Brief Communication

MOLECULAR METABOLISM

β-cell Jagged1 is sufficient but not necessary for islet Notch activity and insulin secretory defects in obese mice

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ABSTRACT

Objective: Notch signaling, re-activated in β cells from obese mice and causal to β cell dysfunction, is determined in part by transmembrane ligand availability in a neighboring cell. We hypothesized that β cell expression of Jagged1 determines the maladaptive Notch response and resultant insulin secretory defects in obese mice.

Methods: We assessed expression of Notch pathway components in high-fat diet-fed (HFD) or leptin receptor-deficient (*db/db*) mice, and performed single-cell RNA sequencing (scRNA-Seq) in islets from patients with and without type 2 diabetes (T2D). We generated and performed glucose tolerance testing in inducible, β cell-specific Jagged1 gain-of- and loss-of-function mice. We also tested effects of monoclonal neutralizing antibodies to Jagged1 in glucose-stimulated insulin secretion (GSIS) assays in isolated islets.

Results: *Jag1* was the only Notch ligand that tracked with increased Notch activity in HFD-fed and *db/db* mice, as well as in metabolically-inflexible β cells enriched in patients with T2D. Neutralizing antibodies to block Jagged1 in islets isolated from HFD-fed and *db/db* mice potentiated GSIS *ex vivo*. To demonstrate if β cell Jagged1 is sufficient to cause glucose tolerance *in vivo*, we generated inducible β cell-specific *Jag1* transgenic (β-Jag1^{TG}) and loss-of-function (iβ-Jag1^{KO}) mice. While forced Jagged1 impaired glucose intolerance due to reduced GSIS, loss of β cell Jagged1 did not protect against HFD-induced insulin secretory defects.

Conclusions: Jagged1 is increased in islets from obese mice and in patients with T2D, and neutralizing Jagged1 antibodies lead to improved GSIS, suggesting that inhibition of Jagged1-Notch signaling may have therapeutic benefit. However, genetic loss-of-function experiments suggest that β cells are not a likely source of the Jagged1 signal.

Keywords Notch; Jagged1; Beta cell; Alpha cell; Insulin secretion; Diabetes

1. INTRODUCTION

Obesity induced type 2 diabetes (T2D) manifests clinically when β cells fail to adapt to insulin resistance [1,2]. Clarifying molecular mechanisms that affect β cell decompensation may result in novel therapeutic interventions to restore endogenous insulin secretion [3], and fundamentally move away from disease management and towards disease modification.

We identified Notch signaling as a novel regulator of β cell function in mouse models [4]. Notch is an evolutionarily-conserved pathway that mediates juxtacrine cell signals to determine cell fate and proliferation/differentiation decisions [5]. In the pancreas, Notch plays a crucial role during embryogenesis to regulate pancreatic progenitor cell proliferation and differentiation of both exocrine and endocrine cell types [6–8]. Once endocrine differentiation was complete, β cell Notch activity was thought to remain at generally low levels [6]. However, we found high levels of Notch activity in β cells from obese mice, and using mouse models of β cell Notch gain- and loss-of-function, determined Notch to be causal to β cell dysfunction [4].

Notch activity is typically regulated by ligand availability in an adjacent cell [9]. In mammals, there are 5 surface-borne ligands (Jagged 1/2, and Delta-like 1/3/4) which bind to one of four Notch receptors (Notch 1–4) [10]. Ligand binding results in γ-secretase-mediated cleavage of Notch receptors, which generates the active signaling moiety, the Notch intracellular domain (NICD) [11]. NICD binds a Mastermind-like/Rbp-Jk complex to activate transcription of *Hes/Hey* and other genes [12] to mark the "Notch-on" state [13]. We hypothesized that islet expression of one or more of the Notch ligands would determine β cell Notch activity, and downstream repercussion. Based on preliminary data suggesting increased Jagged1 in obese mice and patients with T2D, we investigated if β cell Jagged1 was the causal ligand for Notch-induced glucose intolerance.

