Genetics of Type 1 Diabetes: What's Next?

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The discovery of the association between HLA in the major histocompatibility complex (MHC) on chromosome 6p21 with type 1 diabetes, but not with type 2 diabetes, suggested that these disease entities were of different genetic background and pathogenesis. The discovery that some individuals with diabetes had autoantibodies in their blood provided additional evidence that type 1 diabetes had an autoimmune origin. Recently, increasing knowledge of the genome, coupled with rapidly improving genotyping technology and availability of increasingly large numbers of samples, has enabled statistically robust, systematic, genome-wide examinations for discovery of loci contributing to type 1 diabetes susceptibility, including within the MHC itself. Currently, there are over 50 non-HLA regions that significantly affect the risk for type 1 diabetes (http://www.t1dbase.org). Many of these regions contain interesting, but previously unrecognized, candidate genes. A few regions contain genes of unknown function or no known annotated genes, suggesting roles for long-distance gene regulatory effects, noncoding RNAs, or unknown mechanisms. Against a background of ever-improving knowledge of the genome, particularly its transcriptional regulation, and with massive advances in sequencing, specific genes, rather than regions that impinge upon type 1 diabetes risk, will be identified soon. Here we discuss follow-up strategies for genome-wide association (GWA) studies, causality of candidate genes, and genetic association in a bioinformatics approach with the anticipation that this knowledge will permit identification of the earliest events in type 1 diabetes etiology that could be targets for intervention or biomarkers for monitoring the effects and outcomes of potential therapeutic agents. The International Type 1 Diabetes Genetics Consortium (T1DGC) has established significant resources for the study of genetics of type 1 diabetes. These resources are available to the research community and provide a basis for future discovery in the transition from gene mapping to discovery of disease mechanisms.

The T1DGC (http://www.t1dgc.org) is an international research program established in 2002 whose primary aims are to *1*) discover genes that modify risk of type 1 diabetes and *2*) expand on existing genetic resources for type 1 diabetes research (1). Over the last 7 years, the T1DGC has assembled a collection of >4,000 type 1 diabetes affected sib-pair (ASP) families for genetic studies. In addition to building this resource, consortium members have provided access to large case-control collections for specific T1DGC genotyping studies. Building on these assets, four major research projects have been performed: an exhaustive examination of the HLA region by single nucleotide polymorphism (SNP) genotyping and high-resolution HLA typing; a detailed investigation of published candidate genes; a genome-wide linkage scan; and a GWA study and meta-analysis. Importantly, T1DGC data and bio-specimens used in these studies have been made available to the research community. The T1DGC continues to build on these resources to help identify the inherited events in the pathogenesis of type 1 diabetes.

The etiology of human type 1 diabetes is still largely obscure, but it is recognized that both genetic and environmental factors are important in defining disease risk (2). This is supported by observations showing that the proband-wise concordance for monozygotic (MZ) twins is estimated to be ~50% (compared with ~8% for dizygotic [DZ] twins) (3). These MZ twins have the whole range of population genetic risk profiles for type 1 diabetes, and if they were all high-risk DR3/4-DQ8, for example, their concordance for the disease would be much higher. Both animal model and human studies indicate that an autoimmune response to the β-cells of the pancreatic islets occurs in type 1 diabetes. The outcome of this response (health or diabetes) is influenced substantially by an unknown series of stochastic or developmental events in the context of (unknown) environmental factors. The autoimmune process, substantially determined by inherited variation, then progresses through a preclinical phase, leading to destruction of β-cells and a stage of hyperglycemia resulting from reduced β-cell mass and insulin secretory capacity.

Genetic, functional, structural, and animal model studies all indicate that the highly polymorphic HLA class II molecules, namely the DR and DQ α-β heterodimers, are central to susceptibility to type 1 diabetes (4,5). The genes encoding these proteins are located in the HLA region, which spans ~4,000 kb of DNA on human chromosome 6p21.3. The HLA region comprises >200 genes, and 40% of the expressed genes are predicted to have immune re-

See accompanying commentary, p. 1575.

sponse functions (6,7). In addition to the class II genes *HLA-DRB1* and *HLA-DQB1*, any one (or more) of these MHC genes, including the other HLA genes, could contribute to the overall risk for type 1 diabetes. The exact mechanism(s) by which the HLA class II molecules confer susceptibility to immune-mediated destruction of the pancreatic islets is still not known in its entirety, but the binding of key peptides from autoantigens (preproinsulin, GAD, insulinoma-associated 2 antigen, and zinc transporter, ZnT8, so far identified) to HLA class II molecules in the thymus and in the periphery are likely to play an important role. Theoretically, targeting this process of antigen presentation and T-cell activation may be an effective therapeutic approach to preventing type 1 diabetes. In practice, HLA screening is used to identify people at risk for developing type 1 diabetes, for inclusion in, and exclusion from, clinical studies (8) and clinical trials (9).

