The authors performed a meta-analysis of 33 studies examining the association of type 1 diabetes mellitus with polymorphisms in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene, including the A49G (29 comparisons), C(C255)T (three comparisons), and (AT)n microsatellite (six comparisons) polymorphisms. The studies included 5,637 cases of type 1 diabetes and 6,759 controls (4,775 and 5,829, respectively, for analysis of the A49G polymorphism). The random-effects odds ratio for the *G (Ala) allele versus the *A (Thr) allele was 1.45 (95% confidence interval (CI): 1.28, 1.65), with significant between-study heterogeneity ($p < 0.001$). The effect size tended to be higher in type 1 diabetes cases with age of onset $< 20$ years (odds ratio (OR) = 1.61), and there was a significant association between the presence of glutamic acid decarboxylase-65 autoantibodies and the *G allele among type 1 diabetes cases (OR = 1.49). Larger studies showed more conservative results ($p = 0.011$). After exclusion of studies with fewer than 150 subjects and studies with significant deviation from Hardy-Weinberg equilibrium in the controls, the summary odds ratio was 1.40 (95% CI: 1.28, 1.54). Available data showed no strong association for the 106-base-pair allele of the microsatellite polymorphism (OR = 0.99, 95% CI: 0.64, 1.55) or the *T allele of the C(C255)T polymorphism (OR = 0.92, 95% CI: 0.45, 1.89). This meta-analysis demonstrates that the CTLA-4*G genotype is associated with type 1 diabetes.

HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

CTLA-4 Gene Polymorphisms and Susceptibility to Type 1 Diabetes Mellitus: A HuGE Review and Meta-Analysis

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Abbreviations: bp, base pair; CI, confidence interval; CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; GAD-65, glutamic acid decarboxylase-65; IDDM, insulin-dependent diabetes mellitus; OR, odds ratio.

Editor’s note: This article is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).

GENE

The cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene is located on the long arm of chromosome 2q33. It consists of four exons and encodes a costimulatory molecule that is expressed on the surface of activated T cells (1). CTLA-4 and CD28 (also located on 2q33) are members of the immunoglobulin superfamily and bind to the B7 molecule on antigen-presenting cells. This completes the activation initiated when the antigen-specific cell-surface T-cell receptor (CD3 complex) engages the antigen bound to a major histocompatibility complex class II molecule on the surface of an antigen-presenting cell (2). CTLA-4 has a greater...
affinity for the B7 molecule than does CD28, and it down-regulates T-cell function. Therefore, it may play a crucial role in T-cell-mediated autoimmunity and thus in susceptibility to autoimmune diseases, including type 1 diabetes mellitus (3). CTLA-4-deficient mice rapidly develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis, and die 3–4 weeks postpartum (4, 5). CTLA-4 has also been thought to be potentially associated with a wide range of autoimmune disorders, such as autoimmune endocrinopathies, multiple sclerosis, rheumatoid arthritis, celiac disease, and primary biliary cirrhosis (6–10). It is considered the most likely candidate gene for type 1 diabetes susceptibility for the IDDM12 locus on chromosome 2q33 (11).

**GENE VARIANTS**

The human **CTLA-4** gene consists of four exons and three introns (11). There are at least three well-studied polymorphic markers that have drawn the most attention: a C-to-T substitution at position −318 (C(−318)T) of the promoter region (12), an A-to-G transition at position 49 of exon 1 (A49G) which causes a threonine-to-alanine substitution in codon 17 of the leader peptide (A17T) (13), and an (AT)n dinucleotide repeat polymorphism located in the 3′ untranslated region of exon 4 (14). Most epidemiologic studies have addressed the A49G polymorphism. There is evidence for strong linkage disequilibrium between these three polymorphisms (13, 15–17). The *T* allele of the C(−318)T polymorphism tends to occur with the *A* allele of the A49G polymorphism in European populations (15). The 106-base-pair (106bp) allele of the dinucleotide repeat polymorphism is linked with the *G* allele of the A49G polymorphism (13, 16, 17), but the 106bp allele is rather uncommon relative to the *G* allele of the A49G polymorphism. The 86-base-pair (86bp) allele of the dinucleotide repeat polymorphism is linked with the *A* allele of the A49G polymorphism.

Some data on the functional significance of **CTLA-4** polymorphisms are available. The (AT)n repeat may affect RNA stability, as it has been demonstrated for the long repeats product (18). The *G* allele has been related to the strength of down-regulation of T-cell activation (19, 20) and to reduced control of T-cell proliferation (20).

Many molecular epidemiologic studies have evaluated the potential role of A49G (13, 21–47), C(−318)T (31, 34, 44, 48), and the (AT)n repeat (31, 44, 49–52) in susceptibility to type 1 diabetes. Given the amount of accumulated data, it is important to perform a quantitative synthesis of the evidence.

