Brief Genetics Report

Genetic Analysis of Chromosome 2 in Type 1 Diabetes

Analysis of Putative Loci *IDDM7*, *IDDM12*, and *IDDM13* and Candidate Genes *NRAMP1* and *IA-2* and the Interleukin-1 Gene Cluster

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In the spontaneous mouse model of type 1 diabetes, the nonobese (NOD) strain, a type 1 diabetes locus, *Idd5*, has been mapped to chromosome 1 (1). Because mouse chromosome 1 shares a homologous 33-cM region between the genes *col3a1* and *col6a3* with human chromosome 2q31–q35 (data shown at http://www.ncbi.nlm.nih.gov/Omim/Homology/), several groups have searched chromosome 2 for loci affecting human type 1 diabetes. Previous studies have provided evidence for at least three type 1 diabetes loci (*IDDM7*, *IDDM12*, and *IDDM13*) within a 23-cM region of chromosome 2q31–q35 (2–5).

We have now evaluated linkage on chromosome 2, by multipoint analysis using Mapmaker/Sibs, of 34 microsatellite markers and an interleukin-1 (IL-1) β *Taq*I polymorphism in 352 U.K. families (6). The only region showing some positive evidence of linkage (54% 1,0 identical-by-descent [IBD] sharing, maximum logarithm of odds score = 1.08, $P = 0.02$, $Z_0 = 0.21$, $\lambda_s = 1.19$) was at 2q31 between the markers *D2S326–D2S152*, for which a hypothetical locus with a relative risk λ_s of 1.7 could not be excluded (data not shown, available at http://well.ox.ac.uk/~plyons). Previously, the putative *IDDM7* locus was located by linkage and transmission disequilibrium test (TDT) (7) to *D2S152* (2). The *CTLA-4*/*CD28* (*IDDM12*) and *D2S164* (*IDDM13*) regions showed no evidence of linkage. Owerbach and Gabbay (3) also reported linkage in 66 U.K. and 96 U.S. families, many of which were in common with the Copeman et al. study (2), to the chromosome 2q31 region peaking at marker *HOXD8*, only 5 cM centromeric of *D2S152*. In their study, Owerbach and Gabbay found most evidence of linkage to *HOXD8* by conditioning their data by low-risk *IDDM1*/major histocompatibility complex (MHC)/HLA type and high-risk *IDDM2*/Insulin gene (*INS*) variable number of tandem repeats (VNTR) locus genotype (single point $P < 10^{-5}$ in 68 sib pairs) (3). In 205 childhood-onset families from the U.K. and U.S., conditioned in the same way by *IDDM1* and *IDDM2* and including many of the 68 families analyzed previously (3), we did not extend support for the reported linkage results (59%, 151 vs. 106 1,0 IBD sharing; single point $P = 0.005$, P corrected for multiple tests = 0.02, compared with 54%, 409 vs. 344 1,0 IBD, $P = 0.02$ in the total set of 589 families not conditioned by age-of-onset, HLA type, or *INS*). Sib pairs from Denmark, France, and Italy were not included in this analysis, since full HLA and *INS* typing data were not available.

We previously found evidence for association in the presence of linkage of the 228–base pair allele of *D2S152* in three (total $n = 228$ families; Sardinia, $n = 103$, 63% transmission; Italy, $n = 31$, 66%; and U.S., $n = 94$, 63%) of five family data sets (total $n = 447$ families; U.K., $n = 171$, and Denmark, $n = 48$, families showed no increased transmission of the 228 allele) (2). Hence, we have typed more U.S. ($n = 200$; Luo et al. (8) analyzed *D2S152* in 219 families, so we are providing new information here on at least 75 U.S. families), Sardinian ($n =$

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IBD, identical-by-descent; IL-1, interleukin-1; MHC, major histocompatibility complex; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test; T_{sp}, modified transmission disequilibrium testing–based analysis.

