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A Study of Diabetes Mellitus Within a Large Sample of Australian Twins

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Twin studies of diabetes mellitus can help elucidate genetic and environmental factors in etiology and can provide valuable biological samples for testing functional hypotheses, for example using expression and methylation studies of discordant pairs. We searched the volunteer Australian Twin Registry (19,387 pairs) for twins with diabetes using disease checklists from nine different surveys conducted from 1980–2000. After follow-up questionnaires to the twins and their doctors to confirm diagnoses, we eventually identified 46 pairs where one or both had type 1 diabetes (T1D), 113 pairs with type 2 diabetes (T2D), 41 female pairs with gestational diabetes (GD), 5 pairs with impaired glucose tolerance (IGT) and one pair with MODY. Heritabilities of T1D, T2D and GD were all high, but our samples did not have the power to detect effects of shared environment unless they were very large. Weight differences between affected and unaffected cotwins from monozygotic (MZ) discordant pairs were large for T2D and GD, but much larger again for discordant dizygotic (DZ) pairs. The bivariate genetic analysis (under the multifactorial threshold model) estimated the genetic correlation between body mass index (BMI) and T2D to be 0.46, and the environmental correlation at only 0.06.

Diabetes mellitus is an etiologically and clinically heterogeneous group of metabolic diseases defined by hyperglycemia that is caused by defects in insulin secretion and/or insulin action. Depending on the underlying pathogenetic mechanism, the clinical course of the different types varies from a severe, rapidly onset disease with potentially high mortality to a slowly progressing disease that may go unnoticed for many years even while causing damage to target tissues. Diabetes mellitus is associated with micro- and macrovascular complications including nephropathy, retinopathy, autonomic and peripheral neuropathy and cardiovascular disease. The classification of diabetes mellitus into the different subtypes is based on pathogenesis and below follows a description of some of these subtypes.

Type 1 diabetes (T1D) accounts for approximately 10% of all diabetes and consists of the rare variant, idiopathic diabetes and the more common form, autoimmune diabetes (T1aD), which is one of the most common chronic diseases in children and adolescents of European origin. It is characterized by an absolute deficiency of insulin secretion, which results from a T-cell mediated autoimmune destruction of the pancreatic β cell. The patients are characterized by the presence of autoantibodies to β-cell autoantigens and have a lifelong requirement for exogenous insulin.

T1aD is a polygenic disorder resulting from a complex interplay of genetic and environmental risk factors. The dominant genetic susceptibility locus (termed IDDM1) is the human leucocyte antigen (HLA) region on chromosome 6, notably the Class II immune response genes HLA DQ and DR. Susceptibility alleles at the HLA locus (IDDM1) make up approximately 45% of the genetic variance of T1aD. Approximately 20 other loci, some of which may have a modest influence on the development of diabetes and others, which may prove to be false positives, have been reported and given IDDM designation numbers. Associations with T1D risk include the insulin gene-VNTR locus (IDDM2) chromosome 11p15 , CTLA4 (IDDM12) on chromosome 2q33 and PTPN22 on chromosome 1p13. Environmental risk factors implicated in T1D include viruses, dietary factors, nitrates and toxins. As these risk factors for disease are common in the general population, it is considered that known protective alleles as well as low penetrance of susceptibility genes may account for the low overall disease prevalence of 0.05 to 0.3%.

†Juli Condon died on April 1, 2006, aged 46, and this article is dedicated to her memory

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Type 2 diabetes (T2D) is thought to account for approximately 90% of all diabetes cases. T2D is also a heterogeneous and multifactorial disease and is likely to consist of a number of pathogenetic subgroups of currently unknown etiologies. T2D is typified by insulin resistance (i.e., impaired insulin action), which is most often accompanied by varying degrees of impaired insulin secretion. Chronic hyperglycemia may cause insulin resistance, termed 'glucose toxicity'. Chronic exposure to fatty acids, which in the short-term increases insulin secretion mediated by GPR40, a β cell fatty acid-activated receptor, promotes β cell death and diabetes. T2D usually presents with obesity or a high percentage of abdominal fat and is often coupled with hypertension and/or dyslipidemia. In T2D, autoimmune destruction of the β cell does not occur, but there is a decrease in β cell mass thought to result from increased β cell apoptosis.

A number of genes such CAPN10, encoding calpain 19, PPARG encoding peroxisome proliferator–activated receptor gamma and KCNJ11 encoding the potassium channel KIR 6.2. MODY1, MODY3 and the insulin receptor genes, as well as the mitochondrial genome have been implicated in elevated T2D risk (reviewed in Barroso, 2005; O'Rahilly et al., 2005). Several recently published genome-wide association scans have both confirmed these associations, and described novel strong associations to variants in noncoding regions (Diabetes Genetics Initiative of Broad Institute, 2007; Zeggini et al., 2007). Environmental risk factors include the global shift toward more sedentary work and lifestyle practises as well as dietary changes.

Gestational Diabetes (GD) is defined as any degree of glucose intolerance that is initially observed during pregnancy, irrespective of whether the diabetes onset predates, or is maintained beyond, pregnancy. GD results from pregnancy induced insulin resistance and relative diminished insulin secretion. It is a major risk factor for the development of T2D later in life as it as it is believed to result from the same genetic and physiological abnormalities that characterise T2D (Buchanan, 2005; Watanabe et al., 2007). GD patients diagnosed early in pregnancy have a greater risk of developing T2D postpartum than if diagnosed late in pregnancy.

The classical twin study is a powerful method used to determine the relative significance of genetic and environmental components in the etiology of a particular disease or trait. The primary aim of the classical twin study is to compare the concordance rates (the occurrence of the same disease/trait in both members of a twin pair) of identical or monozygotic (MZ) twins, who share a common set of genes, with those of nonidentical or dizygotic (DZ) twins, who, like siblings, share on average 50% of their genes. If MZ concordance rates are significantly higher than DZ rates, then a substantial genetic contribution to the etiology of disease can be inferred. The genetic sharing of MZ and DZ twins, together with a unique degree of environmental sharing beginning *in utero*, allows the separation and quantification of the genetic, shared environmental and unique environmental components of the phenotypic variation of a disease/trait at the population level. Analysis of twins, therefore, allows the estimation of disease heritability (h^2) — the proportion of variance in disease susceptibility due to genetic factors.

