Metformin to treat Huntington disease: a pleiotropic drug against a multi-system disorder

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Highlights

Huntington disease is a multi-system disorder and huntingtin is expressed ubiquitously.

Metformin is a pleiotropic drug which may reach most tissues, and activates a range of targets beneficial to treat HD.

Gut microbiota interplay with metformin substantially increases complexity of using this drug to treat HD

Studying targets of metformin, gut microbiota and using pharmacogenetics, may help design personalised medicine to treat HD

Running title: Pleiotropic metformin to bend ubiquitous mutant huntingtin

ABSTRACT

Huntington disease (HD) is a neurodegenerative disorder produced by an expansion of CAG repeats in the *HTT* gene. Patients of HD show involuntary movements, cognitive decline and psychiatric impairment. People carrying abnormally long expansions of CAGs (more than 35 CAG repeats) produce mutant huntingtin (mHtt), which encodes tracks of polyglutamines (polyQs). These polyQs make the protein prone to aggregate and cause it to acquire a toxic gain of function. Principally affecting the frontal cortex and the striatum, mHtt disrupts many cellular functions. In addition, this protein is expressed ubiquitously, and some reports show that many other cell types are affected by the toxicity of mHtt.

Several studies reported that metformin, a widely-used anti-diabetic drug, is neuroprotective in models of HD. Here, we provide a review of the benefits of this substance to treat HD. Metformin is a pleiotropic drug, modulating different targets such as AMPK, insulin signalling and many others. These molecules regulate autophagy, chaperone expression, and more, which in turn reduce mHtt toxicity. Moreover, metformin alters gut microbiome and its metabolic processes. The study of potential targets, interactions between the drug, host and microbiome, or genomic and pharmacogenomic approaches may allow us to design personalised medicine to treat HD.

Key words: Huntington disease, metformin, AMPK, pleiotropic effects, gut microbiome, pharmacogenetics

INTRODUCTION

Huntington disease (HD) is a rare, progressive monogenic dominant neurodegenerative disorder characterised by involuntary movements, cognitive decline and psychiatric alterations. It is considered a rare disease due to its low prevalence (5-10 per 100,000 people within the Caucasian population) (reviewed by Kay et al., (Kay et al., 2017)). Patients of HD show severe motor defects, such as chorea and dystonia, which usually manifest during adulthood (reviewed by Talukder and co-workers (Talukder et al., 2021). However, before motor diagnosis, these patients may show psychiatric and cognitive defects, as well as mild motor symptoms (Ghosh and Tabrizi, 2018). Huntington is caused by an abnormally long expansion of CAG triplets in the first exon of the *HTT* gene (Macdonald, 1993). This gene encodes huntingtin (Htt), a protein whose function is still the subject of debate (see below and also a review by Jimenez-Sánchez et al., (Jimenez-Sanchez et al., 2017). When *HTT* has 36 or more CAG triplets, mutant huntingtin (mHtt) contains a long polyglutamine (polyQ) track, and acquires a toxic gain-of-function which disrupts a number of cellular processes, from macroautophagy (hereinafter autophagy) to synaptic function (reviewed by Cisbani and Cicchetti (Cisbani and Cicchetti, 2012). Consequently, there is a protein homeostasis imbalance that affects several cellular functions (Krobitsch, S. and Kazantsev; 2011). In this regard, some evidence supports the toxic role of soluble species rather than larger aggregates or inclusion bodies, although this topic is still controversial (Kopito, 2000; Takahashi et al., 2010). Abnormal polyQ expansions are also known to produce other neurodegenerative diseases (Orr, 2012). In HD patients, the age at onset inversely correlates with the length of CAG expansion (Holmans et al., 2017). However, this inverse correlation is far from perfect (Holmans et al., 2017), which reflects the importance of environment and genetic background in the disease.

Although Htt is expressed ubiquitously, neurons seem to be more susceptible to mHtt toxicity and they become impaired, eventually leading to cell death (Cisbani and Cicchetti, 2012). The regions of the brain most affected by mHtt are the striatum and the cortex (Bohanna et al., 2011). However, nuclear resonance imaging of the nervous system shows many affected regions, among which are the cerebellum and hippocampus, in addition to a reduction in the volume of the whole brain (reviewed by Montoya et al., (Montoya et al., 2006)). The affliction of these regions explains many of the symptoms HD patients exhibit. For example, the striatum is principally responsible for motor and

action planning (Provost et al., 2015), which explains motor symptoms. Complex problem solving and abstract thinking lie within some areas of the human cortex. Deterioration of these regions of the brain account for the cognitive decline of HD patients (Provost et al., 2015).

Huntingtin is a ubiquitously expressed protein with many functions

As stated above, the function of Htt is not completely understood, although much effort has gone into attempting to decipher its role in mammals. The structure of Htt provides some clues about its function (reviewed by Saudou and Humbert (Saudou & Humbert, 2016)). For example, Htt is involved in axonal transport (Vitet et al., 2020), vesicle trafficking (Velier et al., 1998) and cell signalling (Luthi-Carter, 2000). In addition, Htt works to regulate the transcription of various genes (Dunah, 2002), including the brain-derived neurotrophic factor (BDNF) (Zuccato, 2001), and it also modulates its own expression (Culver et al., 2016). Htt has been widely conserved throughout evolution: the protein is encoded in some invertebrates and all mammals, with all orthologues showing what are known as HEAT repeats (from Huntingtin, Elongator factor3, PR65/A regulatory subunit of PP2A and Tor1) (Takano and Gusella, 2002). These domains are believed to participate in protein-protein interactions, suggesting that Htt may function as a scaffold, docking molecule or adaptor protein (reviewed by Kobe et al., (Kobe et al., 1999)). In this regard, Htt has been shown to act as a scaffolding protein for selective autophagy (agregophagy, lipophagy and mitophagy) (reviewed by Rui et al., (Rui et al., 2015)). Another clue is provided by a nuclear import signal (located in the N-terminal fragment), which suggests that Htt is translocated from cytoplasm to nucleus (Lunkes et al., 2002). Various researchers have validated this hypothesis in animal models (see for example Hackam and coworkers (Hackam et al., 1999). However, nuclearization of this protein seems to happen only in a cell's neuronal origin (Didiot et al., 2018). Indications are that the function of Htt is essential for life in mammals because Htt-defective mice do not develop a brain (Reiner et al., 2003). Nevertheless, this may not apply to all vertebrates because it does not happen in zebrafish (Sidik et al., 2020).

