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## Genes Involved in Type 1 Diabetes

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Additional information is available at the end of the chapter

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### 1. Introduction

The prevalence of diabetes is increasing worldwide and to date it impacts the lives of approximately 200 million people (Steyn et al., 2009). It is estimated that by 2030, there will be 439 million adults affected by diabetes (International Diabetes Federation/diabetes prevalence: [www.idf.org](http://www.idf.org)). Type 1 diabetes (T1D) represents approximately 10% of these patients and is most prevalent in populations of European ancestry, where there is ample evidence of increased annual incidence during the past five decades (Onkamo et al., 1999; EURODIAB ACE Study Group, 2000).

T1D is a complex trait that results from the interplay between environmental and genetic factors. Much evidence supports a strong genetic component associated with T1D. The epidemiological data showing differences in geographic prevalence is one clear indicator, with populations of European ancestry having the highest presentation rate. T1D has high concordance among monozygotic twins (33 to 42%) (Redondo et al., 2001) and runs strongly in families with sibling risk being approximately 10 times greater than in the general population (Clayton, 2009); this is in clear contrast to the “less genetic” type 2 diabetes, where the sibling risk ratio is relatively modest at 3.5 (Rich, 1990).

T1D develops at all ages and occurs through the autoimmune destruction of pancreatic  $\beta$ -cells with resulting lack of insulin production. The immune system participates in  $\beta$ -cell destruction through several of its components including natural killer (NK) cells, B lymphocytes, macrophages, dendritic cells (DC), and antigen-presenting cells (APCs). Studies in human and animal models have shown that both innate and adaptive immune responses participate in disease pathogenesis, possibly reflecting the multifactorial nature of this autoimmune disorder.

In this review, we provide an update on genome-wide association studies (GWAS) discoveries to date and discuss the latest associated regions added to the growing repertoire of gene networks predisposing to T1D.

## 2. Genetic component in Type 1 diabetes

### 2.1. Before genome-wide association studies

Historically, prior to GWAS, only six loci had been fully established to be associated with T1D. The human leukocyte antigen (HLA) region on chromosome 6p21 was the first known candidate to be strongly associated with T1D in 1970s (Singal & Blajchman, 1973; Nerup et al., 1974; Cudworth & Woodrow, 1975). This cluster of homologous cell-surface proteins is divided into class I (A, B, C) and class II (DP, DQ, RD). The HLA genes encode highly polymorphic proteins, which are essential in self versus non-self immune recognition. The class I molecules are ubiquitously expressed and present intracellular antigen to CD8<sup>+</sup> T cells. Class II molecules are expressed mainly on professional APCs: DCs, macrophages, B-lymphocytes and thymus epithelium. Class II molecules are composed of A and B chains, and present antigens to CD4<sup>+</sup> T cells, which promote inflammation by secreting cytokines upon recognition of their specific targets. Approximately half of the genetic risk for T1D is conferred by the genomic region harboring the HLA class II genes primarily HLA-DRB1, -DQA1 and -DQB1 genes). In 1984, insulin (INS) gene encoded on chromosome 11p15 was identified as second loci linked with T1D (Bell et al., 1984). In 1996, the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) gene encoded on chromosome 2q33 was recognized as third loci (Nistico et al., 1996). In 2004, a protein tyrosine phosphatase, non-receptor type 22 (PTPN22) gene encoded on chromosome 1p13, was found to be associated with susceptibility to T1D in another case-control study (Bottini et al., 2004). Vella et al., 2005 reported interleukin 2 receptor alpha (IL2RA) gene as fifth T1D loci on chromosome 10p15. In 2006, Smyth et al. identified the interferon-induced with helicase C domain 1 (IFIH1) gene on chromosome 2q24.3 as the sixth gene to be strongly associated with T1D.

### 2.2. GWAS of T1D

The advent of GWAS in the mid-2000s has changed the situation dramatically, increasing the pace and efficiency of discovery for the T1D associated loci, by a factor of ten. The critical platform for this work was laid by the HapMap project (International HapMap Consortium, 2003, 2005). The GWAS approach has been made possible by the development of high-density genotyping arrays. The genome is laid out in discrete linkage disequilibrium (LD) blocks with limited haplotype diversity within each of these blocks. Therefore, a minimal set of single nucleotide polymorphisms (SNPs) can detect almost all common haplotypes present, thus improving genotyping accuracy and reducing cost.

The first full-scale GWAS for T1D were published in 2007 by our group (Hakonarson et al., 2007) and The Wellcome Trust Case-Control Consortium (WTCCC, 2007). We examined a large pediatric cohort of European descent using the Illumina HumanHap 550 BeadChip platform.