A number of studies of diabetes from the large population-based Scandinavian twin registries have been published. These include both simple disease concordance studies, and multivariate twin analyses of risk factors such as birth weight or obesity. Hyttinen et al. (2003) report data from 22,650 Finnish twin pairs born since 1958. The risk to a MZ co-twin of a T1D patient was 43%, but only 7.4% to a DZ co-twin, giving an estimated heritability (h^2) of 88% under the multifactorial threshold model. These results are similar to those obtained from 20,888 twin pairs from the Danish Twin Registry (Kyvik et al., 1995), where the crude MZ recurrence risk was 53% (DZ 13%, h^2 = 72%), and in smaller British and American clinic based MZ samples (Redondo et al., 2001), at approximately 50% (after allowing for censoring). In an earlier-born Finnish cohort, the 'Old Twin Cohort' (Kaprio et al., 1992) however, the MZ recurrence risk was significantly lower at 23%, which was interpreted as reflecting improvements in treatment since that era. A Finnish study of 10,168 nontwin siblings of Type 1 diabetics (Harjutsalo et al., 2005) obtained very similar estimated recurrence risks (crude 6.4%) to those seen in the DZ twins, suggesting that effects specific to a twin pregnancy do not greatly influence recurrence risk. By contrast, the Danish study (Kyvik et al., 1995) found DZ twins to be more concordant than ordinary siblings at 13% versus 7%.

With regard to Type 2 diabetes, there are fewer good studies, probably due to the fact that there are so many undiagnosed cases. The only population-based study originates from an Old Finnish Cohort (Kaprio et al., 1992), which gave a crude recurrence risk of 34% for MZ co-twins, and 16% for DZ co-twins. A Danish study of a subsample of 303 twin pairs who were all tested with an oral glucose tolerance test, resulted in crude recurrence risks of 50% for MZ co-twins and 37% for DZ co-twins, while the same risks were 63% and 43% when cases with T2D and impaired glucose tolerance were combined. The heritability estimates were 0.26 for T2D and 0.61 for the combined phenotype. A somewhat similar study was conducted based on male twin pairs in the United States (US), where it was found that 58% of the co-twins of MZ twins with T2D had diabetes (Newman et al., 1987; Poulsen et al., 1999).

Hitherto, there have not been any similar large population-based twin studies from other countries. In the present article, we describe such a study of members of the Australian National Health and Medical Research Council Twin Registry.

Methods

Subjects

The diabetes study participants were all active members of the National Health and Medical Research Council's Australian Twin Registry (ATR), which is a volunteer based twin registry comprising approximately 25,000 twins at the time of ascertainment for the study. The ATR, which was founded in 1978, comprises 10% to 20% of the estimated twin population of Australia and has been shown through extensive analyses to be representative of the Australian population in regard to age, marital status, education (Baker et al., 1996) and social attitudes.

The sampling frame for ascertaining diabetic twins in the present study was derived from nine sources, which span the history of the ATR. All had at least one question relating to diabetes. Five were from studies previously conducted at the Queensland Institute of Medical Research (QIMR), including three mailed Health and Lifestyle Questionnaires (HLQ), one telephone interview and one venepuncture checklist. The other four sources came directly from the ATR and comprised two questionnaires and two recruitment brochures. The sample collected from the QIMR studies was based on individual information and included data for age, sex and body mass index (BMI). The ATR data were based on forms filled out by twin pairs or their parents, so consequently it was not known if both or just one of the twins had diabetes.

QIMR-Based Studies

1. *Cohort 1*. The first HLQ was posted during 1980–1982 to 5967 ATR twin pairs who were born prior to 1965. As with all the HLQs, the Cohort 1 questionnaire covered an extensive range of health issues including questions related to socio-demographic variables, personality measures, health problems and risk factors for disease. Study participants were asked the question: 'Have you any of the following conditions?' Diabetes was included in the list of 13 conditions and twins were required to tick the appropriate box(es) if they or their co-twin were affected. Questionnaires were completed by 3807 twin pairs and 570 single twins, giving a response rate of 69%. Of the 3807 pairs included in the diabetes sampling frame, 99 individuals ticked 'yes' for diabetes (see Table 1).
2. *Aged Cohort*. The HLQ was mailed out to 2281 twin pairs in three waves between November 1993 and July 1995. All twins were over 50 years of age (mean age of respondents was 61.5 ± 8.7 years) and 40% of them had previously participated in the Cohort 1 HLQ. Completed questionnaires were returned by 3116 individuals (1279 complete pairs and 558 singletons). A two-page telephone interview for nonresponders yielded a further 286 individual twins, providing an overall response rate of 75%. For the questionnaire, participants were asked the question: 'Have you or your twin ever had any of the following?' Diabetes was listed amongst 78 diseases and conditions. In the telephone interview, diabetes was listed amongst nine conditions and subjects were required to respond 'yes' or 'no' only for themselves. The Over 50s HLQ yielded 194 individual twins who had self-reported having diabetes.
3. *Cohort 2*. In 1990 a HLQ was mailed out to a younger cohort (mean age 23.2 ± 2.2 years) comprising 4269 twin pairs born between 1964 and 1970. Completed questionnaires were returned by 5058 individuals, giving a response rate of 59%. Participants were asked to respond 'yes' or 'no' to the question: 'Have you ever had any of the following?', which included diabetes amongst 17 diseases and conditions. They were also asked to respond 'yes' or 'no' to the question for their co-twin, as well as whether or not they or their co-twin had acquired diabetes before age 14 or at age 14 or older. Just 34 individuals from this younger cohort responded positively to the questions.
4. *SSAGA Blood*. In 1992–1993 the 3807 twin pair respondents from 'Cohort 1', as well as 64 pairs who had participated in an alcohol challenge study, were approached to undertake a psychiatric telephone interview, which was a modified version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). SSAGA was designed to examine the psychological, physical and social aspects of alcohol and other substance abuse as well as other psychiatric disorders such as anxiety and depression. Of the 6327 individuals who completed the interview, 3386 individuals (54%) provided blood samples during 1993–1996. The SSAGA blood checklist for venepuncture asked for information relating to current medical problems, including the question 'Do you have diabetes? yes/no'; 51 individuals responded 'yes' to diabetes.
5. *Twin 89*. In 1996–2000, a follow-up study to 'Cohort 2' was conducted in the form of a SSAGA telephone interview, with over 6000 individuals interviewed at completion. At the time of ascertainment for the diabetes study, 4824 individuals had been interviewed. Of these, 57 individuals had responded 'yes' to a question asking if they had diabetes.

