hIAPP Amyloidosis in Type 2 Diabetes Mellitus

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Cases of Type 2 Diabetes Mellitus (T2DM) are increasing at an alarming rate due to the rise in obesity, sedentary lifestyles, glucose-rich diets and other factors. Numerous studies have increasingly illustrated the pivotal role that human islet amyloid polypeptide (hIAPP) plays in the pathology of T2DM through damage and subsequent loss of pancreatic β-cell mass. Here researchers provide an up-to-date summary of recent progress in the field, highlighting factors that contribute to hIAPP misfolding and aggregation which have been linked to β-cell cytotoxicity. Understanding the structure of hIAPP and how these factors affect amyloid formation will help better understand how hIAPP misfolds and aggregates and, importantly, help identify potential therapeutic targets for inhibiting amyloidosis so alternate and more effective treatments for T2DM can be developed.

human islet amyloid polypeptide amyloidosis type 2 diabetes mellitus

1. Introduction

Diabetes mellitus is a complex condition linked to increased blood glucose levels, compromised insulin production or action, that can be subdivided into five major types: monogenic diabetes, Type 1 Diabetes mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), gestational diabetes and, more recently, Type 3 Diabetes (T3DM) [1]. Monogenic diabetes is rare and arises from mutations in one of about a dozen genes, including the insulin gene and genes downstream of insulin action, such as glucokinase [2]. T1DM is classified as an autoimmune disorder in which T-cells attack and destroy pancreatic β-cells, which are the site of insulin secretion [3]. Gestational diabetes can occur during pregnancy when the natural production of diabetogenic hormones from the placenta, which induces insulin resistance, cannot be compensated sufficiently by increased insulin production from the mother [4]. In addition to the well categorised conditions T1DM and T2DM, some scientists have recently suggested another type of diabetes called ‘Type 3 Diabetes’. T3DM has been used to describe the neurodegenerative pathologies observed in those with Alzheimer’s Disease, accelerated by insulin resistance in brain tissue, thought to be due to T2DM [5].

T2DM is a complex metabolic condition characterised by hyperglycaemia and peripheral insulin resistance, leading to depletion and dysfunction of pancreatic islet β-cells [6]. A range of co-morbidities has been linked to T2DM including cardiovascular disease, sensory neuropathy, stroke, kidney failure and blindness [7]. The World Health Organization conducted a worldwide study on diabetes in 2016 [8]. The report showed that the number of adults currently living with diabetes is over 422 million worldwide and this has quadrupled since 1980. T2DM accounts for 95% of all these cases, resulting in over 1.5 million deaths. This increase has been linked to rises in obesity that has resulted in a 5% increase in mortality rate in those under the age of 70, with complications linked to blindness, stroke, heart attack, kidney failure and lower limb amputations.

T2DM has been associated with several risk factors that are genetic, epigenetic and environmental [9]. More than 140 genome risk variants have been identified through meta-analysis of genome-wide association studies (GWAS) in European populations [10] and a further 318 novel variants associated with T2DM were found in a trans-ethnic GWAS [11]. Environmental and lifestyle factors have been commonly linked to the rapid increase in T2DM cases worldwide. Obesity has been singled out as one of the most significant factors contributing to the development of T2DM. Almost one-third of the global population can be classified as obese, seeing a meteoric rise in the last century [12]. The term “diabesity” encompasses the co-existence and link between T2DM and obesity, highlighting the fact that being obese is one of the biggest risk factors for T2DM [13]. T2DM was previously assumed to be an adult-onset condition; however, the rise in childhood obesity (which is linked to increasingly sedentary lifestyle and glucose-rich diets) has seen cases of T2DM in under 18-year-olds rising [14]. Diet is therefore also another important contributory factor, with low-fibre, high-glucose diets being associated with a higher risk of T2DM [15].

Social-economic factors are also a risk factor linked to T2DM. Individuals from low-socioeconomic communities are more likely to suffer from chronic stress which is associated with increased blood pressure and elevated blood glucose levels [16]. Those from low-socioeconomic backgrounds are also more likely to have poor diets and be obese, which also accounts for the rise in T2DM in these communities.

Microorganisms have also been associated with T2DM, as highlighted in recent studies looking at whether the gut microbiome is linked to T2DM [17]. Elevated levels of bacteria such as *Fusobacterium* and *Ruminococcus* have been detected in T2DM patients compared to non-diabetic patients. These bacteria form part of the microflora of the intestines that are thought to regulate levels of lipopolysaccharides (LPS), which have been linked to the advancement and development of T2DM [18]. Levels of circulating LPS are increased in T2DM patients and the liver of LPS induced mice has been observed to show insulin resistance. Additionally, healthy weight mice showed fasting glucose levels and weight gain increase in the same range as those of obese and diabetic mice [19].

Prescribed medications to manage T2DM include antihyperglycemic drugs such as metformin and sulfonylureas. Although these have proven to reduce mortality and assist in the control of blood glucose levels, metformin has associated side effects such as nausea and diarrhoea in 50% of patients. This is thought to be as a result of metformin increasing levels of glucagon-like peptide 1 (GLP-1) and increasing intestinal glucose turnover, as well as influencing the microflora of the gut [20]. Sulfonylureas promote insulin release independently of glucose levels but have been reported to cause hypoglycaemia [21]. Scientists are therefore currently researching more effective treatments that focus on reducing and/or reversing the damage to β-cells caused by T2DM and ways to reduce any associated side effects. The development of preventative treatments for pre-diabetic patients would be more effective as it would prevent T2DM and the need for medication and would prevent complications affecting other parts of the body (e.g., eyes, heart) associated with this complex disease.

In order to progress with therapeutic interventions and help prevent disease onset, researchers urgently need a better understanding of T2DM at the molecular level. Despite a plethora of scientific literature on diabetes, exactly what initiates the disease and what factors are responsible for accelerating the condition are still unclear. In this research, researchers have focused on the role of hIAPP and its tendency to misfold and aggregate in the

molecular pathology of T2DM, since accumulating evidence suggests that different conformations of the protein are associated with the initiation and/or acceleration of the disease.