**DISEASE**

Type 1 diabetes mellitus (formerly called insulin-dependent diabetes mellitus [IDDM]) is an organ-specific autoimmune disorder characterized by the T-cell-mediated destruction of the insulin-secreting β cells of the pancreatic islets of Langerhans (53). It affects approximately 35 million people worldwide, and its incidence exhibits significant geographic and racial variation, ranging from more than 35/100,000 population/year in Finland and Sardinia (Italy) to less than 3/100,000 population/year in Asian countries, including Japan, South Korea, and China. In most other Caucasian populations in Europe and America, incidence rates are moderate (10–20/100,000/year) (54). New diagnoses peak around puberty (55). Type 1 diabetes is the second most common chronic childhood disease after asthma (56). Incidence rates are similar for males and females, although a female preponderance has been noted in low-risk populations such as the Japanese (57). There is also seasonal variation, with higher rates being observed in the winter (58). A list of Internet sites pertaining to type 1 diabetes can be found on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).

The pathogenesis of type 1 diabetes includes a combination of multigenic predisposition and environmental factors. Viruses (59–62) such as Coxsackie B, mumps (63), and rubella (64) have been implicated as possible initiators, accelerators, or precipitators of the disease. Among postulated genetic factors, two regions have been well characterized: the major histocompatibility complex on chromosome 6p21 (IDDM1) (65, 66) and the insulin region on chromosome 11p15.5 (IDDM2) (67–70). Many other susceptibility loci have been proposed after complete or partial genome scans (IDDM3–15) (71–73), but the exact genes responsible have not yet been established. IDDM12, located on chromosome 2q33, is one of the confirmed type 1 diabetes susceptibility loci (13). This 300-kilobase region is known to contain at least three genes: **CD28**, **CTLA-4**, and the inducible costimulatory molecule (ICOS) gene. Genetic and physical mapping has suggested that **CTLA-4** or a gene in close proximity to it may be involved in susceptibility to type 1 diabetes (74).

**MATERIALS AND METHODS**

Identification and eligibility of relevant studies

We considered all studies that examined the association of the three major **CTLA-4** polymorphisms with type 1 diabetes. Sources included MEDLINE and EMBASE (search last updated in October 2004). The search strategy was based on combinations of the terms “**CTLA-4**,” “cytotoxic T-lymphocyte-associated antigen-4,” “**CD152**,” and “diabetes.” Reference lists in retrieved articles were also screened.

Nonfamilial case-control studies were eligible if the researchers had determined the distribution of genotypes for any of these polymorphisms in type 1 diabetes cases and disease-free controls. We included only published manuscripts, without any language restriction. We set no restrictions on the source of controls (general population, clinic, or hospital). We excluded studies with family-based designs in which the analysis was based on linkage considerations.

Data extraction

Two investigators independently extracted data, discussed disagreements, and reached consensus on all items. The following information was sought from each report:...
authors, journal and year of publication, country of origin, selection and characteristics of cases and controls, demographic data, racial descent of the study population (European, Asian, North African/Middle Eastern, Sub-Saharan African, or Pacific Asian), numbers of eligible and genotyped cases and controls, and genotype distributions in cases and controls and available subgroups thereof. Furthermore, we examined whether matching had been used; whether there was specific mention of blinding of the genotyping personnel to the clinical status of subjects; whether the genotyping method used had been validated; and whether genotype frequencies in control groups were in Hardy-Weinberg equilibrium.

**Meta-analysis**

For all three polymorphisms, we based the primary analysis on the contrast of alleles in order to detect overall differences. Where sufficient data were available, we also examined the contrast between the two groups of homozygotes and contrasts of each group of homozygotes with the remaining subjects for the *A49G* polymorphism.

We used the odds ratio as the metric of choice. For each genetic contrast, we estimated between-study heterogeneity across all eligible comparisons using the $\chi^2$-based Cochran’s $Q$ statistic (75). Heterogeneity was considered significant at $p < 0.10$ (75). We also report $I^2$ metrics, which quantify heterogeneity irrespective of the number of studies (76). Large heterogeneity is claimed for $I^2$ values of $\geq 75$ percent (76). Data were combined using both fixed-effects (Mantel-Haenszel) and random-effects (DerSimonian and Laird) models. Random effects incorporate an estimate of the between-study variance and provide wider confidence intervals, when the results of the constituent studies differ among themselves. Random effects are more appropriate when heterogeneity is present (75). Unless stated otherwise, random-effects estimates are reported here. In analyses of subgroups, we estimated odds ratios according to racial descent of the study population (European, North African/Middle Eastern, Sub-Saharan African, Asian, or Pacific Asian). Eligibility criteria for patients are shown in table 1. Controls were healthy subjects who were described as normoglycemic and/or non-diabetic, although varying details were presented regarding the extent of testing that had been done to exclude controls with impaired glucose tolerance and diabetes (table 1). Eleven studies (21, 26, 35–37, 39–42, 45, 47) also excluded subjects with a family history of diabetes from the control group.