ATR-Based Studies

6. *Senior Baseline questionnaire*. Health questionnaires were mailed out to both adult and juvenile twins during the period from 1985 to 1993. Questions related to many aspects of health, lifestyle and behavior. Diabetes was included amongst a list of 19 medical conditions and twins were requested to respond both for themselves and their co-twin. For the 'Senior' questionnaire, 69 out of the 5605 twin pairs who responded, reported that one or both twins had diabetes.

Table 1

The Diabetes Mellitus Sampling Frame Showing the Number of Individuals/Pairs, Who Reported Diabetes, in Each of the Datasets

Sample Frame Data Sets	Individuals			Twin Pairs	
	Diabetes	Total	Prevalence (%)	Diabetes	No Diabetes (One or both)
QIMR					
Cohort 1	99	7614	1.3		
Aged	194	3402	5.7		
Cohort 2	34	5058	0.7		
SSAGA	51	3386	1.5		
Twin 89	57	4823	1.2		
Total	375	16,065	2.3	265	8458
ATR					
Senior				69	5605
Junior				6	4943
Registration				28	3228
Newsletter				143	5083
Total				217	15,353
Total unique pairs				384	19,387

Note: Raw data was available for individual twins and for twin pairs for the QIMR and the ATR samples respectively.

7. *Junior Baseline questionnaire*. Six twin pairs reported one or both twins having diabetes from a total of 4943 twin pairs who responded to the 'Junior' questionnaire.
8. *Registration form checklist*. As part of a 'general medical history' checklist, which was included in an ATR registration brochure, twins were asked if they or their co-twin had diabetes. The brochure, which was used during 1991–1995, yielded 28 twin pairs where one or both had diabetes, out of a total of 3228 responding twin pairs.
9. *Newsletter disease checklist*. This checklist was sent out with the 1996 ATR newsletter and included a registration form and a short family health questionnaire. Individuals were asked if they or their co-twin had a variety of diseases including diabetes. One or both twins indicated that they had diabetes in 143 out of the 5083 pairs who completed the checklist.

As described, not all of the QIMR studies were independent. Forty percent of the Aged Cohort participants and nearly all of the SSAGA Blood participants were derived from the 1980 Cohort 1 study. Cohort 2 and Twin 89 had targeted the same younger cohort born between 1964 and 1970. Therefore, there was considerable overlap between the studies in the participants who had reported diabetes. Similarly, as all of the QIMR twins were registered with the ATR, individuals with diabetes were represented in both study groups. The two ATR baseline questionnaires alone represented 80% of all twins registered with the ATR at the time of the initiation of the diabetes study. On resolving the considerable overlap of twins from all the various questionnaires, interviews and checklists, 384 twin pairs had responded positively to diabetes, for one or both twins, in at least one of the nine sources. After removing individuals who were deceased and those who had asked not to be contacted for further studies (thus removing 42 pairs), plus those lost to follow-up (37 pairs), 305 twin pairs from a total pool of 19,387 were found to be eligible for the study.

Diabetes Questionnaires

The 305 twin pairs were initially sent an approach letter asking if they would like to participate in a study, which included taking part in a short telephone interview, on diabetes.

Telephone interview. Interview questions included those related to twin zygosity as well as current age, height and weight. Individuals who identified themselves as having diabetes were questioned as to the diabetes type as well as their age, height and weight at the onset of the disease and treatment since that time, including any hospitalisations and disease complications. All participants were questioned as to the health status and/or cause of death for family members. The diabetes telephone interview was completed by 506 individuals, giving a response rate of 83%. During the interview 266 individuals reported having diabetes. For those individuals who did not complete an interview (n = 104), 44 refused to participate, 8 were incapable of participating, 31 were unable to be contacted and 21 had died. During the telephone interview, participants who identified themselves as having diabetes were also asked for permission to

contact their treating doctor to obtain any additional medical information. They were also asked if they would consent to providing a blood sample. Medical release forms providing written consent for us to contact treating doctor/s were sent out to the 257 individuals who had so agreed. Subjects were asked to provide up to three names and addresses of doctors who had diagnosed or treated their diabetes. On the medical release forms subjects were also given the option of providing any further relevant medical information. The response rate for the return of medical release forms was 97%.

Medical questionnaire. From the information provided, a total of 398 medical questionnaires were posted to treating doctors on behalf of the 249 subjects who had returned the medical release forms. The overall response rate for the return of the medical questionnaires was 87% (n = 347). Of the 249 subjects, 148 had one medical questionnaire per person returned, 86 individuals had two questionnaires returned, 9 had three and 6 had no questionnaires returned from their designated doctor.

Diagnosis Confirmation

For every individual with diabetes, the telephone interview, medical release form and the medical questionnaire/s were examined to verify diabetes diagnosis and type. Verification criteria included, but were not confined to, treatment (at onset of disease as well as current), BMI and age of onset. Approximately 30% of the verified diagnoses were reviewed independently by one of us, who is a diabetologist (J.E.S.). Following this, 255 of the 267 diagnoses were classified as 'definite' and 12 (0.04%) were considered to be 'highly probable'. As such they were included in the analysis.

Zygosity

Opposite-sex pairs are automatically DZ. For same-sex pairs, zygosity was established from standard interview questions regarding twin similarity. The twins were asked if their eye and hair color and their complexion were the same as their co-twin's and whether or not the subjects considered themselves to be identical or nonidentical twins. The twins were also questioned as to the ease with which parents/teachers/strangers could distinguish them. Zygosity derived from questionnaire information has been demonstrated to provide at least 95% agreement with zygosity based on extensive blood typing (Martin & Martin, 1975) and genotyping (Ooki, 1990). For same-sex twin pairs who had participated in parallel studies from which genotyping and blood group information was available, these were used to determine zygosity.

Analysis

Although disease status is a binary trait, for multifactorial disease a useful heuristic is to consider that there is an underlying continuous distribution of liability, or susceptibility to disease. An individual is affected for disease when liability exceeds a threshold on the underlying distribution, which is assumed to be normally distributed. This formulation is computationally convenient but not as arbitrary as it first appears, in that the model is equivalent to one positing risk to continuously increase with liability score. For multifactorial diseases both genetic and environmental factors contribute to liability and the proportion of the total variation of liability resulting from genetic factors gives a measure of 'broad sense' heritability at the population level.