2. Main Text

2.1. The Human Islet Amyloid Polypeptide and Its Link to Diabetes

Deposits of incorrectly folded ("misfolded") hIAPP in the pancreas of people with T2DM have been reported in up to 90% of all T2DM patients [22]. The change in protein conformation from a soluble protein to misfolded forms, which can result in amyloid fibrils in the process of amyloidosis, is associated with several diseases such as Alzheimer's Disease and T2DM, where they aggregate and damage cells and surrounding tissues. Misfolded forms of hIAPP have been linked to pancreatic β-cell damage and as a result, decreased release of insulin and impaired glucose regulation, which can lead to T2DM [23].

hIAPP is a 37 amino acid protein with an element of intrinsically disordered structure and is secreted from β-cells together with insulin [24]. It is classified as an intrinsically disordered protein (IDP) that lacks stable secondary or tertiary structures under physiological conditions, since it contains intrinsically disordered regions within its structure [25].

The gene that encodes hIAPP is found on chromosome 12, includes 3 exons [26] and is initially expressed as an 89 amino acid residue long pre-pro-peptide [27]. A 22-residue signal sequence is cleaved, forming a 67-residue propeptide, which is then processed in the Golgi, followed by the secretory granules of β-cells [28]. Nine amino acids are cleaved from the N-terminus and 16 from the C-terminus of the protein by prohormone convertase enzymes. The remaining 5 mostly basic amino acids are removed by carboxypeptidase E to produce the secreted peptide [28][29].

Several functions have been ascribed to IAPP, including suppression of glucagon release, control of gastric emptying and regulation of satiety [30], all of which can influence glucose homeostasis. IAPP has also been suggested to help regulate blood glucose levels through inhibitory effects on insulin secretion, although there is some ambiguity in the evidence supporting such a role [30][31][32]. Interestingly, a study in mice in which the IAPP gene had been ablated suggested a protective role for IAPP in β-cell function [33]. IAPP has pleiotropic effects and is able to cross the blood–brain barrier [28][29], and hence there is potential for aggregation events occurring around the body that may contribute to the development of diabetes.

3. The Structure of hIAPP

Understanding the structure of hIAPP and its various conformations is important for understanding how it contributes to T2DM and hence for designing effective therapeutics. In T2DM, evidence suggests that hIAPP can adopt different conformations, including pre-IAPP (an un-cleaved form), monomers, dimers, oligomers and fibrils

[34]. Like other protein misfolding disorders (PMDs), it is unclear to what extent each protein conformation contributes to toxicity and disease.

In vitro, synthetic hIAPP monomers dissolved in phosphate buffer or diluted hexafluoro-2-isopropanol form random coil conformations, which misfold into β-sheet structures and fibrils [25]. Analysis of hIAPP fibrils using site-directed spin labelling and electron paramagnetic resonance (EPR) spectroscopy, in combination with simulated annealing molecular dynamics, revealed the fibrils to be composed of stacked misfolded forms of hIAPP. Each hIAPP was bent into a hairpin with each arm having a region of β-sheet structure, opposite and staggered against each other. The fibrils had a left-handed twist and a hydrophobic surface at the fibril ends that is suggested to be the site of fibril growth where subsequent hIAPP monomers can bind, leading to fibril elongation [35]. Molecular dynamics simulations confirmed the random coil overall structure of hIAPP and revealed many metastable conformational states [36]. Some of the states had β-hairpin secondary structure and hydrophobic surfaces, features which facilitate nucleation of aggregation pathways. The formation of insoluble hIAPP fibrils from soluble monomers is proposed to occur via nucleated conformational conversion or conformational selection [36]. Nucleated conformational conversion involves the assembly of fibrils from oligomeric intermediates such as protofibrils [37]. Conformational selection means that the binding of monomers does not involve any change in conformation state but binds to an already formed conformation [38]. NMR results suggest that hIAPP undergoes nucleated conformational selection where the N-terminal α-helical region is involved in monomer “collapse” (hydrophobic or hydrophilic collapse), suggesting that monomers first collapse before further rearranging into ordered proto-fibrils composed of β-sheets [39]. Conformational selection involving this selective collapsing of monomers possessing β-sheet structures has also been supported by molecular dynamic studies [37].

Recently, several cryo-EM structures of recombinant hIAPP have been produced [40][41][42][43] that show fibrils are composed of two protofilaments. The structures observed suggest that the process of fibrillization may be synchronous [43] and the structural similarity of hIAPP fibrils to polymorphs of amyloid-β (Aβ) fibrils [42] may explain how both fibrils can cross-seed one another.

The most detailed, and biologically relevant structures of hIAPP fibrils was published recently by Cao et al., 2021 [44], showing eight morphologically different cryo-EM fibril structures seeded from fibrils extracted from islet cells from a T2DM donor. Four of the structures revealed twisted fibrils comprising two linked protofilament chains each composed of stacked β-strands. Two of the twisted fibrils are homodimers, whilst the other two are heterodimers. Two core folds can be observed in the fibrils which were observed to be capable of interacting in three different ways to form protofibrils, thus allowing the different structures to form. Some of the seeded fibrils do not resemble any of the unseeded ones (derived from peptides only), hence, they could resemble the structure of hIAPP in vivo [44].

Since membranes have been suggested as crucial to hIAPP misfolding and aggregation [45], it is essential to characterise the structure of hIAPP when bound to a membrane-like environment. Studies suggest that membranes accelerate the transition from α-helical to β-sheet pleated structures as revealed by NMR, EPR, TEM and Atomic force microscopy (AFM) studies [46][47][48][49]. NMR and EPR experiments using hIAPP bound to

micelles highlighted residues 9–22 as having an unstable α-helical region, which is assumed to become stabilised during the aggregation process [34]. Lipid binding assays and TEM showed that when bound to anionic or zwitterionic membranes, hIAPP transitions from an α-helical structure to a β-sheet structure within minutes [50]. AFM has also been used to observe membrane-bound pre-fibrillar hIAPP, showing structures resembling ion-channels which could damage membranes by forming pores and changing their fluidity [51]. Other AFM studies have shown hIAPP accumulating on membranes in the form of unstructured aggregates. Membranes containing cholesterol revealed less bound amyloid compared to cholesterol-free membranes [52][53], highlighting the importance of membrane composition to amyloidosis.

