Mechanism of Neonatal Insulin Production Failure in Transcription Factor PDX1 Related MODY4

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Abstract

Maturity-onset diabetes of the young (MODY) is a rare autoimmune disorder. Specifically, MODY4 is a subset of cases stemming from mutations in the proline-rich transactivation domain of the homeobox transcription factor PDX1. This study analyzes the mutation PDX1^{P33T} which substitutes a threonine in the place of a proline in the transactivation domain (Wang et al., 2019). Within pancreatic β-cells, PDX1 is essential for the transcription of the gene INS, which encodes insulin. Further, because of their respectively necessary or augmentary roles in RNA Polymerase (Pol II) recruitment, PDX1 requires the physical binding of cofactors p300 and PSMD9 in order to achieve adequate transcription levels (Qiu et al., 2002, Usher and Showalter, 2022). The PDX1^{P33T} mutation has been characterized by an inadequate transcription of the INS gene, although the physical process has not been documented. From observations of protein modeling and folding tools PyMol and Alphafold2, respectively, the Mahtomedi MaPS Team, in collaboration with the Center for BioMolecular Modeling, suggests that PDX1^{P33T} fails to bind PSMD9 and p300 and thus cannot recruit Pol II itself and transcribe INS because of the removal of proline's atypical sidechain via the substitution of threonine; the substitution relaxes the turn radius of a loop in the transactivation domain from 34.78±5° to 79.68 ±5° (PyMOL, Jumper et al., 2021, Varadi et al., 2021). As a result, PDX1^{P33T} leads to MODY diabetes by tangentially inhibiting β-cell development and their capacity to form insulin, as well as the production of insulin itself.

What is MODY4 and PDX1?

Maturity-onset diabetes of the young (MODY), is a form of diabetes separate from both type 1 (T1) and type 2 (T2). In MODY, insulin production is often prevented or reduced by one of many mutations--the most common being those in the HNF1A, HNF4A, and HNF1B genes (MedlinePlus, n.d.). MODY has eleven different types, all caused by various single gene missense mutations which prevent β-cell insulin secretion. MODY4, the rarest form of MODY, is defined by mutations within the pancreatic and duodenal homeobox 1 (PDX1) gene (Yoshiji et al., 2021). We specifically analyzed the P33T mutation within the transactivation domain (TAD) of PDX1, and how it affects gene expression, because it is the most common source of MODY4 (Wang et al., 2019). PDX1 is commonly expressed in the gastrointestinal tract, predominantly in acinar cells within the islets of Langerhans which are found in the epidermis of the pancreas (Fig. 1) (Egozi et al., 2020).

PDX1 is necessary for both the development of all pancreatic type cells and insulin production within β-cells (Spaeth et al., 2017). A 100-base pair enhancer activates β-cell transcription in the islets of Langerhans and in acinar cells, imparting pancreatic specificity. PDX1 is crucial in the transcription of the insulin coding gene (INS) and pancreatic elastase I gene (ELA1) along with other cofactors specific to pancreatic acinar cells (Viswanath et al., 2000). The INS gene is fundamental to β-cells secreting insulin, while ELA1 is a complement to pancreatic genes encoding digestive enzymes that are expressed selectively in acinar cells (Wang et al., 2019, Mosley et al., 2004).