Probandwise concordance rates (2C/2C+D), where C is the number of concordant twin pairs and D is the number of discordant twin pairs, were estimated for MZ and DZ twins for the three main types of diabetes, T1D, T2D and GD. For GD, as there were no DZ concordant pairs, Haldane's correction was applied in order to estimate concordance. Probandwise concordance rates, which do not vary with ascertainment probability, estimate risk at the individual as opposed to pair level and thus accurately estimate the population casewise rate and may be directly compared with other familial recurrence risk rates. In twins with disease resulting from multifactorial inheritance, probandwise concordance rates can be used to estimate MZ and DZ co-twin correlations (r_{MZ} and r_{DZ}), or correlations of liability. However, as concordance rates are dependent on disease prevalence as well as correlations of liability, if the correlation is high but the disease has a low population prevalence then the concordance will be reduced. A direct comparison of MZ and DZ population risk ratios (PPR, which is the concordance divided by population prevalence) may also be used to determine the underlying genetic structure of a disease; for example, epistatic (hence polygenic) versus additive (either monogenic or polygenic) genetic effects. Both these approaches can be used as a basis for genetic hypothesis testing in twin studies.

Structural equation modeling was used to elucidate the variance components, that is genetic, shared environmental and unique environmental components, attributable to the familial correlations in liability estimated from diabetes concordances. Multifactorial threshold models that incorporate the effects of additive genes (A), common environment (C), and unique environment (E), were applied to the twin data for T1D, T2D and GD using the Mx statistical modeling package. The preferred model was determined from goodness-of-fit statistics and Akaike's information criteria (AIC), which was calculated for each model and is given by χ^2–2df (where df is degrees of freedom). A small AIC provides a measure of the best fitting and more parsimonious model. Co-twin Pearson correlations used as starting values for Mx were determined as:

$\Phi=\rho_{RP}=ad-bc/\sqrt{((a+d)(c+d)(a+c)(b+d))} = \sqrt{\chi^2/N}$, where χ^2 is Pearson's chi-square (Stuart & Ord, 1991).

To examine the genetic and environmental causes of the relationship between BMI and diabetes, a bivariate Cholesky decomposition was applied to the QIMR

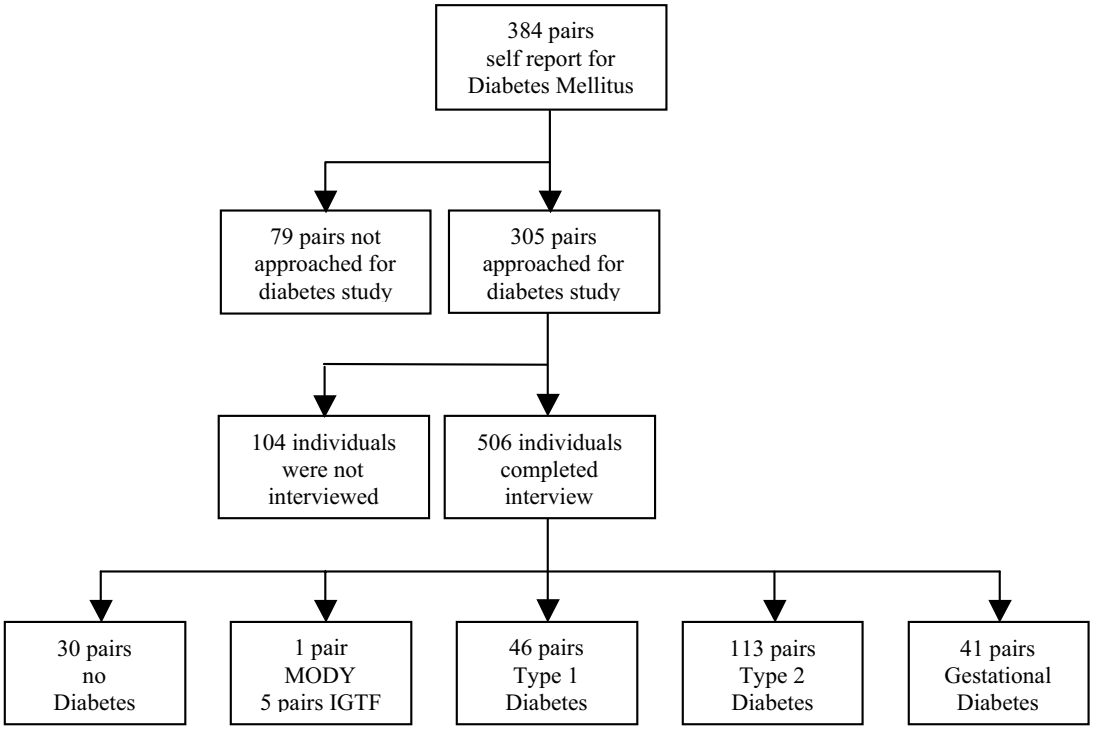

Figure 1

The Australian Twin Diabetes Mellitus Study. Of the 384 twin pairs approached for the Diabetes Study, type 1diabetes was found in 46 twin pairs, type 2 diabetes was found in 113 pairs and gestational diabetes in 41 pairs (see text for details).

raw data for these two variables. Since ATR surveys did not collect height and weight, they could not be included in this analysis. Because diabetes status is a binary variable, BMI was converted to an ordinal variable with four categories to enable bivariate analysis of these measures in Mx, and the model included an adjustment to threshold values for the covariates of age and sex for both variables. As the data were obtained from five sources ranging over a period of 20 years, BMI for twins without disease was predicted at the time of the diabetes interview using a flexible generalized additive regression model implemented in the *R* statistical computing environment (R Core Development Team, 2007). All other analyses were conducted using *R* and SPSS. For GD, all analyses were carried out for same-sex female twin pairs only.