The design involved 561 cases, 1,143 controls and 467 triads in the discovery stage, followed by a replication effort in 939 nuclear families. In addition to finding the “usual” suspects, including an impressive 392 SNPs capturing the very strong association across the major histocompatibility complex (MHC), we identified significant association with variation at the KIAA0350 gene, which we replicated in an additional cohort. The WTCCC study investigated seven common complex diseases including T1D by genotyping 2,000 cases and 3,000 controls with ~500,000 SNPs using the Affymetrix GeneChip, and reported a number of novel T1D loci, including the KIAA0350 genomic region (WTCCC, 2007). Todd et al., 2007 confirmed these findings, using 4,000 cases, 5,000 controls and 3,000 T1D families as well as association reported in the WTCCC study to the 12q13 region. In a separate effort we fast-tracked 24 SNPs at 23 distinct loci from our original study and established association to the 12q13 region with a combined  $P$ -value of  $9.13 \times 10^{-10}$  (Hakonarson et al., 2008); this was the same locus as reported by the WTCCC and Todd et al., 2007. The 12q13 region harbors several genes, including ERBB3, RAB5B, SUOX, RPS26 and CDK2. However, the causative variants at this locus remain unknown. Concannon et al., 2008 reported an association between SNP at the UBASH3A locus on 21q22.3 and T1D by using SNP genotyping data from a linkage study of affected sib pairs in nearly 2,500 multiplex families, a finding also corroborated by our efforts as well as association to the BACH2 gene (Grant et al., 2009).

### 2.3. Meta-analyses of T1D GWAS datasets

In order to get the most from GWAS and to increase the statistical power, several independent research groups carried out meta-analyses using datasets from different investigative groups. Cooper et al., 2008 performed the first meta-analysis by using T1D datasets from the WTCCC, 2007 and the Genetics of Kidneys in Diabetes (GoKind) study (Mueller et al, 2006; Manolio et al., 2007), and confirmed associations for PTPN22, CTLA4, MHC, IL2RA, 12q13, 12q24, CLEC16A and PTPN2. The SNPs with lowest nominal  $P$ -values were taken forward for further genotyping in an additional British cohort of 6,000 cases, 7,000 controls and 2,800 families. As a result, the IL2-IL21 association strengthened further and they found strong evidence for four additional loci: BACH2; a 10p15 region harboring the protein kinase C, theta gene (PRKCE); a 15q24 region harboring nine genes including cathepsin H (CTSH) and a 22q13 region harboring tumor necrosis factor related protein 6 (C1QTNF6). Additional studies are required to elucidate the culprit genes and their mechanism at the 15q24 and 22q13 loci.

Barrett et al., 2009 meta-analysis uncovered in excess of 40 loci, including 18 novel regions, plus they confirmed a number of previously reported (Smyth et al., 2008; Fung et al., 2009; Cooper et al., 2009). The study included samples from WTCCC, 2007, the GoKind study (Mueller et al., 2006) and controls and family sets from Type 1 Diabetes Genetics Consortium (T1DGC). The meta-analysis observed association to 1q32.1 (which harbors the immunoregulatory interleukin genes IL10, IL19 and IL20), 9p24.2 contains only Glis family zinc finger protein 3 (GLIS3; first suggested by us in Grant et al., 2009), 12p13.31 which harbors a number of immunoregulatory genes including CD69 and 16p11.2 harboring IL27. These findings were further supported by our *in silico* replication efforts (Qu et al., 2010).

To identify additional genetic loci for T1D susceptibility, we examined associations in the largest meta-analysis to date between the disease and ~2.54 million genotyped and imputed SNPs in a combined cohort of 9,934 cases and 16,956 controls (Bradfield et al., 2011). Targeted follow-up of 53 SNPs in 1,120 affected trios uncovered three new loci associated with T1D that reached genome wide significance. The most significantly associated SNP (rs539514,  $P = 5.66 \times 10^{-11}$ ) resided in an intronic region of the LMO7 (LIM domain only 7) gene on 13q22. The second most significantly associated SNP (rs478222,  $P = 3.50 \times 10^{-9}$ ) resided in an intronic region of the EFR3B (protein EFR3 homolog B) gene on 2p23; however the region of linkage disequilibrium is approximately 800kb and harbors additional multiple genes, including NCOA1, C2orf79, CENPO, ADCY3, DNAJC27, POMC, and DNMT3A. The third most significantly associated SNP (rs924043,  $P = 8.06 \times 10^{-9}$ ) was in an intergenic region on 6q27, where the region of association is approximately 900kb and harbors additional genes including WDR27, C6orf120, PHF10, TCTE3, C6orf208, LOC154449, DLL1, FAM120B, PSMB1, TBP and PCD2. These latest associations add to the growing repertoire of gene networks predisposing to T1D. Table 1 summarizes all T1D associated loci reported to date.

