polymorphism) as described previously (6).

Statistical analysis. Frequency differences in the IL6 polymorphism between males and females were analyzed overall and according to age at diagnosis using the χ^2 and Fisher's exact tests. For the interaction between age at onset and genotype, a two-way ANOVA was used. Comparison of median age at diagnosis in all groups was analyzed by Kruskal-Wallis testing. All data were stored according to existing data protection regulations within the department.

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