77), and Italian families (n = 131; 116 are new families not previously reported [2,8]) for *D2S152* to test specifically the transmission of the 228 allele: 54% transmission to diabetic offspring in U.S. families (one-tailed P = 0.08), 56% to Sardinian families (P = 0.2), and 52% to Italian families (P = 0.4), totaling, in this new data, 54% transmission (257 transmitted, 219 not transmitted, one-tailed P = 0.05). In addition, in these families, allele 228 was transmitted at only 45% to unaffected siblings (120 transmitted, 147 not transmitted), which was significantly different from the transmission to the affected siblings (2 × 2 contingency test of heterogeneity, P = 0.02). However, we also typed additional U.K. families (n = 189) and newly available sets from Denmark (n = 6), Romania (n = 204), Finland (n = 215), and France (n = 82), all showing transmission of the *D2S152* allele 228 to diabetic siblings close to random expectation (Table 1). Much larger numbers of families will be required to confirm the existence of a putative *D2S152*-linked *IDDM7* locus.

We previously reported (4) 62% transmission of the G allele of a single nucleotide polymorphism (SNP) in codon 49 of *CTLA-4* exon 1 in 187 Italian and 44 Spanish type 1 diabetic families (P = 0.0001), but only 52% in 284 U.K. and 180 U.S. families. To further investigate the negative results in the U.K. and U.S. families, we analyzed a total of 352 U.K. and 236 U.S. families with a second polymorphism located in the 3′ untranslated region of the *CTLA-4* gene (4). Transmission of the two most common alleles was tested initially: alleles 262 and 280 are almost in complete linkage disequilibrium with the A (D′ = 0.97) and G (D′ = 0.92) alleles of the A/G exon 1 SNP, respectively, as reported previously (4,9). In the combined U.K. and U.S. data sets, there was some evidence for increased transmission of the 280 allele (54% transmission; TDT P = 0.02) and decreased transmission of the 262 allele (45.6% transmission; TDT P = 0.001) to affected siblings. Hence, in these U.K. and U.S. families, the *CTLA-4* 3′ untranslated region microsatellite marker is a more informative one than the A/G exon 1 SNP, but this does not necessarily mean that it is closer to the *IDDM12* etiologic variant. In all families (n = 960, including 191 Italian and 180 Sardinian families), we have obtained consistent support for the association, in the presence of linkage, of the *CTLA-4* region with disease (56% transmission to diabetic children for allele 280, P = 0.001, and 46% transmission of 262 allele, P = 0.009, all data shown at http://well.ox.ac.uk/~plyons). We also evaluated *CTLA-4* using a modified TDT-based analysis (T_{sp}) (10) that gives a valid test of association when using multiple affected siblings (P = 0.002, P corrected = 0.004). Taken together with other results, which include previous typing of many of the U.S. families analyzed here (9,11), there is sufficient justification to proceed with cloning, sequencing, and genetic analysis of the *CTLA-4-CD28* region to locate *IDDM12*, which need not necessarily be the *CTLA-4* gene.

Morahan et al. (5) reported evidence of linkage to 2q34–q35 in 114 affected sib pairs (designated *IDDM13*), with the strongest support at adjacent markers *D2S164*, *D2S301*, and *D2S137* in 37–44 informative sib pairs conditioned on both *IDDM1* sharing status ($P = 2.5 \times 10^{-5}$) and sex of the affected offspring. To date, the only additional support for the *IDDM13* region is a reported association of *D2S137*, 3 cM centromeric of *D2S164*, in a Japanese case-control study (12). In 352 U.K. families, we obtained no evidence of linkage of this region to type 1 diabetes. Conditioning by *IDDM1*/MHC/HLA status, as described by Morahan et al. (5), did not improve the negative result, nor was there detectable influence of sex of affected siblings (data not shown). The transmission of the common alleles of seven markers in a ~11 cM region around *D2S164* was analyzed in 352 U.K. and 94 U.S. families using TDT (data not shown). Only allele 180 of marker *D2S157* showed increased transmission in both the U.K. (56% transmission, TDT P = 0.03 and T_{sp} P = 0.02) and U.S. (61% transmission, TDT P = 0.03 and T_{sp} P = 0.03) families, with a total of 57% transmission (correction by the number of loci and alleles tested, 19, P = 0.002, $P_{corrected}$ = 0.05, T_{sp} $P_{corrected}$ = 0.04). Taken together with the results of Morahan et al. (5) and Fu et al. (12), we cannot exclude the presence of a diabetes locus in this region.

