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An Activating Mutation in *STAT3* Results in Neonatal Diabetes Through Reduced Insulin Synthesis

Diabetes 2017;66:1022–1029 | DOI: 10.2337/db16-0867

Neonatal diabetes mellitus (NDM) is a rare form of diabetes diagnosed within the first 6 months of life. Genetic studies have allowed the identification of several genes linked to the development of NDM; however, genetic causes for ∼20% of the cases remain to be clarified. Most cases of NDM involve isolated diabetes, but sometimes NDM appears in association with other pathological conditions, including autoimmune diseases. Recent reports have linked activating mutations in *STAT3* with early-onset autoimmune disorders that include diabetes of autoimmune origin, but the functional impact of *STAT3*-activating mutations have not been characterized at the pancreatic β-cell level. By using whole-exome sequencing, we identified a novel missense mutation in the binding domain of the STAT3 protein in a patient with NDM. The functional analyses showed that the mutation results in an aberrant activation of STAT3, leading to deleterious downstream effects in pancreatic β-cells. The identified mutation leads to hyperinhibition of the transcription factor Isl-1 and, consequently, to a decrease in insulin expression. These findings represent the first functional indication of a direct link between an NDM-linked activating mutation in *STAT3* and pancreatic β-cell dysfunction.

PATHOPHYSIOLOGY

Neonatal diabetes mellitus (NDM) is a rare monogenic form of diabetes characterized by the onset of hyperglycemia within the first 6 months of life (1–3). Although one-half of the cases are transient and can eventually relapse, permanent cases of NDM require lifelong treatment (1–3). Genetic studies have allowed the identification of several causal point mutations and chromosomal aberrations in ∼20 genes implicated in β-cell development, glucose sensing and metabolism, membrane depolarization, and apoptosis (2). The most frequent cases of NDM are caused by aberrations in chromosome 6q24 and missense mutations in the K_{ATP} channel genes (e.g., *KCNJ11*, *ABCC8*) and the insulin gene, but still, genetic causes of at least 20% of NDM cases remain to be clarified (3).

Most cases of NDM involve isolated diabetes, but sometimes NDM appears in association with other pathological conditions, including early-onset autoimmune diseases (4). The most common of these conditions is immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, which is caused by mutations in the *FOXP3* gene (5,6). More recently, activating mutations in the transcription factor STAT3 have been linked to the development of early-onset autoimmune disorder and to isolated permanent NDM with autoimmune etiology (7). STAT3 is a latent transcription factor activated by extracellular signaling ligands, such as cytokines, growth factors, and hormones (8,9). This transducer becomes activated in the cytoplasm by Janus kinases to regulate diverse biological functions, including cell differentiation, proliferation, apoptosis, and inflammation (8,9). STAT3 can act as a transcriptional activator or inhibitor by interacting with other transcription factors and/or coactivators/corepressors, depending on the target promoter (10). In the pancreas, STAT3 signaling is required for the correct development and maintenance of endocrine pancreas

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Received 15 July 2016 and accepted 5 January 2017.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-0867/-/DC1.

(11). In addition, leptin-induced activation of STAT3 plays a crucial role in inhibiting insulin secretion in mice (12).

We identified by whole-exome sequencing (WES) a novel activating mutation in the *STAT3* gene linked to NDM among other autoimmune pathologies. The functional data reveal that the identified mutation in *STAT3* dysregulates insulin synthesis through inhibition of the transcriptional regulator insulin gene enhancer binding protein, factor 1 (Isl-1). Although activating mutations in *STAT3* have been described to lead to early autoimmunity by impairing T-cell development (13), this functional indication is the first of a direct link between an NDM-linked activating mutation in *STAT3* and pancreatic β-cell dysfunction.

RESEARCH DESIGN AND METHODS

Study Participants and Ethics Statement

For WES, we selected a female patient with NDM with detectable islet autoantibodies at the age of 3 months. This patient, whose clinical case has been previously described (14), presented neonatal hypothyroidism with positive thyroid autoantibodies at birth. Watery diarrhea developed in the patient at the age of 13 months, and she was given a diagnosis of celiac disease (positive gliadin autoantibodies and partial duodenal atrophy). Her subsequent course was frequently marked by episodes of watery diarrhea without blood or mucous. The celiac disease diagnosis was corrected to collagenous gastritis and colitis at the age of 15 years. The patient had an appropriate growth rate until age 4 years that remarkably decreased over time, with a final height SD score of −3. Initial genetic testing by Sanger sequencing excluded potential damaging mutations in the KCNJ11, ABCC8, and INS genes. Genomic DNA of the patient was extracted from whole blood by using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The study was approved by the local ethics committee (Cruces University Hospital, Barakaldo, Spain), and written informed consent was obtained from the patient's parents.