Results

Diabetes Datasets

Of the 506 individuals (232 pairs and 42 singletons) who completed the diabetes questionnaire, 342 were female and 164 were male and 200 were MZ and 366 DZ. A total of 267 individuals were determined to have some form of diabetes or impaired glucose tolerance (IGT). Fifty-six individuals (46 twin pairs, either concordant or discordant for disease, and 12 single twins) had Type 1 diabetes, 152 (113 pairs and 17 single twins) had Type 2 diabetes, 45 (41 pairs and 3 single twins) had gestational diabetes. Thirty twin pairs did not have diabetes: 24 pairs self reported as nondiabetic during the interview and a further 6 pairs were demonstrated not to have diabetes from the medical questionnaires (Figure 1). One MZ twin pair was concordant for MODY and six individuals, consisting of one concordant and four discordant twin pairs, had IGT.

The individuals diagnosed as having T1D, T2D or GD are described in Table 2. The mean BMI at onset

Table 2

Age and Body Mass Index (BMI) for Individuals with Diabetes Mellitus: Type 1 Diabetes (T1D), Type 2 Diabetes (T2D) or Gestational Diabetes (GD), at Onset of Disease and at Time of Interview

	T1D	T2D	GD
Total	56	152	45
Sex			
Male	20	61	—
Female	36	91	45
Age at onset			
Mean (*SD*)	18.4 (11.5)	54.4 (13.9)	27.0 (5.0)
Median	15.0	55.0	27.0
Age at interview			
Mean (*SD*)	35.3 (17.0)	63.7 (12.5)	39.4 (9.5)
Median	30.5	65.0	37.0
BMI at onset			
Mean (*SD*)	19.2 (4.1)	29.5 (7.0)	25.9 (5.1)
BMI at interview			
Mean (*SD*)	24.4 (4.2)	28.1 (5.2)	26.3 (4.7)

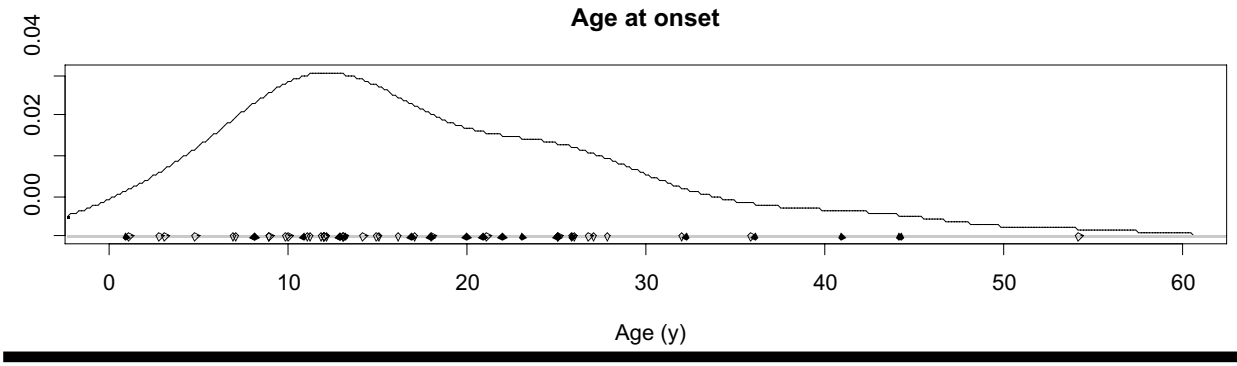

Figure 2

Distribution of age at onset for males (◆) and females (◇) diagnosed with Type 1 diabetes.

of disease for T1D, T2D and GD was 19, 29 and 26 respectively and the mean ages at onset were 18.4, 54.4 and 27.0 years. There was a large, but nonsignificant, sex difference in age of onset for T1D (median age 13.0 years for females and 20.5 years for males). The effect was further increased by removing one female reporting onset at age 54 from the sample (p = .06). However, on further removing two males who reported onset above age 40, resulting in a median age of 17.5 years for men, the sex difference disappeared (p = .45). There was no difference in age of onset in T2D between men (54.0) and women (55.0; p = .21).

The numbers of twin pairs concordant and discordant for T1D, T2D and GD are shown in Table 3. There were four twin pairs who were discordant for T2D where the co-twin had another type of diabetes (for two pairs the co-twin had T1D, for one pair the co-twin had GD and for another the co-twin had IGT). Therefore, in preparing the datasets for the analysis of the three main types of diabetes (T1D, T2D and GD) included in this study, these twin pairs were included in both datasets. That is, for a twin pair where one individual has T2D and one has T1D, the twin pair was included in both datasets; in the T1D data the T2D co-twin, and in the T2D data the T1D co-twin, were classified as unaffected for disease. As such, the total number of twin pairs, as shown in Figure 1, is 236.

Table 3

Monozygotic and Dizygotic Twin pairs Concordant and Discordant for Type 1, Type 2 and Gestational Diabetes Mellitus

Diabetes Type	Concordant		Discordant	
	MZ	DZ	MZ	DZ
T1D	6	2	8	30
T2D	18	4	26	65
GD*	3	0	18	9

Diabetes Concordance

Probandwise concordances (Cp_{MZ}, Cp_{DZ}), with 95% CI, for the various types of diabetes are shown in Table 4. The MZ and DZ concordances are significantly different in both T1D (χ^2_1 = 6.7, p < .01) and T2D (χ^2_1 = 18.9, p < .001). Genetic risk can be inferred from the concordance ratio (Cp_{MZ}/Cp_{DZ}), which was 5.1, 5.3 and 2.5 for T1D, T2D and GD respectively. For T1D, the MZ and DZ recurrence risks (or population risk ratio, PPR = Cp/P, where P is the population prevalence) were 203 and 40 respectively. Similarly, for T2D the PPR_{MZ} and PPR_{DZ} were 8.3 and 0.64 respectively. For GD, the PPR_{MZ} was 5.6 and the PPR_{DZ} was 0.45. All three diseases have PPR_{MZ} greater than 4 $PPR_{DZ,}$ indicating epistasis, thus suggesting these diseases are polygenic in nature.