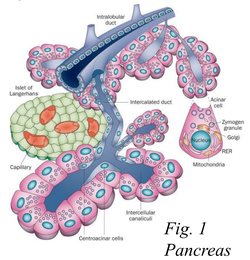


Fig. 1 Pancreas components

P33T Mutation

An unmutated PDX1 maintains a turn radius of 34.78±5°, caused mainly by the amino acid proline's unique sidechain (PyMOL). Proline's presence in the backbone is the fundamental source of this restricted φ angle. Its reconnection to its protein backbone allows proline to assume either trans or cis configuration. Unlike most other amino acids, which all favor trans configurations due to steric clash, proline creates steric clash in both the trans and cis configuration, so depending on other local charge distributions it can take the form of either (LabXchange, 2020). By configuring this way, it often disrupts the secondary structure, causing turns in the beta sheet as we see in the original PDX1 model (Fig. 3). However, when it mutates from a proline to a threonine, it loses that disrupting quality, therefore reducing the turn radius to 79.68±5° (PyMOL) (Fig. 2). Threonine favors a trans configuration, which prevents it from assuming the cis configuration as proline did, preventing the sharp turn found in the non-mutated PDX1 (LabXchange, 2020). P33T mutation occurs in the transactivation domain of PDX1 at the end of a β-turn in the peptide backbone. The transactivation domain is necessary to bind to other transcription cofactors in order to recruit RNA polymerase II and related machinery (Wang et al., 2019).

Transcription

The process of producing proteins from DNA involves two steps. The first step, transcription, is defined by transcribing DNA information into RNA. This process needs the protein cluster RNA Polymerase II (Pol II), the main engine powering transcription. Additionally, in order for the Pol II machinery to access the DNA, the DNA must undergo unraveling from histones (positively charged proteins that DNA wraps around). So, in order to regulate transcription and initiate Pol II, a litany of transcription factors are needed to recruit machinery both to catalyze histones to relax the spooled DNA and recruit Pol II to the specific DNA segment. For INS, both these processes are in large part regulated by PDX1. PDX1 binds both to the promoter and enhancer regions of INS. From there, we identified two cofactors that PDX1 directly interfaces with via its TAD: The 26S proteasome non-ATPase regulatory subunit 9 (PSMD9) and histone acetyltransferase p300 (Mosley et al., 2004, Usher & Showalter, 2022). PSMD9 binds to PDX1 while PDX1 is in the promoter region, facilitating the recruitment of Pol II (UniProt, n.d.). p300, in contrast, binds to PDX1 while it is in the enhancer region and catalyzes the aforementioned DNA unraveling (Ebrahim et al., 2022). As these two cofactors play crucial roles in either facilitating or prepping for transcription, respectively, failure to bind would suggest failure to transcribe INS (Qiu et al., 2002, Mosley et al., 2004, Usher & Showalter, 2022).

Figure 2: Mutated

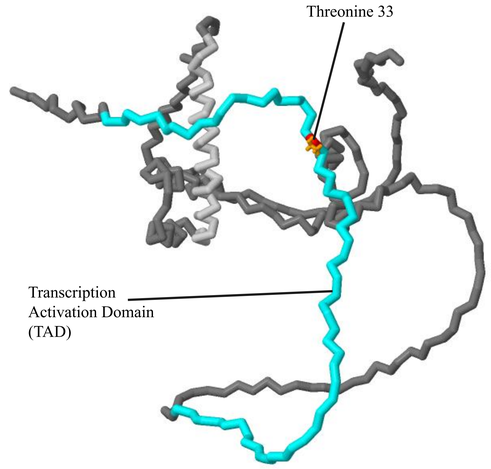
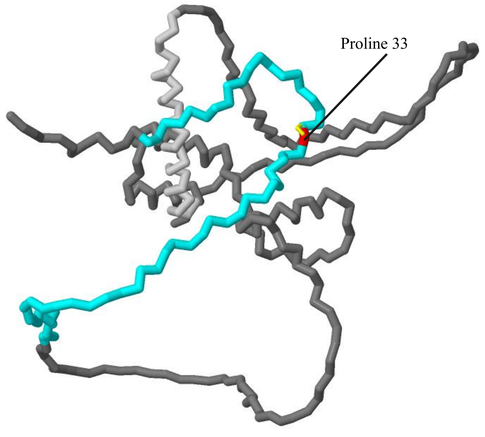


