Brain insulin resistance: an early risk factor for Alzheimer's disease development in Down syndrome

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Down syndrome (DS) is the most frequent chromosomal abnormality that causes intellectual disability, resulting from the presence of an extra complete or segment of chromosome 21 (HSA21) (Tramutola et al., 2020; Lanzillotta et al., 2021). Every year, approximately 6000 children are born with DS and most of them do not have an autonomous life. Thanks to the advancement in medical care, DS individuals live long and often outlive their parents (Lott and Head, 2019). As a consequence, individuals with DS are now experiencing a high incidence of age-associated health problems, especially Alzheimer's disease (AD) dementia (Lott and Head, 2019). In particular, by the age of 40 years, virtually all individuals with DS show AD neuropathology (Lott and Head, 2019). The link between AD and DS is thought to be mainly related to the triplication of the amyloid precursor gene (*APP*), which is encoded on HSA21. However, trisomy of HSA21 results in increased gene dosage for other genes in addition to *APP*, which may also be involved in AD development. These include superoxide dismutase 1, which is involved in redox metabolism; adenosine triphosphate (ATP)-binding cassette sub-family G member 1, which is involved in cholesterol metabolism; cystatin B, beta-secretase 2, and synaptojanin 1 involved in beta-amyloid (Aβ) processing and clearance; the dual-specificity tyrosine phosphorylation-regulated kinase-1A, which is involved in Tau phosphorylation; regulator of calcineurin, which is involved in mitochondrial dysfunction; *S100B* involved in inflammatory responses (Lott and Head, 2019).

In addition to the above-mentioned mechanisms, another potential pathway of AD pathogenesis in DS is represented by the dysregulation of brain insulin signaling (Tramutola et al., 2020; Lanzillotta et al., 2021). Indeed, molecules regulating glucose metabolism, i.e., insulin, are being widely investigated due to their impact on learning and memory, brain development, and aging (Arnold et al., 2018). Brain insulin resistance is referred to as an inadequate response to insulin by brain cells. Interestingly, an increased accumulation of markers for brain insulin resistance was observed early in AD, and brain insulin resistance seems to greatly contribute to the long preclinical period during which often only subtle symptoms are evident in AD (Arnold et al., 2018). Brain insulin resistance impairs synaptic integrity, and Aβ and Tau can also interfere with the actions of insulin at the synapse (Arnold et al., 2018). To note, both synaptic compromise and brain insulin resistance are thought to be present at the earliest stages of AD (Arnold et al., 2018). Mechanistically, brain insulin resistance is due to the downregulation of insulin receptor (IR) or a faulty activation of the insulin signaling cascade mainly driven by the insulin receptor substrate 1 (IRS1) inhibition (Arnold et al., 2018) (**Figure 1**). Indeed, IR downregulation reduces the pool of IR protein at the level of the plasma membrane available to bind insulin. Rather, the inhibition of IRS1 – mediated by the phosphorylation of specific serine residues (307, 312, 636) – leads the uncoupling of IRS1 from the IR, finally responsible for the inability of insulin to promote its downstream effects (Arnold et al., 2018).

Our group recently reported for the first time about the accumulation of markers of brain insulin resistance in the brain of young DS individuals (< 40 years) already before the development of AD pathology (Tramutola et al., 2020). Remarkably, similar to previous observations in AD (Talbot et al., 2012) an overall uncoupling among members of the insulin signaling pathway can be observed in