One study matched for age (43); one study matched for age and gender (27); two studies matched for age, gender, and geographic region (42, 50); and one study matched for geographic region (36). No matching was reported in the other investigations. Polymerase chain reaction methods were used for genotyping in all of the studies. No researchers mentioned explicit blinding of the personnel who performed the genotyping. In five studies, the distribution of genotypes in the control group deviated significantly from Hardy-Weinberg equilibrium (27, 37, 38, 42, 45).

**META-ANALYSIS RESULTS**

**Eligible studies**

A total of 1,050 articles were originally screened. Thirty-three eligible studies examining the relation between *CTLA-4* polymorphisms and susceptibility to type 1 diabetes were identified (13, 21–52) (table 1). Twenty-eight studies contained data for the *A49G* polymorphism (13, 21–47), three contained data for the $C(-318)T$ polymorphism (31, 44, 48), and six contained data for the $AT(n)$ repeats (31, 44, 49–52). One of the eligible studies included subjects from two different racial groups (33), so a total of 29 comparisons were considered for the *A49G* polymorphism. There was considerable diversity of ethnic groups. Eligibility criteria for patients are shown in table 1. Controls were healthy subjects who were described as normoglycemic and/or non-diabetic, although varying details were presented regarding the extent of testing that had been done to exclude controls with impaired glucose tolerance and diabetes (table 1). Eleven studies (21, 26, 35–37, 39–42, 45, 47) also excluded subjects with a family history of diabetes from the control group.

The *A49G* polymorphism

**Meta-analysis database.** The eligible studies for analysis included a total of 5,637 cases with type 1 diabetes and 6,759 controls, of which 4,775 cases and 5,829 controls were available for analysis of the *A49G* polymorphism (table 2). Genotype data were available for 4,615 cases and 5,629 controls, because Ihara et al. (31) provided only allele frequencies. The pooled frequency of the $*G$ allele was 43.3 percent among control subjects (by race, frequencies were 55.4 percent, 36.2 percent, 33.6 percent, 20.6 percent, and 45.2 percent among controls of Asian, European, North African/Middle Eastern, Sub-Saharan African, and Pacific Asian descent, respectively). The overall pooled prevalence of $G/G$ homozygosity was 20.4 percent (33.4 percent, 12.8 percent, 8.9 percent, 5.7 percent, and 22.3 percent in the five racial descent groups, respectively). The overall pooled prevalence of $G/A$ heterozygosity was 44.8 percent (44.1 percent, 46.8 percent, 49.4 percent, 31.2 percent, and 45.7 percent in the five racial descent groups, respectively).

**Data synthesis.** The summary odds ratio suggested a 1.45-fold increase in susceptibility to type 1 diabetes among persons with the $*G$ allele, a finding that was highly statistically significant ($z = 5.72, p < 0.001$), but there was significant between-study heterogeneity ($p < 0.001$ for heterogeneity; $I^2 = 78$ percent) (table 3; figure 1). A sensitivity
TABLE 1. Characteristics of studies included in a meta-analysis of polymorphisms in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and susceptibility to type 1 diabetes mellitus