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2. MATERIAL AND METHODS

2.1. Mice

We obtained 8–10-week-old *db/db* mice (BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db}/*J;* #00642) from Jackson Labs. To generate doxycycline-inducible, β-cell specific Jag1 transgenic mice (β-Jag1^{TG}), we intercrossed tetO-Jag1 (obtained from Ralf Adams at Max Planck, Meunster) [14], ROSA26-rtTA-IRES-EGFP (Jackson Labs #005670) and RIP-Cre^{Herr} [Tg(Ins2-cre)23Herr] [15] mice. To create inducible, β-cell specific Jag1 loss-of-function (iβ-Jag1^{KO}) mice, we intercrossed Jag1 floxed mice (obtained from Kathy Loomes at University of Pennsylvania) [16] with MIP-Cre^{ERT} [Tg(Ins1-Cre/ERT)1Lphi Jackson Labs #0224709] mice. We also generated constitutive β-cell specific Jag1 loss-of-function (β-Jag1^{KO}) mice by intercrossing Jag1 floxed mice with RIP-Cre^{Herr}. After weaning, we maintained mice on standard chow (Purina Mills, #5053) or started 60 % HFD (Research Diets, D12492). Mice were genotyped by PCR primers listed in Table S4A. All mice were maintained on a C57/Bl6 background, and housed three to five per cage in standard 22 °C at 12-hour light/12-hour dark cycles. Adult male mice were used for all experiments, unless indicated otherwise. The Columbia University Institutional Animal Care and Utilization Committee approved all animal procedures.

2.2. Islet isolation

We isolated islets by injecting 1 mg/mL collagenase P (MilliporeSigma) into the common bile duct to digest the whole pancreas, followed by Histopaque (MilliporeSigma) density-gradient fractionation and sedimentation as described in [4]. We sorted islets individually under a stereomicroscope to use for gene expression or *ex vivo* GSIS.

2.3. RNA isolation and RT-qPCR

We extracted RNA from whole islets suspended in TRIzol using NucleoSpin RNA Kit and Columns (Macherey–Nagel) and used High-capacity cDNA Reverse Transcription Kit (Thermo) to make cDNA. Using 10 ng of cDNA, we performed real-time PCR using Power SYBR Green PCR master mix (Thermo) and the CFX96-Real-Time system (Bio-Rad). We determined relative gene expression by the $\Delta\Delta^{CT}$ method, using *Ppia* as the invariant control. qPCR primers are listed in Table S4B.

2.4. Chemicals and inhibitors

We dissolved Tamoxifen (Millipore Sigma) in corn oil and administered 100 mg/kg body weight once daily for 3 consecutive days to each mouse. We administered Doxycycline (Alfa Aesar) in the drinking water at a concentration of 1 mg/mL for 4 or 8 weeks, as indicated. We used IgG1 neutralizing monoclonal antibodies specific for JAG1 (YW167.71.70) or an isotype control (anti-Ragweed) as described in [17].

2.5. Luciferase assay

We co-transfected MIN6 cells with 5 ng of pRL Renilla, 100 ng of pGL4.10 Jag1-promoter-luciferase [18], and 100 ng of pCCL-relA-GFP or -GFP using Lipofectamine 3000 (Invitrogen). We used the Dual-Luciferase Reporter Assay (Promega) to determine reporter activity, normalizing firefly luciferase to Renilla.

2.6. Antibodies and staining protocols

We fixed pancreata in 4 % paraformaldehyde, dehydrated in 30 % sucrose for at least 24 h then embedded in Tissue-Tek O.C.T. compound (VWR, 25608-930) to create frozen sections. We blocked sections in 1 % bovine serum albumin and 5 % donkey serum in phosphate buffered saline with 0.1 % triton X-100 for 1 h at RT, prior to incubation with primary antibodies: Insulin (IR002, Dako Agilent Technologies, ready to use), Hes1 (sc-25392, Santa Cruz Biotechnology, 1:100), and Jagged1 (AF599, R&D Systems, 1:100). Following overnight primary antibody incubation, we used secondary antibodies [Donkey anti-Guinea Pig Alexa Fluor 488 conjugate (706-545-148, Jackson ImmunoResearch, 1:500), Donkey anti-Rabbit Alexa Fluor 555 conjugate (A31572, Thermo Fischer Scientific, 1:500) and Donkey anti-Goat Alex Fluor 647 conjugate (A21447, Thermo Fischer Scientific, 1:500)] with 4′,6-dimidino-2-phenylindole (DAPI) mounting medium to counterstain nuclei. Antibodies used are summarized in Table S4C. Using the Axio Observer Z1 with LSM 710 scanning module (Zeiss Light microscope), we obtained images on a 40X Zeiss Plan-Apochromat oil objective. We conducted a single confocal microscopy session for each experiment, during which the photomultiplier voltage settings (kept below 600 V) and laser transmission (maintained $\leq$ 2 %) for each fluorophore were optimized to enhance the signal's dynamic range. We acquired all images in a 1024 $\times$ 1024-pixel format using Zen (Zeiss) software for microscope operation and image acquisition.