Importantly, decreased insulin degrading enzyme (IDE) levels can lead to hyperinsulinemia, which may contribute to insulin resistance [54]. IAPP is a substrate for the insulin degrading enzyme (IDE) [55], a zinc metalloproteinase expressed in most cell types and also found in the circulation. Modulation of IDE activity could have consequences for amyloid formation by hIAPP and other amyloidogenic proteins [56]. Inhibition of IDE increased amyloidosis and hIAPP cytotoxicity in a cell culture model [57], but not in cultured islets [58]. In mice models, acute administration of a potent IDE inhibitor demonstrated beneficial effects on glucose tolerance [59], indicating potential for therapy of T2DM. However, given its wide distribution and other biochemical functions such as a chaperone and ubiquitin-proteosome pathway modulator, long term or untargeted use of inhibitors may be problematic [57][60].

4. Important Residues Involved in hIAPP Aggregation

Different mechanisms have been proposed explaining how monomers stack together and assemble into oligomers. Identifying exactly how the different conformations form and the key residues involved is important for developing therapeutics to inhibit aggregate formation, especially at the early stages of the process before extensive cellular damage occurs. NMR experiments using hIAPP peptides in solution suggest the initial events may involve adjacent monomers binding via noncovalent interactions involving residues 26–32 before assembling into oligomers [61]. Another study suggests a similar region, residues 29–33, as key to oligomer assembly, and also highlights residues 20–27 as important in fibril formation [62][63].

Several studies indicate that the amyloidogenic properties of human IAPP are linked to residues 20–29, one of the regions previously identified as key to oligomer assembly [64]. Residues 8–18 have also been identified as important in determining the side chain orientations along the β-strand within fibrils that have been shown to differ in fibril polymorphs produced in vitro [28][65]. A comparison of the primary sequence of human IAPP to that in rats (that do not form amyloid or get T2DM) reveals important differences (**Figure 1**). There are no proline residues in hIAPP, whereas rat IAPP has prolines at residues 25, 28 and 29. Molecular dynamics simulations demonstrated that proline residues show a low propensity of forming of β-sheet structures [64] and explains why hIAPP can more easily form β-sheet structures compared to rats. This provides compelling evidence associating IAPP aggregation with development of diabetes. This has been further corroborated in studies with transgenic rodent models, where rats that do not normally develop diabetes can become naturally diabetic if the human IAPP gene is inserted [66]. Researchers compared the tertiary structures of human (PDB 5MGQ) and rat IAPP (PDB 2KJ7) solved by NMR

and highlighted where in these 3D structures residues differed. Residues 18, 23, 25, 26, 28 and 29 differ between the two species.

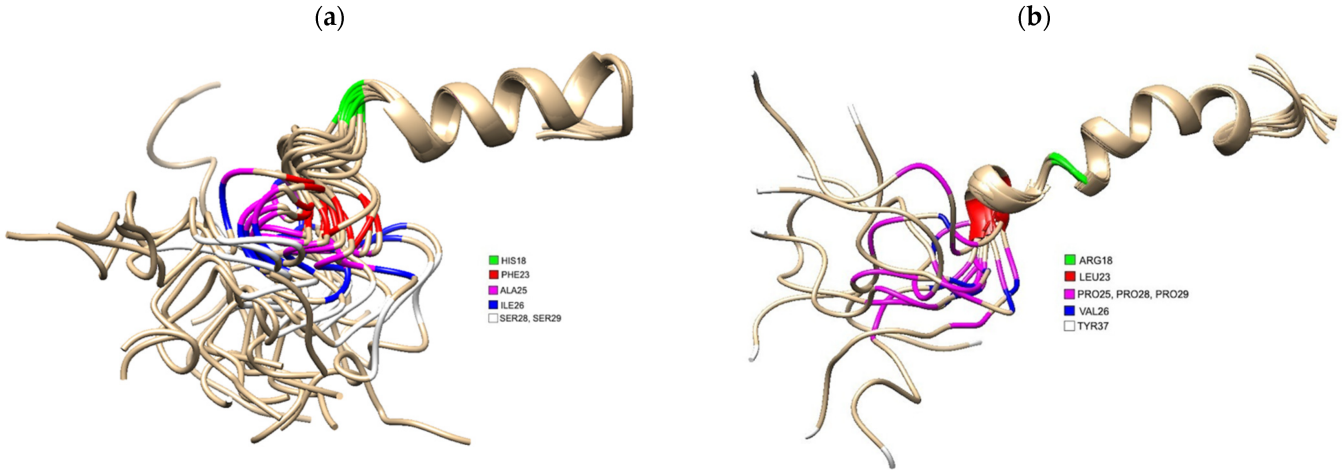

Figure 1. (**a**) 3D Structure of human IAPP residues 1–37 of the mature protein, solved by NMR (PDB 5MGQ) with key residues highlighted to show the difference compared to rat IAPP. The C and N terminus are located on the left and right, respectively. Residues were highlighted using USCF Chimera. HIS18 is highlighted in green, PHE23 is red, ALA25 is magenta, ILE26 is blue and SER28 and SER 29 are white.; (**b**) 3D Structure of rat IAPP residues 1–37, solved by NMR (PDB 2KJ7). Residues differing from hIAPP were highlighted using USCF Chimera. ARG18 is highlighted in green, LEU23 is red, PRO25, PRO28 and PRO 29 are magenta, VAL26 and TYR37 is white.

A Ser20Gly mutation found in Chinese and Japanese populations has been linked to an increased rate of amyloid aggregation, and an increased propensity to T2DM, further supporting the importance of the 20–29 region in amyloidosis [67]. The quantity of amylin produced is unaffected by the mutation, since mRNA levels observed in individuals with and without the mutation were comparable [68]. Cryo-EM structures reveal differences between wild type and S20G fibrils [40][41][42], both showing two protofilaments and a left-handed twist but differences in features such as repeat and crossover lengths. Differences observed in the backbone conformations could explain how the early onset T2D IAPP genetic polymorphism S20G can aggregate more readily than wildtype and may provide a structural explanation for surface-templated fibril assembly [41].