<table>
<thead>
<tr>
<th>Country(ies)</th>
<th>Racial descent</th>
<th>Selection/characteristics of cases and controls</th>
<th>No. of eligible subjects</th>
<th>Authors and year of study (reference no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>European</td>
<td>Persons with type 1 diabetes from the Belgian Diabetes Registry Ethnically matched subjects</td>
<td>483</td>
<td>Nistico et al., 1996 (13)</td>
</tr>
<tr>
<td>United States</td>
<td>European</td>
<td>Persons with type 1 diabetes; age at onset &lt; 18 years Nondiabetic persons aged &gt; 18 years who were HLA-DR3*- or HLA-DR4-positive; absence of HLA-DR2</td>
<td>158</td>
<td>Owerbach et al., 1997 (52)</td>
</tr>
<tr>
<td>Germany and Canada</td>
<td>European</td>
<td>Persons with type 1 diabetes; age at onset 2–33 years (mean = 17.9 years) Healthy persons from Germany and Canada with no family history of diabetes or autoimmune disease</td>
<td>293</td>
<td>Donner et al., 1997 (21)</td>
</tr>
<tr>
<td>Belgium</td>
<td>European</td>
<td>Persons with type 1 diabetes aged &lt; 40 years from the Belgian Diabetes Registry who fulfilled NDDG* criteria; mean age = 20 years Blood donors, seemingly healthy paramedical personnel, and children with minor trauma seen in the emergency department (mean age = 20 years (SD*, 11))</td>
<td>525</td>
<td>Van der Auwera et al., 1997 (22)</td>
</tr>
<tr>
<td>Poland</td>
<td>European</td>
<td>Children with type 1 diabetes; mean age at onset = 9.5 years (range, 0.4–17.5 years) Healthy blood donors from the same area</td>
<td>192</td>
<td>Krokowski et al., 1998 (23)</td>
</tr>
<tr>
<td>France</td>
<td>European</td>
<td>Adult-onset patients with insulin-dependent diabetes mellitus; 62.5% males; mean age at onset = 24.9 years Healthy blood donors</td>
<td>112</td>
<td>Djilali-Saiah et al., 1998 (24)</td>
</tr>
<tr>
<td>Japan</td>
<td>Asian</td>
<td>Persons with type 1 diabetes per clinical features and laboratory data (islet cell antibodies and postprandial serum C-peptide); mean age at onset = 24.5 years (range, 1–69 years) Randomly selected healthy subjects aged 18–71 years</td>
<td>173</td>
<td>Awata et al., 1998 (25)</td>
</tr>
<tr>
<td>Japan</td>
<td>Asian</td>
<td>Unrelated persons with type 1 diabetes who had sudden onset of severe symptoms or rapid progress to overt diabetes and were dependent on exogenous insulin; mean age at onset = 25.9 years Unrelated persons with no clinical evidence or family history of diabetes mellitus or any autoimmune disease</td>
<td>110</td>
<td>Yanagawa et al., 1999 (26)</td>
</tr>
<tr>
<td>Japan</td>
<td>Asian</td>
<td>Persons with insulin-dependent type 1 diabetes according to 1997 ADA* criteria; 42.7% males; mean age at onset = 34 years (SD, 2); mean age = 44 years (SD, 2) Healthy persons matched on age and sex</td>
<td>117</td>
<td>Hayashi et al., 1999 (27)†</td>
</tr>
<tr>
<td>Japan</td>
<td>Asian</td>
<td>Persons with type 1 diabetes aged 1–71 years who were diagnosed according to NDDG criteria Randomly selected healthy subjects aged 18–71 years</td>
<td>111</td>
<td>Abe et al., 1999 (28)</td>
</tr>
<tr>
<td>Japan</td>
<td>Asian</td>
<td>Persons with type 1 diabetes per clinical and laboratory features (anti-GAD-65* level &gt; 1.2 U/ml, urinary C-peptide level &lt; 20 µg/day); 38.7% males; mean age at onset = 21.8 years (SD, 18.8); age ≤ 26.4 years Healthy subjects; 44.9% males</td>
<td>74</td>
<td>Takara et al., 2000 (29)</td>
</tr>
</tbody>
</table>