Huntington disease is a multi-organ disorder

As previously stated, Htt is ubiquitously expressed in both the nervous system and peripheral tissues (DiFiglia et al., 1995; Macdonald, 1993) (“Tissue expression of HTT -

Summary - The Human Protein Atlas," n.d.); (Uhlen et al., 2015). Although *HTT* is widely expressed in neurons, in the brain, mHtt is particularly detrimental for the corpus striatum and the cortex (Saudou & Humbert, 2016). This is probably because these areas are more sensitive to mHtt-induced toxicity. It is due to this neuronal vulnerability that HD is usually considered a purely neurodegenerative disease. However, work performed in animal models and observations of patients have revealed disturbances caused by mHtt toxicity in the skin (Aladdin et al., 2019; Seo et al., 2004), liver (Chiang et al., 2011), testicles (Van Raamsdonk et al., 2007), metabolism (Goodman et al., 2008), heart (Abildtrup and Shattock, 2013; Melkani, 2016; Pattison et al., 2008; Pattison and Robbins, 2008), digestive system (Stan et al., 2020), adipose tissue (Fain, 2001), lungs (Jones et al., 2016), and many other tissues and organs (Figure 1). Therefore, HD must be considered a multi-system disorder, as Mielcarek suggests (Mielcarek, 2015).

Based on this literature, it seems that therapeutic approaches to fight HD should be systemically directed rather than exclusively targeting the nervous system. For instance, one clinical trial (Tabrizi et al., 2019) has researched a strategy based on silencing the expression of mHtt in HD patients using intrathecally injected antisense oligonucleotides (ASOs). The trial was a success, meaning that it managed to reduce huntingtin in cerebrospinal fluid and it was well tolerated by patients (Tabrizi et al., 2019). Two different clinical trials subsequently employed these strategies to test the efficacy of ASOs were, unfortunately, unsuccessful (Kingwell, 2021). However, in the future it is possible that technical issues will be overcome to achieve successful silencing mHtt. If this were to occur, mHtt might be successfully blocked within the nervous system and neurons would be safer from the harm caused by mHtt, but HD patients would still suffer from other non-neuronal symptoms. Therefore, research into systemic treatments that reach all organs and tissues is a line of investigation that is guaranteed.

Metformin to treat systemic dysregulation of the metabolism

The herb *Galega officinalis,* which contains high levels of guanidines with anti-diabetic effects, has been in use since the Middle Ages to treat a number of conditions, including type 2 diabetes (Bailey and Day, 2004). Galegine, one of the guanidines present in *G. officinalis*, was used as a scaffold to synthesise other compounds (Bailey and Day, 2004). Of these, metformin proved to be the best of the antidiabetic compounds (Bailey and Day, 2004). Currently, metformin is widely used to treat type 2 diabetes (Rojas and

Gomes, 2013), in which it improves insulin resistance and glucose uptake by hepatic gluconeogenesis suppression (He and Wondisford, 2015). However, metformin is also able to modulate metabolism in other ways. For example, metformin modulates lipid metabolism by increasing fatty acid uptake in adipose tissue and inhibiting lipid accumulation in skeletal muscle among type 2 diabetes patients (Virtanen et al., 2003; Wang et al., 2014). A disruption of metabolism was also observed in *Drosophila* HD models, which exhibit an alteration of body weight and lipid levels, contributing to an energy deficiency (Aditi et al., 2016). Moreover, it is likely that metformin can reach most organs and tissues, based on the data from mouse models, and research in humans (Gormsen et al., 2016; Sundelin et al., 2020; Zake et al., 2021). Taken as a whole, these data suggest that metformin modulates several metabolic pathways in a systemic manner in non-insulin dependent diabetes patients, and it may also restore the metabolic dysregulation associated with other diseases, thus providing evidence of a clear relationship between HD and type 2 diabetes. Patients of HD show a tendency to develop glucose intolerance (reviewed by Farrer (Farrer, 1985)) and also metabolic dysregulation (Patassini et al., 2016). Patassini and co-workers identified the dysregulation of several metabolites from the tricarboxylic-acid and urea cycles in addition to amino acid metabolism in the post-mortem brains of HD patients (Patassini et al., 2016). This has also been observed in mice and *in vitro* models of HD-altered lactate metabolism, which can be restored after overexpression of a neuronal glucose transporter, GLUT3 (Solís-Maldonado et al., 2018). In accordance with these data, transcriptomic analysis shows the altered expression of glucose-related genes such as the glucose receptors GLUT1 and GLUT4, in striatal cell lines from HD mice (Chaves et al., 2017). Another interesting aspect of the study also shows upregulation of *Sorcs1*, which encodes a protein of the vacuolar protein sorting-10 (Vps10) family (Chaves et al., 2017). This gene is required for insulin secretion and lipid metabolism, but also plays a role in β-amyloid secretion (Lane et al., 2012; Savas et al., 2015) and synaptic function (doi: 10.1371/journal.pbio.3000466), therefore linking lipid metabolism and neurodegenerative diseases (Chaves et al., 2017). Before this, it had been observed that HD patients show a disruption in cholesterol metabolism, which produces lower levels of 24S-hydroxycholesterol in plasma and is directly proportional to disease progression (Leoni and Caccia, 2015). Finally, a cross-sectional study showed that HD patients who

take metformin to treat type 2 diabetes show better cognitive function than metformin-untreated HD patients (Hervás et al., 2017).

These data support the hypothesis that HD progression runs parallel to metabolic dysregulation and, therefore, drugs that modulate or restore this metabolic failure could be potential therapeutic strategies to alleviate mHtt associated toxicity. Thus, the use of metformin may constitute a good approach to treat dysregulated metabolism in HD patients simultaneously with many other issues (see below). Among the many targets of metformin, the AMP-activated protein kinase is one of the most investigated. Moreover, this enzyme is expressed in all tissues and organs in the human body, making it a systemic target to treat HD (Figure 1).

AMPK as a target of metformin to treat HD

The AMP-activated protein kinase (AMPK) is one of the best studied targets of metformin as a potential therapy to treat HD (Figures 1 and 2) (Hervás et al., 2017; Jin et al., 2016; Sanchis et al., 2019; Tang, 2020; Vázquez-Manrique et al., 2016; Walter et al., 2016). This heterotrimeric serine-threonine kinase is a master regulator of energy homeostasis in cells (Barnes et al., 2002; Kim et al., 2016). It is activated allosterically by AMP when ATP levels drop, but also by many other substances and signals (Figure 2). Composed of a catalytic (AMPKα) and two regulatory (AMPKβ and AMPKγ) subunits (Barnes et al., 2002; Sanz et al., 2013), AMPK is an obligate heterotrimer and can be activated by endogenous signals (metabolites, protein kinases, hormones, and many others) or synthetic drugs (Kim et al., 2016). A common mechanism of AMPK activation involves the LKB1 kinase (Liver kinase B1/serine threonine kinase 11) when raising AMP levels is required (Ramamurthy and Ronnett, 2012). In addition, the Ca^{2+}/calmodulin-dependent kinase, kinase β (CaMKKβ) also activates AMPK by raising intracellular Ca^{2+} levels (Ramamurthy and Ronnett, 2012). AMPK requires the phosphorylation of threonine-172 residue to catalyse several substrates related to cell growth, autophagy, and metabolism pathways (Mihaylova and Shaw, 2011).