A conserved disulphide bond found outside the cross β-core is located between residues Cys 2 and Cys 7 of hIAPP. Removal of this bond through mutation or Cys-Cys disulphide reduction was shown to increase hIAPP amyloidosis while also decreasing hIAPP-induced membrane leakage, highlighting the importance of this region in fibril formation and related cytotoxicity [69].

5. The Role of hIAPP Oligomeric Intermediates in T2DM

Accumulating evidence suggests that hIAPP oligomers play a crucial role in cytotoxicity. Due to their soluble nature and short lifespan, it has been extremely difficult to isolate them to characterise their structure. Altamirano-Bustamante et al. [70] used various techniques (Immunoblotting, TEM, ultrastructural immunolocalization and CD

spectroscopy) to confirm the presence of oligomers in sera of healthy, T1DM, T2DM and obese children, utilising specific anti-oligomer antibodies. Samples from all groups showed the presence of fibrils and trimers, hexamers and dodecamers and oligomers of varying size were also observed (detected with anti-oligomer antibodies). Surprisingly, oligomers were also detected in sera from healthy children, but they had the lowest number of small oligomers out of all the groups, which are thought to be more toxic than the larger counterparts. The fact that oligomers have also been seen in healthy individuals is confusing, demonstrating that they are present in asymptomatic individuals and may not always contribute to disease. The observation of hIAPP oligomers in sera now requires further experiments to optimise purification methods to try to isolate them from sera so that they can be further characterised.

6. Mechanisms of hIAPP Cytotoxicity

HIAPP has been linked to cytotoxicity of pancreatic β-cells, but more recently other cells such as neuronal cells [71]. Whilst several mechanisms linking hIAPP and toxicity have been suggested (as summarised in **Figure 2**), the forms of hIAPP that mediate this toxicity is still debated, as are the precise mechanisms involved. Some studies propose that it is the fibrils that directly damage β-cells [72], while others suggest oligomeric intermediates are responsible [67]. However, more than one form of hIAPP may contribute to cytotoxicity, possibly to different extents that may be via a variety of mechanisms. Monomeric hIAPP has been observed to increase membrane fluidity, leading to membrane destabilisation [73], and has also been observed to increase production of reactive oxygen species (ROS) in β-cells [74]. Oligomeric hIAPP has been shown to decrease cell viability as well as increase membrane fluidity. Cell damage involving fibril growth at the membrane is linked to membrane leakage [75].

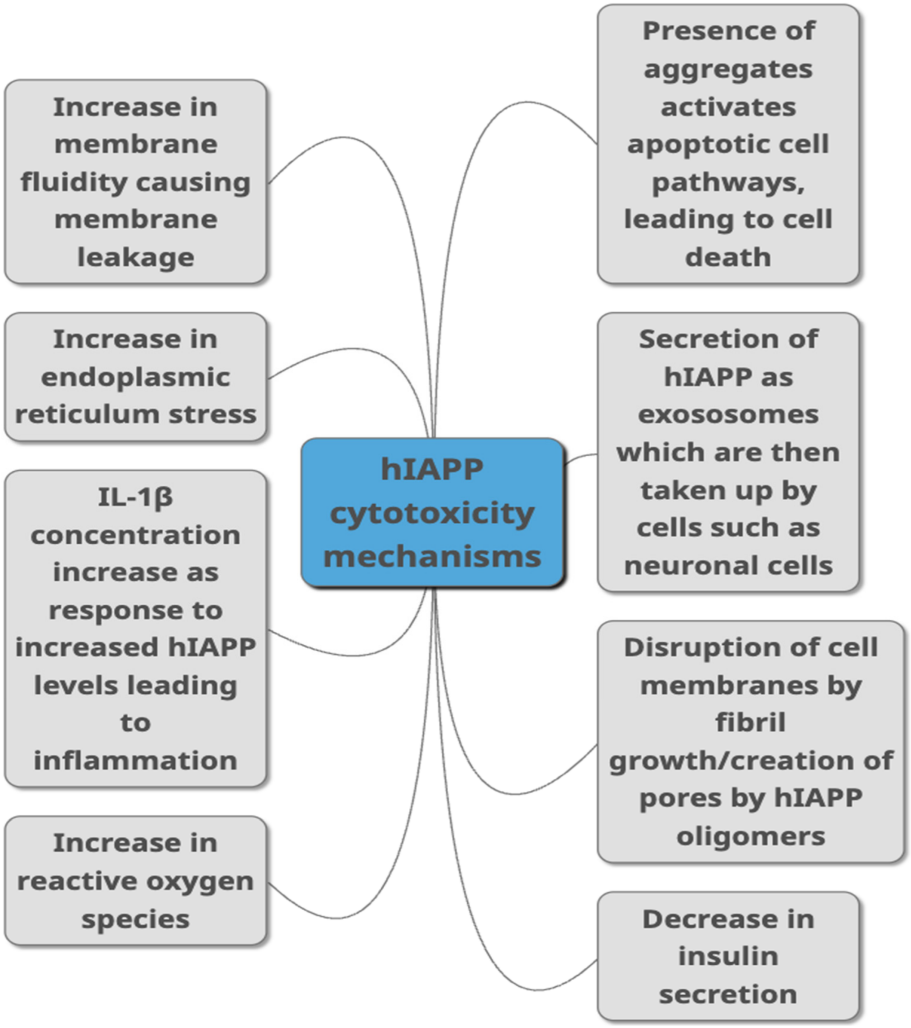

Figure 2. Proposed mechanisms through which hIAPP is linked to toxicity in T2DM as summarised from studies published in peer-reviewed journals. They include different ways of damaging the cells such as causing damage to the membrane, causing cell inflammation, damaging cells through aggregate build-up, by increasing ROS production and ER stress. Decreased insulin secretion, activation of apoptopic pathways and secretion via exosomes are other pathways suggested to link hIAPP with cytotoxicity.