Table continues
### TABLE 1. Continued

<table>
<thead>
<tr>
<th>Country(ies)</th>
<th>Racial descent</th>
<th>Selection CHARACTERISTICS OF CASES AND CONTROLS</th>
<th>No. of eligible subjects</th>
<th>Authors and year of study (reference no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>China</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per NDDG criteria; 50.6% males; age at onset 0.3–15.0 years (mean = 7.1 years (SD, 3.7))</td>
<td>253</td>
<td>91</td>
</tr>
<tr>
<td><strong>Sweden</strong></td>
<td>European</td>
<td>Persons with type 1 diabetes aged 0–34 years  Age-, region-, and sex-matched normal subjects</td>
<td>616</td>
<td>502</td>
</tr>
<tr>
<td><strong>Japan</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes Healthy controls</td>
<td>236</td>
<td>390</td>
</tr>
<tr>
<td><strong>Japan</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per NDDG criteria; mean age at onset = 7.9 years (SD, 5.5) (range, 0–22 years); 60.6% males</td>
<td>160</td>
<td>200</td>
</tr>
<tr>
<td><strong>Tunisia</strong></td>
<td>North African</td>
<td>Persons with type 1 diabetes aged 1–15 years (mean age = 10.3 years) Normal children with no autoimmune disorders</td>
<td>74</td>
<td>48</td>
</tr>
<tr>
<td><strong>China</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per NDDG criteria; 70.3% males</td>
<td>350</td>
<td>420</td>
</tr>
<tr>
<td><strong>Ghana</strong></td>
<td>Sub-Saharan African</td>
<td>Persons with type 1 diabetes recruited from pediatric units; 52.7% males Healthy persons aged 0.3–15 years (mean age = 6.9 years (SD, 4.2)); 50.2% males</td>
<td>182</td>
<td>201</td>
</tr>
<tr>
<td><strong>Northern Ireland</strong></td>
<td>European</td>
<td>Persons with type 1 diabetes with age at onset &lt;15 years from a prospective register (diagnosis 1997–1999) Randomly sampled healthy schoolchildren aged 12–15 years</td>
<td>130</td>
<td>307</td>
</tr>
<tr>
<td><strong>China</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per NDDG criteria; 42.9% males; age at diagnosis 0.3–15.8 years (mean = 7.2 years (SD, 3.8)) Healthy siblings of patients; 46.2% males</td>
<td>347</td>
<td>260</td>
</tr>
<tr>
<td><strong>Japan</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per WHO* Study Group criteria; 40.0% males; age at onset 0.6–16 years</td>
<td>125</td>
<td>200</td>
</tr>
<tr>
<td><strong>Japan</strong></td>
<td>Asian</td>
<td>Persons with type 1 diabetes per NDDG criteria; median age at onset 9.0 years (range, 0–64 years) Healthy schoolchildren aged ~10 years</td>
<td>206</td>
<td>200</td>
</tr>
<tr>
<td><strong>Italy</strong></td>
<td>European</td>
<td>Adults with latent autoimmune diabetes; 25.5% males; mean age at diagnosis = 51 years (range, 25–68 years) Healthy subjects with no family history of diabetes, matched geographically; 49.4% males; median age, 48 years (range, 22–62 years)</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td><strong>France</strong></td>
<td>European</td>
<td>Persons with type 1 diabetes per WHO criteria; 46.3% males; mean age = 29 years (range, 5–66 years); mean disease duration = 12 years Nondiabetic subjects with no family history of type 1 diabetes; 28.6% males; mean age = 35 years (range, 20–63 years)</td>
<td>134</td>
<td>273</td>
</tr>
<tr>
<td><strong>Czech Republic</strong></td>
<td>European</td>
<td>Persons with type 1 diabetes per WHO criteria from an outpatient pediatric clinic; age at diagnosis &lt;15 years (mean = 7.6 years (SD, 3.8)); 48.9% males Nondiabetic children undergoing minor surgical interventions or surgery for hernias; mean age = 8.5 years (SD, 3.9)</td>
<td>305</td>
<td>289</td>
</tr>
</tbody>
</table>
analysis excluding one study that was clearly an outlier (42) yielded a summary odds ratio of 1.36 (95 percent confidence interval (CI): 1.24, 1.51; p < 0.001). Effect sizes were consistent across subgroups of differing racial descent (for patients of Asian descent, odds ratio (OR) = 1.41, p < 0.001; for patients of European descent, OR = 1.54, p < 0.001; data on other racial groups were sparse). There was still highly significant between-study heterogeneity within racial descent subgroups. When the five studies that deviated significantly from Hardy-Weinberg equilibrium were excluded, the random-effects odds ratio remained 1.44 (95 percent CI: 1.31, 1.59), but heterogeneity decreased (Q = 52.84, \( I^2 = 56 \) percent), even though the heterogeneity was still formally statistically significant (p < 0.001).

Analyses of genotypes suggested that G/G homozygosity more than doubled the risk of type 1 diabetes (p < 0.001, with substantial heterogeneity; \( I^2 = 71 \) percent), which is consistent with a codominant (per-allele) model. We found similar effect sizes in recessive and dominant models, with considerable heterogeneity (\( I^2 = 68 \) percent and \( I^2 = 90 \) percent, respectively). Results for all of these comparisons were consistent across racial descent subgroups (table 3).

In studies or subgroups of patients with type 1 diabetes and age of onset <20 years (15 contrasts with 11,768 alleles;...
see Web table 1, available on the websites of the *Journal* (http://www.aje.oupjournals.org) and the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/), the random-effects odds ratio was 1.61 (95 percent CI: 1.28, 2.02; $Q = 92.99; I^2 = 85 percent$) (figure 2). In studies or subgroups of patients with late onset (13 contrasts

### Table 2. Distribution of genotypes of the A49G polymorphism in studies of the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and susceptibility to type 1 diabetes mellitus