Reference	Sample Size	Replication Sample Size	Ethnic Group	Study Type	Main Findings
Hakonarson et al., 2007	467 trios, 561 cases, 1,143 controls	2,350 individuals in 549 families; 390 trios	European ancestry	GWAS	HLA-DRB1, HLA-DQA2, CLEC16A, INS, PTPN22
WTCCC 2007	1,963 cases, 2,938 controls	see Todd et al., 2007	European, British	GWAS	HLA-DRB1, INS, CTLA4, PTPN22, IL2RA, IFIH1, PPARG, KCNJ11, TCF7L2
Todd et al., 2007	see WTCCC 2007	2997 trios, 4,000 cases, 5,000 controls	European British	GWAS	PHTF1-PTPN22, ERBB3, CLEC16A, C12orf30
Hakonarson et al., 2008	467 trios, 561 cases, 1,143 controls	549 families, 364 trios	European ancestry	GWAS	SUOX - IKZF4
Concannon et al., 2008	2,496 families	2,214 trios, 7,721 cases, 9,679 controls	European ancestry	GWAS	INS, IFIH1, CLEC16A, UBASH3A
Cooper et al., 2008	3,561 cases, 4,646 controls	6,225 cases, 6,946 controls, 3,064 trios	European ancestry	GWAS meta- analysis	PTPN22, CTLA4, HLA, IL2RA, ERBB3, C12orf30, CLEC16A, PTPN2
Grant et al., 2009	563 cases, 1,146 controls, 483 case- parents trios	636 families, 3,303 cases, 4,673 controls	European ancestry	GWAS	EDG7, BACH2, GLIS3, UBASH3A, RASGRP1

Reference	Sample Size	Replication Sample Size	Ethnic Group	Study Type	Main Findings
Awata et al., 2009	735 cases, 621 controls	-	Japanese	TaqMan genotyping	ERBB3, CLEC16A
Zoledziwska et al., 2009	1037 cases, 1706 controls	-	European, Sardinian	TaqMan genotyping	CLEC16A
Fung et al., 2009	8010 cases, 9733 controls	-	European, British	TaqMan genotyping	STAT4, STAT3, ERAP1, TNFAIP3, KIF5A/ PIP4K2C
Wu et al., 2009	205 cases, 422 controls	-	Han Chinese	TaqMan genotyping	CLEC16A
Barrett et al., 2009	7,514 cases, 9,045 controls	4,267 cases, 4,670 controls, 4,342 trios	European	GWAS meta- analysis	MHC, PTPN22, INS, C10orf59, SH2B3, ERBB3, CLEC16A, CTLA4, PTPN2, IL2RA, IL27, C6orf173, IL2, ORMDL3, GLIS3, CD69, IL10, IFIH1, UBASH3A, COBL, BACH2, CTSH, PRKCQ, C1QTNF6, PGM1
Wallace et al., 2010	7,514 cases, 9,045 controls	4,840 cases, 2,670 controls, 4,152 trios	European ancestry	GWAS meta- analysis	DLK1, TYK2
Wang et al., 2010	989 cases, 6197 controls	-	European ancestry	GWAS	PTPN22, IL10, IFIH1, KIAA0746, BACH2, C6orf173, TAGAP, GLIS3, L2R, INS, ERBB3, C14orf181, IL27, PRKD2, HERC2, CLEC16A, IFNG, IL26,
Reddy et al., 2011	1434 cases, 1864 controls	-	European ancestry, southeast USA	TaqMan genotyping	PTPN22, INS, IFIH1, SH2B3, ERBB3, CTLA4, C14orf181, CTSH, CLEC16A, CD69, ITPR3, C6orf173, SKAP2, PRKCQ, RNLS, IL27, SIRPG, CTRB2
Bradfield et al., 2011	9,934 cases, 16,956 controls	1,120 trios	European ancestry	GWAS meta- analysis	LMO7, EFR3B, 6q27, TNFRSF11B,



Reference	Sample Size	Replication Sample Size	Ethnic Group	Study Type	Main Findings
					LOC100128081, FOSL2
Asad et al., 2012	424 families, 3078 cases, 1363 controls	-	European, Scandinavians	Genotyping and sequencing	HTR1A, RFN180
Huang et al., 2012	16,179 individuals	-	European ancestry	Genomes- based imputation	CUX2, IL2RA

**Table 1.** T1D susceptibility loci identified to date.

**2.4. Immune components in T1D**

The immune system is well organized and well regulated with a basic function of protecting the host against pathogens. This places the immune system in a vital position between healthy and diseased states of the host. Its protective task is regulated by a complex regulatory mechanism involving a diverse army of cells and molecules of humoral and cellular factors working in concert to protect the body against invaders. The human immune system has two components: innate and adaptive. Innate immunity is comprised of physical, chemical, and microbiological barriers to the entry of antigen, and the elements of immune system (DC, macrophages, mast cells, NK cells, neutrophils, monocytes, complements, cytokines and acute phase proteins), which provide immediate host defense. Adaptive immunity is the hallmark of the immune system of higher animals with T and B cells as the key cellular players that provide more specific life-long immunity.