WES

Exome enrichment was carried out by using the Nextera Rapid Capture Expanded Exome Enrichment kit followed by high-throughput next-generation sequencing in a HiScanSQ (Illumina, San Diego, CA) to generate WES in 75-base pair (bp) paired-end reads. Sequence reads were mapped to the reference human genome (UCSC GRCh37/hg19) by using the WES pipeline Web tool (15). The same Web tool was used for duplicate removal, base quality recalibrations, single nucleotide polymorphism (SNP) and deletion/insertion polymorphism calling, and annotation. Variants were filtered by the following quality criteria: coverage ≥10, ambiguously mapped reads ≥5, quality ≥50, variant confidence ≥1.5, and strand bias ≤60. To find potential etiological variants, we filtered out all variants located outside exonic regions, synonymous variants, and variants with a minor allele frequency >0.05. In addition, we followed a de novo prioritization strategy and excluded all inherited variants on the basis of WES of both parents. Evaluation of the possible functional significance of the identified mutations was performed with prediction software, including SIFT, PolyPhen-2, and MutationTaster. The candidate variant (p.P330S) identified by WES in the *STAT3* gene (NM_139276) was confirmed by Sanger sequencing.

Culture of INS-1E and EndoC-βH Cells and Cytokine Treatment

The rat INS-1E cell line was cultured in RPMI medium as previously described (16). The same medium but without antibiotics was used for transfection experiments. The EndoC-βH1 cell line (provided by R. Scharfmann, University of Paris) was cultured in DMEM as previously described (17). The same medium but with 1% FBS and without BSA and antibiotics was used for transfection experiments. INS-1E cells were treated with rat IL-6 (R&D Systems, Abingdon, U.K.) at a concentration of 20 ng/mL for 12 h.

Expression Plasmids

Expression plasmid with the identified mutation (P330S) was generated by using an overexpression plasmid containing the wild-type (WT) STAT3 cDNA (OriGene Technologies, Rockville, MD), and mutation was introduced by using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA).

Transfection Experiments and Luciferase Reporter Assay

Empty, WT, or mutant STAT3 plasmids were transfected into INS-1E or EndoC-βH1 cells by using Lipofectamine 2000 lipid reagent (Thermo Fisher Scientific, Chicago, IL) (18). For STAT3 luciferase studies, INS-1E cells were cotransfected with 200 ng STAT3-responsive dual firefly/*Renilla* luciferase Cignal Reporter (QIAGEN). After 24 h, cells were left untreated or treated with IL-6 (20 ng/mL) for 12 h, and luciferase activities were assayed by using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

Western Blot and Real-Time PCR

Protein expression was evaluated by Western blot by using antibodies that target total STAT3 (sc-482; Santa Cruz Biotechnologies, Dallas, TX), phospho-STAT3 (#9507; Cell Signaling, Danvers, MA), Isl-1 (sc-390793; Santa Cruz Biotechnologies), insulin (#8138; Cell Signaling), and α-tubulin (T9026; Sigma-Aldrich) (used as loading control). Immunoreactive bands were revealed by using the SuperSignal West Femto chemiluminescent substrate (Thermo Fisher Scientific) and detected by using a Molecular Imager ChemiDoc XRS+ System with ImageLab Software (Bio-Rad, Hercules, CA).

RNA extraction was performed by using the RNeasy Mini Kit (QIAGEN), and cDNA was synthesized with the QuantiTect Reverse Transcription Kit (QIAGEN). Expression of Isl-1, PAX6, LMX1A, rat INS-1 and INS-2, and human INS was assayed by real-time PCR by using

TaqManGene Expression Assays (Thermo Fisher Scientific) in an Eco Real Time PCR machine (Illumina). Expression values were corrected for the housekeeping genes GAPDH (rat) or β-actin (human) and for STAT3 expression to control for differences in transfection efficiency.

Chromatin Immunoprecipitation Assay

WT and mutant STAT3-transfected cell extracts were precleared by a 1-h incubation with protein A/herring sperm DNA at 4°C. Samples were incubated overnight at 4°C with BSA-blocked protein A and anti-STAT3 antibody (Santa Cruz Biotechnologies) or rabbit anti-IgG antibody (Abcam, Cambridge, U.K.) as negative control. Samples were then eluted, proteinase K treated, and incubated for 3 h at 62°C in high-salt solution to reverse the cross-link reaction. DNA fragments were analyzed by quantitative PCR, and input DNA was analyzed simultaneously for normalization. Quantitative PCR was performed by using SYBR Green (Bio-Rad) with the following primer pair for the STAT3 binding site −1262 to −1143 bp of the rat Isl-1 promoter: 5′-CTCAGAAAGACGGTAGATTT-3′ and 5′- AGCTTTCTAATTTGTTTCC-3′.