MHC FINE MAPPING

Although the highly polymorphic HLA class II genes clearly play the most important single role in susceptibility to type 1 diabetes, variation at these loci alone cannot explain all of the evidence of genetic association and linkage of the MHC with type 1 diabetes. To better define genes within the MHC that may affect type 1 diabetes risk and would therefore merit further studies, the T1DGC undertook a comprehensive study of the genetics of the classic 4-Mb MHC region. More than 3,000 SNPs and 66 microsatellite markers were genotyped in 2,300 type 1 diabetes ASP families (~10,000 individuals) (10). HLA typing using immobilized probes was also performed on these samples for *HLA-A, -B, -C, -DRB1, -DQA1, -DQB1, -DPA1,* and *-DPB1.* These data (available for viewing at http://www.t1dbase.org) represent the largest collection of families with type 1 diabetes genotyped at such a detailed level.

Specific combinations of alleles, or haplotypes, of the *DRB1, DQA1,* and *DQB1* genes (in *cis* and in *trans*) determined the extent of risk and a distribution of DR-DQ haplotypes and genotypes ranging from highly susceptible to highly protective have been observed (11). Odds ratios (ORs) >40 were observed for some genotypes (e.g., *DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0401-DQA1*0301-DQB1*0302*). High genetic risks have been reported for islet autoimmunity and type 1 diabetes in DR3/4-DQ8 siblings who shared both HLA haplotypes with their diabetic sibling, although this has yet to be confirmed (12). Further, independent effects of *HLA-A, HLA-B,* and *HLA-DPB1* (13) were also demonstrated. Following adjustment for linkage disequilibrium to haplotypes at the DR-DQ region, both susceptible and protective alleles were found at *HLA-B* (e.g., *B*3906,* susceptible, and *B*5701,* protective), *HLA-A* (e.g., *A*2402,* susceptible, and *A*1101,* protective), and *HLA-DPB1* (e.g., *DPB1*0301* and **0202,* susceptible, and **0402,* protective) (13,14).

Other features of the HLA–type 1 diabetes association were also examined; however, only support for an HLA effect by age at diagnosis was found (15–18). Presumably, the risk conferred by specific HLA class I and class II alleles and haplotypes reflects the specificity of peptide binding and presentation (19,20). New genomic knowledge will better define the naturally processed peptides from autoantigens in type 1 diabetes. Intriguingly, a decrease in high-risk HLA genetic contribution in new-onset cases over the last decades has been observed in several studies, suggesting a change in environmental impact on penetrance as the incidence of type 1 diabetes increases (21–23).

The T1DGC MHC fine mapping data and results were published as a supplement to *Diabetes, Obesity and Metabolism* (10). The T1DGC has made the data available to the scientific community for additional analyses, by request to the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK) Central Repository (https://www.niddkrepository.org/niddk/home.do). The T1DGC is also probing the MHC in greater genomic detail, including a collaboration with the Federation of Clinical Immunology Societies (FOCiS) and with DNA sequence analysis to investigate the association of the "secondary" *DRB3, DRB4,* and *DRB5* variation in the context of the *DRB1* haplotypes on which these alleles are found.

CANDIDATE GENE STUDIES

Insulin gene (*INS*). The importance of variation at or near the insulin gene (*INS*) on chromosome 11p15.5 was originally suggested by early association studies (24). The genetic risk conferred by the *INS* locus is generally ascribed to differing size classes of alleles at a region with a variable number of tandem repeats (VNTR, mini-satellite polymorphisms) flanking the insulin gene. The class I alleles of the *INS VNTR,* which increase risk of type 1 diabetes, have been associated with lower insulin mRNA and protein expression in the thymus, compared with the dominant protective class III alleles. Decreased central tolerance allows more autoreactive T-cells to escape into the periphery, increasing susceptibility to disease (25,26). Recent studies that highlight insulin and its precursors as the major initiating autoantigen in human type 1 diabetes (27,28) provide support for this hypothesis.

CTLA4. The variants associated with type 1 diabetes in the cytotoxic T-lymphocyte–associated protein 4 (*CTLA4*) gene were identified by association mapping using both NOD mouse and human samples (29–31). The *CTLA4*-encoded molecule is a co-stimulatory receptor that inhibits T-cell activation and functions in CD4 T regulatory cells. Several human autoimmune diseases are associated at the same genomic region (2q33) that contains *CTLA4.* Narrowing down the list of candidate causal variants and their effect on *CTLA4* gene splicing has been aided by using samples from patients with Graves' disease (31) and point to variants in the 3′ region of the gene, altering the level of a soluble form of the receptor. *CTLA4* genetic variation has a strong effect, presumably via its role in regulation of peripheral tolerance (32), in which the disease-associated *CTLA4* haplotype is predisposing to a failure in tolerance to multiple organs or tissues. In the NOD mouse in which convincing statistical gene-gene interactions can be observed, the effect of allelic variation of *CTLA4* depends on different combinations of other susceptibility loci, including complete masking of the effect (that is, no association with disease) (33).