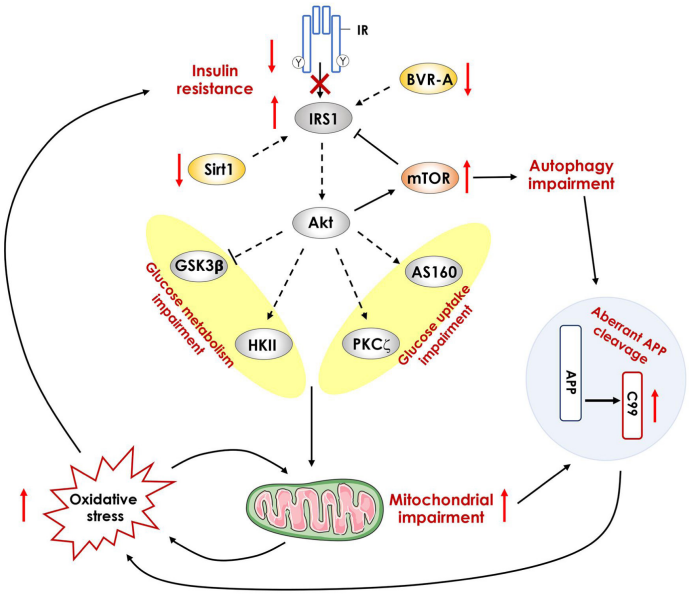

Figure 1 | **Schematic representation of insulin signaling pathway with highlighted proteins found to be altered in DS brain.**
Under physiological conditions, binding of insulin to the insulin receptor (IR) leads to IR autophosphorylation on Tyr residues (e.g., Tyr1158/1162/1163), which regulate IR activation. In turn, once activated, IR promotes the phosphorylation of its substrate (insulin receptor substrate-1, IRS1) on specific Tyr residues (e.g., 632). This event drives the activation of the phosphoinositide 3-kinase/Akt (PI3K-Akt) pathways downstream from IRS1. Akt promotes the phosphorylation of several targets, among which are: (1) Akt substrate of 160 (AS160) (on Thr642, activating site) that together with the atypical protein kinase C zeta (PKC), are responsible for the translocation of glucose transporter-4 (GLUT4)-containing vesicles to the plasma membrane to mediate glucose uptake; (2) Hexokinase-II (HKII) a rate-limiting enzyme involved in glucose metabolism and mitochondrial functions; (3) Glycogen synthase kinase 3 beta (GSK3) (on Ser9, inhibitory site), which has a role in energy production; and (4) the mammalian target of rapamycin (mTOR) (on Ser2448, activating site), which regulates protein synthesis and autophagy. Under pathological conditions, IRS1 can be phosphorylated on Ser residues (e.g., Ser307 and Ser636) and this event is responsible for the uncoupling between IR and IRS1. This molecular event is at the base of the insulin resistance phenomenon and means that even if insulin can bind to IR, this latter is not able to promote IRS1 activation. Hence, pathways downstream from IRS1 cannot be activated in response to insulin. Brain insulin resistance is further associated with an impairment of mitochondrial bioenergetics. Mitochondrial failure leads to increased oxidative stress as well as favors the amyloid precursor protein (APP) amyloidogenic cleavage, responsible for APP C-terminal fragment 99 (APP-C99) and eventually beta amyloid (Aβ) accumulation. Increased production of APP-C99 and Aβ can be sustained by the hyperactivation of mTOR, which inhibits autophagy. Finally, among the proteins known to regulate insulin signalling both biliverdin reductase-A (BVR-A) and NAD-dependent deacetylase sirtuin (Sirt1) are known to favor the correct activation of IRS1 and thus the consequent activation of the insulin signalling pathway. Dotted arrows: decreased activity in young Down syndrome (DS); plain arrows/lines: increased activity in DS; red arrows highlight proteins/pathways upregulated or downregulated in DS brain and Ts65dn mice.

the young DS brain (Tramutola et al., 2020). In particular, reduced IR protein levels and increased IRS1 inhibition were found (Tramutola et al., 2020) (**Figure 1**). These alterations parallel the loss of Akt-mediated regulation of IRS1 downstream targets including (i) glycogen synthase kinase 3 beta (GSK3β) inhibition, (ii) AS160 activation; (iii) PKCζ levels; and (iv) HKII levels (Tramutola et al., 2020) (**Figure 1**).