7. Acceleration of hIAPP Misfolding and Aggregation

A crucial question in the understanding of hIAPP aggregate formation is what initiates and accelerates the formation of the fibrils that have been linked to T2DM. If researchers knew what triggered the misfolding cascade in the first place, then T2DM (and possibly other PMDs) may be preventable. Distinguishing between what initiates and what accelerates the process of amyloidosis is key but may involve the same factors. Several factors may contribute to the formation of these misfolded aggregates, and researchers have summarised these in **Figure 3**. These include the concentration of hIAPP, cell stress, the role of proteoglycans, the immune response and the association with other amyloidogenic proteins such as cross-seeding (**Figure 3**).

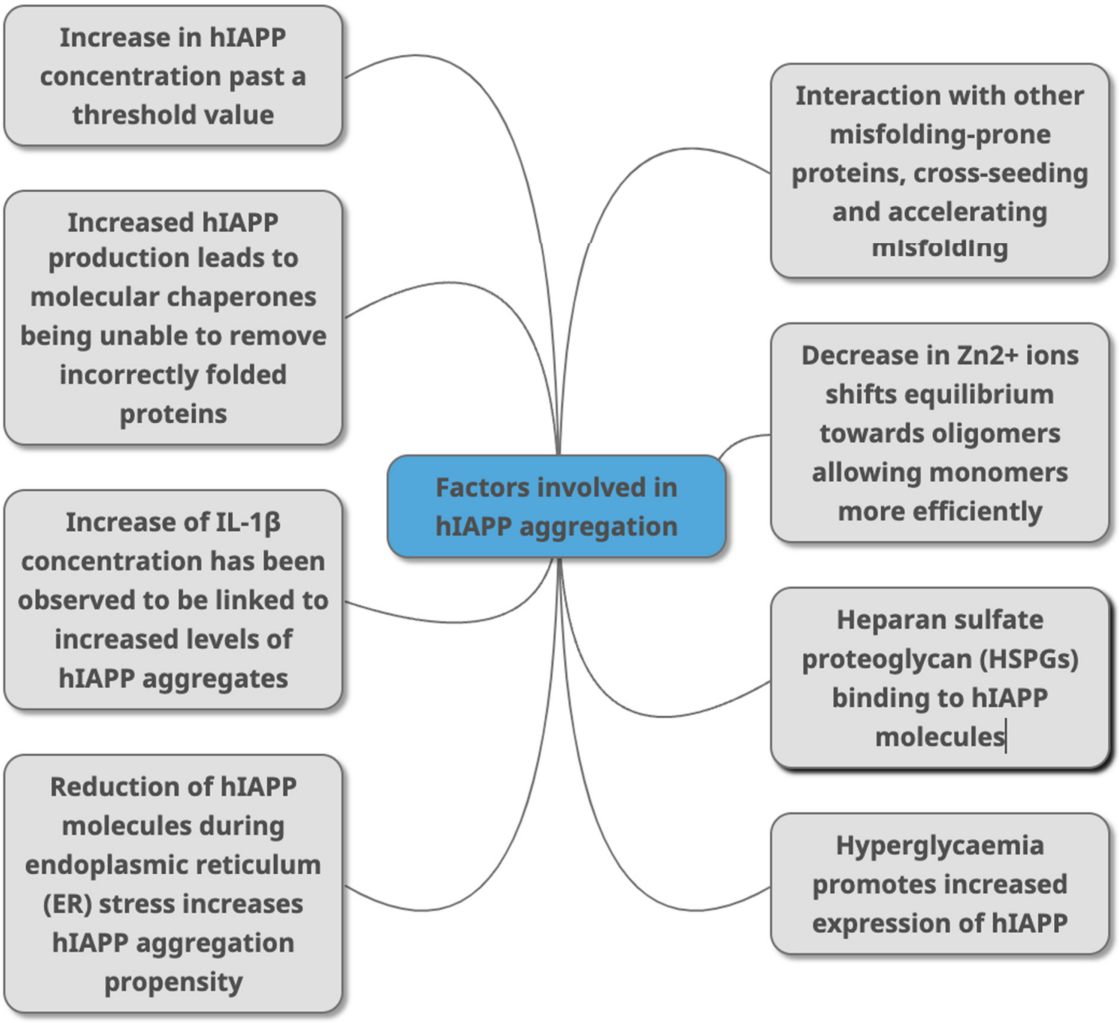

Figure 3. Factors that have been identified in peer-reviewed journals as contributing to hIAPP aggregation. This can occur due to change of concentrations of different molecules (HSPGs, Zn^{2+} IL-1β, Aβ, alpha synuclein) that directly or indirectly interact with hIAPP as part of various metabolic mechanisms (molecular chaperones, and through conditions linked with T2DM (hyperglycaemia, ER stress).

References

1. Mittal, K.; Mani, R.; Katare, D. Type 3 Diabetes: Cross Talk between Differentially Regulated Proteins of Type 2 Diabetes Mellitus and Alzheimer's Disease. Sci. Rep. 2016, 6, 25589.
2. Steck, A.; Winter, W. Review on monogenic diabetes. Curr. Opin. Endocrinol. Diabetes Obes. 2011, 18, 252–258.
3. Tan, S.; Mei Wong, J.; Sim, Y.; Wong, S.; Mohamed Elhassan, S.; Tan, S.; Ling Lim, G.; Rong Tay, N.; Annan, N.; Bhattamisra, S.; et al. Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. Diabetes Metab. Syndr. Clin. Res. Rev. 2019, 13, 364–372.