PTPN22. A functional variant of the lymphoid-specific protein tyrosine phosphatase (*PTPN22*) gene on chromosome 1p13 is strongly associated with type 1 diabetes as well as other autoimmune diseases (34,35). LYP, encoded by the *PTPN22* gene, is an inhibitor of T-cell activation, acting by dephosphorylating T-cell receptor-proximal signaling molecules such as LCK and ZAP70. A variant of *PTPN22* resulting in an amino acid substitution (R620W)

TABLE 1
Regions with evidence of linkage to type 1 diabetes

Chromosome	Position (cM)	LOD	P	LOD-1 support interval	Flanking markers for the LOD-1 interval
2	194.5	3.28	5×10^{-5}	191.3–197.8	rs1882395/rs1369842
6	52.0	213.2	8×10^{-216}	51.0–52.5	rs11908/rs412735
11	2.5	3.16	7×10^{-5}	0–8.5	rs741737/rs1609812
19	9.5	2.84	1.5×10^{-4}	7.5–26	rs887270/rs1044250
19	58.0	2.54	3×10^{-4}	52–63	rs1019937/rs1878926

Adapted from Concannon et al. (57). LOD, logarithm of odds.

has been shown to have functional consequences for *PTPN22* function in vitro and in vivo, and may be the causal variant in this region. Provocatively, in the current in vitro immunoassays of β- and T-cell activation and cytokine production, the R620W variant is a gain-of-function allele, suggesting that inhibition of LYP might be a therapeutic target in type 1 diabetes.

IL2RA. The IL-2R α-subunit of the IL-2 receptor complex locus (*IL2RA*) was found to be associated with type 1 diabetes using a tag SNP approach (36). The gene *IL2RA* is found on chromosome 10p15.1 and encodes the expression of *CD25* on regulatory naive T-cells, memory T-cells, and activated monocytes (37). The regulated expression of the CD25 protein is important for suppressing T-cell proliferation by an immunogenic stimulus. *IL2RA* has been identified as an associated gene in multiple autoimmune diseases (38–40). Recent fine mapping and functional studies have identified several variants that make independent contributions to risk for type 1 diabetes, indicating that *IL2RA* is the causal gene in the region. Different *IL2RA* variants influence the risk for development of multiple sclerosis, another autoimmune disease (41). In type 1 diabetes, the noncoding variants in *IL2RA* alter gene transcription, affecting expression of CD25 on the surface of naive and memory T-cells, and IL-2 production by stimulated memory T-cells (42). These human results parallel those observed in mouse studies, in which the CD25 ligand, *Il2* (the gene encoding the key cytokine IL-2), has been identified as the major non-MHC risk gene (43).

Other candidate genes. Previous studies using candidate gene approaches have suggested many additional loci contributing to susceptibility of type 1 diabetes susceptibility (44). However, numerous early studies were underpowered, owing to limitations in genomic information and genotyping technology, as well as small sizes of available cohorts.

The T1DGC, using the same samples as in the MHC and candidate gene investigations, reevaluated 382 SNPs from 21 recently reported candidate genes, assembling nearly 4,000 ASP families and fully characterizing (through tagging SNPs and reported variants) the genetic contributions to type 1 diabetes risk. These results suggest that, aside from the MHC, 11p15 (*INS*), 2q33 (*CTLA* and other genes), 10p15.1 (*IL2RA*), and 1p13 (*PTPN22*), few of these published candidate genes can be replicated. In addition, a total of 1,715 SNPs were selected from the Wellcome Trust Case Control Consortium (WTCCC) GWA study of type 1 diabetes, and 581 SNPs were selected that exhibited association with autoimmune disease and type 2 diabetes loci (45,46). These studies confirmed established loci (above) (47,48) and suggested additional risk conferred by loci on chromosomes 5q31 (*TCF7* [P19T], transcription

factor 7, T-cell specific, HMG-box), 18q12 (*FHOD3*, formin homology two domain containing 3), and Xp22 (*TLR8/ TLR7* toll-like receptor 8/toll-like receptor 7). Type 1 diabetes has many susceptibility loci and therefore pathways in common with autoimmune diseases. With the recent exception of *GLIS3* (49), no genetic overlap was found between type 1 diabetes and type 2 diabetes loci (45,46,50). The dataset established by the T1DGC from its Candidate Gene Workshops is available from the NIDDK Central Repository.

Genome-wide linkage. A number of genome-wide scans for linkage to type 1 diabetes have been reported (4,51–55). All these studies consistently demonstrated linkage of type 1 diabetes to the MHC and specifically to the HLA genes on human chromosome 6p21.3. Additional regions with evidence of linkage have been identified, but many of these regions have not been reproduced in independent studies.

The T1DCG has completed genome-wide linkage studies, including a meta-analysis of data from previous linkage studies with a subset of T1DGC families (4), as well as the largest ASP linkage study in type 1 diabetes (56). Five non-HLA regions (Table 1), and a distinct locus located in the broad HLA linkage peak, showed some evidence of linkage to type 1 diabetes. In general, the peaks delineated broad regions with multiple identified associated loci (www.t1dbase.org). Both *INS* and *CTLA4* are included among the identified regions from linkage. By applying family-based association testing to the linkage data from T1DGC families, one novel region associated with type 1 diabetes was identified, the *UBASH3A* region on chromosome 21, which has been confirmed in additional datasets (57). *UBASH3A* is expressed exclusively in T-cells, and animal studies implicate it in T-cell signaling.