2.7. Glucose tolerance tests (GTT) and glucose-stimulated insulin secretion (GSIS) assays

After a 16-hour fast, we injected mice intraperitoneally with 2 g/kg body weight glucose for chow fed or 1 g/kg glucose for HFD-fed mice. We measured blood glucose concentrations at 0-, 15-, 30-, 60-, 90- and 120-minutes after injection. For *in vivo* GSIS assays, we collected plasma via tail bleeding at 0-, 15-, and 30-minutes post glucose injection as above, unless indicated otherwise. For *ex vivo* GSIS, we maintained isolated islets in 5.5 mM (chow fed mice) or 11.1 mM (HFD or *db/db* mice) glucose RPMI 1640 supplemented with 10 % FBS and 1 % penicillin-streptomycin at 37 °C in 5 % CO_2 [19]. After an overnight recovery, we collected batches of 5 islets of similar size, washed and preincubated each batch in Krebs-Ringer-HEPES Bicarbonate (KRBH) buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 2 mM $NaHCO_3$, 10 mM HEPES (pH 7.4), 1.5 mM $CaCl_2$, 0.1 % BSA) in 2.8 mM glucose at 37 °C in 5 % CO_2 for 60 min. Following low glucose conditioning, we transferred islets to fresh 2.8 mM glucose KRBH for 60 min, followed by stimulatory 16.8 mM glucose KRBH for 60 min at 37 °C in 5 % CO_2, and collected supernatant at low- and high-glucose. For Notch ligand neutralizing antibody experiments, we used the same protocol, but after overnight recovery in 11.1 mM glucose, we "treated" islets with control and experimental antibodies at 25 μg/mL for an additional 18–20 h, then performed *ex vivo* GSIS the following day, with KRBH buffer solution containing respective antibodies. We measured insulin from plasma and supernatant by ELISA (Mercodia).

2.8. RNA-seq

We isolated RNA from islets of 3 chow-fed control (*Cre*$^{+}$, *Rosa26-rtTA*$^{+}$, *tetO-Jag1*$^{-}$) and 3 β-Jag1^{TG} (*Cre*$^{+}$, *Rosat26-rtTA*$^{+}$, *tetO-Jag1*$^{+}$) mice after 8 weeks of Dox exposure. Islets selected showed RNA integrity number (RIN) 8 or above by an Agilent 2100 Bioanalyzer. As in [20], we conducted RNA-Seq by generating a library prep using a poly-A pulldown method with the Illumina NovaSeq6000 instrument, which generated at least 20 M raw sequencing reads per sample. We performed differential gene expression analyses with *limma,* implemented in the BioJupies application [21]. We analyzed RNA-Seq datasets from GSE107489 [22] and GSE153222 [23], by normalizing the count for each gene of interest to the total number of gene counts per mouse for a million reads and by comparing the FPKMs for each gene, respectively. We processed and visualized the single-cell RNA seq (scRNA-Seq) dataset from GSE200531 [24] using Cellenic [25].

2.9. Regulatory networks and transcriptional regulator activity analysis

We analyzed scRNA-Seq from nondiabetic and T2DM islet donors from [26]. Described in detail in [20,26], we used ARACNe [27] to generate islet specific regulatory networks. We then used metaVIPER [28] to generate protein activity profiles for RPBJ and JAG1. Activity analysis for cell surface proteins is validated in [29]. For subpopulation analysis focusing on Hβ (healthy β cells) and MIβ (metabolically inflexible β cells), we performed dimension reduction, using gene expression and metaVIPER and clustering analysis, using iterClust [30].

2.10. Statistics

We presented results as the mean +/− SEM. We calculated differences between 2 groups using a 2-sided Student's t-test. Differences among multiple groups and a control were calculated by 1 way ANOVA, followed by Tukey's multiple comparison post hoc test. We considered a p-value of less than 0.05 to be significant.