Insulin Release Assay

For determination of insulin release, INS-1E cells and EndoC-βH1 were incubated for 1 h in glucose-free RPMI GlutaMAX-I medium and HEPES-buffered Krebs-Ringer solution, respectively. INS-1E cells were then incubated for 30 min in Krebs-Ringer solution supplemented with 1.67 mmol/L glucose, 16.7 mmol/L glucose, or 16.7 mmol/L glucose + 10 μmol/L forskolin, and EndoC-βH1 cells were incubated for 1 h in Krebs-Ringer solution with 0 mmol/L glucose, 20 mmol/L glucose, or 20 mmol/L glucose + 45 μmol/L isobutylmethylxanthine (IBMX). Results were normalized by the insulin content measured in lysed cells and by STAT3 expression to control for differences in transfection efficiency. Insulin accumulation in the medium of INS-1E cells and EndoC-βH1 cells was measured by rat and human insulin ELISA kit (Mercodia, Uppsala, Sweden), respectively. Total protein was measured in cell lysates with a BCA Protein Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

WES Identifies a Missense Mutation in STAT3 Linked to NDM

After filtering for quality control parameters, 64,951 targeted DNA variants from the reference human genome sequence UCSC GRCh37/hg19 were identified by WES in a patient with NDM (Table 1). Comparison with the public database Single Nucleotide Polymorphism database (dbSNP135) and the 1000 Genomes Project revealed that of 7,473 nonsynonymous exonic variants, 1,286 were novel (i.e., not previously annotated). By following a de novo strategy, we identified 21 noninherited variants present in the affected proband and absent in both parents. We next performed an in silico prediction analysis and observed that 4 of the 21 mutations had a potential deleterious effect. The potentially deleterious mutations were located in *STAT3*, *LGR4* (leucine-rich repeat containing G protein–coupled receptor 4), *PTPN21* (protein tyrosine phosphatase, nonreceptor type 21), and *MEX3B* (Mex-3 RNA binding family member B) genes. Finally, to identify potential etiological variants, a bibliographic search in PubMed was performed to assess whether the identified genes were related to an interesting function for the pathogenesis of the disease (i.e., β-cell function, pancreas development, β-cell differentiation).

Table 1—Number and characteristics of identified variants through the WES analysis of a patient with NDM

	Index	Mother	Father
All identified variants (raw data)	316,186	284,847	274,380
Variants fulfilling quality parameters	64,951	60,496	56,722
Located in exonic regions	16,413	16,120	15,621
Nonsynonymous variants	7,473	7,332	7,101
Nonannotated variants (based on 1000 Genomes Project and dbSNP135) and MAF <0.05	1,286	1,298	1,412
Noninherited variants	21	—	—
Predicted to be deleterious	4	—	—

MAF, minor allele frequency.

We finally ended up with a candidate mutation in *STAT3*, a heterozygous C to T transition in exon 10 of the *STAT3* gene at position 988 of the coding DNA sequence predicted to replace a proline with a serine residue at position 330 (c.988C>T; p.Pro330Ser). The mutation was validated by Sanger sequencing in the proband and excluded in both parents. The identified variant is located at the DNA binding domain of the STAT3 protein (Fig. 1*A*), where genetic variants have been reported in patients with early-onset multiorgan autoimmune disease and lymphoproliferation (13,19,20). A comparison across species showed that Pro330 and the surrounding region is highly conserved and suggested potential pathogenicity of the identified mutation (Fig. 1*B*). Genetic alterations in the DNA binding domain of STAT3 have been shown to provoke anomalous activation of the transcription factor, resulting in downstream effects in the expression of its target genes. Thus, gain-of-function mutations in *STAT3* lead to increased SOCS3 expression, a major downstream target of STAT3 (13).