Data from T1DGC genome-wide linkage experiments are available to the scientific community by request. Linkage studies in complex human disease are now recognized to have limited sensitivity due to the typical small locus-specific effect sizes. A major focus of current research is on the identification of putative risk genes with rarer or structural variants that could contribute to disease, and it is possible that the regions showing some evidence of linkage harbor variants that are not common SNPs well covered by the currently available genotyping platforms (58).

Genome-wide association (GWA). During the past few years, GWA studies have represented a paradigm shift in strategies for identifying risk genes for complex (multifactorial) human diseases, including type 1 diabetes (Fig. 1). This research has been made possible by the developments of high-density SNP genotyping arrays, analytical methods that build on the synthesis of population genetics, statistical genetics and genetic epidemiology, and the
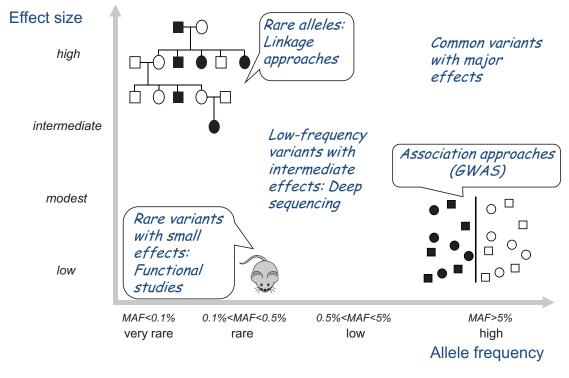
FIG. 1. Contribution and frequency of risk alleles dictate mapping strategies. Linkage studies have demonstrated that multifactorial disorders, including type 1 diabetes, cannot be explained by a limited number of rare variants with large effects, and GWA studies have shown that they cannot be explained by a limited number of common variants of moderate effects. Hence, the most significant gap is currently in detecting low-frequency variants with intermediate effects. MAF, minor allele frequency. Adapted from McCarthy et al. (62).

use of large clinically well-characterized case and control populations, as well as family collections, as that provided by the T1DGC. Careful attention to study design has been essential to eliminate or minimize bias (59–62). Type 1 diabetes genetic research has benefited from the collaboration among investigators, since T1DGC members have provided access to their own large collections to complement the T1DGC collection of families, case subjects, and control subjects. The family collections have proved invaluable not only for replicating case-control results but also for providing additional validation of the selection of control cohorts and their geographical and ethnic group matches to case subjects and investigating parent-of-origin effects.

The recently completed T1DGC GWA study, meta-analysis, and replication study included data from >30,000 individuals (47; http://www.t1dbase.org). Excluding HLA, there were 41 regions in the human genome that provided evidence of association with type 1 diabetes ($P < 10^{-6}$) (Table 2 and Fig. 2). Fifteen of these 41 regions were previously reported. Of the 26 novel regions, 18 were replicated in independent case-control and family collections (overall $P < 5 \times 10^{-8}$). Four additional SNPs were associated ($P < 0.05$) in the replication study but failed to reach genome-wide significance (overall $P < 5 \times 10^{-8}$) (Table 2). Over 100 other regions had SNPs that achieved associations with type 1 diabetes at borderline levels of significance ($10^{-6} < P < 10^{-5}$). Overall, the T1DGC GWA study and meta-analysis (48,63) provided convincing evidence for >40 non-HLA type 1 diabetes risk loci, with effect sizes of alleles ranging from OR = 2.38 (11p15.5, INS) to OR = 1.05 (17q21.2; SMARCE1). Many of these loci contain genes that affect the immune response (Table 2 and Fig. 2), although alternative, and as yet unknown, pathways may be implicated, including, for example, several genes such as IFIH1, GLIS3, and PTPN2 strongly expressed in β-cells.

In type 1 diabetes, initial analyses suggest that the risk conferred by non-HLA loci appears to be lower in ASP families (already enriched for high-risk HLA genes) than in sporadic cases (47). For example, it has been observed that some non-HLA SNPs (e.g., TCF7 P19T) show evidence of association with type 1 diabetes only in families that are not HLA-DR3/DR4, the highest HLA risk (64). This result suggests the presence of interaction, or a departure from the multiplicative model (statistical independence of the distributions of genotypes at two nonlinked loci). Further analysis is needed to fully clarify this observation (65). It seems likely that these interactions are small and, as a result, the biological interpretation and impact of such interactions will be difficult.