Altered brain insulin signaling also is associated with defects of glucose uptake in DS. Our data show the existence of defects in the mechanisms responsible for GLUT4 translocation to the plasma membrane (Tramutola et al., 2020). Although most glucose uptake in neurons mainly occurs via GLUT3, insulin-regulated GLUT4 is also co-expressed with GLUT3 in brain regions related to cognitive behaviors (Arnold et al., 2018). These regions include the basal forebrain, hippocampus, amygdala and, to lesser degrees, the cerebral cortex and cerebellum. Under physiological conditions, insulin induces GLUT4 translocation to the neuronal cell membrane via an Akt-dependent mechanism (White and Copps, 2016) and is thought to improve glucose flux into neurons during periods of high metabolic demand, such as learning and memory (Arnold et al., 2018). Rather, we observed reduced AS160 activation and reduced PKCζ protein levels, both proteins regulating GLUT4-containing vesicles translocation to the plasma membrane (White and Copps, 2016) (**Figure 1**).

In addition to that, we hypothesized a reduction of glucose utilization and oxidative metabolism based on the observation that HKII and mitochondrial complexes levels are reduced in DS brain (Tramutola et al., 2020). Mitochondria are essential for the control of energy metabolism and their dysfunction plays a key role in the pathophysiology of AD both in the general population and in DS individuals (Di Domenico et al., 2017; Arnold et al., 2018; Lott and Head, 2019). Indeed, neurons are highly dependent on energy supply; thus, even slight changes in the process of ATP generation (through glycolytic and/or mitochondrial pathways) can interfere with their viability (Di Domenico et al., 2017). Increased oxidative stress levels driven by mitochondrial dysfunction, associated with a drop in ATP production, will trigger neuronal degeneration and death (Di Domenico et al., 2017). Since mitochondria are neurons' main power source, even slight alterations in their function can result in vital perturbations (Di Domenico et al., 2017). Results collected in DS brain agree with this paradigm and are further strengthened by previous observations coming from redox proteomics studies performed by our group showing the irreversible oxidative modification of enzymes regulating energy metabolism (i.e. glyceraldehyde 3-phosphate dehydrogenase (GAPDH), enolase, malate dehydrogenase, creatine kinase, ATP synthase), that likely impairs their activity, both in DS and AD post mortem brain (Di Domenico et al., 2017).

Notwithstanding with that, while mitochondrial dysfunction has been extensively reported both in AD and DS, whether brain insulin resistance is among the driving mechanisms responsible for mitochondrial defects in the brain is not known. Several works, including ours, proposed an association between brain insulin resistance and defects of mitochondrial machinery/bioenergetics. Indeed, a number of proteins within the insulin siganling cascade also are known to have a role in regulating mitochondrial functions, i.e., Akt, GSK3β, mammalian target of rapamycin (mTOR), and adenosine 5′-monophosphate-activated protein kinase (AMPK) among the others (Di Domenico et al., 2017; Arnold et al., 2018; Lott and Head, 2019). For that reason, one could speculate that development of brain insulin resistance, by impairing the activation of the above-mentioned proteins might lead to mitochorial alterations, finally responsible for reducing energy production and increased oxidative stress. However, mechanistic insights are missing and represent an intriguing aspect that warrants further analyses.

The concept of early-onset brain insulin resistance in DS was strengthened by the results collected in a recent longitudinal study performed in Ts65dn mice (a well-known model to study DS) (Lanzillotta et al., 2021). This study describes potential molecular mechanisms responsible for brain insulin resistance development in DS. We proposed the existence of crosstalk among insulin resistance, oxidative stress, and mitochondrial defects in Ts65dn mice brain, likely contributing to brain alterations at the base of intellectual disability and the development of dementia in DS (Lanzillotta et al., 2021).

In the above-mentioned longitudinal study, we identified two phases, i.e., 1 month and 9 months, during which accumulation of brain insulin resistance markers are more evident in Ts65dn mice (Lanzillotta et al., 2021). These two phases reflect two different stages of brain physiology: an early phase (1 month) during which brain is still at a developmental stage, and a late phase (9 months), which is likely the time during which the risk of AD-like neurodegeneration becomes higher (Lanzillotta et al., 2021).

In agreement with data collected in humans, results from Ts65dn mice reveal that proteins of the insulin signaling mainly present with perturbations in phosphorylation (an index of their activation), that are not consistent among adjacent kinases – and thus consecutive steps – in the insulin signaling cascade (Lanzillotta et al., 2021) (**Figure 1**). Our idea is that proteins are uncoupled from each other and fail in adequately responding to insulin stimulation.