The Identified STAT3 Mutation Leads to Increased STAT3 Activity but Does Not Affect STAT3 Phosphorylation

The transcription factor STAT3 is activated by phosphorylation at tyrosine 705, which induces dimerization, nuclear translocation, and DNA binding (21). Phosphorylation changes in some serine residues of the STAT3

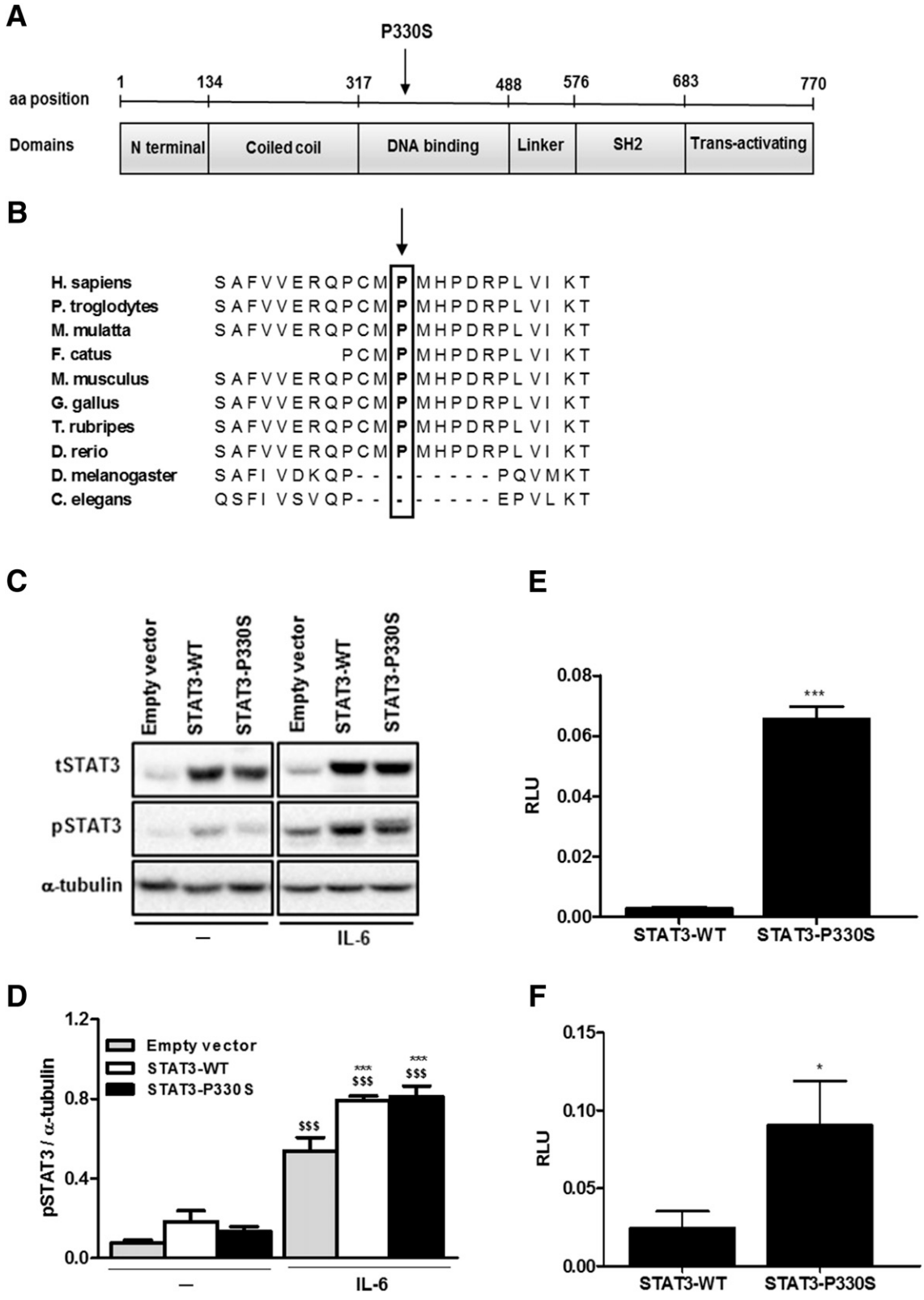

Figure 1—A missense mutation in the STAT3 DNA binding domain is linked to NDM and leads to increased STAT3 transcriptional activity without affecting its phosphorylation. *A*: Schematic representation of the human STAT3 protein structure. A de novo mutation (P330S) identified in one individual with NDM is located in the DNA binding domain. *B*: Alignment of the multiple STAT3 protein sequences across species. The altered amino acid (P330) is highly evolutionarily conserved among species. *C* and *D*: INS-1E cells were transfected with an empty plasmid (empty vector) or transfected with a plasmid encoding the human STAT3-WT or the mutant STAT3 (STAT3-P330S). Cells were left untreated (−) or treated with recombinant IL-6 (20 ng/mL) for 12 h. Total and phosphorylated STAT3 expression was analyzed by Western blot, and α-tubulin was used as the loading control. The results are representative of three independent experiments (*C*), and

protein have been shown to affect the phosphorylation state of tyrosine 705, eventually affecting its activation (22). The newly identified mutation in STAT3 led to a change from a nonphosphorylatable amino acid (proline) to a phosphorylatable amino acid (serine), creating a potential phosphorylation site that might affect the phosphorylation state and activation of STAT3.