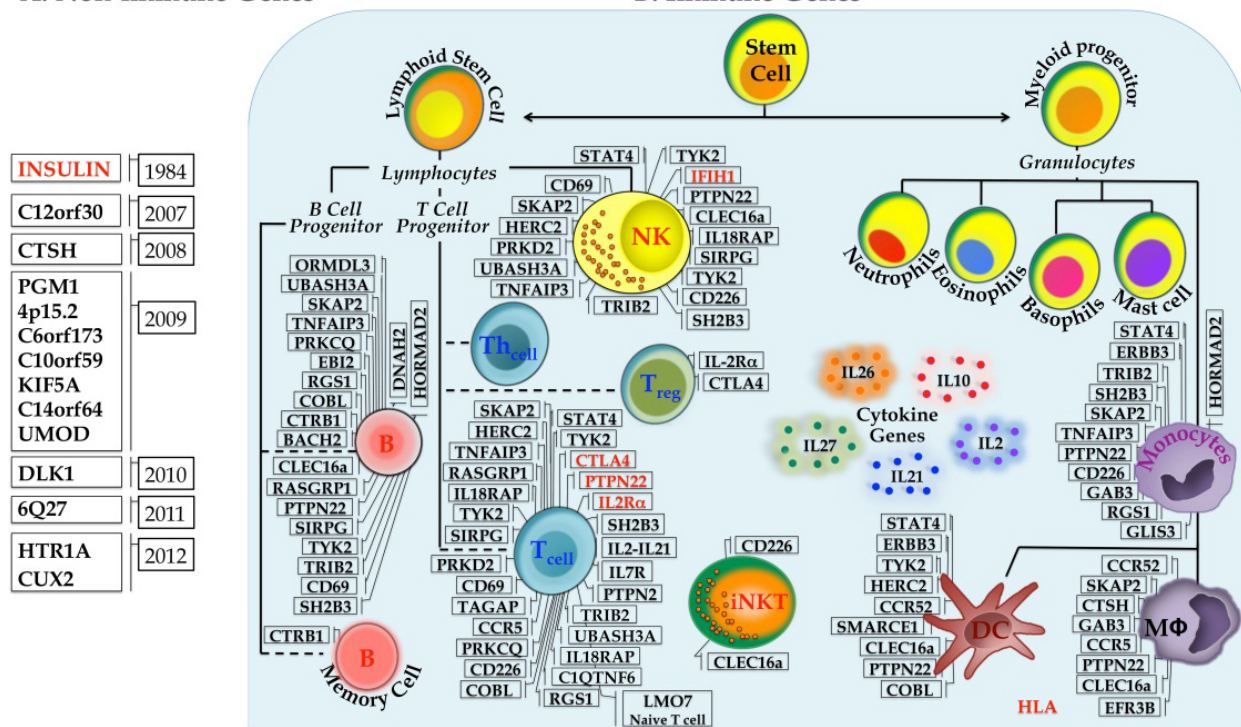
In T1D this system breaks down: insulin-producing  $\beta$ -cells are subjected to specific attack by the host immune system. To better understand the etiology of T1D, a plethora of research has been done to link the systematic destruction of  $\beta$ -cells and the role of the immune system. Linkage studies in 1970s revealed MHC as the first key contributor to T1D susceptibility. Further linkage analysis and candidate gene association studies revealed additional loci associated with T1D. Starting in 2007, GWAS have increased the number of loci be associated with T1D to almost 60. In Figure 1 we present 59 T1D susceptibility loci as where we have classified them into loci harboring non-immune (14) vs. immune (45) genes. Functional aspects of some genes are discussed below.

The complex crosstalk between innate and adaptive immune cells has major impact on the pathogenesis and development of T1D as illustrated in Figure 2. The initiation phase (Phase I) of T1D development takes place in the pancreas where conventional dendritic cells (cDCs) capture and process  $\beta$ -cell antigens. Apoptosis (‘natural cell death’) or viral infection can lead to  $\beta$ -cell death. Antiviral responses are mediated by invariant natural killer T (iNKT) cells; crossplay between iNKT and plasmacytoid DCs (pDCs) controls viral replication thus prevents subsequent inflammation, tissue damage, and downregulating T1D pathogenesis.

Migration of activated cDCs to the draining lymph node primes pathogenic islet antigen-specific T cells. This activation is promoted by macrophages through IL12 secretion. B cells present  $\beta$ -cell antigen to diabetogenic T cells and secrete autoantibodies in response. The activation of islet antigen-specific T cells can be inhibited by cDCs through engagement of programmed cell death ligand 1 (PDL1). The expansion phase (Phase II): iNKT cells can further promote the recruitment of tolerogenic cDCs and pDCs. These DCs promote expansion of regulatory T (TReg) cells through the production of indoleamine 2,3-dioxygenase (IDO), IL10, transforming growth factor- $\beta$  (TGF $\beta$ ) and inducible T cell co-stimulator ligand (ICOSL). Phase III: In the pancreas,  $\beta$ -cell can be killed by diabetogenic T cells and NK cells through the release of interferon- $\gamma$  (IFN $\gamma$ ), granzymes and perforin, as well as by macrophages through the production of tumour necrosis factor (TNF), IL-1 $\beta$  and nitric oxide (NO). IL12 produced by cDCs sustains the effector functions of activated diabetogenic T cells and NK cells. TReg cells that inhibit diabetogenic T cells and innate immune cells through IL10 and TGF $\beta$  can prevent  $\beta$ -cell damage. Tolerogenic pDCs stimulated by iNKT cells could also control diabetogenic T cells through IDO production. Lastly,  $\beta$ -cells can inhibit diabetogenic T cells by expressing PDL1 and escape the cell death.