Similar to what observed in the human brain, increased IRS1 inhibition both at 1 and 9 months of age are associated with defects of mitochondrial machinery (reduced levels of Complex I, III and IV) and increased oxidative stress markers, i.e. protein-bound 4-hydroxy-2-nonenal and 3-nitrotyrosine in Ts65dn mice brain (Lanzillotta et al., 2021) (**Figure 1**). Together, these alterations contribute to the impairment of brain energy metabolism, ultimately resulting in neuronal damage in DS.

To understand "who does what" our study also identified specific molecular targets, whose fault activation may favor the development of brain insulin resistance at 1 month and the further upswing observed at 9 months. Increased oxidative stress levels and reduced Sirt1 levels may have a role in the development of brain insulin resistance in juvenile mice (Lanzillotta et al., 2021) (**Figure 1**). Reduced biliverdin reductase-A (BVR-A) activation and the hyper-activation of the mTOR may have a role in the development of brain insulin resistance in older mice (Lanzillotta et al., 2021) (**Figure 1**).

In agreement, elevated levels of oxidative stress markers were found in amniotic fluid from mothers currying DS fetuses as well as in fibroblast isolated from DS fetuses (Di Domenico et al., 2017). Moreover, both hydrogen peroxide and peroxynitrite administration lead to IRS1 inhibition and insulin resistance in neuronal cells *in vitro* (Barone et al., 2019). Furthermore, Sirt1 was shown to favor IRS1 Tyr phosphorylation and to safeguard cells from mitochondrial impairment and oxidative stress (Lanzillotta et al., 2021). Therefore, it is conceivable to think that increased oxidative stress levels and reduced Sirt1 levels favor the onset of brain insulin resistance early in life in Ts65dn mice, contributing to an altered energy metabolism responsible for defects in brain development observed in DS.

Similarly, we believe that BVR-A and mTOR have a role at 9 months. We previously demonstrated either *in vitro* or *in vivo* that reduced BVR-A activation leads to IRS1 inhibition through the hyper-activation of mTOR (White and Copps, 2016; Lanzillotta et al., 2020). As consequence, we observed the accumulation of AD neuropathological hallmarks including Aβ and phosphorylated Tau (Barone et al., 2019). To note, mTOR

hyperactivation is a well-recognized alteration found both in DS and AD brain (Di Domenico et al., 2018). The importance of mTOR relies upon the fact that other than promoting brain insulin resistance, mTOR hyperactivation is responsible for the inhibition of degradative systems, i.e., autophagy, which results in the accumulation of neurotoxic aggregates both DS and AD brain (Di Domenico et al., 2018). One key aspect of our research regards the co-existence of brain insulin resistance with the accumulation of toxic amyloid precursor protein (APP) by-products, i.e., APP-C99, observed both in young DS individuals (Tramutola et al., 2020) and in Ts65dn mice (Lanzillotta et al., 2021) (**Figure 1**). Increased APP-C99 may contribute to the buildup of intraneuronal Aβ and other oxidized substrates that are responsible for damaging cellular components in DS brain thus triggering the development of AD-like neuropathology. Then, as in a vicious cycle, Aβ generation further exacerbates brain insulin resistance possibly through different mechanisms including TNFα-mediated inhibition of IRS1 (Barone et al., 2019).

Finally, our results are of particular interest in interpreting the molecular basis of deficits in learning and memory observed in DS population. Insulin plays a role in synaptic plasticity mechanisms and memory formation in rodents and humans, and, importantly, the development of brain insulin resistance impairs cognitive and learning functions (Arnold et al., 2018). In agreement with that, the accumulation of brain insulin resistance markers was associated with loss of synaptic proteins, e.g. syntaxin-1, post-synaptic density protein 95, and reduced brain-derived neurotrophic factor levels both in human and Ts65dn mice brain. Of great interest appears the consistent reduction of syntaxin-1 observed already at a young age. Loss of syntaxin-1 severely compromised neuronal viability *in vivo* and *in vitro*, indicating an obligatory role of syntaxin-1 for the maintenance of developing and mature neurons (Vardar et al., 2016).