4. Mack, L.; Tomich, P. Gestational Diabetes. Obstet. Gynecol. Clin. N. Am. 2017, 44, 207–217.
5. Nguyen, T.; Ta, Q.; Nguyen, T.; Nguyen, T.; Van Giau, V. Type 3 diabetes and its role implications in Alzheimer's disease. Int. J. Mol. Sci. 2020, 21, 3165.
6. Giugliano, D.; Ceriello, A.; Esposito, K. Glucose metabolism and hyperglycemia. Am. J. Clin. Nutr. 2008, 87, 217S–222S.
7. Stratton, I. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): Prospective observational study. BMJ 2000, 321, 405–412.
8. World Health Organization. Available online: https://www.who.int (accessed on 6 April 2022).
9. Ling, C.; Rönn, T. Epigenetics in Human Obesity and Type 2 Diabetes. Cell Metab. 2019, 29, 1028–1044.
10. Xue, A.; Wu, Y.; Zhu, Z.; Zhang, F.; Kemper, K.E.; Zheng, Z.; Yengo, L.; Lloyd-Jones, L.R.; Sidorenko, J.; Wu, Y.; et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. Nat. Commun. 2018, 9, 2941.
11. Vujkovic, M.; Keaton, J.M.; Lynch, J.A.; Miller, D.R.; Zhou, J.; Tcheandjieu, C.; Huffman, J.E.; Assimes, T.L.; Lorenz, K.; Zhu, X.; et al. Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis. Nat. Genet. 2020, 52, 680–691.
12. Chooi, Y.C.; Ding, C.; Magkos, F. The epidemiology of obesity. Metabolism 2019, 92, 6–10.
13. Toplak, H.; Leitner, D.; Harreiter, J.; Hoppichler, F.; Wascher, T.; Schindler, K.; Ludvik, B. "Diabesity"—Adipositas und Typ-2-Diabetes (Update 2019). Wien. Klin. Wochenschr. 2019, 131, 71–76.
14. Pulgaron, E.R.; Delamater, A.M. Obesity and Type 2 Diabetes in Children: Epidemiology and Treatment. Curr. Diabetes Rep. 2014, 14, 508.
15. Weickert, M.O.; Pfeiffer, A.F. Impact of Dietary Fiber Consumption on Insulin Resistance and the Prevention of Type 2 Diabetes. J. Nutr. 2018, 148, 7–12.
16. Hill, J.; Nielsen, M.; Fox, M.H. Understanding the Social Factors That Contribute to Diabetes: A Means to Informing Health Care and Social Policies for the Chronically Ill. Perm. J. 2013, 17, 67–72.
17. Li, W.-Z.; Stirling, K.; Yang, J.-J.; Zhang, L. Gut microbiota and diabetes: From correlation to causality and mechanism. World J. Diabetes 2020, 11, 293–308.
18. Gurung, M.; Li, Z.; You, H.; Rodrigues, R.; Jump, D.B.; Morgun, A.; Shulzhenko, N. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine 2020, 51, 102590.

19. Cani, P.D.; Amar, J.; Iglesias, M.A.; Poggi, M.; Knauf, C.; Bastelica, D.; Neyrinck, A.M.; Fava, F.; Tuohy, K.M.; Chabo, C.; et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007, 56, 1761–1772.
20. Foretz, M.; Guigas, B.; Viollet, B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Nat. Rev. Endocrinol. 2019, 15, 569–589.
21. Sola, D.; Rossi, L.; Schianca, G.P.C.; Maffioli, P.; Bigliocca, M.; Mella, R.; Corlianò, F.; Fra, G.P.; Bartoli, E.; Derosa, G. State of the art paper Sulfonylureas and their use in clinical practice. Arch. Med. Sci. 2015, 4, 840–848.
22. Bishoyi, A.K.; Roham, P.H.; Rachineni, K.; Save, S.; Hazari, M.A.; Sharma, S.; Kumar, A. Human islet amyloid polypeptide (hIAPP)—A curse in type II diabetes mellitus: Insights from structure and toxicity studies. Biol. Chem. 2021, 402, 133–153.
23. Pandey, A.; Chawla, S.; Guchhait, P. Type-2 diabetes: Current understanding and future perspectives. IUBMB Life 2015, 67, 506–513.
24. Hanabusa, T.; Kubo, K.; Oki, C.; Nakano, Y.; Okai, K.; Sanke, T.; Nanjo, K. Islet amyloid polypeptide (IAPP) secretion from islet cells and its plasma concentration in patients with non-insulin-dependent diabetes mellitus. Diabetes Res. Clin. Pract. 1992, 15, 89–96.
25. Scollo, F.; La Rosa, C. Amyloidogenic Intrinsically Disordered Proteins: New Insights into Their Self-Assembly and Their Interaction with Membranes. Life 2020, 10, 144.
26. Castillo, M.J.; Scheen, A.J.; Lefèbvre, P.J. Amylin/islet amyloid polypeptide: Biochemistry, physiology, patho-physiology. Diabete Metab. 1995, 21, 3–25.
27. Westermark, P.; Engström, U.; Westermark, G.T.; Johnson, K.H.; Permerth, J.; Betsholtz, C. Islet amyloid polypeptide (IAPP) and pro-IAPP immunoreactivity in human islets of Langerhans. Diabetes Res. Clin. Pract. 1989, 7, 219–226.
28. Akter, R.; Cao, P.; Noor, H.; Ridgway, Z.; Tu, L.-H.; Wang, H.; Wong, A.G.; Zhang, X.; Abedini, A.; Schmidt, A.M.; et al. Islet Amyloid Polypeptide: Structure, Function, and Pathophysiology. J. Diabetes Res. 2016, 2016, 2798269.
29. Wang, J.; Xu, J.; Finnerty, J.; Furuta, M.; Steiner, D.F.; Verchere, C.B. The Prohormone Convertase Enzyme 2 (PC2) Is Essential for Processing Pro-Islet Amyloid Polypeptide at the NH2-Terminal Cleavage Site. Diabetes 2001, 50, 534–539.
30. Westermark, P.; Andersson, A.; Westermark, G.T. Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. Physiol. Rev. 2011, 91, 795–826.
31. Cao, P.; Abedini, A.; Raleigh, D.P. Aggregation of islet amyloid polypeptide: From physical chemistry to cell biology. Curr. Opin. Struct. Biol. 2012, 23, 82–89.