There were no significant differences between concordances of male and female MZ pairs for either T1D (MZF: 0.52 [0.14–0.82]; MZM: 0.78 [0.26–0.97]), or T2D (MZF: 0.76 [0.53–0.91]; MZM: 0.62 [0.33–0.82]). Similarly, there was no significant difference in MZ concordance if we stratified for age of onset for either T1D (age of onset ≤ 11: 0.89 [0.44–0.99]; age of onset > 11: 0.71 [0.13–0.97]) or

Table 4

Concordance Rates for Monozygotic and Dizygotic Twins with Type 1, Type 2 or Gestational Diabetes Mellitus

Diabetes Type	Probandwise Concordance				
	MZ	DZ	$\chi^2$1	*P* value	MZ/DZ Ratio
T1D	.61 (.30–.83)	.12 (.02–.34)	6.7	< .01	5.1
T2D	.58 (.42–.72)	.11 (.03–.24)	18.9	< .001	5.3
GD*	.28 (.08–.55)	.11 (.00–.55)	0.3	.60	2.5

Table 5

Co-Twin Correlations for Liability to T1D, T2D and GD: Chi-Square Tests for Differences Between MZ and DZ Correlations Estimated by Maximum Likelihood

Diabetes type	Co-twin correlations (95%CI)		
	MZ	DZ	(χ^2_1)
T1D	.96 (.86–.99)	.61 (.32–.81)	11.21, $p < .001$
T2D	.93 (.86–.97)	.56 (.34–.72)	22.70, $p < .001$
GD	.75 (.52–.90)	–.26 (–1.0–.74)	6.65, $p < .05$

T2D (age of onset ≤ 54: 0.75 [0.50–0.90]; age of onset > 54: 0.46 [0.24–0.67]).

Heritability

Variance component threshold models were fitted to contingency tables for MZ and DZ twins constructed from the combined QIMR-ATR Contingency Data (Table 3). Prior to this, assumptions regarding the homogeneity of thresholds across birth order and zygosity were tested within a maximum likelihood framework using the chi-square likelihood ratio test as the statistical fit index. No effect of birth order or zygosity (MZ vs. DZ) on thresholds ($p > .05$) was found for any type of diabetes, resulting in the estimate of a single threshold for each classification.

MZ and DZ co-twin correlations in liability to disease were significantly different for T1D and T2D (both $p < .001$) and for GD ($p < .05$,) and are shown in Table 5.

A saturated model including ACE components of variance was initially fitted to the data, with subsequent models testing the significance of A and C parameters in explaining the trait variance. These nested models were compared using the likelihood ratio test, with fit indices given in Table 6.

Although the AE model is the more parsimonious for T1D (AIC = –6.53) and T2D (AIC = –5.67), this is considered to result from the small sample size as illustrated by the broad and overlapping confidence intervals of the MZ and DZ twin correlations (Table 5). The crude genetic variance for the AE model accounts for 96% and 93% of the total variance in T1D and T2D respectively. However, it is well recognized that a significant portion of the variance of these diseases is due to environmental factors. The heritabilities from the ACE models for T1D and T2D are 0.69 (0.26–0.99) and 0.73 (0.38–0.96) respectively, while the estimates of shared environmental variance are 0.27 (0.00–0.67) and 0.20 (0.00–0.53). Thus, while our sample size is too small to detect significant estimates of genetic and shared environmental variance simultaneously (Martin et al., 1978), shared environmental factors could be making a substantial contribution to variance in liability to both diseases, which we do not have to power to detect. For GD, our sample size is even smaller and the absence of any concordant DZ pairs ensures that the DZ correlation is negative. While liability to GD is best explained by an AE model with a heritability of 0.75 (0.00–0.90), our data are not incompatible with a shared environmental component as large as 76% of variance (Table 7).

Because age and BMI are potentially important covariates, we repeated the variance components analysis using raw data observations rather than summed contingency tables, reading in individual values of these variables as modifiers of the disease threshold for each type of diabetes. However, since these variables were not available for the ATR data, this analysis was carried out on the QIMR data alone. Therefore, the contingency table analysis described above was repeated using the QIMR dataset alone to allow a direct comparison of the effects of the covariates on variance component estimates. For T1D there was no effect of age or BMI on thresholds. However, for T2D there were large effects of age ($\chi^2_1 = 86.4$, $p = .00$) and BMI ($\chi^2_1 = 77.3$, $p = .00$) and therefore these were included in the model. Similarly for GD, for which the raw data analysis was carried out on female twins only ($n = 9741$ individuals), age ($\chi^2_1 =$

Table 6

Threshold Model Fit Indices for QIMR–ATR Contingency Data for T1D, T2D and GD

Diabetes Type	Model	χ^2	*df*	*P*	AIC	χ^2	*df*	*p*
T1D	ACE	0.54	3	.91	–5.46			
	AE	1.47	4	.83	–6.53	0.94	1	.33
	CE	11.21	4	.02	3.21	10.68	1	.00
	E	86.34	5	.00	76.34	85.80	2	.00
T2D	ACE	1.51	3	.68	–4.50			
	AE	2.37	4	.67	–5.67	0.86	1	.35
	CE	22.70	4	.00	14.7	21.20	1	.00
	E	195.18	5	.00	185.09	193.58	2	.00
GD	ACE	3.89	3	.27	–2.11			
	AE	3.89	4	.42	–4.11	0.00	1	1
	CE	6.65	4	.16	–1.36	2.76	1	—
	E	30.29	5	.00	20.29	26.39	2	.00

Table 7

Proportions of Additive Genetic, Common and Unique Environmental Variance for T1D, T2D and GD, Including 95% Confidence Intervals

Dataset	Model	Diabetes Type	Point Estimates (95% CI)		
			A (Additive Genes)	C (Shared Environment)	E (Unique Environment)
QIMR + ATR (Contingency table data)	ACE	T1D	.69 (.26-.99)	.27 (.00-.67)	.04 (.01-.14)
		T2D	.73 (.38–.96)	.20 (.00–.53)	.07 (.03–.14)
		GD	.75 (.00–.90)	.00 (.00–.76)	.25 (.10–.49)
	AE	T1D	.96 (.87–.99)	—	.04 (.01–.13)
		T2D	.93 (.87–.97)	—	.07 (.03–.13)
		GD	.75 (.51–.90)	—	.25 (.10–.49)
QIMR (Contingency table data)	ACE	T1D	.56 (.05–.99)	.39 (.00–.80)	.05 (.01–.23)
		T2D	.70 (.30–.94)	.18 (.00–.56)	.11 (.05–.20)
		GD	.76 (.00–.91)	.00 (.00–.81)	.24 (.09–.50)
QIMR (Raw data)	ACE	T1D	.56 (.05–.99)	.39 (.00–.80)	.05 (.01–.23)
		T2D	.72 (.20–.88)	.07 (.00–.53)	.21 (.12–.34)
		GD	.71 (.00–.88)	.00 (.00–.84)	.30 (.12–.58)

Note: The estimates for the QIMR raw data are derived from a model which includes the effects of age and BMI on thresholds for T2D and GD.