Under basal and IL-6 treatment conditions, endogenous levels of total STAT3 protein were very low compared with cells transfected with plasmids overexpressing WT and mutant STAT3 (Fig. 1*C*). Under the nontreated condition, phosphorylation of STAT3 was insignificant in all conditions and did not differ between WT and mutant STAT3-transfected cells (Fig. 1*C* and *D*). Treatment with IL-6 (20 ng/mL) led to an increase in STAT3 phosphorylation in all experimental conditions, but phosphorylation levels did not differ between WT and mutant STAT3-transfected cells (Fig. 1*C* and *D*). These results show that P330S mutation does not affect the phosphorylation status of STAT3 either in the basal condition or under IL-6 treatment.

In nontreated cells, STAT3 mutation resulted in an increase in reporter activity by ~23-fold compared with WT STAT3 (Fig. 1*E*). Overexpression of the mutant STAT3 in INS-1E cells also resulted in increased reporter activity relative to WT after stimulation with IL-6 for 12 h; however, the increase was less pronounced than in the basal condition (~3.8-fold) (Fig. 1*F*).

These results demonstrate that the P330S mutation induces a constitutive hyperactivation of STAT3 and suggest that independently of its phosphorylation status, the mutant STAT3 has an increased DNA binding capacity that leads to enhanced transcriptional activity. These results are in line with previous studies in which polyautoimmune syndrome–related missense mutations in the DNA binding domain of STAT3 have been shown to significantly increase STAT3 activity (7). Other missense mutations located in the SH2 domain or in the transactivation domain of STAT3 also lead to a less marked increased STAT3 activity (7). Of note, inactivating mutations in the DNA binding domain of STAT3 have been identified in patients with hyper-IgE syndrome (23). Although molecular modeling of mutations located in the SH2 domain of the STAT3 protein suggest that alterations in this domain are likely to result in enhanced DNA binding affinity because of increased electrostatic interaction with the DNA backbone (7), the exact mechanism by which activating mutations in the binding domain lead to enhanced transcriptional activity remains to be clarified.

The Activating STAT3 Mutation Results in Decreased Insulin Synthesis Through Inhibition of Isl-1 Expression

The STAT molecules participate in the regulation of T-cell differentiation and play a crucial role in cytokine signaling (24). Consequently, mutations in STAT genes lead to various but overlapping phenotypes, ranging from susceptibility to infections to multiorgan autoimmunity (7,13,19,20,23).

By taking into account that STAT3 plays a crucial role in basal β-cell functions, such as regulation of insulin secretion (11,12), we evaluated the possible impact of the identified mutation at the pancreatic β-cell level. Insulin secretion is regulated by several transcription factors, such as Pax4, Nkx6.1, Maf, Foxa, Pdx1, and Isl-1, most of which are required for pancreatic development and basal β-cell functions (25). Bioinformatic prediction of potential transcription factor binding sites revealed that three transcription factors known to regulate insulin expression harbor transcription factor binding sites for STAT3 in their promoters (Isl-1, PAX6, and LMX1A). We examined the effect of the identified *STAT3*-activating mutation in the endogenous expression of Isl-1, PAX6, and LMX1A in rat and human pancreatic β-cells. To this aim, Isl-1, PAX6, and LMX1A mRNA expression was analyzed in WT and mutant STAT3-transfected rat and human β-cells. Although expression of PAX6 and LMX1A did not differ between WT and mutant STAT3-transfected β-cells (Supplementary Fig. 1), Isl-1 mRNA expression was decreased by 75% and 32% in mutant STAT3 rat and human β-cells, respectively (Fig. 2*A* and *D*). As shown in Fig. 2*F*, reduced Isl-1 expression was confirmed at the protein level in both mutant STAT3 rat and human β-cells.