3. RESULTS

3.1. Jagged1 expression tracks with Notch activity in obese mice

To determine the upstream signal transducing β cell Notch activation, we surveyed the most abundant Notch ligands (*Jag1, Dll1,* and *Dll4*) and Notch receptors (*Notch1* and *Notch3*) [4] in islets isolated from high-fat diet (HFD)-fed (Figure 1A) and leptin receptor deficient (*db/db*, Figure 1B) mice. In both models, we observed increased *Jag1* expression, without a change in other Notch ligands or receptors. Increased *Jag1* expression tracked with increased Notch activity, as assessed by expression of canonical Notch target genes such as *Hairy and enhancer of split-1* (*Hes1*) ([4] and Figure 1C). We confirmed these findings by analyzing publicly available bulk RNA-Seq data in *db/db* [22] and HFD-fed mice [23], which also showed higher islet *Jag1* expression and Notch target genes (Tables S1A, B). Next, to assess responsible cell type within the islet, we analyzed single-cell RNA sequencing (scRNA-Seq) from islets isolated from *db/db* mice (GSE200531) [24]. In these data, we observed higher β cell Notch activity (Fig. S1C), as well as increased β cell *Jag1* (Figs. S1D and E). These data suggested the potential of intra-β cell Jagged1-Notch signaling. We next queried mechanism of increased β cell *Jag1* in obesity. We had previously identified an NF-κB binding site in the *Jag1* promoter [18], but whether this was accessible and active in β cells was unclear. Active NF-κB led to increased *Jag1*-luciferase in mouse insulinoma (MIN6) cells (Figure 1D), suggesting that inflammatory stimuli can activate *Jag1* expression in β cells, an observation consistent in other cell types and diseases [31,32].

3.2. JAGGED1 is associated with Notch activity in patients with T2D

To evaluate potential translational relevance of these findings, we used scRNA-Seq profiles obtained from donors with and without T2D [26]. Using the metaVIPER algorithm [28], which allows transformation of mRNA expression data to infer protein activity, we found increased JAGGED1 activity in patients with T2D (Figure 1E, left). To localize the cellular source and confirm our findings in *db/db* mice, we revisited previous subpopulation analyses where we observed higher Notch/RBPJ activity in metabolically-inflexible (MIβ) β cells, which are enriched relative to healthy β cells (Hβ) in patients with T2D [20]. Hβ are defined by robust INS/MAFA activity, while MIβ show increased α cell and metabolic inflexibility markers [26]. JAGGED1 and RBPJ activity were highly correlated at the single-cell level, with highest JAGGED1 activity in MIβ cells (Figure 1E, right). Overall, these data suggest that in disease states associated with β cell metabolic stress and insulin demand, JAGGED1 is likely responsible for the increased Notch activity in neighboring β cells.

3.3. Jagged1 neutralizing antibodies improve GSIS

To test the functional repercussions of these observations, we used a validated Jagged1-specific blocking antibody [17]. We "treated" islets