32. Mulder, H.; Ahren, B.; Sundler, F. Islet amyloid polypeptide and insulin gene expression are regulated in parallel by glucose in vivo in rats. Am. J. Physiol. Metab. 1996, 271, E1008–E1014.
33. Mulder, H.; Gebre-Medhin, S.; Betsholtz, C.; Sundler, F.; Ahrén, B. Islet amyloid polypeptide (amylin)-deficient mice develop a more severe form of alloxan-induced diabetes. Am. J. Physiol. Endocrinol. Metab. 2000, 278, E684–E691.
34. Moore, S.J.; Sonar, K.; Bharadwaj, P.; Deplazes, E.; Mancera, R.L. Characterisation of the Structure and Oligomerisation of Islet Amyloid Polypeptides (IAPP): A Review of Molecular Dynamics Simulation Studies. Molecules 2018, 23, 2142.
35. Higham, C.E.; Jaikaran, E.T.; Fraser, P.E.; Gross, M.; Clark, A. Preparation of synthetic human islet amyloid polypeptide (IAPP) in a stable conformation to enable study of conversion to amyloid-like fibrils. FEBS Lett. 2000, 470, 55–60.
36. Bedrood, S.; Li, Y.; Isas, J.M.; Hegde, B.G.; Baxa, U.; Haworth, I.S.; Langen, R. Fibril Structure of Human Islet Amyloid Polypeptide. J. Biol. Chem. 2012, 287, 5235–5241.
37. Qiao, Q.; Bowman, G.R.; Huang, X. Dynamics of an Intrinsically Disordered Protein Reveal Metastable Conformations That Potentially Seed Aggregation. J. Am. Chem. Soc. 2013, 135, 16092–16101.
38. Fu, Z.; Aucoin, D.; Davis, J.; Van Nostrand, W.E.; Smith, S.O. Mechanism of Nucleated Conformational Conversion of Aβ42. Biochemistry 2015, 54, 4197–4207.
39. Paul, F.; Weikl, T.R. How to Distinguish Conformational Selection and Induced Fit Based on Chemical Relaxation Rates. PLOS Comput. Biol. 2016, 12, e1005067.
40. Cao, Q.; Boyer, D.; Sawaya, M.; Ge, P.; Eisenberg, D. Cryo-EM structure and inhibitor design of humanIAPP (amylin) fibrils. Nat. Struct. Mol. Biol. 2020, 27, 653–659.
41. Gallardo, R.; Iadanza, M.G.; Xu, Y.; Heath, G.R.; Foster, R.; Radford, S.E.; Ranson, N.A. Fibril structures of diabetes-related amylin variants reveal a basis for surface-templated assembly. Nat. Struct. Mol. Biol. 2020, 27, 1048–1056.
42. Röder, C.; Kupreichyk, T.; Gremer, L.; Schäfer, L.U.; Pothula, K.R.; Ravelli, R.B.G.; Willbold, D.; Hoyer, W.; Schröder, G.F. Cryo-EM structure of islet amyloid polypeptide fibrils reveals similarities with amyloid-β fibrils. Nat. Struct. Mol. Biol. 2020, 27, 660–667.
43. Zhang, X.; Li, D.; Zhu, X.; Wang, Y.; Zhu, P. Structural characterization and cryo-electron tomography analysis of human islet amyloid polypeptide suggest a synchronous process of the hIAPP1−37 amyloid fibrillation. Biochem. Biophys. Res. Commun. 2020, 533, 125–131.
44. Cao, Q.; Boyer, D.R.; Sawaya, M.R.; Abskharon, R.; Saelices, L.; Nguyen, B.A.; Lu, J.; Murray, K.A.; Kandeel, F.; Eisenberg, D.S. Cryo-EM structures of hIAPP fibrils seeded by patient-

extracted fibrils reveal new polymorphs and conserved fibril cores. Nat. Struct. Mol. Biol. 2021, 28, 724–730.

45. Li, M.S.; Klimov, D.K.; Straub, J.E.; Thirumalai, D. Probing the mechanisms of fibril formation using lattice models. J. Chem. Phys. 2008, 129, 175101.
46. Brender, J.; Dürr, U.H.; Heyl, D.; Budarapu, M.B.; Ramamoorthy, A. Membrane fragmentation by an amyloidogenic fragment of human Islet Amyloid Polypeptide detected by solid-state NMR spectroscopy of membrane nanotubes. Biochim. Biophys. Acta (BBA) 2007, 1768, 2026–2029.
47. Apostolidou, M.; Jayasinghe, S.A.; Langen, R. Structure of α-Helical Membrane-bound Human Islet Amyloid Polypeptide and Its Implications for Membrane-mediated Misfolding. J. Biol. Chem. 2008, 283, 17205–17210.
48. Zhang, X.; Clair, J.R.S.; London, E.; Raleigh, D.P. Islet Amyloid Polypeptide Membrane Interactions: Effects of Membrane Composition. Biochemistry 2017, 56, 376–390.
49. Sasahara, K. Membrane-mediated amyloid deposition of human islet amyloid polypeptide. Biophys. Rev. 2018, 10, 453–462.
50. Caillon, L.; Hoffmann, A.R.F.; Botz, A.; Khemtemourian, L. Molecular Structure, Membrane Interactions, and Toxicity of the Islet Amyloid Polypeptide in Type 2 Diabetes Mellitus. J. Diabetes Res. 2016, 2016, 5639875.
51. Bhowmick, D.C.; Kudaibergenova, Z.; Burnett, L.; Jeremic, A.M. Molecular Mechanisms of Amylin Turnover, Misfolding and Toxicity in the Pancreas. Molecules 2022, 27, 1021.
52. Knight, J.D.; Miranker, A.D. Phospholipid catalysis of diabetic amyloid assembly. J. Mol. Biol. 2004, 341, 1175–1187.
53. Cho, W.-J.; Trikha, S.; Jeremic, A.M. Cholesterol Regulates Assembly of Human Islet Amyloid Polypeptide on Model Membranes. J. Mol. Biol. 2009, 393, 765–775.
54. Pivovarova, O.; Höhn, A.; Grune, T.; Pfeiffer, A.F.; Rudovich, N. Insulin-degrading enzyme: New therapeutic target for diabetes and Alzheimer’s disease? Ann. Med. 2016, 48, 614–624.
55. Bennett, R.G.; Duckworth, W.C.; Hamel, F.G. Degradation of Amylin by Insulin-degrading Enzyme. J. Biol. Chem. 2000, 275, 36621–36625.
56. Sousa, L.; Guarda, M.; Meneses, M.J.; Macedo, M.P.; Miranda, H.V. Insulin-degrading enzyme: An ally against metabolic and neurodegenerative diseases. J. Pathol. 2021, 255, 346–361.
57. Bennett, R.G.; Hamel, F.G.; Duckworth, W.C. An Insulin-Degrading Enzyme Inhibitor Decreases Amylin Degradation, Increases Amylin-Induced Cytotoxicity, and Increases Amyloid Formation in Insulinoma Cell Cultures. Diabetes 2003, 52, 2315–2320.