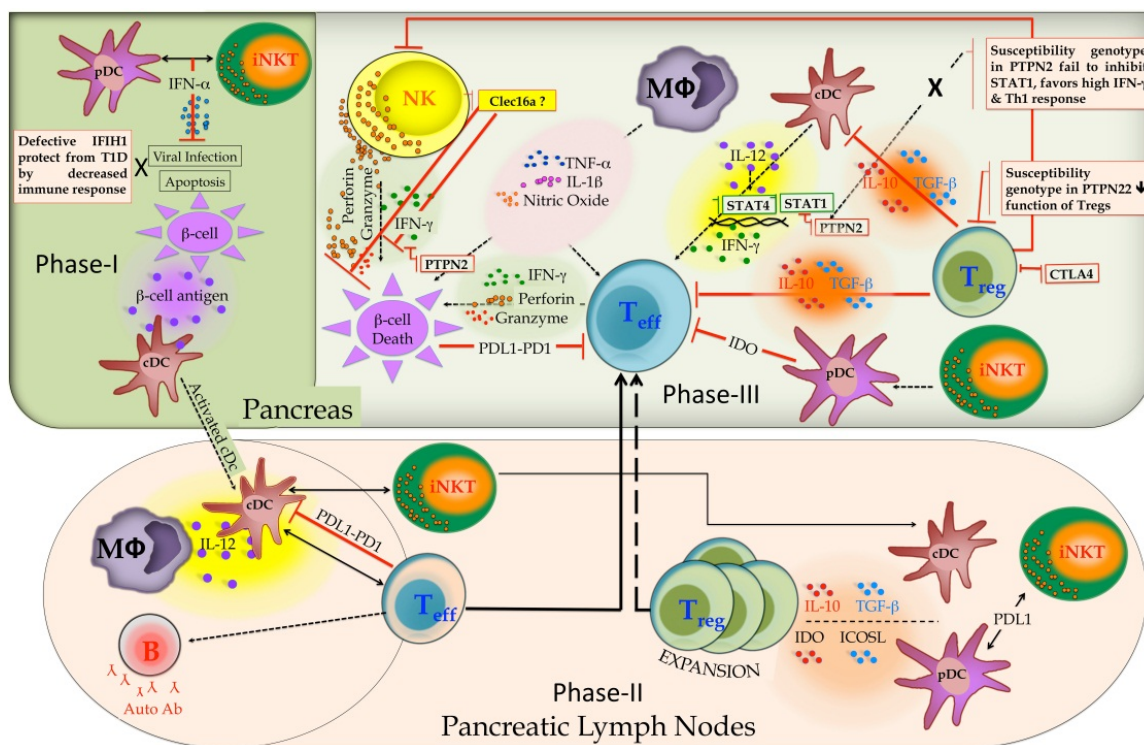
### A. Non-immune Genes

### B. Immune Genes



**Figure 1.** Immune and Non-immune T1D genes are depicted in a concept map representing the components of the immune system. The discovery of T1D susceptibility genes started as early as 1974 with just six genes identified by 2006 shown in red. The advent of GWAS led to flurry of novel genes associated with T1D reaching the excess of 40 by 2009 and almost 60 by 2012.





**Figure 2.** Pathogenesis model of T1D involves complex interactions between innate and adaptive immune cell types.

## 2.5. CLEC16A (16p13)

The C-type lectin domain family 16, member A (CLEC16A) gene encodes protein with C-type lectin domain structure, which makes it potentially related to the immune response (Robinson et al., 2006). It is established that C-type lectins function both as adhesion and pathogen recognition receptors (PPRs) (Cambi & Figdor, 2003). In addition, CLEC16A is almost exclusively expressed in immune cells including DCs, B-lymphocytes and NK cells. Our 2007 GWAS in a large pediatric cohort of European descent identified CLEC16A as a novel T1D susceptibility gene within a 233-kb linkage disequilibrium block on chromosome 16p13. Three common non-coding variants of the CLEC16A gene (rs2903692, rs725613 and rs17673553) reached genome-wide significance for association with T1D (Hakonarson et al., 2007). Subsequent replication studies in an independent cohort confirmed the association. Importantly, the allele of CLEC16A linked to protection from T1D was also associated with higher levels of CLEC16A expression in NK cells (Hakonarson et al., 2007).

The 2007 WTCCC study independently discovered CLEC16A (formally known as KIAA0350) as a T1D susceptibility locus associated with the non-coding variant rs12708716. This finding was confirmed immediately for T1D in populations of European descent (Todd et al., 2007, Cooper et al., 2008). To date, several SNPs (rs2903692, rs17673553, rs725613, rs12708716, rs12921922, rs12931878) within the CLEC16A gene have been reported to be associated with T1D in several populations: Sardinian (Zoledziewska et al., 2009), Spanish (Martinez et al., 2010), southeast USA (Reddy et al., 2011), Chinese (Wu et al., 2009; Sang et al., 2012), and

Japanese (Yamashita et al., 2011). Recently CLEC16A was also associated with adult-onset of autoimmune diabetes (Howson et al., 2011).