None of the alleles of microsatellites associated with the candidate genes of the IL-1 gene cluster (2q12), the *IA-2* autoantigen gene (2q35) (13), or *NRAMP1* (2q35) (forward 5′-ACTCGCATTAGGCCAACGAG-3′; reverse 5′-TTCTGTGC-CTCCCAAGTTAGC-3′ polymerase chain reaction primer sequences), a host resistance locus that has a key role in macrophage function (14), tested using TDT showed any evidence of increased allele transmission to diabetic children in the U.K. data set (data not shown). Genetic analysis of the *IA-2* gene was performed using the previously described intron polymorphism (*D2S1753ea*; Genbank ID Z48226 b; forward 5′-GGGAAGAG-TAGATGATGGCCT-3′; reverse 5′-CATAATCTCTATGAACGCT-TTTTG-3′) and a novel microsatellite (D2S31F23) isolated from

TABLE 1
Transmission of allele 228 of *D2S152* to affected and unaffected siblings in families from Sardinia, U.S., Italy, U.K., Denmark, Finland, France, and Romania

	Previous families					New families					Total families					Unaffected		
Data sets	n	T	NT	%T	$P \le 0.05$	n	T	NT	%T	$P \le 0.05$	n	T	NT	%T	$P \le 0.05$	T	NT	%T
Sardinia	103	55	32	63	0.01	77	29	23	56	—	180	84	55	60	0.01	54	58	48
U.S.	94	85	50	63	—	200	187	162	54	—	294	268	207	56	0.005	24	31	44
Italy	31	29	15	66	0.035	131	41	34	55	—	162	70	53	57	—	42	58	42
U.K.	171	142	150	49	—	189	136	138	50	—	360	278	288	49	—	—	—	—
Denmark	48	34	50	40	—	6	6	5	—	—	54	40	55	42	—	—	—	—
Finland	—	—	—	—	—	215	92	97	49	—	215	92	97	49	—	54	51	51
France	—	—	—	—	—	82	64	51	56	—	82	64	51	56	—	50	39	56
Romania	—	—	—	—	—	204	65	70	48	—	204	65	70	48	—	43	47	48
Total	447	341	292	54	0.05	1104	615	575	52	—	1551	961	876	52	0.05	267	284	48

NT, not transmitted; T, transmitted; %T, percent transmission.

a P1 artificial chromosome clone containing the *IA-2* gene (forward 5′-AATTGCATCACTGCACTCCA-3′; reverse 5′-GCAACATTTTACCATCATAGTT-3′). As reported previously, allele 206 of *D2S160*, which maps near the IL-1 gene cluster, showed increased transmission in the U.S. families (2); in the 352 U.K. families, however, no evidence of increased transmission to diabetic siblings was observed (49% transmission), and the total result failed to reach $P \leq 0.05$. Transmission analysis of haplotypes of markers in the IL-1 gene cluster, as well as haplotypes from the *NRAMP1* (*NRAMP1-D2S1471*) and *IA-2* (*D2S1753EA*-D2S31F23) regions, revealed no significant deviation from 50% (data not shown). Several associations of the IL-1 gene cluster with type 1 diabetes (15,16) and other autoimmune diseases have been previously reported, but all of these studies have been carried out using a case-control design. Family-based association analysis, such as TDT, avoids possible problems of artifactual results owing to population stratification effects (7). Nevertheless, our negative findings do not rule out a role for the 2q12 region in type 1 diabetes, but a gene with $\lambda_s \geq 1.7$ is excluded (data not shown).

Because allele A3 of the *NRAMP1* microsatellite promoter polymorphism in the putative *IDDM13*/2q34–q35 region has been linked and associated in families with rheumatoid arthritis (14), we analyzed the transmission of this allele to type 1 diabetic siblings in those U.K. families ($n = 116$) that have a first- or second-degree relative with rheumatoid arthritis. Allele A3 was transmitted at 58% (TDT = 0.04, $T_{sp} = 0.04$) to type 1 diabetic siblings, indicating that follow-up analysis is warranted.

In conclusion, our results illustrate the difficulties of genetic analysis of common disease, even when quite large numbers of families are available with parents for a disease that is relatively unambiguous to diagnose and that shows a strong familial clustering. Future studies should be carried out in large data sets from ethnically homogeneous populations.