Follow-up of confirmed genes and variants. As suggested by the results in Table 2 and Fig. 2, many of the identified non-HLA regions contain candidate genes that are plausible by functional considerations. The median size of the identified regions is 255 kb (range 68 kb to 1.9 Mb), and they contain between 0 and 27 known genes. This suggests that there are >300 candidate genes, if we assume that the causal gene(s) is in the linkage disequilibrium, LD, region. However, since a causal variant in an associated region could affect transcriptional regulation of a gene several thousand base pairs away, owing to the existence of long-range regulatory elements or enhancers, including the number of candidate genes within 0.5 Mb on either side of an associated region brings the number of candidates in the order of 1,000 protein coding genes and ~500 non-protein coding pseudogenes and RNA-encoding sequences (http://www.t1dbase.org). It is evident that a combination of further more detailed genetic mapping, and genotype-phenotype correlation studies, are neces-

TABLE 2
Type 1 diabetes–associated loci from GWA studies

SNP	Chromosome	Position	LD region*	OR minor allele	Gene of interest or containing most associated SNP†
rs2476601‡	1p13.2	114179091	113.62–114.46	2.05	*PTPN22*
rs2269241§‖	1p31.3	63881359	63.87–63.94	1.10	*PGM1*
rs2816316‡	1q31.2	190803436	190.73–190.82	0.89	*RGS1*
rs3024505§	1q32.1	205006527	204.87–205.12	0.84	*IL10 (CNTN2)*
rs1534422§‖	2p25.1	12558192	12.53–12.60	1.08	*(gene desert)*
rs917997‡	2q12.1	102437000	102.22–102.58	0.83	*IL18RAP*
rs1990760‡	2q24.2	162832297	162.67–163.10	0.86	*IFIH1*
rs3087243‡	2q33.2	204447164	204.38–204.53	0.88	*CTLA4*
rs11711054‡	3p21.31	46320615	45.96–46.63	0.85	*CCR5*
rs10517086§	4p15.2	25694609	25.64–25.75	1.09	*(gene desert)*
rs4505848§	4q27	123351942	123.13–123.83	1.13	*IL2*
rs6897932‡	5q13.2	35910332	35.84–36.07	0.89	*IL7R*
rs9268645‡	6p21.32	32516505	24.70–34.00	6.8	*MHC*
rs11755527‡	6q15	91014952	90.86–91.10	1.13	*BACH2*
rs9388489§	6q22.32	126740412	126.48–127.46	1.17	*C6orf173*
rs2327832‡	6q23.3	138014761	137.80–138.40	0.90	*TNFAIP3*
rs1738074‡	6q25.3	159385965	159.13–159.62	0.92	*TAGAP*
rs7804356§	7p15.2	26858190	26.62–27.17	0.88	*SKAP2*
rs4948088§	7p12.1	50994688	50.87–51.64	0.77	*COBL*
rs7020673§	9p24.2	4281747	4.22–4.31	0.88	*GLIS3*
rs12251307‡	10p15.1	6163501	6.07–6.24	1.61	*IL2RA*
rs11258747‡	10p15.1	6512897	6.48–6.59	0.84	*PRKCQ*
rs10509540§	10q23.31	90013013	90.00–90.27	0.75	*RNLS*
rs7111341‡	11p15.5	2169742	2.02–2.26	2.38	*INS (TH)*
rs4763879§	12p13	9801431	9.51–9.80	1.09	*CD69*
rs2292239‡	12q13.2	54768447	54.64–55.09	1.31	*ERBB3*
rs1678536‡	12q13.3.	56265457	55.27–56.82		*Multiple (MMP19-LOCx-GSTPP)*
rs3184504‡	12q24.12	110368991	109.77–111.72	1.28	*SH2B3*
rs1465788§	14q24.1	68333352	68.24–68.39	0.86	*C14orf181*
rs4900384§	14q32.2	97568704	97.43–97.60	1.09	*(0; gene desert)*
rs3825932‡	15q25.1	77022501	76.77–77.05	0.86	*CTSH*
rs12708716‡	16p13.13	11087374	10.92–11.56	0.81	*CLEC16A*
rs12444268§‖	16p12.3	20250073	20.17–20.28	1.10	*UMOD*
rs4788084§	16p11.2	28447349	28.19–28.94	0.86	*IL27 (NUPR1)*
rs7202877§	16q23.1	73804746	73.76–74.09	1.28	*CTRB1*
rs16956936§‖	17p13.1	7574417	7.56–7.66	0.92	*DNAH2*
rs2290400§	17q12	35319766	34.63–35.51	0.87	*ORMDL3 (GSDML3)*
rs7221109§	17q21.2	36023812	35.95–36.13	0.95	*SMARCE1*
rs1893217‡	18p11.21	12799340	12.73–12.92	1.28	*PTPN2*
rs763361‡	18q22.2	65682622	65.63–65.72	1.16	*CD226*
rs425105§	19q13.32	51900321	51.84–52.02	0.86	*PRKD2*
rs2281808§	20p13	1558551	1.44–1.71	0.90	*SIRPG*
rs11203203‡	21q22.3	42709255	42.68–42.76	1.13	*UBASH3A*
rs5753037§	22q12.2	28911722	28.14–29.00	1.10	*LOC729980/HORMAD2*
rs229541‡	22q13.1	35921264	35.90–36.00	1.12	*C1QTNF6*
rs2664170§	Xq28	153598796	153.48–154.10	1.16	*GAB3*