58. Hogan, M.F.; Zeman-Meier, D.; Zraika, S.; Templin, A.; Mellati, M.; Hull, R.L.; Leissring, M.A.; Kahn, S.E. Inhibition of Insulin-Degrading Enzyme Does Not Increase Islet Amyloid Deposition in Vitro. Endocrinology 2016, 157, 3462–3468.
59. Maianti, J.P.; McFedries, A.; Foda, Z.H.; Kleiner, R.E.; Du, X.Q.; Leissring, M.A.; Tang, W.-J.; Charron, M.J.; Seeliger, M.A.; Saghatelian, A.; et al. Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones. Nature 2014, 511, 94–98.
60. Tang, W.-J. Targeting Insulin-Degrading Enzyme to Treat Type 2 Diabetes Mellitus. Trends Endocrinol. Metab. 2016, 27, 24–34.
61. Luca, S.; Yau, W.-M.; Leapman, R.; Tycko, R. Peptide Conformation and Supramolecular Organization in Amylin Fibrils: Constraints from Solid-State NMR. Biochemistry 2007, 46, 13505–13522.
62. Wiltzius, J.J.; Sievers, S.A.; Sawaya, M.R.; Cascio, D.; Popov, D.; Riekel, C.; Eisenberg, D. Atomic structure of the cross-β spine of islet amyloid polypeptide (amylin). Protein Sci. 2008, 17, 1467–1474.
63. Raleigh, D.; Zhang, X.; Hastoy, B.; Clark, A. The β-cell assassin: IAPP cytotoxicity. J. Mol. Endocrinol. 2017, 59, R121–R140.
64. Hoffmann, K.Q.; McGovern, M.; Chiu, C.-C.; De Pablo, J.J. Secondary Structure of Rat and Human Amylin across Force Fields. PLoS ONE 2015, 10, e0134091.
65. Wineman-Fisher, V.; Atsmon-Raz, Y.; Miller, Y. Orientations of Residues along the β-Arch of Self-Assembled Amylin Fibril-Like Structures Lead to Polymorphism. Biomacromolecules 2015, 16, 156–165.
66. Matveyenko, A.V.; Butler, P.C. β-Cell Deficit Due to Increased Apoptosis in the Human Islet Amyloid Polypeptide Transgenic (HIP) Rat Recapitulates the Metabolic Defects Present in Type 2 Diabetes. Diabetes 2006, 55, 2106–2114.
67. Krotee, P.; Rodriguez, J.A.; Sawaya, M.R.; Cascio, D.; Reyes, F.E.; Shi, D.; Hattne, J.; Nannenga, B.; Oskarsson, M.E.; Philipp, S.; et al. Atomic structures of fibrillar segments of hIAPP suggest tightly mated β-sheets are important for cytotoxicity. eLife 2017, 6, e19273.
68. Zeman-Meier, D.; Entrup, L.; Templin, A.; Hogan, M.F.; Mellati, M.; Zraika, S.; Hull, R.L.; Kahn, S.E. The S20G substitution in hIAPP is more amyloidogenic and cytotoxic than wild-type hIAPP in mouse islets. Diabetologia 2016, 59, 2166–2171.
69. Ridgway, Z.; Zhang, X.; Wong, A.G.; Abedini, A.; Schmidt, A.M.; Raleigh, D.P. Analysis of the Role of the Conserved Disulfide in Amyloid Formation by Human Islet Amyloid Polypeptide in Homogeneous and Heterogeneous Environments. Biochemistry 2018, 57, 3065–3074.

70. Altamirano-Bustamante, M.M.; Altamirano-Bustamante, N.F.; Larralde-Laborde, M.; Lara-Martínez, R.; Leyva-García, E.; Garrido-Magaña, E.; Rojas, G.; Jiménez-García, L.F.; Monsalve, M.C.R.; Altamirano, P.; et al. Unpacking the aggregation-oligomerization-fibrillization process of naturally-occurring hIAPP amyloid oligomers isolated directly from sera of children with obesity or diabetes mellitus. Sci. Rep. 2019, 9, 18465.
71. Burillo, J.; Fernández-Rhodes, M.; Piquero, M.; López-Alvarado, P.; Menéndez, J.; Jiménez, B.; González-Blanco, C.; Marqués, P.; Guillén, C.; Benito, M. Human amylin aggregates release within exosomes as a protective mechanism in pancreatic β cells: Pancreatic β-hippocampal cell communication. Biochim. Biophys. Acta 2021, 1868, 118971.
72. Hiddinga, H.J.; Eberhardt, N.L. Intracellular Amyloidogenesis by Human Islet Amyloid Polypeptide Induces Apoptosis in COS-1 Cells. Am. J. Pathol. 1999, 154, 1077–1088.
73. Bag, N.; Ali, A.; Chauhan, V.S.; Wohland, T.; Mishra, A. Membrane destabilization by monomeric hIAPP observed by imaging fluorescence correlation spectroscopy. Chem. Commun. 2013, 49, 9155–9157.
74. Pilkington, E.; Gurzov, E.; Kakinen, A.; A Litwak, S.; Stanley, W.J.W.; Davis, T.P.T.; Ke, P.C. Pancreatic β-Cell Membrane Fluidity and Toxicity Induced by Human Islet Amyloid Polypeptide Species. Sci. Rep. 2016, 6, 21274.
75. Engel, M.F.M.; Khemtémourian, L.; Kleijer, C.C.; Meeldijk, H.J.D.; Jacobs, J.; Verkleij, A.J.; de Kruijff, B.; Killian, J.A.; Höppener, J.W.M. Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane. Proc. Natl. Acad. Sci. USA 2008, 105, 6033–6038.