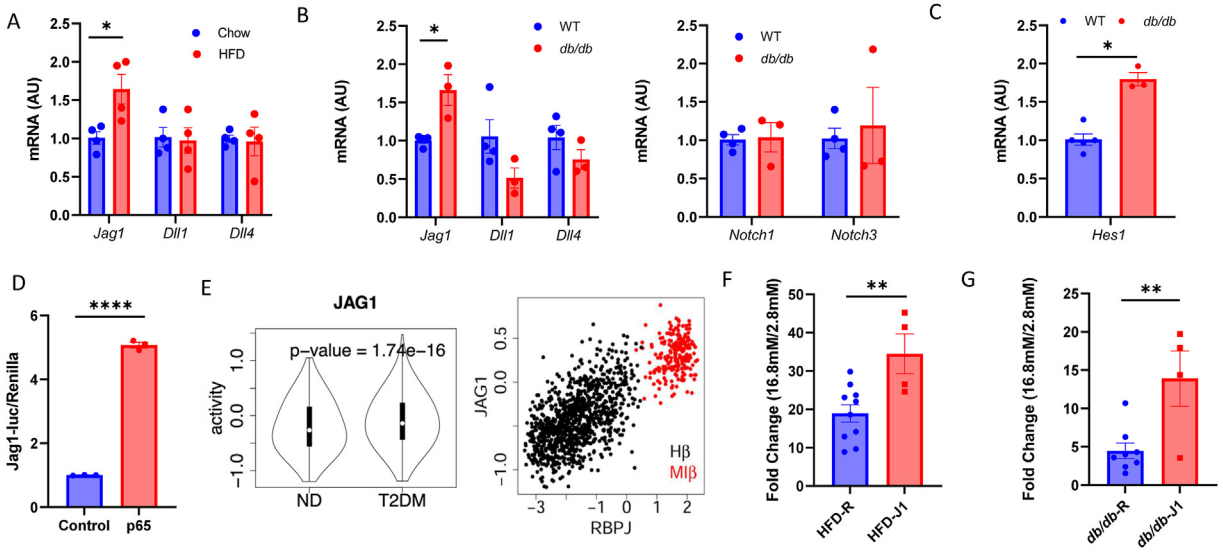

Figure 1: Blocking increased Jagged1 in obesity potentiates insulin secretion. (**A**) Gene expression of Notch ligands in islets isolated from WT mice fed normal chow or HFD for 24 weeks (n = 4 mice/group). (**B, C**) Gene expression of Notch ligands, receptors and the Notch target *Hes1* in islets isolated from 8 to 10-week-old *db/db* mice as compared with WT littermate controls (n = 3–5 mice/group). (**D**) Jag1 promoter luciferase activity in MIN6 cells co-transfected with p65 (n = 3/group). (**E**) Single-cell RNA sequencing of β cells from patients with and without T2D, comparing JAG1 and RBPJ activity in metabolically healthy β (Hβ) cells, compared to metabolically inflexible (MIβ) cells. (**F**) *Ex vivo* GSIS performed in islets isolated from WT mice fed HFD for 24 weeks and (**G**) 8-10-week-old *db/db* mice after overnight treatment with anti-ragweed (R) control or Jag1 blocking antibody (J1). All data are shown with group means +/− SEM. *p < 0.05; **p < 0.01, ****p < 0.0001, 2 tailed t-test.

isolated from HFD-fed (Figure 1F) and *db/db* mice (Figure 1G) with anti-Jagged1 or an isotype control antibody, and found a significant increase in glucose stimulated insulin secretion (GSIS) in both models.

3.4. Forced β cell Jagged1 activation impairs GSIS likely through a reduction in MODY genes

These results provided strong rationale to generate inducible, β cell-specific Jag1 gain-of-function (β-Jag1^{TG}) mice. We leveraged a doxycycline (dox)-inducible Jagged allele, to bypass potential interference with normal pancreatic endocrine development, and crossed tetO-Jag1 [14], ROSA26-rtTA-IRES-EGFP, and RIP-Cre^{Herr} mice. We analyzed β-Jag1^{TG} and littermate control mice fed either chow or HFD from weaning (Figure 2A). In the absence of dox-containing drinking water, β-Jag1^{TG} mice showed normal glucose tolerance (Fig. S2A), but in response to dox, β-Jag1^{TG} mice displayed progressive glucose intolerance (Figure 2B,C), despite unchanged body weight as compared to control animals (Fig. S2B). HFD-fed β-Jag1^{TG} mice showed similar glucose intolerance (Figure 2D). To understand this abnormality in glucose homeostasis, we performed *in vivo* (Figure 2E) and *ex vivo* (Fig. S2C) GSIS, both of which were blunted in β-Jag1^{TG} mice. Consistent with predictions, islets isolated from β-Jag1^{TG} mice showed increased *Jag1* and Notch activity as assessed by gene expression (Figure 2F) and immunofluorescence of fixed pancreata (Figure 2G). Glucose intolerance and increased Notch activity were also observed in chow-fed female β-Jag1^{TG} mice as compared to Cre- controls (Figs. S2D and E). Taken together, these data suggest that forced β cell Jagged1 expression phenocopied β-cell specific Notch gain-of-function (β-NICD) mice [4].

To understand molecular mechanisms underlying phenotypes of β-Jag1^{TG} mice, we performed RNA-Seq from islets derived from chow-fed control (*Cre*$^{+}$, *Rosa26-rtTA*$^{+}$, *tetO-Jag1*$^{-}$) and β-Jag1^{TG} mice. As expected, some of the most highly upregulated genes in β-Jag1^{TG} mice were those known to be affected by Notch signaling, including *Hes1* and *Spp1* [33] (Figures 2H and S2F, H). We next performed KEGG pathway analysis to determine systematic changes induced by Jagged1/Notch signaling. Although we observed several interesting differences associated with decreased β cell function (i.e. decreased FoxO signaling [34] and increased TGF-β signaling [35]), the most striking change was reduced Mature Onset Diabetes of the Young (MODY) genes (Figure 2I,J and S2G, I). These data suggest that Jagged1/Notch-induced β cell dysfunction is likely multifactorial, including some element of reduced β cell maturity.