Significant observations from Barrett et al. (47) are listed. *The size of the GWA regions is defined by the linkage disequilibrium, LD, of the region. LD regions were calculated with the HapMap CEU Founders dataset in snpMatrix (http://www.bioconductor.org/packages/release/bioc/html/snpMatrix.html) using different D' and r^2 thresholds. †The gene physically closest to the marker position is listed. For candidate genes suggested for specific regions, if not closest to the marker, these are listed in brackets. Adapted from www.t1dbase.org. ‡The SNP marker represents a known susceptibility locus for type 1 diabetes from previous studies. §The marker represents a newly identified type 1 diabetes risk locus that was confirmed in the replication part of the study. ‖Marker that was significant in the GWA study and replication study but did not reach genome-wide significance in the combined analysis.

sary for identification of the causal genes within these regions. Some of these studies are underway—a recent initiative on Fine Mapping and Gene Function in Type 1 Diabetes, supported by the National Institutes of Health, supports several different approaches.

Studies to evaluate the molecular differences in gene regulation or function that are due to the supposed causative genetic risk variants (e.g., protein expression level and differences in cellular function between case and control subjects) are needed to explore the mechanisms through which the causal variants generate disease risk. Even when a gene has an obvious potential to explain pathogenesis and to be a component in the disease mechanism, inferences concerning function may be limited. Furthermore, several of the identified loci do not suggest genes with known functions: in fact, some of the associated regions do not contain annotated genes, pointing to potential contribution of long-range gene expression reg-
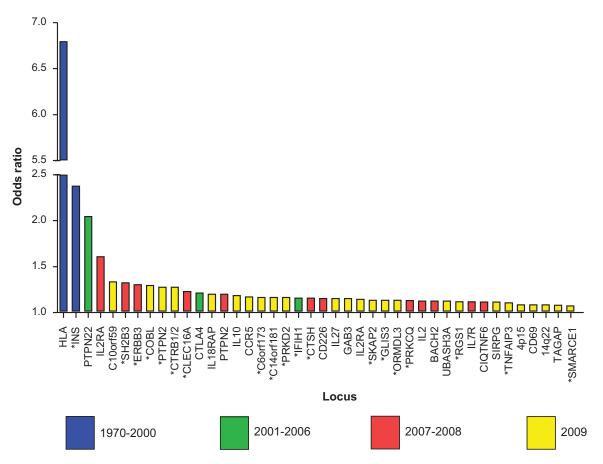
FIG. 2. GWA studies have significantly accelerated the pace of gene discovery in type 1 diabetes. However, most genetic associations discovered currently are weak. Color-coding designates year of discovery of these candidate genes. The *y*-axis indicates the best estimate of the OR for risk alleles at each of the indicated loci on the basis of currently published data (47). For each genomic region where convincing association with type 1 diabetes has been reported, the gene of interest or containing the most associated SNP is indicated on the x-axis. The majority of these genes are implicated in the immune response, but several of the non-HLA genes are expressed in human pancreatic islets (marked with *) (www.t1dbase.org) (82). (A high-quality digital representation of this figure is available in the online issue.)

ulatory elements and/or nonprotein coding RNA genes. The vast majority of the currently reported associations also do not point unambiguously to a particular gene, but to several within, and outside, a block of linkage disequilibrium. Thus, genes that may clearly be implicated are often not annotated with respect to function.

In silico analyses and experimental data indicate that up to 50% of conserved cis-acting elements in the human genome may be 1 Mb from target genes, sometimes in the introns of neighboring genes, although most regulatory sequences are within 50 kb of the gene (66). Thus, "true" type 1 diabetes genes may be some distance from the association signal, although the reported association provides an anchor point on which to base functional studies.

Recent data (67) and ongoing investigations indicate that other types of common genetic variation (e.g., copy number or structural variants, such as deletions and duplications) may contribute little to the observed familial clustering of type 1 diabetes risk. However, rare loss-of-function structural gene variants could still make an important contribution to type 1 diabetes risk, through identification of which particular gene in a region of association could harbor a causal variant. With further advances in array and sequencing technologies, it is anticipated that such loss-of-function variants will be identified that influence susceptibility to type 1 diabetes (68).

Inferences from genetic studies. Each newly identified association of a candidate locus with type 1 diabetes

presents new challenges. Finding the causal genes and the causal variants, understanding how they affect disease pathophysiology, and dissecting their contribution to type 1 diabetes risk remain the major undertakings. For some genes, the effect sizes of risk alleles are such that larger collections of patients will be needed to identify the causal genes and limit the number of potential causal variants. Genotype-phenotype fine-mapping studies, however, can be performed with much smaller sample sizes while still achieving convincing statistical evidence (e.g., 42). Each confirmed gene, based on both statistical and functional evidence, provides a key piece of the etiology of type 1 diabetes, regardless of the magnitude of the odds ratio as a measure of the population association.