Several GWAS in different autoimmune diseases such as multiple sclerosis (MS) (Zuvich, 2011; Nischwitz et al., 2011), Addison's disease (Skinningrud et al., 2008), systemic lupus erythematosus (SLE) (Gateva et al., 2009; Zhang et al., 2011), Celiac disease (Dubois et al., 2010), Crohn's disease (Márquez et al., 2009), selective immunoglobulin A deficiency (Jagielska et al., 2012), alopecia areata (Jagielska et al., 2012), rheumatoid arthritis (Martinez et al., 2010) and primary biliary cirrhosis (Mells et al., 2011; Hirschfield et al., 2012) also demonstrated association of the 16p13 loci with disease risk, implying that the 16p13 region contains a key regulator of the self-reactive immune response.

Recently, Davison et al., 2012 reported intron 19 of the CLEC16A gene behaves as a regulatory sequence, which affects the expression of a neighboring gene dexamethasone-induced (DEXI). While it is clear that intron 19 of CLEC16A is highly enriched for transcription-factor-binding events, more functional studies are needed to advance from GWAS to candidate causal genes and their biological functions.

To find causal variant of CLEC16A gene we sequenced the 16p13 region in 96 T1D patients and found 10 new non-synonymous SNPs resulting in one stop-codon, two splice site mutations, and 7 amino acid changes (unpublished data). The studies are under way to examine if these changes are correlated with CLEC16A expression and if these SNPs are present in control group.

Little is yet proven about CLEC16A functions. Kim et al., 2010 characterized ema as an endosomal membrane protein is required for endosomal trafficking and promotes endosomal maturation. Expression of human orthologue of ema 'CLEC16A' rescued the *Drosophila* mutant demonstrating conserved function of the protein. A recent study by the same group also reported its requirement for the growth of autophagosomes and proposed that the Golgi is a membrane source for autophagosomal growth, and that ema facilitates this process (Kim et al., 2012). Expression of CLEC16A rescued the autophagosome size defect in the ema mutant, suggesting that regulation of autophagosome morphogenesis may be one of the fundamental functions of CLEC16A. Another recent study elucidated the dynamic expression changes and localization of CLEC16A in lipopolysaccharide (LPS) induced neuroinflammatory processes in adult rats. CLEC16A expression was strongly induced in active astrocytes in inflamed cerebral cortex. *In vitro* studies indicated that the up-regulation of CLEC16A might be involved in astrocyte activation following LPS challenge (Wu et al., 2012).

## 2.6. Other novel T1D susceptibility loci (2011-2012)

In our latest effort to identify additional genetic loci for T1D, we examined associations in the largest meta-analysis to date between T1D and ~2.54 million SNPs in a combined cohort of 9,934 cases and 16,956 controls. Targeted follow-up of 53 SNPs in 1,120 affected trios uncovered three novel loci associated with T1D that reached genome-wide significance (Bradfield et al., 2011).

**LMO7 (13q22):** The most significantly associated SNP (rs539514,  $P = 5.66 \times 10^{-11}$ ) resides in an intronic region of the LMO7 (LIM domain only 7) gene on 13q22 (Bradfield et al., 2011). LMO7

is a multi-domain mammalian protein with a calponin homology (CH) domain, a discs-large homologous regions (DHR) domain, and a LIM domain. Proteins of this family are involved in protein-protein interactions, regulation of cell adhesion and signaling (Ooshio et al., 2004; Yamada et al., 2004). The expression of LMO7 is cell type specific (Furuya et al., 2002; Kang et al., 2000; Lindvall et al., 2005; Bradfield et al., 2011; Rozenblum et al., 2002; Sasaki et al., 2003) and is essential for the development of muscle and heart tissues. Mice with homozygous deletions of LMO7 display retinal, muscular, and growth retardation (Semenova et al., 2003). LMO7 is upregulated in multiple cancers, especially at the metastatic stage; however under normal conditions its expression is low and limited to very few tissues (Furuya et al., 2002; Kang et al., 2000; Sasaki et al., 2003; Perou et al., 2000). In cultured rat ascites hepatoma cells, the upregulation of LMO7 correlates with the ability of transforming growth factor  $\beta$  (TGF $\beta$ ) to enhance the invasiveness of these cells (Nakamura et al., 2005). Recent GWAS meta-analysis by Bradfield et al., 2011 identified LMO7 association with T1D. Although the function of LMO7 does not clearly relate to the etiology of T1D, LMO7 is expressed in pancreatic islets and thus is a possible biological candidate at this locus (Kutlu et al., 2009).