3.5. β cell Jagged1 is not necessary for HFD-induced glucose intolerance

To determine if β cell Jagged1 is necessary for HFD-induced metabolic defects, we generated tamoxifen-inducible, β cell-specific, Jagged1 loss-of-function (iβ-Jag1^{KO}) mice using the *MIP-Cre*^{ERT} driver crossed with *Jag1* floxed mice. To mimic conditions where Notch activity is highest, we delayed tamoxifen until 24 weeks of life (Fig. S3A), a protocol that also mitigates known effects of *MIP-Cre*^{ERT} on body weight and β cell proliferation [4]. Consistently, baseline metrics prior to tamoxifen treatment confirmed normal body weight, glucose tolerance and insulin tolerance in iβ-Jag1^{KO} or MIP-Cre+ Jag1/flox mice, as compared to Cre- controls (Figure S3B,C,F,G) [36]. However, we also observed no difference in glucose tolerance or *in vivo* GSIS even after tamoxifen injections in iβ-Jag1^{KO} and heterozygote mice (Figure 3A,B and S3H). Consistent with a lack of phenotype, despite a reduction in *Jag1* expression, Notch activity was not affected (Figure 3C,D). Preservation of Notch activity was not due to a compensatory increase in other Notch ligands or receptors (Figs. S3D and E).

To confirm these data, we also generated constitutive β-Jag1^{KO} mice by intercrossing *RIP-Cre*^{Herr} [15] and *Jag1* floxed mice. We were cognizant that this model may interfere with normal Notch signaling in early β cell development and proliferation [37,38]. Nevetheless, similar

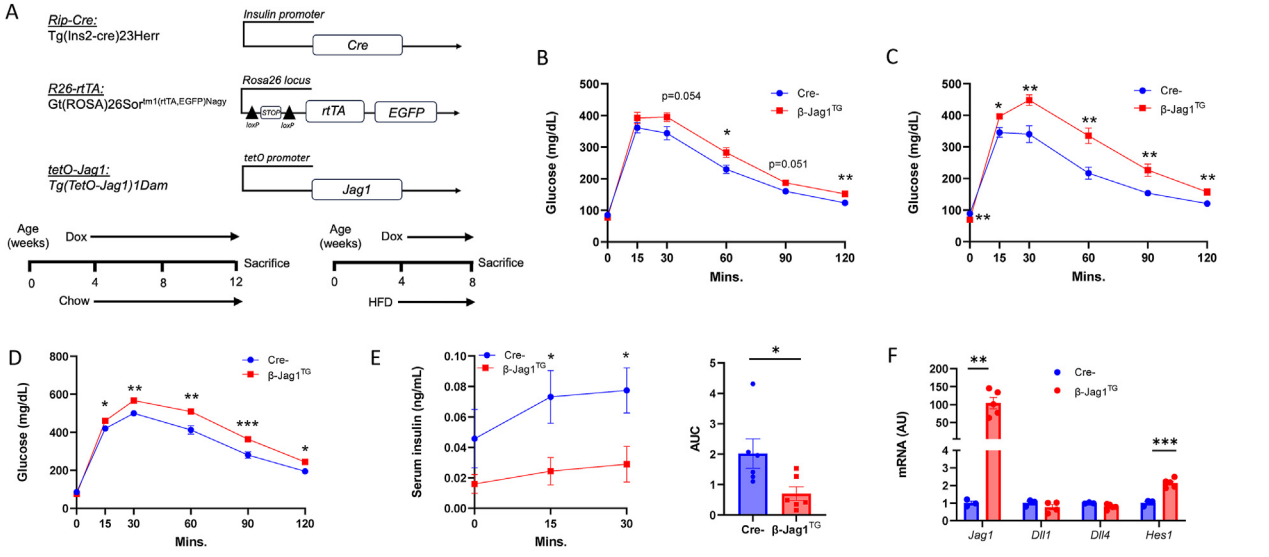

Figure 2: β cell-specific Jagged1 transgenic mice show increased Notch activity and glucose intolerance due to reduced GSIS. (**A**) Generation and experimental schematic used for β-Jag1^{TG} mice. (**B**) GTT in chow-fed, β-Jag1^{TG} and Cre- male mice with 4 weeks and (**C**) 8 weeks of doxycycline administration (n = 7–9 mice/group). (**D**) GTT in HFD-fed β-Jag1^{TG} male mice with 4 weeks of doxycycline administration. (**E**) Plasma insulin post intraperitoneal glucose injection and area under the curve (AUC) in HFD-fed β-Jag1^{TG} and Cre- control mice (n = 6–7 mice/group). (**F**) Gene expression in islets isolated from chow-fed β-Jag1^{TG} and Cre- control male mice (n = 3–5 mice/group). (**G**) Representative images of pancreatic sections from chow-fed β-Jag1^{TG} and Cre- control mice stained with Hes1 and Jag1. (n = 3–4 mice/group). Scale bars 20uM. (**H**) RNA sequencing of islets isolated from β-Jag1^{TG} and controls, including a volcano plot of notable differentially expressed genes and (**I**) upregulated and (**J**) downregulated KEGG pathways in β-Jag1^{TG} islets after Gene Ontology enrichment analysis using Enrichr. All data are shown with group means +/− SEM. *p < 0.05; **p < 0.01; ***p < 0.001, 2 tailed t-test.