The transcription factor Isl-1 is expressed in all four principal cell types of the endocrine pancreas and has been shown to regulate expression of insulin in pancreatic β-cells. In French Caucasian morbidly obese individuals, one SNP in the Isl-1 gene might modulate the risk for type 2 diabetes and increase body weight (26). Of note, STAT3 is implicated in the transcriptional regulation of Isl-1, and in rodent pancreatic β-cells, leptin-induced STAT3 phosphorylation has been shown to lead to decreased Isl-1 activity and reduced insulin expression (12). To assess whether STAT3 mutant–triggered Isl-1 inhibition leads to a decrease in insulin expression, we examined insulin mRNA expression in WT and mutant STAT3-transfected pancreatic β-cells. Expression of INS-1 and INS-2 was decreased in STAT3 mutant rat cells by ~70% (Fig. 2*B* and *C*), whereas human INS was decreased

densitometry data (*D*) are mean ± SEM. $$$*P* < 0.001 vs. nontreated (−) and transfected with the same plasmid; ****P* < 0.001 vs. empty vector treated with IL-6; Student *t* test followed by Bonferroni correction. *E* and *F*: Cells were transfected as in *C* and cotransfected with a STAT3-responsive luciferase plus a constitutively expressing *Renilla* construct (used as internal control). After 24 h of recovery, cells were left untreated (*E*) or treated (*F*) with IL-6 (20 ng/mL) for 12 h, and luciferase activity was measured. Data are mean ± SEM of six independent experiments shown as relative luciferase units (RLUs). ****P* < 0.001; **P* < 0.05 vs. STAT3-WT–transfected cells; Student *t* test. aa, amino acid.