Assays of AMPK as a potential treatment for HD used the nematode *Caenorhabditis elegans*. In these animals, the activation of AMPK by genetic means or using metformin reduced the neuronal toxicity induced by the first exon of *HTT*-carrying expanded CAG triplets (Vazquez-Manrique et al., 2016). In the same work, using murine

models, the authors showed that activation of this enzyme in early phases of HD reduced mHtt aggregation in brains (*in vivo*), and reduced toxic unfolded mHtt (*in vitro*) (Vázquez-Manrique et al., 2016). In parallel, other authors showed that AMPK-mediated activation of autophagy reduced HD phenotypes (Walter et al., 2016). Previous work had used metformin as a therapeutic agent, in the R6/2 mouse model of HD, showing that male mice lived longer than controls (Ma et al., 2007) although the authors did not investigate the mechanisms involved. Later, a preclinical trial in the zQ175 mouse model of HD (Menalled et al., 2012) showed that activation of AMPK using metformin, may be a good strategy to fight this disease (Sanchis et al., 2019). Three months of metformin treatment reduced motor and psychiatric symptoms of zQ175 mice, reduced mHtt aggregation, in parallel with activation of autophagy, in the striatum and cortex, and decreased neuroinflammation (Menalled et al., 2012). Recently, other authors have shown that AMPKβ-dependent activation of AMPK, using salicylate, induces neuroprotection against polyQ and alpha-synuclein-associated stress in *C. elegans* (Gómez-Escribano et al., 2020).

In contrast with the findings described above, Ju and co-workers showed that overactivation of AMPK by AICAR (5-aminoimidazole-4-carboxamide-1-d-ribofuranoside) contributed to brain atrophy, neuronal loss, and increased mHtt aggregates in the R6/2 mouse model of HD (Ju et al., 2011). They showed that striatal neurodegeneration is caused by the nuclear translocation of AMPKα1, in R6/2 mice and post-mortem samples of HD patients (Ju et al., 2011). However, these apparently conflicting results may be explained by differences in the models and biological samples used. Firstly, Ju and co-workers used R6/2 mice, which show the earliest disease onset and fastest disease progression of all HD mouse models(Reviewed by Farshim and Bates (Farshim and Bates, 2018). In contrast, zQ175 mice remain healthy much longer and begin to have mild behavioural defects at three months of age (Menalled et al., 2012). Secondly, the post-mortem samples from HD patients that show nuclearized AMPKα1 represent probably the most extreme endpoint to be investigated, and may not show physiologically relevant features of AMPK function. We believe that is fair to hypothesise that activation of AMPK, in early phases of the disease, may activate autophagy, which in turn reduces toxic molecules from neurons, thus promoting survival. In contrast, activation or overactivation of AMPK in later phases, either by endogenous means or by drugs, may produce autophagy-induced apoptosis.

Whatever the case, we strongly believe that activation of AMPK using metformin needs further exploration, which is why members of our team registered a clinical trial to use this drug against cognitive decline in HD patients (NCT04826692).

Metformin is a pleiotropic drug

Besides characterisation of the beneficial effects of AMPK-mediated metformin in HD models, other authors have considered the different pharmacological pathways of metformin. Arnoux and co-workers described how treating the Hdh150 mouse model of HD with metformin from a pre-symptomatic age restored hyperactivity in neuronal networks of the visual cortex and affected the anxiety of mice (Arnoux et al., 2018). Some earlier work had shown that the MID1/PP2A/mTOR protein complex may regulate translation of mHtt (Krauß et al., 2013). Based on that, Arnoux et al., studied whether treatment with metformin, which is known to interfere with this complex (Kickstein et al., 2010a), may rescue HD phenotypes through this pathway. As expected by the authors, *in vitro* and *in vivo* experiments showed that this was the case (Arnoux et al., 2018).

How can we reconcile that metformin seems to act to reduce symptoms in HD models through what are in principle two different mechanisms? Both mechanisms show that the action of metformin can decrease the amount of mHtt aggregated, either by an mTOR-dependent pathway or through AMPK signalling. However, these pathways are not independent, since AMPK phosphorylates mTOR to reduce protein synthesis. In fact, it has been shown that there is a metformin-induced interplay between AMPK and mTOR in anti-cancer activity (Chomanicova et al., 2021). Moreover, the MID1/PP2A complex is a direct inhibitor of AMPK activity (Joseph et al., 2015); hence, if metformin destabilises this complex, it may also maintain higher levels of AMPK activity. To conclude, we believe that both are compatible, and could possibly be cooperating to reduce the toxicity of mHtt.

It has recently been shown that metformin may modulate RAN (Repeat associated Non-AUG) translation through inhibition of the PKR (RNA-dependent protein kinase) pathway in ALS/FTD mice (Zu et al., 2020). This has therapeutic implications for HD, since research has described how toxic RAN peptides are produced in models of this disease (Bañez-Coronel et al., 2015).

Many more targets of metformin exist, although they have not been characterized in biological models of HD. For example, metformin induces pleiotropic effects to attenuate atherosclerosis in mice (Forouzandeh et al., 2014). This process involves activation of AMPK and repression of ATR1 (angiotensin II type 1 receptor) and SOD-1 (Superoxide dismutase-1) (Forouzandeh et al., 2014). In addition, metformin antagonizes glucagon signalling by inhibiting adenylate cyclase, which reduces PKA activity (Figure 2), resulting in a reduction of glucose during fasting (Miller et al., 2013a). Furthermore, metformin activates degradation of Hypoxia Inducible Factor 1 Subunit Alpha (HIF-1α) in hepatocellular carcinoma, hence reducing tumour growth (X. Zhou et al., 2016).Metformin is therefore a highly pleiotropic drug that induces modulation of a wide range of pathways and molecules (Figure 2, Table 1). Moreover, it may be possible that more molecules modulated by this drug emerge in the future as potential druggable targets to fight HD. To summarise, metformin is able to tune the function of a wide range of targets, in many cells and tissues to modulate all sorts of pathways and metabolic processes (Table 1).