In conclusion, our studies underlie that increasing evidence from biomarker studies suggests that the pathophysiology of AD in DS is similar to that of the sporadic and autosomal dominant forms of AD in the general population. Notwithstanding with that, while longitudinal studies have shown the reliability of measurements of brain insulin resistance markers, i.e. inhibited IRS1, in predicting AD development in the general population (Kapogiannis et al., 2019), the history of biomarker changes in DS has not been established. Conversely, the characterization of the preclinical phases of AD in DS would provide insights for early diagnosis in this susceptible group of people. Considering that the fields of DS and AD research have many points of synergy, including genetics, pathogenesis and clinical manifestations, our results suggest that understanding age-associated changes of brain insulin resistance markers could be crucial for the design of trials in DS aimed at preventing or moderating AD progression.

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References

Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, Ahima RS, Craft S, Gandy S, Buettner C, Stoeckel LE, Holtzman DM, Nathan DM (2018) Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol 14:168-181.

Barone E, Tramutola A, Triani F, Calcagnini S, Di Domenico F, Ripoli C, Gaetani S, Grassi C, Butterfield DA, Cassano T, Perluigi M (2019) Biliverdin reductase-A mediates the beneficial effects of intranasal insulin in Alzheimer disease. Mol Neurobiol 56:2922-2943.

Di Domenico F, Barone E, Perluigi M, Butterfield DA (2017) The triangle of death in Alzheimer's disease brain: the aberrant cross-talk among energy metabolism, mammalian target of rapamycin signaling, and protein homeostasis revealed by redox proteomics. Antioxid Redox Signal 26:364-387.

Di Domenico F, Tramutola A, Foppoli C, Head E, Perluigi M, Butterfield DA (2018) mTOR in Down syndrome: role in Ass and tau neuropathology and transition to Alzheimer disease-like dementia. Free Radic Biol Med 114:94-101.

Kapogiannis D, Mustapic M, Shardell MD, Berkowitz ST, Diehl TC, Spangler RD, Tran J, Lazaropoulos MP, Chawla S, Gulyani S, Eitan E, An Y, Huang CW, Oh ES, Lyketsos CG, Resnick SM, Goetzl EJ, Ferrucci L (2019) Association of extracellular vesicle biomarkers with Alzheimer disease in the baltimore longitudinal study of aging. JAMA Neurol 76:1340-1351.

Lanzillotta C, Zuliani I, Vasavda C, Snyder SH, Paul BD, Perluigi M, Di Domenico F, Barone E (2020) BVR-A deficiency leads to autophagy impairment through the dysregulation of AMPK/mTOR axis in the brain-implications for neurodegeneration. Antioxidants (Basel) 9:671.

Lanzillotta C, Tramutola A, Di Giacomo G, Marini F, Butterfield DA, Di Domenico F, Perluigi M, Barone E (2021) Insulin resistance, oxidative stress and mitochondrial defects in Ts65dn mice brain: a harmful synergistic path in down syndrome. Free Radic Biol Med 165:152-170.

Lott IT, Head E (2019) Dementia in Down syndrome: unique insights for Alzheimer disease research. Nat Rev Neurol 15:135-147.

Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, Fuino RL, Kawaguchi KR, Samoyedny AJ, Wilson RS, Arvanitakis Z, Schneider JA, Wolf BA, Bennett DA, Trojanowski JQ, Arnold SE (2012) Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest 122:1316-1338.

Tramutola A, Lanzillotta C, Di Domenico F, Head E, Butterfield DA, Perluigi M, Barone E (2020) Brain insulin resistance triggers early onset Alzheimer disease in Down syndrome. Neurobiol Dis 137:104772.

Vardar G, Chang S, Arancillo M, Wu YJ, Trimbuch T, Rosenmund C (2016) Distinct functions of syntaxin-1 in neuronal maintenance, synaptic vesicle docking, and fusion in mouse neurons. J Neurosci 36:7911-7924.

White MF, Copps KD (2016) The mechanisms of insulin action. In: Endocrinology: adult and pediatric, 7th ed (Saunders WB, ed), pp556-585. Amsterdam: Elsevier.

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