**EFR3B (2q23):** The second most significantly associated SNP among the new loci (rs478222,  $P=3.50 \times 10^{-9}$ ) resides in an intronic region of the EFR3B (protein EFR3 homolog B) gene on 2p23; however, the region of linkage disequilibrium is approximately 800 kb and harbors additional multiple genes, including NCOA1, C2orf79, CENPO, ADCY3, DNAJC27, POMC, and DNMT3A. EFR3B is an 817 amino acid protein that exists as three alternatively spliced isoforms and belongs to the EFR3 family. The gene encoding EFR3B maps to human chromosome 2p23.3. A number of genetic diseases have been linked to genes on chromosome 2 including Harlequin ichthyosis, lipid metabolic disorder sitosterolemia, and Alstrom syndrome. Our recent study shows novel association of 2q23 locus with T1D risk. Location of SNP rs478222 in the intronic region of EFR3B gene makes it a good candidate, however the 2q23 region harbors additional multiple genes, including NCOA1, C2orf79, CENPO, ADCY3, DNAJC27, POMC, and DNMT3A.

Nuclear receptor coactivator 1 (NCOA1) protein is a member of the p160/steroid receptor coactivator (SRC) family. The product of this gene binds to a variety of nuclear hormone receptors in a ligand-dependent manner, suggesting that NCOA1 may play a role as a bridging molecule between nuclear hormone receptors and general transcription factors (Onate et al., 1995; Torchia et al., 1997).

C2orf79 is peptidyl-tRNA hydrolase domain containing 1 (PTRHD1) predicted protein with unknown function.

Centromere protein O (CENPO) gene encodes a component of the interphase centromere complex. The protein is localized to the centromere throughout cell division and is required for bipolar spindle assembly, chromosome segregation and checkpoint signaling during mitosis (Okada et al., 2006).

Adenylate cyclase 3 (ADCY3) gene encodes a membrane-associated enzyme. This protein catalyzes the formation of the secondary messenger cyclic adenosine monophosphate (cAMP) and is highly expressed in human placenta, testis, ovary, and colon (Ludwig & Seuwen,

2002). Wong et al., 2000 reported the presence of adenylyl cyclase 2, 3, and 4 in olfactory cilia. ADCY3 mutants failed olfaction-based behavioral tests indicating that ADCY3 and cAMP signaling are critical for olfactory-dependent behavior.

DnaJ/Hsp40 homolog, subfamily C, member 27 (DNAJC27) gene encodes 273 amino acid protein with RAB-like GTPase and DNAJ domains. EST database suggests high expression in nervous system and reproductive organs (Nepomuceno-Silva et al., 2004).

Pro-opiomelanocortin (POMC) gene encodes a polypeptide hormone precursor protein synthesized mainly in corticotroph cells of the anterior pituitary. POMC is essential for normal steroidogenesis and maintenance of adrenal weight. Mutations in this gene have been associated with early onset obesity, adrenal insufficiency, and red hair pigmentation (Krude et al., 1998; Hung et al., 2012).

DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A) gene encodes a protein that functions as a *de-novo* methyltransferase that can methylate unmethylated and hemimethylated DNA with equal efficiencies (Yanagisawa et al., 2002).

Additional fine gene mapping and functional studies are needed to determine causal variants for 2q23 region and their role in T1D.

**Intergenic region 6q27:** Intergenic region on 6q27 contained the third most significantly associated SNP (rs924043,  $P=8.06 \times 10^{-9}$ ) in our recent study (Bradfield et al., 2011). The region of association is approximately 900kb and harbors multiple genes including PHF10, TCTE3, DLL1, FAM120B, PSMB1, TBP, and PDCD2. The 6q27 region also includes several genes of unknown function: C6orf208/LINC00574 (long intergenic non-protein coding RNA 574), T-complex-associated-testis-expressed 3 (TCTE3), LOC154449, WD repeat domain 27 (WDR27) and chromosome 6 open reading frame 120 (C6orf120).

Plant Homeo Domain (PHD) finger protein 10 (PHF10) encodes a subunit of an ATP-dependent chromatin-remodeling complex that functions in neural precursor cells (Yoo et al., 2009).

Delta-like 1-Drosophila (DLL1) is a human homolog of the Notch Delta ligand and a member of the delta/serrate/jagged family. It plays a role in mediating cell fate decisions during hematopoiesis and cell communication (Santos et al., 2007; Dontje et al., 2006). The protein is expressed in heart, pancreas and brain. Su et al., 2006 reported pancreatic regeneration in chronic pancreatitis requires activation of the notch-signaling pathway.

Family with sequence similarity 120B (FAM120B) gene encodes protein belonging to the constitutive coactivator of peroxisome proliferator-activated receptor gamma (PPARG) family. FAM120B functions in adipogenesis through PPARG activation in a ligand-independent manner (Li et al., 2007).

Proteasome (prosome, macropain) subunit, beta type, 1 (PSMB1) gene encodes a member of the proteasome B-type family, also known as the T1B family, that is a 20S core beta subunit (Trachtulec et al., 1997). This gene encodes TBP, the TATA-binding protein a transcription factor that functions at the core of the DNA-binding multiprotein transcription factor IID (TFIID). Binding of TFIID to TBP is the initial transcriptional step of the pre-initiation complex



(PIC) and plays a role in the activation of eukaryotic genes transcribed by RNA polymerase II (Keutgens et al., 2010).