Synergistic use of metformin enhances its therapeutic potential

Metformin may have detrimental and/or collateral undesirable effects. For example, Espada and co-workers described a negative effect of metformin in aged worms, which reduced their lifespan and survival, due to mitochondrial impairment (Espada et al., 2019). Moreover, metformin treatment in 20-month-old mice alters cardiac metabolism and longevity, compared to 2-month-old metformin-treated mice (Zhu et al., 2021). Therefore, it is important to consider undesired pleiotropic effects and the time of treatment when designing therapies using metformin. In this regard, it has been shown that AMPK may be activated synergistically using metformin and salicylate (or derivatives of salicylate) (Ford et al., 2015; Gómez-Escribano et al., 2020; O'Brien et al., 2015; Talarico et al., 2016; Ye et al., 2016). This pharmacological synergistic action of the drugs activates AMPK, with an approximately ten-fold reduction in the dose of each of the two drugs (Gómez-Escribano et al., 2020). Hence, reducing the amount of the drugs offers the advantage of avoiding activation of undesired targets, but still reducing polyQ-induced toxicity, improving healthspan and restoring neuronal function in *C. elegans* (Gómez-Escribano et al., 2020). Therefore, these strategies that use a synergistic combination of metformin with other natural or synthetic drugs to treat diseases should be further explored.

Pharmacogenetics and metformin: personalised medicine in HD

Personalised medicine or precision medicine provides tools to improve the way that patients are treated, based on their genetic background and environmental context. Treatment is customised based on genetics and on people's lifestyles, and directed for an individual according to their specific features. In this regard, metformin may not be equally effective for every HD patient. In some cases it may produce mild side effects, for example gastric discomfort for a few patients or low blood sugar levels in patients treated with other antidiabetic compounds (Nasri and Rafieian-Kopaei, 2014). Moreover, the drug may cause more serious side effects, such as lactic acidosis in limited specific contextual conditions (patients with infections, kidney malfunction, etc.) (Nasri and Rafieian-Kopaei, 2014). In contrast, metformin is sometimes not sufficient to provide glycaemic control in diabetic patients (Kahn et al., 2006; Turner et al., 1999) due to factors that may have an impact on its response and strongly depend on a patient's genetic background. In pharmacogenetic studies, allelic variants in genes related to metformin absorption, distribution, and excretion (e.g. membrane transport proteins encoded by the solute carrier (SLC) gene family) have been found to modify pharmacodynamics and pharmacokinetics, which ultimately affect response to the drug (Florez, 2017; Gong et al., 2012; Shikata et al., 2007; Shu et al., 2008, 2007; Song et al., 2008; Tzvetkov et al., 2009; Wang et al., 2011). Table 2 shows all the variants that the Pharmacogenomics Knowledge Base (PharmGKB, www.pharmgkb.org, last accessed 17 January 2022) contains in its clinical annotations section regarding metformin within known genes. These variants are mainly single nucleotide polymorphisms (SNPs). PharmGKB is the main source of information on pharmacogenomics (Cita PharmGKB), and its website offers all the information compiled and curated from publications and other relevant sources (drug labels, e.g.) by a team of experts. It is financially supported by NIH/NHGRI/NICHD, managed at Stanford University, and the data is under a Creative Commons Attribution-ShareALike 4.0 license. In the clinical annotations section, a phenotypic impact is given to a genetic variant regarding a concrete drug. PharmGKB curators assign a level of evidence to each "drug-variant" pair according to predefined criteria about the quality of the studies and sources analysed. The table shows the SNPs, the genes where they are located, the possible alleles and their mean frequency among all the populations according to the Genomes Aggregation Database (gnomAD), also included in the PharmGKB website (the frequency of the different alleles can vary

slightly depending on the database). PharmGKB also shows the proposed effect of different genotype variants on metformin results. Hence, it would be logical to treat HD patients with metformin, as long as they fit the criteria for inclusion/exclusion within the clinical trial, since the possible benefits seem to clearly surpass the minor side effects and small direct costs. By genotyping the most relevant SNPs influencing metformin effects, clinicians would be able to adjust the treatment in a personalized manner, increasing efficacy and minimizing toxicity, thus achieving the best possible results.

Various studies have highlighted several SNPs in the SLC22A1, SLC22A2, and SLC22A3 genes which codify organic cation transporters 1, 2 and 3 (OCT1, OCT2 and OCT3, respectively). These membrane proteins can transport metformin from the intestinal lumen, distribute it through the bloodstream and facilitate its intake into cells (reviewed by Koepsell, (Koepsell, 2013)). They have also been linked to the availability and effect of metformin (Al-Eitan et al., 2019; Florez, 2017; Santoro et al., 2018; Shu et al., 2008, 2007; Tzvetkov et al., 2009). In various studies involving healthy volunteers, several genetic variants of OCT1 wielded a significant effect on pharmacokinetics and the therapeutic response to metformin after oral administration (Shu et al., 2008, 2007; Tzvetkov et al., 2009). Individuals carrying hypomorphic alleles of OCT1 showed a lower assimilation of the drug than those with wild-type alleles and therefore the treatment had a reduced effect. Moreover, it is reported that reduced function alleles encoding OCT1, showed less activation of AMPK by metformin in mice (Shu et al., 2007). Similar results were observed in patients with a reduced function in OCT2 and OCT3, suggesting the importance of this family of cation carriers in the response to metformin treatment (Becker et al., 2009; Tzvetkov et al., 2009).

Other SLC-encoded carrier proteins, such as the multi-drug and toxin extrusion proteins 1 and 2-K (MATE1/SLC47A and MATE2-K/SLC47A2), have also been proposed as potential modulators of metformin pharmacological effectiveness (Becker et al., 2009; Choi et al., 2011; Mousavi et al., 2017; Pedersen et al., 2018). In this regard, an allele of an intronic variant of MATE1/SLC47A1, rs2289669 G>A, was significantly associated with a greater reduction in haemoglobin A1c (HbA1c) in a cohort of 116 metformin users, suggesting that this allele encodes a less effective MATE1 efflux transporter, which translates to a reduced transport of metformin (Becker et al., 2009). Likewise, an impaired MATE1 transporter, which results in a reduced efflux of metformin through renal and hepatic cells, leads to an increase in metformin plasma

levels, and possibly to a larger undesirable decrease in glucose levels (Santoro et al., 2018). Later studies in type II diabetes mellitus (T2DM) patients further supported the association between SNP rs2289669 in SLC47A1 and the glucose-lowering effect of metformin (He et al., 2015; Mousavi et al., 2017; Tkáč et al., 2013). Furthermore, homozygous individuals for 130G>A rs12943590 in MATE2-K had a significantly lower response to metformin therapy (Choi et al., 2011). Moreover, variants in the plasma membrane monoamine transporter (PMAT) (SLC29A4) can influence metformin pharmacokinetics, as reported in a study by Moon *et al*. (Dawed et al., 2019; Moon et al., 2018).