Figure 3: Unmutated



P33T and MODY4

The PDX1^{P33T} mutation is characterized by the substitution of a threonine for a proline in PDX1's transactivation domain. Proline's sidechain creates a uniquely tight turn, so when threonine takes its place, the turn radius of a loop in the TAD relaxes significantly--from 34.78±5° to 79.68±5° (PyMOL, Jumper et al., 2021). Due to this radius change, PDX1^{P33T} cannot properly match up with cofactors PSMD9 and p300, and fails to bind to them. Therefore, it is unable to recruit the necessary machinery, meaning transcription of INS cannot occur. Under normal conditions, insulin regulates the mass of β-cells, which ensures they function properly and can themselves produce insulin (Rachdaoui, 2020). Therefore, when INS is not transcribed due to the PDX1^{P33T} mutation, both β-cell development and their capacity to form insulin are negatively impacted. This inability to produce insulin, in turn, leads to and is a core symptom of, MODY diabetes (MedlinePlus, n.d., Rachdaoui, 2020).

Conclusion

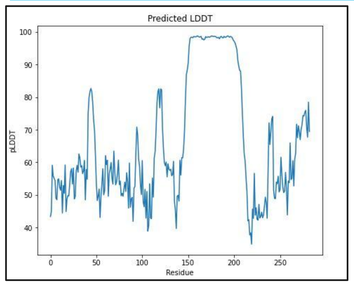


Fig. 4 pLDDT is the measure AlphaFold2 gives for its per-residue confidence given by the 1DDT-Cα metric described in Mariani et al., 2013. Generally, <50 is unusable, 50-70 is tentative, 70-90 is generally applicable, and 90-100 is excellent

In total, our research points to the unique nature of proline's sidechain within PDX1 as the catalyst for MODY4 by way of disrupting the recruitment of the machinery necessary to both unravel histones and initiate transcription via Pol II. We substantiated this hypothesis using modeling tools such as AlphaFold2 and ChimeraX. However, two provisions must be acknowledged: first, the predictions produced by AlphaFold2 within the TADs were significantly uncertain (Fig. 4). Within the TAD of PDX1^{P33T}, there was an average per-residue confidence score (pLDDT) of 55. This low confidence is likely due to the TAD itself being chaotic. However, it must be acknowledged that the exact change in the TAD is unclear. We can be certain it changed, but to what exact degree is uncertain. This leads into, second provision: we are substantiating our predictions with predictions. There is a nonzero possibility that because proline has historically caused this shape, both us and AlphaFold2 predict this same phenomenon without any true backing. Trust in exact results has to come from, then, trust in (1) AlphaFold2's attention to nuance and (2) that proline would act normally. So, looking forward, the next steps that should be taken are to model both the wild-type and mutated PDX1 alongside p300 and PDX1 in AlphaFold2 to increase confidence in the behavior.

References

Ebrahim, N., Shakirova, K., & Dashinimaev, E. (2022). PDX1 is the cornerstone of pancreatic β-cell functions and identity. *Frontiers in Molecular Biosciences*, *9*. https://doi.org/10.3389/fmolb.2022.1091757

Egozi, A., Bahar Halpern, K., Farack, L., Rotem, H., & Itzkovitz, S. (2020). Zonation of Pancreatic Acinar Cells in Diabetic Mice. *Cell Reports*, *32*(7), 108043. https://doi.org/10.1016/j.celrep.2020.108043

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., & Back, T. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596*(7873), 583–589. https://doi.org/10.1038/s41586-021-03819-2

LabXchange. (2020, January 19). *LabXchange*. Www.labxchange.org. https://www.labxchange.org/library/pathway/lx-pathway:573b19de-1dda-48de-b82c-7cf4c3524f3c/items/lx-pb:573b19de-1dda-48de-b82c-7cf4c3524f3c:html:aafcc3d3

Logsdon, C. D., & Ji, B. (2013). The role of protein synthesis and digestive enzymes in acinar cell injury. *Nature Reviews Gastroenterology & Hepatology*, *10*(6), 362–370. https://doi.org/10.1038/nrgastro.2013.36

Mariani, V., Biasini, M., Barbato, A., & Schwede, T. (2013). lDDT: a local superposition-free score for comparing protein structures and models using distance difference tests. *Bioinformatics*, *29*(21), 2722–2728. https://doi.org/10.1093/bioinformatics/btt473