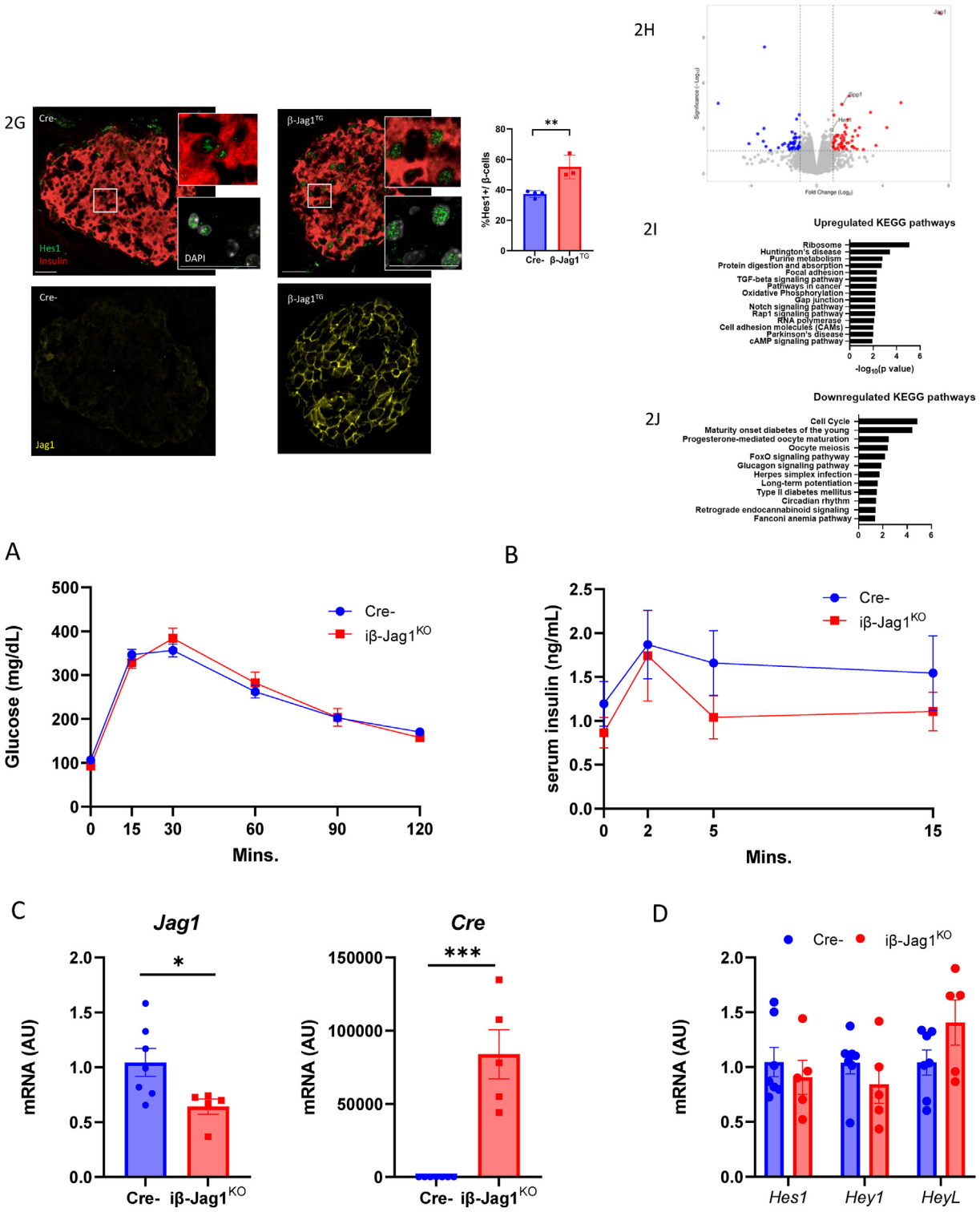

Figure 3: Ablation of β cell Jagged1 did not affect Notch activity, glucose tolerance or GSIS. (**A**) GTT and (**B**) plasma insulin after intraperitoneal glucose in HFD-fed, male iβ-Jag1^{KO} and Cre- control mice 6 weeks after tamoxifen injections (n = 9–13 mice/group). (**C, D**) Gene expression in islets isolated from HFD-fed iβ-Jag1^{KO} and Cre- control mice (n = 5–7 mice/group). All data are shown with group means +/− SEM. *p < 0.05; ***p < 0.001, 2 tailed t-test.

to iβ-Jag1^{KO} mice, β-Jag1^{KO} mice also showed unchanged islet Notch activity and glucose tolerance as compared to littermate controls (Figs. S3I and J). Overall, these data suggest that β cell Jagged1 is not required for islet Notch activity and effects on GSIS.