Combinations of many alleles, possibly hundreds, combine with effects of environmental factors (probably numerous and ubiquitous) to establish the risk profile for type 1 diabetes. Each common variant in isolation has a subtle effect on disease risk, but each may alter a key function in the immune system and its interaction with pancreatic β-cells. Recent discussion of "missing heritability" for complex human traits has considered the source of this variation and appropriate research strategies to detect these genetic effects (61). Studies in populations that are distinct from Europeans or European ancestry, such as populations of recent African ancestry or from Asian countries, are likely to narrow the large chromosomal regions of association identified in current studies and to

increase the yield of rare variants (69). Future studies examining rare variants, structural variation, and polymorphisms not well imputed should be helpful in uncovering the remaining missing heritability in type 1 diabetes.

A recent sequencing study provides an example of detection of rare variants in type 1 diabetes. Targeted sequencing in a series of candidate coding regions resulted in *IFIH1* being identified as the causal gene in a region associated with type 1 diabetes by GWA studies (58). *IFIH1* encodes a cytoplasmic helicase that mediates induction of the interferon response to viral RNA. The discovery of *IFIH1* as a contributor to susceptibility to type 1 diabetes has strengthened the hypothesis (70) about a mechanism of disease pathogenesis involving virus-genetic interplay and raised type 1 interferon levels as a cofactor in β-cell destruction. Nonetheless, it should be recognized that a component of the missing heritability (familial aggregation) in type 1 diabetes could well be due to unrecognized intra-familial environmental factors.

Disease pathogenesis. Contemporary models of pathogenesis of type 1 diabetes support the involvement of two primary *dramatis personae*: the immune system and the β-cell. The known and newly identified genetic risk factors for type 1 diabetes present exciting opportunities to build on to the current cast of disease mechanisms and networks. Most of the listed genes of interest (Table 2) and those in extended regions are assumed to regulate immune function. Some of these genes, however, may also have roles in the β-cell (insulin being the most obvious example). Another gene, *PTPN2*, encoding a protein tyrosine phosphatase, was identified as affecting the risk for type 1 diabetes as well as for Crohn disease (47,71). *PTPN2* is expressed in immune cells, and its expression is highly regulated by cytokines. However, *PTPN2* is expressed also in β-cells, where it modulates interferon (IFN)-γ signal transduction and has been shown to regulate cytokine-induced apoptosis (72). Other candidate genes, such as *NOS2A*, *IL1B*, reactive oxygen species scavengers, and candidate genes, identified in large GWA studies of type 2 diabetes, have not been found to be significant contributors to the susceptibility of type 1 diabetes (73).

Recently, new relationships between type 1 diabetes and other autoimmune and inflammatory diseases have been uncovered (63,71,74) (Table 3). Certain HLA haplotypes have long been known to strongly influence genetic predisposition to autoimmunity (75). The contribution of the specific HLA component differs considerably among different autoimmune diseases, but most relate to the function of the adaptive immune response and the binding and presentation of specific peptides. The results of GWA studies have reinforced the belief that type 1 diabetes is an autoimmune disease and that HLA is the major genetic determinant of risk for type 1 diabetes. Importantly, there is a substantial overlap in non-HLA susceptibility loci between type 1 diabetes and other autoimmune diseases (76). This overlap in genetic susceptibility locus (although not necessarily the same causal variant [41]) supports the concept that genetic risk in autoimmunity is determined in part by variation in genes that act on control mechanisms of the immune system. It will be important to identify loci that are distinct to type 1 diabetes (such as the *INS* locus), since these loci may illuminate type 1 diabetes–specific pathways.

The T1DGC is participating in a follow-up study of multiple autoimmune disease consortia. This project identifies significant loci from GWA studies to develop the

TABLE 3

Type 1 diabetes loci showing overlap with risk loci of other immune diseases

Gene of interest	Immune diseases
PTPN22 (1p13.2)	AITD, Crohn disease, MS, RA, SLE
RGS1 (1q31.2)	Celiac disease
IL10 (1q32.1)	Crohn disease
IL18RAP (2q12.1)	Celiac disease
IFIH1 (2q24.2)	AITD
CTLA4 (2q33.2)	RA
CCR5 (3p21.31)	Celiac disease
IL2 (4q27)	AITD, RA, Celiac disease
IL7R (5p13.2)	MS
TNFAIP3 (6q23.3)	RA, SLE
TAGAP (6q25.3)	Celiac disease
IL2RA (10p15.1)	MS, SLE
SH2B3 (12q24.12)	Celiac disease
CLEC16A (16p13.13)	MS
ORMDL3 (17q12)	Asthma
PTPN2 (18p11.21)	Celiac disease, Crohn disease
CD226 (18q22.2)	MS, RA

From http://www.t1dbase.org and http://www.genome.gov/GWAstudies. The loci are from Table 1, where overlap to risk loci in other autoimmune or inflammatory diseases have been reported. AITD, autoimmune thyroid disease; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

ImmunoChip, a 200,000-SNP custom array that will provide dense SNP mapping of regions that have been associated (at genome-wide significance) with autoimmune diseases. Both individual and shared regions of the genome will be assayed across autoimmune diseases. These results will be made available through T1DBase, and the data will be made available from the NIDDK Central Repository.