7.11, $p = .03$) and BMI ($\chi^2_1 = 15.41$, $p = .00$) were included in the model.

The proportions of additive genetic, common and unique environmental variance for T1D, T2D and GD, with 95% confidence intervals, are given in Table 7 for the combined contingency data, the QIMR-only contingency data, and the QIMR raw data to which age, sex and BMI covariates were fitted. The QIMR-only data showed a smaller heritability than the combined QIMR–ATR for T1D, but given the small sample sizes (there were only 31 individuals with T1D in the QIMR data), this is considered to be an issue of power rather than a real effect, as illustrated by the very wide 95% confidence intervals. However, for T2D liability the variance components are very similar for the combined (A = 0.73, C = 0.20, E = 0.07) and QIMR-only (0.70, 0.18, 0.11) contingency data, and the same is true for GD.

The effects of including BMI and age as covariates in the model for the T2D disease threshold can be seen by comparing the variance components for the ACE model in the QIMR contingency data (0.70, 0.18, 0.11) versus those for the raw data analysis (0.72, 0.07, 0.21). Since co-twins are the same age, removing its effects reduces the shared environmental variance from 18% to 7% of the total. Since BMI is influenced by both genetic and environmental factors, removing its effects will have unpredictable consequences for the variance components of liability to T2D, in this case increasing E from 11 to 21% and leaving the genetic influence almost unchanged (70% versus 72%). Similarly, by correcting GD thresholds for age and BMI, the genetic variance estimate decreases from 76% to 71%.

Diabetes and Body Mass Index

The association between diabetes and BMI is further explored in Table 8, which shows that the affected twin–unaffected twin difference in weight of DZ twin pairs who are discordant for either T2D or GD is significantly larger than the corresponding difference for discordant MZ twin pairs. For T1D this contrast was not significant ($t = 1.16$, $p = .25$). Table 8 confirms the importance of weight as a risk factor for T2D and GD, but goes further in showing that both genetic and unique environmental influences mediate this risk. In all twins, the heavier twin is at elevated risk. If this were mediated only by environmental factors unique to the individual, we would see the same difference within MZ and DZ discordant pairs. That the DZ difference is so much larger than for MZ discordant pairs confirms that genetic factors influencing weight also influence T2D and GD risk.

Table 8

Weight Differences Between Twin1 and Twin 2 (Affected Twin–Unaffected Twin) for Twin Pairs Discordant for Either T2D or GD and Test of Significance of This Difference Between MZ and DZ Pairs

Diabetes Type	Zygosity	Discordant Pairs (*n*)	Mean Weight Differences (Aff-Unaff; Kg)	*t*	*p* value
T2D	MZ	25	5.3	6.30	.000
	DZ	64	18.2		
GD	MZ	17	8.1	2.56	.017
	DZ	11	16.2		

Table 9

Estimates of Disease Prevalence From Our Sample Compared With the Australian Population

Dataset	Diabetes Type	Threshold	Prevalence (Per 10,000)	Population Prevalence (Per 10,000)
QIMR+ATR (contingency tables)	T1D	−2.9857	14	30
	T2D	−2.7048	34	700
	GD	−3.0590	11	500
QIMR (contingency tables)	T1D	−2.8411	22	
	T2D	−2.4098	80	
	GD	−2.8615	21	
QIMR (raw data)	T1D	−2.8518	22	
	T2D	−2.9703	23	
	GD	−2.8299	23	

Bivariate Cholesky decomposition analyses were carried out to quantify the proportion of genetic variance contributing to the shared variance between BMI and disease status for T2D and GD. The results of each analysis showed strong genetic mediation of the relationship between T2D and both GD and BMI, with no significant influence of the common environment supported in either analysis. In the saturated model (i.e., ACE), for T2D the relationship with BMI could be entirely explained by additive genes, and for GD, 77% of the covariance with BMI was explained by additive genes. To simplify the model, all sources of common environmental variance were removed (in both analyses $p = 1$); additive genetic variance was significant ($p < .01$) and retained in both models. Figure 3 depicts the standardized path coefficients influencing the traits in the AE model; 95% confidence intervals that include zero indicate that the path coefficient is not significant. In both analyses, the unique environmental pathway between BMI and diabetes was not significant. Genes influencing BMI explained 18% of variance in T2D and 7% of variance in GD.

Diabetes Prevalence

T2D and GD prevalences, which were taken from thresholds generated from the ACE models for the diabetes sample, are very low compared with disease prevalences from the Australian population (Dunstan et al., 2002); these comparisons are shown in Table 9. T1D, however, is comparable with the estimated population prevalence. The prevalences of the three diseases taken from the QIMR data are approximately double that of the total data (QIMR+ATR) suggesting that the screening for diabetes was more effective using just the QIMR population. This is demonstrated by examining the double sampled twins (Table 10);