<table>
<thead>
<tr>
<th>Authors and year of study (reference no.)</th>
<th>Racial descent</th>
<th>G/G No. of cases</th>
<th>G/A No. of cases</th>
<th>A/A No. of cases</th>
<th>Frequency of the <em>G</em> allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nistico et al., 1996 (13)</td>
<td>European</td>
<td>74</td>
<td>248</td>
<td>161</td>
<td>40.9</td>
</tr>
<tr>
<td>Donner et al., 1997 (21)</td>
<td>European</td>
<td>55</td>
<td>147</td>
<td>91</td>
<td>43.9</td>
</tr>
<tr>
<td>Van der Auwera et al., 1997 (22)</td>
<td>European</td>
<td>75</td>
<td>269</td>
<td>181</td>
<td>39.9</td>
</tr>
<tr>
<td>Krokowski et al., 1998 (23)</td>
<td>European</td>
<td>60</td>
<td>95</td>
<td>37</td>
<td>56.0</td>
</tr>
<tr>
<td>Djilali-Saiah et al., 1998 (24)</td>
<td>European</td>
<td>34</td>
<td>41</td>
<td>37</td>
<td>48.7</td>
</tr>
<tr>
<td>Awata et al., 1998 (25)</td>
<td>Asian</td>
<td>72</td>
<td>80</td>
<td>21</td>
<td>64.7</td>
</tr>
<tr>
<td>Yanagawa et al., 1999 (26)</td>
<td>Asian</td>
<td>45</td>
<td>46</td>
<td>19</td>
<td>61.8</td>
</tr>
<tr>
<td>Hayashi et al., 1999 (27)</td>
<td>Asian</td>
<td>54</td>
<td>42</td>
<td>21</td>
<td>64.1</td>
</tr>
<tr>
<td>Abe et al., 1999 (28)</td>
<td>Asian</td>
<td>50</td>
<td>45</td>
<td>16</td>
<td>65.3</td>
</tr>
<tr>
<td>Takara et al., 2000 (29)</td>
<td>Asian</td>
<td>33</td>
<td>25</td>
<td>16</td>
<td>61.5</td>
</tr>
<tr>
<td>Lee et al., 2000 (30)</td>
<td>Asian</td>
<td>150</td>
<td>85</td>
<td>18</td>
<td>76.1</td>
</tr>
<tr>
<td>Ihara et al., 2001 (31)</td>
<td>Asian</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>70.6</td>
</tr>
<tr>
<td>Kamoun Abid et al., 2001 (32)</td>
<td>North African</td>
<td>32</td>
<td>38</td>
<td>4</td>
<td>68.9</td>
</tr>
<tr>
<td>Osei-Hyiaman et al., 2001 (33)</td>
<td>Sub-Saharan African</td>
<td>9</td>
<td>67</td>
<td>106</td>
<td>23.4</td>
</tr>
<tr>
<td>McCormack et al., 2001 (34)</td>
<td>European</td>
<td>21</td>
<td>69</td>
<td>40</td>
<td>42.7</td>
</tr>
<tr>
<td>Kikuoka et al., 2001 (35)</td>
<td>Asian</td>
<td>57</td>
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*ND, no data (no genotype data available).*
with 9,260 alleles; Web table 2), it was 1.31 (95 percent CI: 1.28, 1.54; $Q = 83.12$, $I^2 = 86$ percent) (figure 3). Patients with type 1 diabetes were separated according to the presence or absence of GAD-65 autoantibodies in only six studies (962 alleles; Web table 3). Carriage of the *G allele was significantly associated with the presence of these autoantibodies, without any between-study heterogeneity (random-effects OR = 1.49, 95 percent CI: 1.05, 2.13; $Q = 1.99$; $I^2 = 0$ percent) (figure 4).

**Bias and heterogeneity diagnostics.** There was no evidence that the magnitude of the overall odds ratio estimates changed in the same direction over time. For the allele comparison, the random-effects odds ratio was 1.44 in 1996, 1.41 in 1997, 1.39 in 1998, 1.25 in 1999, 1.30 in 2000, 1.36 in 2001, 1.48 in 2002, 1.45 in 2003, and 1.45 through October 2004.

There was evidence that larger studies (those with smaller variance) showed more conservative results for the association of the *G allele with type 1 diabetes than smaller studies ($\tau$ rank correlation = 0.34, $p = 0.011$). This was primarily due to the three smallest studies (each with fewer than 150 subjects), which were also the ones that showed the most prominent odds ratio estimates. After exclusion of these three studies, there was no longer any significant correlation between the variance and the effect size ($\tau = 0.17$, $p = 0.22$), and the summary odds ratio by random effects was 1.33 (95 percent CI: 1.21, 1.46), with diminished between-study heterogeneity ($Q = 62.79$, $I^2 = 60$ percent).

After exclusion of both the three smallest studies and those with significant deviation from Hardy-Weinberg equilibrium, the summary random-effects odds ratio was 1.40 (95 percent CI: 1.28, 1.54), and heterogeneity decreased further ($Q = 43.62$ (21 df), $I^2 = 52$ percent). Between-study heterogeneity also diminished in the subgroups with age of onset < 20 years (OR = 1.48, 95 percent CI: 1.27, 1.73; $I^2 = 57$ percent) and late onset (OR = 1.23, 95 percent CI: 0.96, 1.57; $I^2 = 79$ percent).

### Other polymorphisms

The 106bp allele of the (AT)n microsatellite polymorphism (four studies (2,958 alleles); random-effects OR = 0.99, 95 percent CI: 0.64, 1.55; $Q = 9.37$, $I^2 = 68$ percent) and the T allele of the C(−318)T polymorphism (three studies (2,398 alleles); random-effects OR = 0.92, 95 percent CI: 0.45, 1.89; $Q = 8.85$, $I^2 = 77$ percent) were not significantly associated with type 1 diabetes (Web table 4). Two studies (31, 49) suggested a protective effect against type 1 diabetes susceptibility for the 86bp allele of the (AT)n microsatellite, but four other studies examining the (AT)n microsatellite (44, 50–52) did not report on this allele contrast, so the postulated association should be viewed with caution.