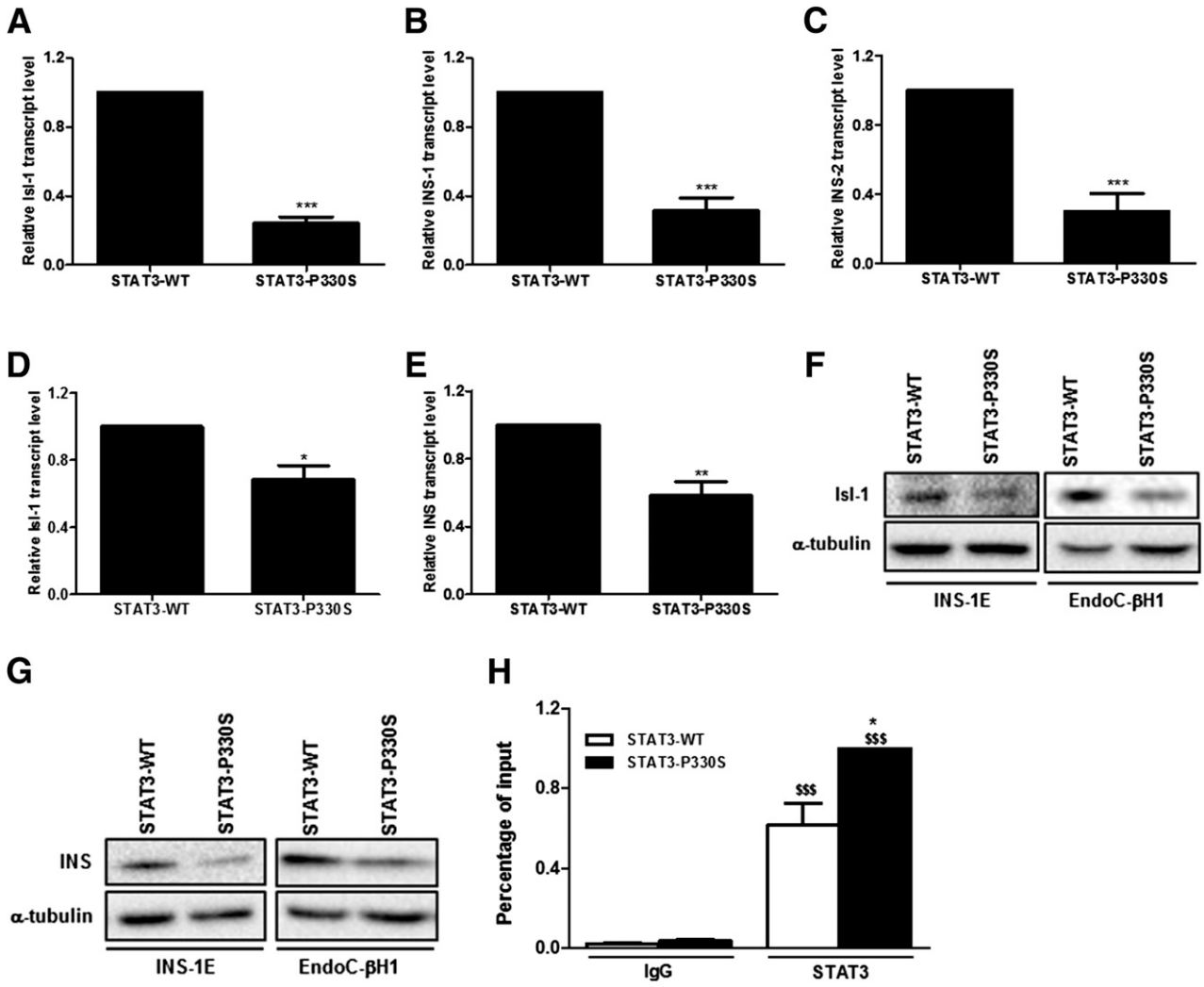

Figure 2—Activating mutation in STAT3 decreases Isl-1 and insulin expression in rat and human pancreatic β-cells by increasing STAT3 ability to bind to the Isl-1 promoter. INS-1E and EndoC-βH1 cells were transfected with a plasmid encoding the human STAT3-WT or the mutant STAT3 (STAT3-P330S). After 36 h of recovery, mRNA expression of rat Isl-1 (*A*), INS-1 (*B*), INS-2 (*C*), or human Isl-1 (*D*) and INS (*E*) was assayed by real-time PCR and normalized for the housekeeping gene GAPDH (*A–C*) or β-actin (*D* and *E*) and for STAT3 expression to control for differences in transfection efficiency. Data are mean ± SEM of four (*A–C*) and six (*D* and *E*) independent experiments. ***$P <$ 0.001, **$P <$ 0.005, *$P <$ 0.05 vs. STAT3-WT; Student *t* test. *F* and *G*: Isl-1 and INS protein expression in STAT3-WT– and STAT3-P330S–transfected INS-1E and EndoC-βH1 cells was analyzed by Western blot, and α-tubulin was used as the loading control. *H*: ChIP analysis revealed an increased ability of mutant STAT3 to bind to the Isl-1 promoter region in positions −1,262 to −1,143 bp (previously shown to be a transcription factor binding site for STAT3 in mouse [12]). IgG antibody was used as a negative control. Data are mean ± SEM of four independent experiments shown as percentage of input. *$P <$ 0.05 vs. STAT3-WT–transfected cells; $$$$P <$ 0.001 vs. IgG and transfected with the same plasmid; ANOVA followed by Student *t* test with Bonferroni correction.

by 42% in mutant STAT3-transfected human β-cells compared with WT cells (Fig. 2*E*). Decreased insulin mRNA expression in mutant STAT3 rat and human β-cells was confirmed at the protein level by Western blot (Fig. 2*G*). To determine whether mutant STAT3 had an increased ability to bind the Isl-1 promoter, we performed a quantitative chromatin immunoprecipitation (ChIP) analysis. INS-1E cells were transfected with WT- or mutant STAT3-overexpressing plasmids, and by using DNA fragments precipitated with anti-STAT3 as templates, the Isl-1 promoter region in positions −1,262 to −1,143 bp (previously shown to be a transcription factor binding site for STAT3 in mouse [12]) was amplified with quantitative PCR. As shown in Fig. 2*H*, mutant STAT3 showed an increased ability to bind to the Isl-1 promoter region compared with the WT STAT3 protein.

To determine whether mutant STAT3 cause impaired insulin secretion, we analyzed glucose-stimulated insulin secretion in WT and mutant STAT3-transfected rat and human β-cells. As shown in Fig. 3*A*, in WT STAT3-transfected INS-1E cells, high-glucose (16.7 mmol/L) and high-glucose + forskolin exposure increased insulin secretion by nearly two- and fivefold, respectively. In mutant STAT3 cells, glucose-stimulated insulin secretion was