Programmed cell death 2 (PDCD2) gene encodes a nuclear protein highly expressed in placenta, heart, pancreas, lung, and liver, and lowly expressed in spleen, lymph nodes, and thymus. Expression of this gene is known to be repressed by B-cell CLL/lymphoma 6 (BCL6); a transcriptional repressor (Agata et al., 1996).

In addition, despite not reaching the genome wide significance, our study observed evidence for association at three additional loci containing the candidate genes LOC100128081, TNFRSF11B and FOSL2 (Bradfield et al., 2011). Of these, it is notable that the tumor necrosis factor receptor superfamily, member 11B (TNFRSF11B) is a strongly associated locus with bone mineral density, also discovered in GWAS, and the locus harboring LOC100128081 has also been reported in the context of a GWAS of SLE. FOS-like antigen 2 (FOSL2) gene encodes a leucine zipper protein that dimerizes with the JUN family proteins and forms the transcription factor complex activator protein 1 (AP-1). The FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation (Cohen et al., 1989).

**CUX2 (12q24):** Huang et al., 2012 re-analyzed the original 2007 WTCCC study by using the 1000 Genomes imputation and reported refined variant rs1265564 in Cut-like homeobox 2 (CUX2) region for association with T1D. CUX2 is expressed exclusively in neural tissues. The protein belongs to the CUT homeobox family and contains three CUT domains and a homeodomain; both domains are DNA-binding motifs (Gingras et al., 2005). CUX2 gene has been shown to directly regulate the expression of NeuroD (Iulianella et al., 2008). NeuroD/BETA2, a transcription factor of the insulin gene, is reported to be associated with T1D in Asian descent (Iwata et al., 1999; Kavvoura & Ioannidis, 2005). Thus CUX2 is a plausible candidate for exploration in T1D pathogenesis.

**HTR1A (5p13-q13):** Asad et al., 2012 confirmed the previously suggested association between the chromosome 5p13-q13 regions and T1D in Scandinavian families (Nerup et al., 2001). None of the previous GWAS have reported any association of 5p13-q13 with T1D. This recent study identified the 5-hydroxytryptamine receptor 1A (HTR1A) and the ring finger protein 180 (RFN180) genes to be associated with T1D in multiplex (Swedish and Danish) families. However, the conditional analysis indicated HTR1A has as a primary association with T1D. Both quantitative PCR and immunohistochemical analysis confirmed the presence of the HTR1A in human pancreas (Asad et al., 2012). The study suggests that HTR1A may affect T1D susceptibility by modulating the initial autoimmune attack or either islet regeneration, insulin release, or both. The HTR1A gene is known to encode for a G-protein coupled receptor specific for serotonin, which mediates cellular signaling via the amine serotonin (Barnes & Sharp, 1999). The HTR1A receptor is mainly known to mediate signal transduction in neurons in the central nervous system (Lesurtel et al., 2008). However, serotonin is also produced in pancreatic islets of several different species (Sundler et al., 1980). Studies in rodent islets show inhibition of insulin secretion by serotonin (Zawalich et al., 2004). Sumatriptan (serotonin agonist) has an inhibitory effect on insulin secretion in humans (Coulie et al., 1998). Mohanan et al., 2006 reported a decrease in expression of HTR1A with increased insulin release during pancreatic regeneration. HTR1A also plays a role in the immune system. High level of protein expression



has been reported in activated T-cells and low in resting T-cells; down regulates adenylate cyclase, which in turn regulates T-cell cytokine production and cytotoxicity (Aune et al., 1993). Hence polymorphisms in the HTR1A gene may affect insulin release and T-cell activity, thereby increases the risk of developing T1D.

### 3. Conclusions

This chapter provides a summary of recent advances in the identification of multiple variants associated with T1D. Genome wide association studies have revolutionized the field of autoimmune mediated disorders. In T1D only six genetic factors were well established before GWAS. GWAS has contributed greatly by expanding the number of established genetic variants to 57 genes. Most of these genes are novel and were not in any investigator's favorite list. For the first time there is real consensus on the role of specific genetic factors underpinning T1D pathogenesis.

The discoveries of genetic factors involved in the pathogenesis of T1D through GWAS present the first step in a much longer process leading to cure. Genes uncovered using this approach are indeed fundamental to disease biology and will define the key molecular pathways leading to cure of T1D. However, such genome wide scans can lack coverage in certain regions where it is difficult to genotype so it is possible that other loci with reasonable effect sizes remain to be uncovered.

To date most of T1D-associated variants have been discovered utilizing cohorts of European ancestry because the SNP arrays were designed to optimally capture the haplotype diversity in this ethnicity. Novel SNP arrays are needed with the same degree of capture in diverse populations to elucidate the full role of each locus in a worldwide context.

The next challenge is to resolve the specific causal variants and determine how they affect the expression and function of these gene products. The Next-Generation Sequencing (NGS) technology has opened new avenues to elucidate the role of coding and noncoding RNAs in health and disease and would speed up the identification of causative gene variants in T1D.

No doubt, the *in vitro* and *in vivo* biology of these genes will be fascinating areas of exploration for many scientists. Only after fully uncovering the functional context of T1D associated genes; these findings will show promise of use for preventive strategies.

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