### DISCUSSION

This meta-analysis included data from 33 studies with approximately 12,400 type 1 diabetes cases and controls. Over 10,500 subjects were from 29 studies concerning the A49G polymorphism. The A49G polymorphism is clearly associated with type 1 diabetes. We caution the reader that there was significant heterogeneity in the results of the analyzed studies. Exclusion of the smallest studies, which were also the ones that gave the most impressive estimates of association, and studies in which controls deviated from
Hardy-Weinberg equilibrium diminished the heterogeneity considerably and suggested a 40 percent relative increase in the risk of type 1 diabetes conferred by this polymorphism. There were hints that the effect may be stronger in type 1 diabetics with age of onset < 20 years and that the *G allele may also be associated with the presence of GAD-65.

FIGURE 1. Odds ratios for the association between the *G allele of the A49G polymorphism in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and susceptibility to type 1 diabetes mellitus. Individual studies (circles) are listed by increasing sample size from top to bottom. The diamond shows the summary random-effects odds ratio estimate from a meta-analysis. Horizontal lines, 95% confidence interval. (For individual studies, see reference list.)

FIGURE 2. Odds ratios for the association between the *G allele of the A49G polymorphism in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and susceptibility to type 1 diabetes mellitus in studies or subgroups with age at onset < 20 years. Individual studies (circles) are listed by increasing sample size from top to bottom. The diamond shows the summary random-effects odds ratio estimate from a meta-analysis. Horizontal lines, 95% confidence interval. (For individual studies, see reference list.)
autoantibodies in type 1 diabetes patients. These latter associations should be viewed with some reservation, given the relatively wide confidence intervals and the selective reporting of information regarding these parameters in the study reports. The association of *G allele carriage with anti-GAD-65 autoantibodies may also reflect undetected confounding with duration of disease or age of onset. However, these observations further strengthen the notion that the A49G polymorphism may be associated with the more typical, autoimmune variant of diabetes mellitus.

Data on other CTLA-4 polymorphisms were sparse. We saw no effect for the 106bp allele of the \((AT)\n\) polymorphism, while the 86bp allele seemed to be associated with protection from type 1 diabetes in two studies. This would

![FIGURE 3](https://academic.oup.com/aje/article-abstract/162/1/3/166364)  
**FIGURE 3.** Odds ratios for the association between the *G allele of the A49G polymorphism in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and susceptibility to type 1 diabetes mellitus in studies or subgroups with late onset. Individual studies (circles) are listed by increasing sample size from top to bottom. The diamond shows the summary random-effects odds ratio estimate from a meta-analysis. Horizontal lines, 95% confidence interval. (For individual studies, see reference list.)

![FIGURE 4](https://academic.oup.com/aje/article-abstract/162/1/3/166364)  
**FIGURE 4.** Odds ratios for the association between carriage of the *G allele of the A49G polymorphism in the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene and the presence of glutamic acid decarboxylase-65 autoantibodies among patients with type 1 diabetes mellitus. Individual studies (circles) are listed by increasing sample size from top to bottom. The diamond shows the summary random-effects odds ratio estimate from a meta-analysis. Horizontal lines, 95% confidence interval. (For individual studies, see reference list.)
be consistent with the linkage of the latter allele with the *A allele of the A49G polymorphism. At least one linkage study has suggested that the polymorphism actually involved in the disease is not A49G but a polymorphism in linkage disequilibrium with CTLA-4 markers that are probably closer to (AT)n than to A49G (80). Finally, because of linkage disequilibrium with the *A allele of A49G, one might expect a small amount of protection to be conferred by the *T allele of the (C–318)T polymorphism, and our data are compatible with this possibility.

The observed between-study heterogeneity should lead to some caution. The smallest published studies tended to produce more impressive effect sizes than the larger studies. This may be due to publication or time-lag bias, wherein smaller studies with statistically significant results tend to be published more quickly than studies of similar sample size and quality with “negative” results (81–83). Moreover, in five studies, genotype frequencies in controls significantly deviated from Hardy-Weinberg equilibrium. Reassuringly, after exclusion of studies that deviated from Hardy-Weinberg equilibrium as well as very small studies, the heterogeneity decreased while the risk remained the same. Still, there was considerable heterogeneity even with these exclusions. This may suggest some residual bias and/or some genuine diversity of the strength of the association, depending on the specific clinical and laboratory characteristics of type 1 diabetes. Type 1 diabetes is quite a heterogeneous syndrome, with considerable variability in age of onset, abruptness of onset, and autoantibody profile. Alternatively, heterogeneity may also point to some other “causal” polymorphism in variable linkage disequilibrium with the *G allele across different populations in the same gene or in neighboring genes. Nevertheless, fine mapping analyses also suggest that peak linkage and association are observed in the CTLA-4 region (76).

