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EDITORIAL

Clarifying the function of genes at the chromosome 16p13 locus in type 1 diabetes: *CLEC16A* and *DEXI*

Morgan A. Gingerich[1] · Vaibhav Sidarala[1] · Scott A. Soleimanpour[1,2]

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Abstract
More than a decade after the discovery of a novel type 1 diabetes risk locus on chromosome 16p13, there remains complexity and controversy over the specific gene(s) that regulate diabetes pathogenesis. A new study by Nieves-Bonilla et al. shows that one of these genes, DEXI, is unlikely to contribute to type 1 diabetes pathogenesis and positions the endolysosomal E3 ubiquitin ligase CLEC16A as the primary culprit by which this gene locus influences diabetes risk.

Type 1 diabetes (T1D) is characterized by the destruction of insulin-producing pancreatic β cells by infiltrating cells of the immune system. Although the precise mechanisms underlying this disease remain elusive, it has become evident that T1D is a complex polygenic disease with both genetic and environmental components owing to defects in the immune system, the gut microbiome, and pancreatic β cells [1–3]. Despite the complex nature and familial aggregation of T1D, knowledge of its genetic etiology is valuable in identifying potential therapeutic targets in predisposed individuals for early intervention.

Several high-density genome-wide association studies (GWAS) have discovered genetic loci that contribute to T1D risk, including both well-known and recently identified loci such as the major histocompatibility complex (MHC) class II genes, the insulin locus *INS*, the protein tyrosine phosphatase-22 *PTPN22*, the cytotoxic T-lymphocyte-associated protein 4 *CTLA4*, and the interleukin-2 receptor alpha *IL2RA* [1]. However, these associations only account for half of the genetic predisposition to T1D suggesting the existence of other genetic determinants that remain unexplored. Over a decade ago, a Wellcome Trust Case Control Consortium study of ~2000 patients with T1D to identify novel genetic risk alleles identified several new regions of interest to T1D, including at chromosome 16p13 [4]. This was later confirmed by several other T1D cohorts [5, 6], concluding the presence of a disease-associated single-nucleotide polymorphism (SNP; rs12708716) to be associated with T1D and multiple sclerosis (MS) [5].

The chromosome 16p13.13 locus is a ~530 kb long region consisting mainly of four genes. A majority of the SNPs reported to be associated with T1D are located in the intronic regions of the *CLEC16A* gene (Fig. 1). Formerly known as KIAA0350, CLEC16A regulates mitochondrial autophagy and endosomal maturation [7–10]. *CLEC16A* is flanked by two neighboring genes: *CIITA*, which is required for the expression of MHC Class II, and *SOCS1*, a negative modulator of cytokine signaling. The 16p13 genetic locus is also comprised of *DEXI*, a gene of unknown function and activated by immune suppressors [11].

Several GWAS and fine-mapping analyses have identified additional SNPs at the chromosome 16p13.13 locus that are associated with both autoimmune and non-autoimmune disorders. However, the implications of these variants on gene expression (and their contribution to disease phenotypes) were initially unknown [4–6]. *CLEC16A* was initially considered to be the primary candidate T1D susceptibility gene as most of the disease-associated SNPs reside within its 238 kb gene sequence. Indeed, a number of gene expression studies have reported regulation of CLEC16A expression by these SNPs. We previously

These authors contributed equally: Morgan A. Gingerich, Vaibhav Sidarala

✉ Scott A. Soleimanpour
ssol@med.umich.edu

1 Division of Metabolism, Endocrinology & Diabetes, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48105, USA

2 Veterans Affairs Ann Arbor Health Care System, Ann Arbor, MI 48105, USA

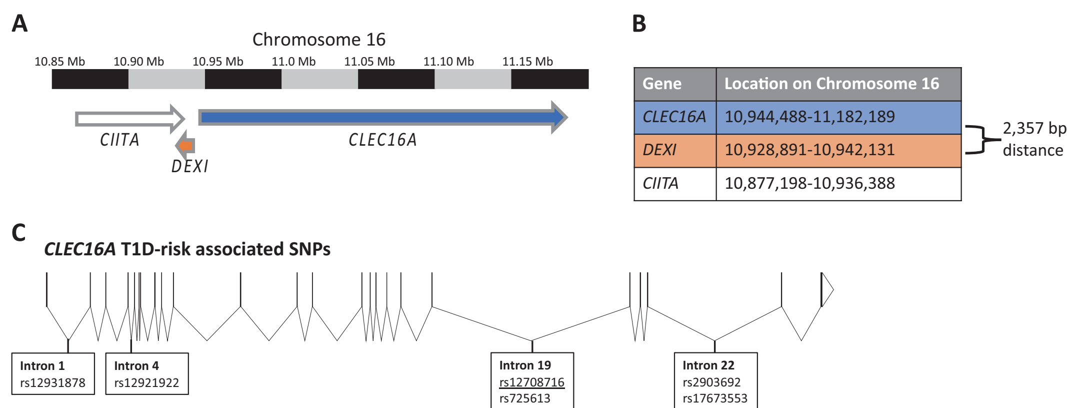

Fig. 1 A deeper view of the chromosome 16p13.13 locus in type 1 diabetes. **a** Schematic of *Homo sapiens* chromosome region 16p13.13. Arrows indicate gene locations. **b** Location of *CLEC16A, DEXI, and CIITA* on *Homo sapiens* chromosome 16. **c** Schematic of *CLEC16A* exons (vertical bars), and introns (angled lines connecting exons). *CLEC16A* T1D risk associated SNPs are indicated in boxes. All SNPs are intronic. Underlined SNP indicates SNP that is associated with reduced CLEC16A expression in human islets [7]