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REFERENCES

1. Cornall RJ, Prins JB, Todd JA, Pressey A, DeLarato NH, Wicker LS, Peterson LB: Type 1 diabetes in mice is linked to the interleukin-1 receptor and *Lsh/Ity/Bcg* genes on chromosome 1. *Nature* 353:262–265, 1996
2. Copeman JB, Cucca F, Hearne CM, Cornall RJ, Reed PW, Rønningen KS, Undlien DE, Nistico L, Buzzetti R, Tosi R, Pociot F, Nerup J, Cornélis F, Barnett AH, Bain SC, Todd JA: Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (*IDDM7*) to human chromosome 2q31-q33. *Nat Genet* 9:80–85, 1995
3. Owerbach D, Gabbay KH: The HOXD8 locus (2q31) is linked to type I diabetes: interaction with chromosome 6 and 11 disease susceptibility genes. *Diabetes* 44:132–136, 1995
4. Nistico' L, Buzzetti R, Pritchard LE, Van der Auwera B, Giovannini C, Bosi E, Larrad MT, Rios MS, Chow CC, Cockram CS, Jacobs K, Mijovic C, Bain SC, Barnett AH, Vandewalle CL, Schuit F, Gorus FK, Registry BD, Tosi R, Pozzilli P, Todd JA: The *CTLA-4* gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. *Hum Mol Genet* 5:1075–1080, 1996
5. Morahan G, Huang D, Tait BD, Colman PG, Harrison LC: Markers on distal chromosome 2q linked to insulin-dependent diabetes mellitus. *Science* 272:1811–1813, 1996
6. Bain SC, Todd JA, Barnett AH: The British Diabetic Association: Warren repository. *Autoimmunity* 7:83–85, 1990
7. Spielman R, McGinnis R, Ewens W: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
8. Luo D-F, Buzzetti R, Rotter JI, Maclaren NK, Raffael LJ, Nistico' L, Giovannini C, Pozzilli P, Thomson G, She J-X: Confirmation of three susceptibility genes to insulin-dependent diabetes mellitus: *IDDM4*, *IDDM5* and *IDDM8*. *Hum Mol Genet* 5:693–698, 1996
9. Marron MP, Raffel LJ, Garchon HJ, Jacob CO, Serrano-Rios M, Martinez Larrad MT, Teng WP, Park Y, Zhang ZX, Goldstein DR, Tao YW, Beaurain G, Bach JF, Huang HS, Luo DF, Zeidler A, Rotter JI, Yang MC, Modilevsky T, Maclaren NK, She J-X: Insulin-dependent diabetes mellitus (IDDM) is associated with *CTLA-4* polymorphisms in multiple ethnic groups. *Hum Mol Genet* 6:1275–1282, 1997
10. Martin ER, Kaplan NL, Weir BS: Tests for linkage and association in nuclear families. *Am J Hum Genet* 61:438–448, 1997
11. Awata T, Kurihara S, Iitaka M, Takei S, Inoue I, Ishii C, Negishi K, Izumida T, Yoshida Y, Hagura R, Kuzuya N, Kanazawa Y, Katayama S: Association of *CTLA-4* gene A-G polymorphism (*IDDM12* locus) with acute-onset and insulin-depleted IDDM as well as autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis) in Japanese population. *Diabetes* 47:128–129, 1998
12. Fu J, Ikegami H, Kawaguchi Y, Fujisawa T, Kawabata Y, Hamada Y, Ueda H, Shintani M, Nojima K, Babaya N, Shen Q-J, Uchigata Y, Urakami T, Omori Y, Shima K, Ogihara T: Association of distal chromosome 2q with IDDM in Japanese subjects. *Diabetologia* 41:228–232, 1998
13. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E: Identification of protein tyrosine phosphatase-like *IA2* (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol* 155:5419–5426, 1995
14. Shaw M-A, Clayton D, Atkinson SE, Williams H, Miller N, Sibthorpe D, Blackwell JM: Linkage of rheumatoid arthritis to the candidate gene *NRAMP1* on 2q35. *J Med Genet* 33:672–677, 1996
15. Pociot F, Ronningen KS, Bergholdt R, Lorenzen T, Johannesen J, Ye K, Dinarello CA, Nerup J: Genetic susceptibility markers in Danish patients with type 1 (insulin-dependent) diabetes: evidence for polygenicity in man: Danish Study Group of Diabetes in Childhood. *Autoimmunity* 19:169–178, 1994
16. Blakemore AIF, Cox A, Gonzalez A-M, Maskill JK, Hughes ME, Wilson RM, Ward JD, Duff GW: Interleukin-1 receptor antagonist allele (*ILRN*2*) associated with nephropathy in diabetes mellitus. *Hum Genet* 97:369–374, 1996

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