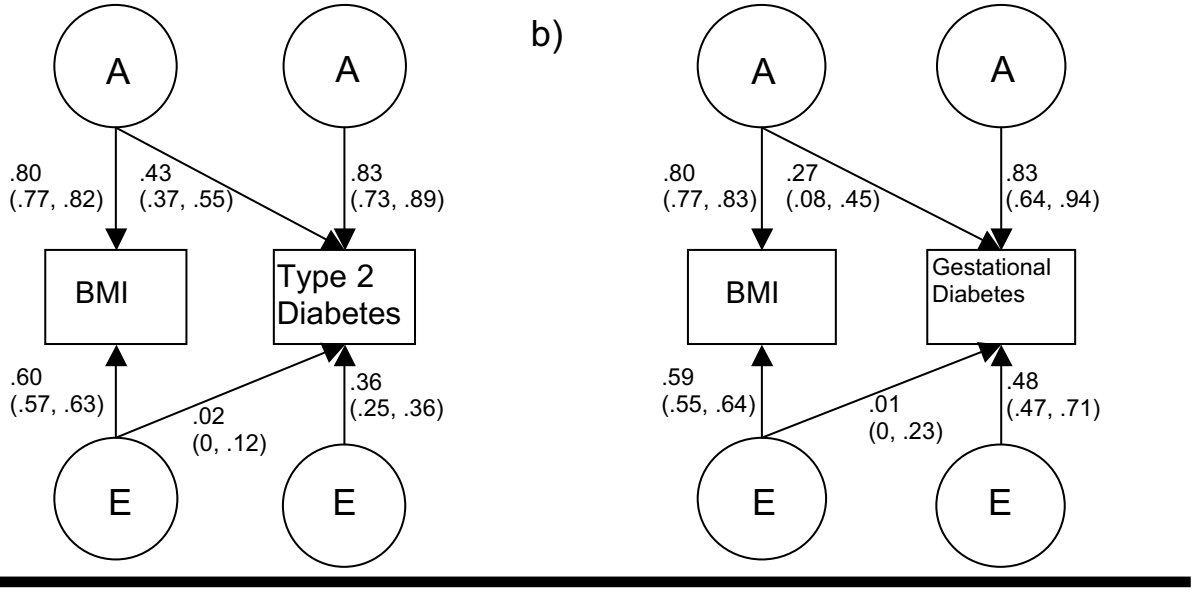

Figure 3

Bivariate Cholesky models depicting the standardized path coefficients for additive genetic and unique environmental influences on (a) BMI and Type 2 Diabetes, and (b) BMI and Gestational Diabetes.

Table 10

Capture-Recapture: Twin Pairs Present in Both the QIMR and the ATR Datasets

		ATR		Total
		+	–	
QIMR	+	98	72	170
	–	34	4219	4253
Total		132	4291	4423

that is twin pairs who were screened for diabetes in both the QIMR data and the ATR data.

The specificity, that is, the proportion of truly nondiabetics identified by the sampling frame, was ~99% for both QIMR and ATR datasets. The sensitivity of ATR and QIMR datasets to identify twin pairs where one or both has diabetes was 0.58 and 0.74 respectively. Thus, based on the double sampled twins, it is estimated that 181 twin pairs with diabetes were missed from the ATR sampling frame and 25 from QIMR.

Comorbidity with Asthma

All the surveys that probed diabetes also asked about asthma, so we were able to see whether there was any comorbidity. Contrary to some data suggesting an inverse relationship between type 1 diabetes and asthma, we found no support for such a relationship in our data (p = .39).

Discussion

The nosology and etiology of diabetes remain obscure and there are intensive international efforts underway to dissect the genetic and environmental risk factors influencing the different subtypes (Salonen et al., 2007; Sandhu et al., 2007; Todd et al., 2007; Sladek et al., 2007; Zeggini et al., 2007; Pascoe et al., 2007), and the extent to which these overlap between subtypes. Just as twins have been important in the past in demonstrating the major role of genetic factors in diabetes (Kyvik et al., 1995; Hyttinen et al., 2003; Benyamin et al., 2007), once the principal genetic variants have been identified they will be even more useful in helping to dissect the interactions of genetic and environmental risk factors in disease causation and the role of epigenetic factors. Discordant MZ pairs will be particularly useful in examining G x E interaction. Our primary goal, therefore, was to use the large Australian Twin Registry to identify a sample of well-characterised diabetic twin pairs who might be a useful resource for more detailed molecular and G x E investigations. In the course of doing so, we have been able to confirm and extend a number of key findings about the genetic epidemiology of diabetes.

The Australian Twin Registry was founded almost thirty years ago and has been funded continuously since 1980 by the National Health and Medical Research Council as a resource for biomedical research. About 30,000 pairs, of both sexes and zygosity groups, and all ages, have enrolled voluntarily, or been enrolled by their parents. At frequent intervals, subsets of these twins have been surveyed by various researchers, or by the ATR itself. We have taken advantage of disease checklists included in surveys on nine different occasions during the life of the ATR, to identify twins with diabetes. Follow-up questionnaires to the twins and their doctors have confirmed diagnoses and allowed us to classify the twins into disease subtypes.

Our analyses found prevalences well below those reported for the population for T2D and GD (although comparable for T1D) and suggest that the surveys conducted by QIMR have been reasonably efficient (74%) in identifying diabetic twin pairs, while those conducted by the ATR, mainly in the course of recruiting the twins, have been less efficient (58%). Based on the Australian MZ twinning rate, and the incidence of T1D at the time of the study, we would expect a total of ~400 MZ twin pairs containing one or two cases of T1D in the Australian population. The deficiency of T2D cases will reflect the more cryptic nature of this disease and the relatively young age of the ATR catchment, while the lower than population prevalence of GD may reflect women's poor recall, or confusion about complications of their pregnancies. It is known that T2D is underdiagnosed in Australia, as in other Western countries.

As a result, although we were recruiting from a pool of almost 20,000 pairs, the number of twins identified with any subtype was not large, and consequently the power to discriminate between models of causation was not high. Although we could reject purely environmental models of causation and estimated very high heritabilities for all three major subtypes, we had little power to simultaneously detect shared environmental influences, even if these had been large. For T2D, correction for increasing incidence with advancing age reduced the estimate of shared environmental variance, but did not increase the heritability, rather increasing the variance due to unique environmental factors.

The involvement of excess body weight in T2D risk was seen quite dramatically in twin pairs discordant for the disease. In discordant MZ pairs, the twin affected with T2D was some 5kg heavier than the unaffected twin, and in discordant DZ pairs this difference rose to 18kg, confirming that both genetic and environmental factors are involved in the comorbidity of high BMI and T2D. The full bivariate genetic analysis of BMI and T2D found that while the genetic correlation was .46, the environmental correlation was only .06. That is, 20% of the genetic influence on T2D was contributed by genes with a joint influence on BMI. The corresponding figure for unique environmental factors was only 0.3%. The size of the genetic correlation is in keeping with the roles of the newly

discovered genes controlling susceptibility to T2D such as FTO (see the review of Frayling, 2007).

In contrast to some other epidemiological studies (Tirosh et al., 2006), we did not demonstrate a negative correlation between asthma and T1D. This may reflect the sample size recruited, as the reported relative risks are not large.

In conclusion, genetic analysis of this large sample of twins confirms the current broad model of diabetes, especially with respect to pleiotropic genes influencing T2D. Twins ascertained through this work are undergoing more detailed follow-up, which will include molecular genetic studies, allowing assessment of specific genetic contributions to overall familial resemblance

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