These data are extremely relevant because these proteins are expressed within the nervous system ("The Human Protein Atlas," n.d.). Since these transporters show a broad substrate specificity, they can also deliver certain pharmacological compounds to the brain. For instance, it has been reported that SLC22A family members are expressed in the brain, OCT1 and OCT2 are found in the blood-brain barrier (BBB) while OCT3 is found in neurons and glia (Reviewed by Aykac and Sehirli (Aykac & Sehirli, 2020)). Moreover, SLC47A1 and SLC47A2 encoded proteins MATE1 and MATE-2K were detected in isolated human brain micro-vessels (Geier et al., 2013). Finally, PMAT (Slc29a4) is strongly expressed in the brain, with some areas such as the dentate gyrus and choroid plexus particularly enriched (Dahlin et al., 2007; Engel et al., 2004). For this reason, reduced function of these transporters will affect metformin entry to the brain, one of the most damaged organs in HD, lessening the effectiveness of a possible future treatment.

These variants, which are located on genes that modulate the transport and metabolism of metformin, may be used as pharmacogenetic markers to determine which patients may likely be better responders to this drug. As for its use in HD therapy, analysis of these variants in patients prior to a treatment proposition would avoid subjecting patients to ineffective therapy and prevent delays in adopting a more adequate approach in a situation where time is a key factor. Moreover, as HD is a systemic disorder, the presence of some allelic variants in genes that code for tissue-specific cation transporters may alter the effect of metformin in certain tissues. Furthermore, some authors suggest that these variants worsen some side effects and intolerance derived from metformin treatment due to reduced excretion therefore causing accumulation of the drug (Dawed et al., 2019;

Dujic et al., 2016). In such cases, lower doses of the compound may be considered to treat these specific patients. Overall, these data highlight the importance of considering each patient's genetic context when it comes to metformin treatment, so personalised medicine can be applied. Currently, we have a large body of evidence about strategies to guide treatments with pre-emptive pharmacogenetics testing (García-Alfonso et al., 2021; Theken et al., 2020; Whirl-Carrillo et al., 2021). In this regard, all HD patients should be treated with metformin, independently of their genetic background since the possible benefits surpass the minor side effects of this drug. Hence, it is worthwhile genotyping the most relevant SNPs that influence the effects of metformin in clinical studies. With this, clinicians would expand their knowledge of the association between the genetic background of patients and the side effects and/or benefits of using this drug. This in turn will bring use of metformin closer to personalised medicine.

Microbiota alteration and HD

The human gut microbiota is a complex and metabolically active system formed by microorganisms of different phyla that colonise the digestive tract after birth. The symbiotic relationship established between gut microbiota and the organism is essential for human health and alterations in its composition or function, also called dysbiosis, may induce a pathological context (reviewed by DeGruttola et al., 2016).

Gut dysbiosis has been reported in several neurological and psychiatric disorders (Scheperjans et al., 2015; Settanni et al., 2021; Vogt et al., 2017). For instance, a gut-driven regulation of brain inflammatory pathways might be linked to some of the clinical signs or brain abnormalities seen in Huntington disease. Some studies reflect a communication axis between the metabolites produced from bacteria present in the gut microbiome and the nervous and immune systems (Wang and Wang, 2016). This communication system, known as the gut-brain axis, allows the flow of information between the cells that compose the microbiota and the central nervous system, and alterations of this signalling pathway have profound consequences for the progression of many neurodegenerative diseases (reviewed by Ma and co-workers (Q. Ma et al., 2019)). Communication between the gut microbiota and the nervous system is bidirectional, and when the composition or function of the microbiome is not within healthy ranges, it has a detrimental impact on cognition and behaviour (reviewed by Wang and Kasper (Y. Wang & Kasper, 2014).

Recent findings suggest that gut dysbiosis may have a regulatory role in the onset and progression of HD (Du et al., 2020; Kong et al., 2020; Wasser et al., 2020). Kong *et al.*, found evidence of gut dysbiosis in a mouse model of HD, with a substantial change in the microbiome of the R6/1 mice at 12 weeks of age, compared to wildtype animals (Kong et al., 2020). These authors observed an increase in the *Bacteroidetes* phylum and a decrease in the *Firmicutes* family of eubacteria (Kong et al., 2020). It is widely accepted that the ratio between these phyla provides information about intestinal homeostasis (Ley et al., 2005; Turnbaugh et al., 2006). Moreover, when analysing microbiome sequencing data, it is common to use terms such as alpha and beta diversity. Alpha diversity measures microbiome diversity (richness) within a sample, whereas beta diversity indicates the similarity or dissimilarity between two groups. On this subject, although Kong and co-workers found no differences in female HD mice, there was higher microbial alpha-diversity in male HD mice compared to wild type animals, with significantly higher Shannon and Inverse Simpson diversity indices in the male HD specimens (Kong et al., 2020). These two indices inform about the number of species and their relative abundance, and the average proportional abundance, respectively. Concerning beta-diversity, the authors identified a signature of bacteria, which allowed them to discriminate between HD and wild type mice from the same sex, using principal coordinates analysis (PCoA). For male HD mice, this signature consisted of bacterial families from the Clostridiales, Bacteroidales and Lactobacillales orders, whereas the bacterial families present in the female HD group's signature were Coriobacteriales, Clostridiales, Erysipelotrichales, Bacteroidales, and Burkholderiales. This microbiota alteration finally correlated with weight gain impairment despite higher food intake and the shift in the microbiome composition coincided with the onset of motor impairments tested on the rotarod (Kong et al., 2020).

In 2020, Wasser *et al*. provided the first evidence of gut dysbiosis in HD patients, and found a link between gut bacteria, cognitive ability and clinical outcomes (Wasser et al., 2020). Using faecal samples, they investigated whether the gut microbiota of carriers of mutant CAG expansion differed from that of age- and gender-matched healthy controls. The HD patients showed a lower alpha diversity, indicating less species richness within each participant and lower relative abundance; as well as variations in beta diversity, indicating a different microbial community structure between participants (Wasser et al., 2020). Moreover, these researchers identified changes in gut microbes, gut functional pathways and levels of enzymes in the Huntington disease gene expansion