Our data are also consistent with a dose-response genetic effect: G/G homozygosity more than doubled the risk of type 1 diabetes. Although selecting the best genetic model is difficult, the findings of this meta-analysis are most consistent with a codominant model. Moreover, the results of the subgroup analyses for different racial descent groups were compatible with this possibility. The methods used for CTLA-4 A49G genotyping in the studies analyzed here were based on the polymerase chain reaction technique, but several different variants were used, and primers differed across studies. The most common primers were forward 5'-GCTCTACCTCTGAAGACCT-3' and reverse 5'-AGTCTCAGCTACCTTGTAG-3' (used in eight studies); forward 5'-GCTCTACCTCTGAAGACCT-3' and reverse 5'-AACCCAGGTAGGAAGACAC-3' (used in six studies); and forward 5'-CCACGGCTTCTTGTCTGTA-3' and reverse 5'-AGTCTCAGCTACCTTGTAG-3' (used in four studies). Other primers were used in six studies, and in four studies the researchers did not specify the primers used. Although the genotyping error rate is likely to be small for a biallelic polymorphism, none of the studies included in the meta-analysis reported any data on validation of the genotyping or estimation of the measurement error rate. Interlaboratory standardization and consistent reporting of results may be an even greater issue for the microsatellite polymorphism. Future studies should also ensure and clearly report that the personnel who performed the genotyping were blinded as to the clinical status of participants.

**LABORATORY TESTS**

The methods used for CTLA-4 A49G genotyping in the studies analyzed here were based on the polymerase chain reaction technique, but several different variants were used, and primers differed across studies. The most common primers were forward 5'-GCTCTACCTCTGAAGACCT-3' and reverse 5'-AGTCTCAGCTACCTTGTAG-3' (used in eight studies); forward 5'-GCTCTACCTCTGAAGACCT-3' and reverse 5'-AACCCAGGTAGGAAGACAC-3' (used in six studies); and forward 5'-CCACGGCTTCTTGTCTGTA-3' and reverse 5'-AGTCTCAGCTACCTTGTAG-3' (used in four studies). Other primers were used in six studies, and in four studies the researchers did not specify the primers used. Although the genotyping error rate is likely to be small for a biallelic polymorphism, none of the studies included in the meta-analysis reported any data on validation of the genotyping or estimation of the measurement error rate. Interlaboratory standardization and consistent reporting of results may be an even greater issue for the microsatellite polymorphism. Future studies should also ensure and clearly report that the personnel who performed the genotyping were blinded as to the clinical status of participants.

**POPULATION TESTING**

To date, no population testing for CTLA-4 polymorphisms is in use. The results of this meta-analysis do not suggest that such testing is indicated on a population-wide basis, given the relatively modest increase in risk conferred by the *G allele. Nevertheless, in future studies, investigators should consider including screening for the CTLA-4 *G allele in randomized clinical trials that enroll children at moderate or high perceived risk of developing type 1 diabetes. Family history remains the strongest risk factor for diabetes as type 1 diabetes (especially in Asian youths) cannot be fully excluded. This being acknowledged, overall misclassification of cases and controls is probably not a major issue in this meta-analysis.

Because of the various and serious lifelong complications of type 1 diabetes, it is crucial to identify etiologic factors for the pathogenesis of this disease. The major histocompatibility complex region explains approximately half of the genetic susceptibility to type 1 diabetes (65, 66), suggesting that additional determinants exist, and such determinants are suggested repeatedly by different genome scans (67–73, 85). However, while the list of identified candidate susceptibility loci is continuously expanding, most of the reported associations to date remain nonreplicable or at least controversial after subsequent investigation. Even when genetic associations are replicated (86), they usually have a minor public health impact that would not lead to routine screening recommendations (87). Nevertheless, such knowledge could improve our understanding of complex and multivariate diseases such as type 1 diabetes. Allowing for these caveats, the current meta-analysis demonstrates that CTLA-4 is a genetic determinant of type 1 diabetes.
type 1 diabetes susceptibility, but trials have also used immunologic testing for the presence of autoantibodies, metabolic screening, and human leukocyte antigen genotyping in various combinations (88–91). CTLA-4 genotyping may also provide some useful additional information for identifying high-risk children. On the basis of the frequency of the *G* allele in the analyzed studies and the estimated odds ratio, one can calculate that the attributable fraction of type 1 diabetes due to this polymorphism is 18 percent in populations of Asian descent and 16 percent in populations of European descent. We acknowledge that prior randomized trials on primary prevention using other parameters for risk stratification have either had largely negative results (88, 89) or are ongoing (91), but the concept of early intervention should be probed further with different preventive interventions and better risk stratification.

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