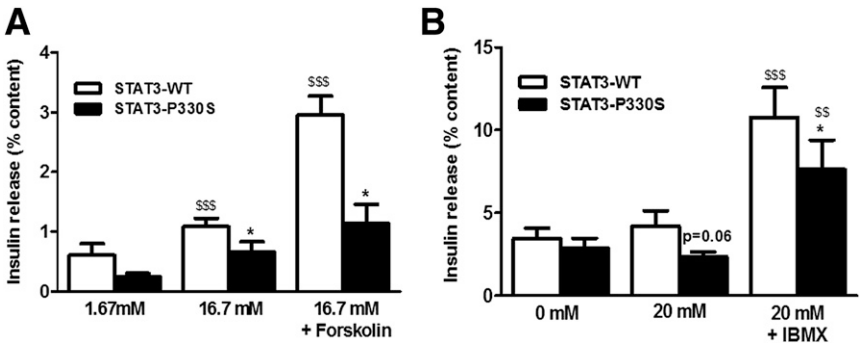

Figure 3—Mutant STAT3 reduces glucose-stimulated insulin secretion in rat and human pancreatic β-cells. INS-1E (*A*) and EndoC-βH1 (*B*) cells were transfected with a plasmid encoding the human STAT3-WT or the mutant STAT3 (STAT3-P330S). After 36 h of recovery, glucose-stimulated insulin secretion was measured after stimulation with 1.67 mmol/L glucose, 16.7 mmol/L glucose, or 16.7 mmol/L glucose + 10 μmol/L forskolin in INS-1E cells (*A*) or 0 mmol/L glucose, 20 mmol/L glucose, or 20 mmol/L glucose + 45 μmol/L IBMX in EndoC-βH1 cells (*B*). Insulin release values were normalized for STAT3 expression to control for differences in transfection efficiency and are represented as percentage of insulin content. Data are mean ± SEM of five to six independent experiments. $^*P < 0.05$ vs. STAT3-WT–transfected cells under the same stimulation condition; $\$\$\$P < 0.001$ and $\$\$P < 0.01$ vs. 1.67 mmol/L glucose (*A*) or 0 mmol/L glucose (*B*) stimulation and transfected with the same plasmid; ANOVA followed by Student *t* test with Bonferroni correction.

decreased by ~60% in the low-glucose and high-glucose + forskolin conditions and by 40% in the high-glucose condition compared with WT STAT3-transfected cells (Fig. 3*A*). These results were confirmed in the human pancreatic β-cell line EndoC-βH1 in which overexpression of mutant STAT3 led to a decrease in glucose-stimulated insulin secretion compared with WT cells (Fig. 3*B*). Total insulin content measured by ELISA in cell lysates showed that mutant STAT3 overexpression decreased intracellular insulin content compared with WT cells (Supplementary Fig. 2), further confirming the results obtained by insulin protein determination by Western blot (Fig. 2*G*). In line with the results obtained by insulin mRNA expression analysis, the decrease in insulin protein content in mutant STAT3-transfected cells was more pronounced in rat β-cells than in human β-cells.

These results showed that P330S mutation in STAT3 leads to decreased insulin synthesis in pancreatic β-cells through enhanced inhibition of the transcription factor Isl-1. Although we have not observed an effect of mutant STAT3 on β-cell viability (data not shown), we cannot exclude other potential impacts of hyperactive STAT3 at the pancreatic β-cell level. Indeed, constitutive STAT3 activation has been linked to dysregulation of the cell cycle control and apoptosis genes in other cell types (27). At the pancreas level, STAT3 modulates the β-cell cycle and protects β-cells from DNA damage (28), and β-cell–specific STAT3 knockout mice exhibit insulin secretory defects and impaired pancreatic islet architecture (29).

Although STAT3 gain-of-function mutations have been shown to lead to early-onset autoimmunity by affecting regulation of immune-related cells (7,13,19,20), the current findings demonstrate that activating mutations in *STAT3* affect insulin secretion and may thus explain pancreatic β-cell dysfunction in some forms of diabetes, including NDM. These results suggest, therefore, that activating mutations in the *STAT3* gene can result in NDM because of two contrasting mechanisms: through dysregulation of immune-related cells or through dysfunction of basal pancreatic β-cell function.

Acknowledgments. The authors thank J.R. Bilbao, from the Immunogenetics Research Laboratory, BioCruces Research Institute, UPV-EHU, for help and advice in the analysis of expression data; A. Castellanos-Rubio, from the Immunogenetics Research Laboratory, BioCruces Research Institute, UPV-EHU, for advice in ChIP experiments; and A.M. Aransay, from the Genome Analysis Platform, CIC bioGUNE, for the use of the sonicator.

Funding. This work was supported by Instituto de Salud Carlos III PI14/01104, integrated into the State Plan for Research and Development 2013–2016 and cofinanced by the European Regional Development Fund. T.V. is the recipient of a predoctoral fellowship from the University of the Basque Country (IF/UPV/12/135).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.V. and I.S. contributed to the general idea and design of experiments, researched data, contributed to the discussion, and wrote, revised, and edited the manuscript. R.M., K.G.-E., I.U., and I.M.d.L. researched data and revised and edited the manuscript. M.A., A.A., C.C., R.B., and L.C. contributed to the original idea, contributed to the discussion, and revised and edited the manuscript. I.S. and L.C. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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