carrier (HDGEC) group in comparison to healthy controls (HCs) (Wasser et al., 2020). The authors also discovered affected functional gut pathways in the HDGEC group, including the superpathway of serine and glycine biosynthesis, the starch degradation V pathway, methylerythritol phosphate pathways I and II and the NAD biosynthesis I pathway, whose relative abundance increases. Regarding enzyme analysis, the relative abundance of glutathione transferase was significantly lower in the HDGEC group compared to HCs (Wasser et al., 2020). This change is related to increased oxidative stress and neuroinflammation and has been observed in Parkinson's and Alzheimer's disease (Mazzetti et al., 2015). In addition, in comparison with non-HD people, the authors found a correlation between a reduced presence of the *Eubacterium hallii* bacteria and more severe motor symptoms in the HDGEC group. Among pre-symptomatic individuals, there was an inverse correlation between the abundance of *E. hallii* in their microbiome and the age-at-onset of symptoms (Wasser et al., 2020). In another study, Du *et al*. compared the microbiota and peripheral cytokine levels of 33 HD patients and 33 HCs (Du et al., 2020). According to their analysis, the faecal microbiota of the HD and healthy control group differed significantly, suggesting that certain gut microbiome components are linked to HD. Consistent with the preclinical study by Kong et al. (2018) but contrary to the observations of Wasser et al. (2020) in HD patients, Du's group found that alpha diversity in the gut microbiota of HD patients was significantly higher than that of HCs. They argued that this disparity could be due to different ethnic origins, geography, host, genetics, age, and other subject-related factors. Their research found a higher abundance of the *Intestinimonas* and *Bilophila* genus in HD patients, which positively correlated with plasma concentrations of IL-4 (an anti-inflammatory cytokine) and negatively correlated with proinflammatory IL-6 concentrations, respectively. This suggests the presence of a systemic chronic inflammatory condition linked to altered gut microbiota (Du et al., 2020). These authors also found correlations between microbiota and clinical scores. A greater abundance of the *Intestinimonas* genus in HD patients correlated with total functional capacity (TFC) scores (Du et al., 2020), which measure the ability to work, manage finances, complete household duties, undertake self-care tasks and live independently. Higher scores indicate better performance (Shoulson and Fahn, 1979). In contrast, the higher abundance of the *Lactobacillus* genus observed in HD patients negatively correlated with Mini-Mental State Examination (MMSE) scores, which measure general cognitive function (Folstein et al., 1975).

The evidence stated above suggests that there is a link between gut dysbiosis and HD. However, there is insufficient data to understand how the gut microbiome affects the course of this disease. Longer-term studies with a higher number of patients and more advanced technology (shotgun metagenome analysis and more advanced association analysis) are necessary to understand whether microbiomes modulate HD. If microbiomes play an important role in the progression of HD, investigating them may uncover gut biomarkers in HD patients that will help in early diagnosis of the disease. Finally, this avenue of research suggests the intriguing possibility that the gut might constitute a future target for therapeutic intervention in HD and other neurodegenerative diseases.

Crosstalk between microbiome and metformin modulates progression of the disease

Commonly used non-antibiotic drugs influence the composition of gut microbiota (Jackson et al., 2018; Vich Vila et al., 2020), and inversely, the microbiome influences the effects of drugs on people (see for example (Klünemann et al., 2021; Rinse K Weersma et al., 2020; Zimmermann et al., 2021)). The notion that metformin has an impact on microbiota has been shown in the nematode worm *C. elegans*. As previously mentioned, this drug was known to extend the lifespan and fitness of the worms, in an AMPK-dependent manner (Onken and Driscoll, 2010a). Cabreiro and co-workers found that the beneficial effects of metformin in worms required the administration of live bacteria (Cabreiro et al., 2013). *C. elegans* feeds on different species of bacteria in the wild, and usually on *E. coli* in the laboratory (Zečić et al., 2019). Treating the worms with metformin in the presence of dead bacteria unexpectedly had the opposite effect, reducing the lifespan of the animals (Cabreiro et al., 2013). The researchers found that metformin impacted the metabolism of folate and methionine of E. coli, which in turn extended the worms' lifespan (Cabreiro et al., 2013). This was the first mechanism provided for the microbiota-mediated beneficial effects of metformin in an animal, and *C. elegans* became a very useful system , to study the relationships between microbiota and animal aging and health thanks to the simplicity of the animal and its genetic tractability (reviewed by Cabreiro and Gems (Cabreiro and Gems, 2013)). Using this microbe-animal system model, Pryor et al. performed a screening for host-microbe-drug-nutrient interactions between metformin, *E. coli* and *C. elegans* (Pryor et al., 2019), providing the proof-of-concept that this system may be of interest to further explore such complex relationships.

The interaction between microbiota and metformin can also be found in mammals. Treating obese mice with metformin increased their gut population of the mucin-degrading bacterium *Akkermansia spp.* (Shin et al., 2014), which in turn resulted in better glucose tolerance and reduced inflammation of the adipose tissue of the mice (Shin et al., 2014). In diabetic rats, intravenous was less effective than intraduodenal metformin administration at lowering blood glucose levels (Stepensky et al., 2002). This strongly suggests that the benefits of treatment with metformin require the drug to interact with the microbiota (Stepensky et al., 2002), as is the case in worms (Cabreiro et al., 2013). In addition, Bauer *et al*. discovered a shift in the composition of microbiota following upper small intestine metformin treatment of high-fat diet rats (Bauer et al., 2018), with an increase in the relative abundances of the *Lactobacillaceae* family and *Lactobacillus* genus. In mice fed with a diet rich in fat, metformin restores the abundance of *Lactobacillus* and *A. muciniphila*, known to have positive effects on the host (Z.-Y. Zhou et al., 2016) and metformin treatment also increases the population of the mucin-degrading bacteria *Akkermansia muciniphila* (Shin et al., 2014)., As the study of Ma *et al*. (Ma et al., 2018) revealed, microbiome alteration also occurs in healthy conditions: metformin treatment in healthy mice alters the abundance of microbes in faecal samples, compared to non-treated controls. A human study reported similar findings of metformin treatment resulting in a significant change in the abundance of more than 80 bacterial strains in individuals with type-2-diabetes (T2D), with the *Firmicutes* and *Proteobacteria* phyla most affected (Wu et al., 2017). Moreover, according to other authors, metformin enhances the number of bacteria that generate short chain fatty acids, thus mediating its therapeutic effects (Forslund et al., 2015). A different study corroborated this conclusion observing the presence of higher faecal levels of these lipids in metformin users (Zhernakova et al., 2016).

However, interaction between gut microbes and drugs is bi-directional, and increasing evidence suggests that the gut microbiota may have a direct impact on the response of an individual to a treatment by enzymatically modifying drug structure and altering its disposition, action or toxicity; a phenomena known as pharmacomicrobiomics (reviewed by Weersma (Weersma et al., 2020)). On balance, it seems that the particular gut microbial population of each person may affect their response to metformin treatment, and modification of the microbiome could be a particularly appealing target to enhance metformin effectiveness. Given the evidence supporting the idea that metformin targets

the gut microbiota to mediate its beneficial effects, it is not unfair to speculate that metformin may mitigate the alterations in microbiota seen in HD patients, contributing to amelioration of the disease. Taken together, future strategies for HD should consider analysis of the modulating effect of metformin on gut microbiota and the specific microbiome population of each patient, as this would allow selection of the most suitable therapy for each person, further empowering the capacity of personalized medicine in HD.