observed that the rs12708716 SNP acted as an expression quantitative trait locus (eQTL) with associated decreases in CLEC16A mRNA expression in human pancreatic islets, as well as impaired pancreatic β-cell function and dysglycemia, linking this SNP to relevant functional outcomes in human T1D [7]. A fine-mapping and gene expression analysis by Mero et al. showed strong association of rs12708716 with MS and decreased CLEC16A expression in the thymus, but not in peripheral blood samples, suggesting a cell type-specific regulation [12]. Risk alleles in the *CLEC16A* locus also acted as an eQTL for CLEC16A in CD4+ T-lymphocytes, while NK cell lines bearing *CLEC16A* SNPs have differential expression of CLEC16A [6, 13]. Beyond studies on CLEC16A, recent studies have indicated that SNPs within chromosome 16p13 regulate transcription of DEXI, with disease risk negatively correlated to DEXI expression [14]. Using chromosome conformation capture, Davison et al. demonstrated the formation of a DNA enhancer loop between intron 19 of *CLEC16A* and the *DEXI* proximal promoter to control DEXI transcription [15]. Together, these contrasting studies indicate the crucial need for in vivo assessments to clarify the causal autoimmune candidate gene(s) residing at this locus.

Disease-associated SNPs may modify DEXI expression, yet functional studies are critical to determine whether dysregulation of DEXI contributes to T1D. Functional assessments of DEXI have been limited to date. To address this gap and determine a potential causal role for DEXI in T1D risk, Nieves-Bonilla et al. generated a whole-body genetic DEXI knockout (KO) in the nonobese diabetic (NOD) mouse model of T1D [16]. Importantly, DEXI loss does not affect diabetes occurrence, indicating that DEXI is dispensable for autoimmunity and the maintenance of β-cell function. Notably, DEXI deficiency in vivo does not replicate recent ex vivo observations suggesting a role for DEXI in type I interferon signaling in β cells [17]. The apparent differences between these two recent studies may stem from use of different systems (in vivo NOD genetic KOs vs. ex vivo cultured islets/β-cell lines), thus additional studies may be necessary to clarify DEXI function. However, the important study by Nieves-Bonilla et al., with a rigorous in vivo genetic loss of function approach in an established model of autoimmune diabetes, provides strong in vivo evidence that DEXI does not affect the risk for autoimmune diabetes and suggests a different focus for the role of the chromosome 16p13 locus in T1D.

Whereas in vivo functional studies of DEXI do not support a role in T1D, a dearth of studies in numerous models indicate crucial functional roles for CLEC16A. Originally thought to be a C-type lectin, *CLEC16A* in fact encodes an endolysosomal E3 ubiquitin ligase [10]. CLEC16A was first characterized in *Drosophila* to regulate endosomal trafficking and autophagosome function [18, 19]. CLEC16A plays a similar role in *C. elegans*, by regulating the formation and function of phagolysosomes to degrade apoptotic cells [20].

Studies in mammalian systems also support a role for CLEC16A in the development of T1D. Indeed, we previously identified that CLEC16A maintains insulin secretion and glucose homeostasis by regulating the formation of a ubiquitin-dependent mitophagy complex within pancreatic β cells [7, 8, 10]. Beyond studies demonstrating a role for *CLEC16A* SNPs in human β-cell function, we also observed that pharmacological inhibition of the CLEC16A-mitophagy pathway impaired β-cell function in humans [10]. Several recent studies have demonstrated key roles for CLEC16A in T-cell selection by modifying thymic epithelial autophagy as well as in NK cells, splenocytes, and B-lymphocytes at least in part through mitophagy and/or MEK

signaling [21–23]. CLEC16A was also shown to control late endosome formation and MHC class II presentation in antigen presenting cells [9]. Moreover, Nieves-Bonilla et al. assessed potential interaction between CLEC16A and DEXI by generating CLEC16A and DEXI double deficient NOD mice, finding DEXI had no combinatorial or synergistic effect on autoimmune diabetes related to CLEC16A deficiency [16]. Collectively, these studies define key roles for CLEC16A within cell types of importance to T1D.

Nieves-Bonilla et al. address a critical debate regarding the causal T1D gene within the chromosome 16p13.13 locus [16]. Using a novel in vivo genetic mouse model, the authors demonstrate that DEXI deficiency does not affect the risk of diabetes in NOD mice. This study also underscores the critical importance of relevant model systems to better clarify the role of unknown contributors to complex polygenic diseases such as T1D. Indeed, there is now a strength of data to date supporting functional and multisystem roles of CLEC16A in cell types of importance to the pathogenesis of T1D. Together, these data suggest that *CLEC16A*, not *DEXI*, is the etiological T1D gene within chromosome region 16p13.13. Many questions remain regarding the specific molecular mechanism(s) and tissue-specific contributions by which CLEC16A affects T1D susceptibility. Finally, the translatability of all studies of CLEC16A performed in model organisms should be confirmed in human T1D to better comprehend the mechanisms underlying T1D pathogenesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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