Clinical implications of GWAS results. Recently, Clayton (65) evaluated the genetic architecture of type 1 diabetes from the GWA meta-analysis study conducted by the T1DGC. It was concluded that the principal value of the newly discovered SNPs would be to increase our understanding of disease pathogenesis, rather than increase our ability to predict disease development on an individual level. Even if we could explain all the familial clustering of the disease (genetic and environmental factors), of which the largest contributor is the HLA, receiver operator curve analyses showed that the positive predictive value is limited, where a trial designed to capture 80% of all future cases has to treat 20% of the general population, of whom only <0.5% will develop type 1 diabetes.

The ultimate objective of genetic research is the translation of genetics findings into advances in clinical care. An obvious question is, "What can a risk gene with an OR in the range of 1.05–1.2 add to clinical treatments for type 1 diabetes?" However, a low OR does not disqualify the encoded protein as a potential drug target. Both *PPARG* and *KCNJ11* are genes that have a low OR for type 2 diabetes risk, yet they encode for major drug targets. A major contribution of genetics to type 1 diabetes will be the identification of important disease pathways that can be examined for new therapeutic targets or biomarkers, including the stratification of subjects at risk for interventions or patients for effective treatment (and prevention of complications).

From GWAS to integrative genomics. Redefining and stratifying human disease, especially with regard to pharmacological response, in the post-GWA era is essential. A

new approach to classifying human disease that both appreciates the uses and limits of reductionism and incorporates the tenets of the nonreductionist approach of complex systems analysis is necessary. Disease phenotypes reflect consequences of variation in complex genetic networks operating within a dynamic environmental framework. Further genetic and functional evaluations, conducted at the highest levels of experimental rigor and repeatability and reproducibility, are necessary to establish and confirm involvement of such networks in type 1 diabetes, to fully elucidate the biological mechanisms of the networks and to identify the strongest risk phenotypes (77,78). Some phenotypes will be regulated by several of the type 1 diabetes genes and may well be precursors of disease, appearing at the earliest stages of the development of type 1 diabetes and perhaps even preceding aggressive autoimmunity (79).

Recently, it was suggested that since the vast majority of disease genes show no tendency to encode highly connected protein hubs but are localized to the functional periphery of networks (80), they are not essential for explaining disease pathogenesis. The counterargument is to consider that cellular networks are modular, consisting of groups of highly interconnected proteins responsible for specific cellular functions. Disease pathogenesis represents the perturbation of probably many specific functional modules caused by a variation in one or more of the components producing recognizable developmental and/or physiological dynamic instability (81). Such a model offers a hypothesis for the emergence of complex or polygenic disorders—a phenotype often correlates with the inability of a particular functional module to carry out its basic function. For extended modules, many different combinations of gene variants might incapacitate the module and lead to the same clinical phenotype. The correlation between disease pathogenesis and functional modules can improve our understanding of cellular networks by helping us to identify which genes are involved in the same cellular function or network module. Pathogenic processes may progress to clinical disease such as type 1 diabetes; alternatively, these processes may be interrupted at subclinical levels. The identification of such phenotypes or disease precursors is therefore a key aim. Comprehensive gene expression studies in cells and tissues relevant to type 1 diabetes will help lead to identification of relevant networks. Importantly, the association of disease with functional networks may also influence our choice of new therapeutic targets.

CONCLUSION

The greatest genetic risk (both increased risk, *susceptible*, and decreased risk, *protective*) for type 1 diabetes is conferred by specific alleles, genotypes, and haplotypes of the HLA class II (and class I) genes. There are currently about 50 non-HLA region loci that also affect the type 1 diabetes risk. Many of the assumed functions of the non-HLA genes of interest suggest that variants at these loci act in concert on the adaptive and innate immune systems to initiate, magnify, and perpetuate β-cell destruction. The clues that genetic studies provide will eventually help lead us to identify how β-cell destruction is influenced by environmental factors. While there is extensive overlap between type 1 diabetes and other immune-mediated diseases, it appears that type 1 and type 2 diabetes are

genetically distinct entities. These observations may suggest ways to help identify causal gene(s) and, ultimately, a set of disease-associated variants defined on specific haplotypes. Unlike other complex human diseases, relatively little familial clustering remains to be explained for type 1 diabetes. The remaining missing heritability for type 1 diabetes is likely to be explained by as yet unmapped common variants, rare variants, structural polymorphisms, and gene-gene and/or gene-environmental interactions, in which we can expect epigenetic effects to play a role. The examination of the type 1 diabetes genes and their pathways may reveal the earliest pathogenic mechanisms that result in the engagement of the innate and adaptive immune systems to produce massive β-cell destruction and clinical disease. The resources established by the international T1DGC are available to the research community and provide a basis for future discovery of genes that regulate the earliest events in type 1 diabetes etiology—potential targets for intervention or biomarkers for monitoring the effects and outcomes of potential therapeutic agents.

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