Conclusion and future perspectives

In the future, a cleverly designed gene therapy may offer us a cure for HD. To date, this strategy faces many challenges, due to the difficulty of delivering nucleic acids within the brain. Another hurdle is that once the nervous system has been cured, many other organs and tissues will suffer the devastating effects of mHtt. In the meantime, therefore, there is a justification for exploring the use of conventional drugs, such as metformin. This drug has shown tremendous beneficial effects in patients for many age-related conditions like diabetes, cancer, cognitive decline, etc. (reviewed by Novelle et al., (Novelle et al., 2016)). Work on animal HD models, and cross-sectional studies of patients with the condition, suggest that metformin may benefit people suffering HD. Moreover, metformin is a pleiotropic drug that activates many potential targets to treat HD, and reaches nearly every organ and tissue of the human body. Further studies about these targets, together with the study of the microbiome, using genomic approaches, and the use of pharmacogenetics, will allow the design of precision metformin therapies.

ACKNOWLEDGEMENTS

RPVM used grants (PI17/00011 and PI20/00114) funded by the Instituto de Salud Carlos III (ISCIII, Madrid, Spain). These grants are co-financed by the European Development Regional Fund "A way to achieve Europe" (ERDF). Funds from the Fundación Ramón Areces were also used (CIVP19S8119). CTR holds a grant by the Generalitat Valenciana and the European Social Fund (ACIF/2020/366). RVM received an Ayuda Miguel Gil grant (VII Convocatoria Ayudas a la Investigación MHER, 2019, cofinanced by Colegio Oficial de Farmacéuticos de Sevilla and Fundación Cajasol). RVM has also received funds from the Asociación Valenciana de Enfermedad de Huntington (AVAEH).

Bibliography

Abildtrup, M., Shattock, M., 2013. Cardiac Dysautonomia in Huntington's Disease. Journal of Huntington's Disease 2, 251–261. https://doi.org/10.3233/JHD-130054

Aditi, K., Shakarad, M.N., Agrawal, N., 2016. Altered lipid metabolism in Drosophila model of Huntington's disease. Sci Rep 6, 31411. https://doi.org/10.1038/srep31411

Aladdin, A., Király, R., Boto, P., Regdon, Z., Tar, K., 2019. Juvenile Huntington's Disease Skin Fibroblasts Respond with Elevated Parkin Level and Increased Proteasome Activity as a Potential Mechanism to Counterbalance the Pathological Consequences of Mutant Huntingtin Protein. IJMS 20, 5338. https://doi.org/10.3390/ijms20215338

Al-Eitan, L.N., Almomani, B.A., Nassar, A.M., Elsaqa, B.Z., Saadeh, N.A., 2019. Metformin Pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 Polymorphisms on Glycemic Control and HbA1c Levels. J Pers Med 9, E17. https://doi.org/10.3390/jpm9010017

Arnoux, I., Willam, M., Griesche, N., Krummeich, J., Watari, H., Offermann, N., Weber, S., Narayan Dey, P., Chen, C., Monteiro, O., Buettner, S., Meyer, K., Bano, D., Radyushkin, K., Langston, R., Lambert, J.J., Wanker, E., Methner, A., Krauss, S., Schweiger, S., Stroh, A., 2018. Metformin reverses early cortical network dysfunction and behavior changes in Huntington's disease. Elife 7. https://doi.org/10.7554/eLife.38744

Aykac, A., Sehirli, A.O., 2020. The Role of the SLC Transporters Protein in the Neurodegenerative Disorders. Clin Psychopharmacol Neurosci 18, 174–187. https://doi.org/10.9758/cpn.2020.18.2.174

Bailey, C., Day, C., 2004. Metformin: its botanical background. Practical Diabetes International 21, 115–117. https://doi.org/10.1002/pdi.606

Bañez-Coronel, M., Ayhan, F., Tarabochia, A.D., Zu, T., Perez, B.A., Tusi, S.K., Pletnikova, O., Borchelt, D.R., Ross, C.A., Margolis, R.L., Yachnis, A.T., Troncoso, J.C., Ranum, L.P.W., 2015. RAN Translation in Huntington Disease. Neuron 88, 667–677. https://doi.org/10.1016/j.neuron.2015.10.038

Barnes, K., Ingram, J.C., Porras, O.H., Barros, L.F., Hudson, E.R., Fryer, L.G.D., Foufelle, F., Carling, D., Hardie, D.G., Baldwin, S.A., 2002. Activation of GLUT1 by metabolic and osmotic stress: potential involvement of AMP-activated protein kinase (AMPK). J. Cell. Sci. 115, 2433–2442.

Bauer, P.V., Duca, F.A., Waise, T.M.Z., Rasmussen, B.A., Abraham, M.A., Dranse, H.J., Puri, A., O'Brien, C.A., Lam, T.K.T., 2018. Metformin Alters Upper Small Intestinal Microbiota that Impact a Glucose-SGLT1-Sensing Glucoregulatory Pathway. Cell Metab 27, 101-117.e5. https://doi.org/10.1016/j.cmet.2017.09.019

Becker, M.L., Visser, L.E., van Schaik, R.H.N., Hofman, A., Uitterlinden, A.G., Stricker, B.H.Ch., 2009. Genetic Variation in the Multidrug and Toxin

Extrusion 1 Transporter Protein Influences the Glucose-Lowering Effect of Metformin in Patients With Diabetes: A Preliminary Study. Diabetes 58, 745–749. https://doi.org/10.2337/db08-1028

Bohanna, I., Georgiou-Karistianis, N., Egan, G.F., 2011. Connectivity-based segmentation of the striatum in Huntington's disease: Vulnerability of motor pathways. Neurobiology of Disease 42, 475–481. https://doi.org/10.1016/j.nbd.2011.02.010

Cabreiro, F., Au, C., Leung, K.-Y., Vergara-Irigaray, N., Cochemé, H.M., Noori, T., Weinkove, D., Schuster, E., Greene, N.D.E., Gems, D., 2013. Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell 153, 228–239. https://doi.org/10.1016/j.cell.2013.02.035

Cabreiro, F., Gems, D., 2013. Worms need microbes too: microbiota, health and aging in *Caenorhabditis elegans*. EMBO Mol Med 5, 1300–1310. https://doi.org/10.1002/emmm.201100972

Cacicedo, J.M., Yagihashi, N., Keaney, J.F., Ruderman, N.B., Ido, Y., 2004. AMPK inhibits fatty acid-induced increases in NF-κB transactivation in cultured human umbilical vein endothelial cells. Biochemical and Biophysical Research Communications 324, 1204–1209. https://doi.org/10.1016/j.bbrc.2004.09.177

Chaves, G., Özel, R.E., Rao, N.V., Hadiprodjo, H., Costa, Y.D., Tokuno, Z., Pourmand, N., 2017. Metabolic and transcriptomic analysis of Huntington's disease model reveal changes in intracellular glucose levels and related genes. Heliyon 3, e00381. https://doi.org/10.1016/j.heliyon.2017.e00381

Chiang, M.-C., Chern, Y., Juo, C.-G., 2011. The dysfunction of hepatic transcriptional factors in mice with Huntington's Disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1812, 1111–1120. https://doi.org/10.1016/j.bbadis.2011.05.006

Choi, J., Yee, S., Ramirez, A., Morrissey, K., Jang, G., Joski, P., Mefford, J., Hesselson, S., Schlessinger, A., Jenkins, G., Castro, R., Johns, S., Stryke, D., Sali, A., Ferrin, T., Witte, J., Kwok, P.-Y., Roden, D., Wilke, R., McCarty, C., Davis, R., Giacomini, K., 2011. A Common 5′-UTR Variant in MATE2-K Is Associated With Poor Response to Metformin. Clin Pharmacol Ther 90, 674–684. https://doi.org/10.1038/clpt.2011.165

Chomanicova, N., Gazova, A., Adamickova, A., Valaskova, S., Kyselovic, J., 2021. The role of AMPK/mTOR signaling pathway in anticancer activity of metformin. Physiol Res.