4. DISCUSSION

Identifying molecular determinants of β cell dysfunction is essential to understand a disease that has reached pandemic proportions.

Despite long-standing efforts to improve insulin secretory defects, and more recent advances to treat obesity and subsequent insulin resistance with incretins, the root cause of β cell failure remains unclear.

In this study, we found that Jagged1 tracked with higher Notch activity in islets isolated from human donors with T2D and in obese mouse models. But while β cell Jagged1 was sufficient to impair insulin secretion and glucose tolerance, likely through increased Notch activity in adjacent β cells [10,39,40], leading to β cell immaturity — consistent with our prior observations in Notch gain-of-function mice [4] — we also observed that mice lacking β cell Jagged1 showed normal Notch activity and glucose tolerance. Of note, *Jag1* expression in these islets was decreased only modestly in two different loss-of-function models. This suggests that another islet cell may be the predominant source of Jagged1, a hypothesis that dovetails with improved GSIS with Jagged1 neutralizing antibody treatment of islets from HFD-fed and *db/db* mice. Along these lines, Rubey et al. showed high Jagged1 staining in α cells [41], but whether this may be causal to Notch activity and downstream effects on β cells was not studied. Islets from patients with T2D are relatively enriched in α cells, due to an increase in α cell proliferation [42,43] or a relative loss of β cells [44]. However, scRNA-seq of islets from *db/db* mice [24] did not show difference is *Jag1* expression or prevalence of *Jag1*+ α cells. Although the potential for novel α/β cell Jag1/Notch circuit still exists and is the subject of current work, this suggests that *Jag1* in a different non-α/β cell type within the islet may be responsible to transduce signal to β cell Notch receptors.

There are several limitations of this work, in particular with the β cell Cre driver alleles. *MIP-Cre*^{ERT} is an effective and inducible β cell-specific Cre line without ectopic expression in hypothalamus or other tissues, but we cannot exclude that the null finding found in iβ-Jag1^{KO} mice may be due to expression of the human growth hormone minigene embedded in this line [36]. We attempted to confirm these findings using another inducible model, *Ins1-Cre*^{ERT} mice, but were unable to get efficient recombination in this line (not shown), perhaps due to hypermethylation of the *Ins1* promoter leading to genetic silencing [45]. We thereby confirmed data from iβ-Jag1^{KO} mice using the *RIP-Cre*^{Herr} line [15]. Null effects in resulting mice suggest that β cell Jagged1 plays a likely minor role in islet Notch activity, but again, these results could be confounded by potential interference with normal endocrine development due to constitutive expression and/or hypothalamic expression of this transgene.

Patients with T2D have β cell dysfunction at the time of diagnosis, but there is substantial heterogeneity in disease course. Some patients progress quickly to a state of substantial β cell insufficiency, necessitating exogenous insulin therapy. Mechanisms underlying this heterogeneity are unclear. Our scRNA-Seq analysis suggests that Jagged1-induced Notch activity may be a "late" mechanism of T2D pathogenesis, taking an already metabolically stressed state and further impairing insulin secretion in a feed-forward mechanism to worsen β cell dysfunction. Understanding this pathway to leverage currently available inhibitors may potentially alter the disease course.

5. CONCLUSIONS

β cell Jagged1 is sufficient but not necessary for Notch-induced GSIS defects and glucose intolerance. However, Jagged1 neutralizing antibody treatment improves GSIS, suggesting the possibility that a different islet cell type modulates β cell Notch activity.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Nina Suda: Writing — review & editing, Writing — original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alberto Bartolomé:** Writing — review & editing, Methodology, Conceptualization. **Jiani Liang:** Writing — review & editing, Investigation. **Jinsook Son:** Writing — review & editing, Resources. **Yoko Yagishita:** Formal analysis, Writing — review & editing. **Christian Siebel:** Resources. **Domenico Accili:** Writing — review & editing, Methodology, Funding acquisition. **Hongxu Ding:** Resources, Formal analysis, Data curation. **Utpal B. Pajvani:** Writing — review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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DECLARATION OF COMPETING INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

DATA AVAILABILITY

Data will be made available on request.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2024.101894.

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