MedlinePlus. (n.d.). *Maturity-onset diabetes of the young: MedlinePlus Genetics*. Medlineplus.gov. https://medlineplus.gov/genetics/condition/maturity-onset-diabetes-of-the-young/#:~:text=MODY%20can%20be%20caused%20by

Mosley, A. L., Corbett, J. A., & Ozcan, S. (2004). Glucose Regulation of Insulin Gene Expression Requires the Recruitment of p300 by the β-Cell-Specific Transcription Factor Pdx-1. *Molecular Endocrinology*, *18*(9), 2279–2290. https://doi.org/10.1210/me.2003-0463

Pettersen, E. F., Goddard, T. D., Huang, C. C., Meng, E. C., Couch, G. S., Croll, T. I., Morris, J. H., & Ferrin, T. E. (2020). UCSF ChimeraX : Structure visualization for researchers, educators, and developers. *Protein Science*, *30*(1), 70–82. https://doi.org/10.1002/pro.3943

Qiu, Y., Guo, M., Huang, S., & Stein, R. (2002). Insulin Gene Transcription Is Mediated by Interactions between the p300 Coactivator and PDX-1, BETA2, and E47. *Molecular and Cellular Biology*, *22*(2), 412–420. https://doi.org/10.1128/MCB.22.2.412-420.2002

Rachdaoui, N. (2020). Insulin: The Friend and the Foe in the Development of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, *21*(5), 1770. https://doi.org/10.3390/ijms21051770

Spaeth, J. M., Gupte, M., Perelis, M., Yang, Y.-P., Cyphert, H., Guo, S., Liu, J.-H., Guo, M., Bass, J., Magnuson, M. A., Wright, C., & Stein, R. (2017). Defining a Novel Role for the Pdx1 Transcription Factor in Islet β-Cell Maturation and Proliferation During Weaning. *Diabetes*, *66*(11), 2830–2839. https://doi.org/10.2337/db16-1516

UniProt. (n.d.). *UniProt*. Www.uniprot.org. Retrieved March 13, 2023, from https://www.uniprot.org/uniprotkb/Q9WTV5/entry

Usher, E. T., & Showalter, S. A. (2022). Biophysical insights into glucose-dependent transcriptional regulation by PDX1. *Journal of Biological Chemistry*, 102623. https://doi.org/10.1016/j.jbc.2022.102623

Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., Yuan, D., Stroe, O., Wood, G., Laydon, A., Žídek, A., Green, T., Tunyasuvunakool, K., Petersen, S., Jumper, J., Clancy, E., Green, R., Vora, A., Lutfi, M., & Figurnov, M. (2021). AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Research*, *50*(D1). https://doi.org/10.1093/nar/gkab1061

Viswanath, R. L., Rose, S. D., Swift, G. H., & MacDonald, R. J. (2000). A Binary Mechanism for the Selective Action of a Pancreatic β-Cell Transcriptional Silencer*. *Journal of Biological Chemistry*, *275*(51), 40273–40281. https://doi.org/10.1074/jbc.M007021200

Wang, X., Sterr, M., Ansarullah, Burtscher, I., Böttcher, A., Beckenbauer, J., Siehler, J., Meitinger, T., Häring, H.-U., Staiger, H., Cernilogar, F. M., Schotta, G., Irmler, M., Beckers, J., Wright, C. V. E., Bakhti, M., & Lickert, H. (2019). Point mutations in the PDX1 transactivation domain impair human β-cell development and function. *Molecular Metabolism*, *24*, 80–97. https://doi.org/10.1016/j.molmet.2019.03.006

Yoshiji, S., Horikawa, Y., Kubota, S., Enya, M., Iwasaki, Y., Keidai, Y., Aizawa-Abe, M., Iwasaki, K., Honjo, S., Hosomichi, K., Yabe, D., & Hamasaki, A. (2021). First Japanese Family With *PDX1*-MODY (MODY4): A Novel *PDX1* Frameshift Mutation, Clinical Characteristics, and Implications. *Journal of the Endocrine Society*, *6*(1). https://doi.org/10.1210/jendso/bvab159

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