Cisbani, G., Cicchetti, F., 2012. An in vitro perspective on the molecular mechanisms underlying mutant huntingtin protein toxicity. Cell Death Dis 3, e382–e382. https://doi.org/10.1038/cddis.2012.121

Culver, B.P., DeClercq, J., Dolgalev, I., Yu, M.S., Ma, B., Heguy, A., Tanese, N., 2016. Huntington's Disease Protein Huntingtin Associates with its own mRNA. JHD 5, 39–51. https://doi.org/10.3233/JHD-150177

Cuyàs, E., Verdura, S., Llorach-Parés, L., Fernández-Arroyo, S., Joven, J., Martin-Castillo, B., Bosch-Barrera, J., Brunet, J., Nonell-Canals, A., Sanchez-Martinez, M., Menendez, J.A., 2018a. Metformin Is a Direct SIRT1-Activating Compound: Computational Modeling and Experimental Validation. Front. Endocrinol. 9, 657. https://doi.org/10.3389/fendo.2018.00657

Cuyàs, E., Verdura, S., Llorach-Pares, L., Fernández-Arroyo, S., Luciano-Mateo, F., Cabré, N., Stursa, J., Werner, L., Martin-Castillo, B., Viollet, B., Neuzil, J., Joven, J., Nonell-Canals, A., Sanchez-Martinez, M., Menendez, J.A., 2018b. Metformin directly targets the H3K27me3 demethylase KDM6A/UTX. Aging Cell 17, e12772. https://doi.org/10.1111/acel.12772

Dahlin, A., XIA, L., KONG, W., HEVNER, R., WANG, J., 2007. Expression and Immunolocalization of the Plasma Membrane Monoamine Transporter in the Brain. Neuroscience 146, 1193–1211. https://doi.org/10.1016/j.neuroscience.2007.01.072

Dawed, A.Y., Zhou, K., van Leeuwen, N., Mahajan, A., Robertson, N., Koivula, R., Elders, P.J.M., Rauh, S.P., Jones, A.G., Holl, R.W., Stingl, J.C., Franks, P.W., McCarthy, M.I., 't Hart, L.M., Pearson, E.R., IMI DIRECT Consortium, 2019. Variation in the Plasma Membrane Monoamine Transporter (PMAT) (Encoded by SLC29A4) and Organic Cation Transporter 1 (OCT1) (Encoded by SLC22A1) and Gastrointestinal Intolerance to Metformin in Type 2 Diabetes: An IMI DIRECT Study. Diabetes Care 42, 1027–1033. https://doi.org/10.2337/dc18-2182

DeGruttola, A.K., Low, D., Mizoguchi, A., Mizoguchi, E., 2016. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis 22, 1137–1150. https://doi.org/10.1097/MIB.0000000000000750

Didiot, M.-C., Ferguson, C.M., Ly, S., Coles, A.H., Smith, A.O., Bicknell, A.A., Hall, L.M., Sapp, E., Echeverria, D., Pai, A.A., DiFiglia, M., Moore, M.J., Hayward, L.J., Aronin, N., Khvorova, A., 2018. Nuclear Localization of Huntingtin mRNA Is Specific to Cells of Neuronal Origin. Cell Reports 24, 2553-2560.e5. https://doi.org/10.1016/j.celrep.2018.07.106

DiFiglia, M., Sapp, E., Chase, K., Schwarz, C., Meloni, A., Young, C., Martin, E., Vonsattel, J.-P., Carraway, R., Reeves, S.A., Boyce, F.M., Aronin, N., 1995. Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. Neuron 14, 1075–1081. https://doi.org/10.1016/0896-6273(95)90346-1

Du, G., Dong, W., Yang, Q., Yu, X., Ma, J., Gu, W., Huang, Y., 2020. Altered Gut Microbiota Related to Inflammatory Responses in Patients With

Huntington's Disease. Front Immunol 11, 603594. https://doi.org/10.3389/fimmu.2020.603594

Dujic, T., Causevic, A., Bego, T., Malenica, M., Velija-Asimi, Z., Pearson, E.R., Semiz, S., 2016. Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. Diabet Med 33, 511–514. https://doi.org/10.1111/dme.13040

Dunah, A.W., 2002. Sp1 and TAFII130 Transcriptional Activity Disrupted in Early Huntington's Disease. Science 296, 2238–2243. https://doi.org/10.1126/science.1072613

El-Mir, M.-Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoulet, M., Leverve, X., 2000. Dimethylbiguanide Inhibits Cell Respiration via an Indirect Effect Targeted on the Respiratory Chain Complex I. Journal of Biological Chemistry 275, 223–228. https://doi.org/10.1074/jbc.275.1.223

Engel, K., Zhou, M., Wang, J., 2004. Identification and characterization of a novel monoamine transporter in the human brain. J Biol Chem 279, 50042–50049. https://doi.org/10.1074/jbc.M407913200

Espada, L., Dakhovnik, A., Chaudhari, P., Martirosyan, A., Miek, L., Poliezhaieva, T., Schaub, Y., Nair, A., Döring, N., Rahnis, N., Werz, O., Koeberle, A., Kirkpatrick, J., Ori, A., Ermolaeva, M.A., 2019. Late life metformin treatment limits cell survival and shortens lifespan by triggering an aging-associated failure of energy metabolism (preprint). Physiology. https://doi.org/10.1101/863357

Fain, J.N., 2001. Abnormalities in the functioning of adipocytes from R6/2 mice that are transgenic for the Huntington's disease mutation. Human Molecular Genetics 10, 145–152. https://doi.org/10.1093/hmg/10.2.145

Farrer, L.A., 1985. Diabetes mellitus in Huntington disease. Clin Genet 27, 62–67. https://doi.org/10.1111/j.1399-0004.1985.tb00185.x

Farshim, P.P., Bates, G.P., 2018. Mouse Models of Huntington's Disease, in: Precious, S.V., Rosser, A.E., Dunnett, S.B. (Eds.), Huntington's Disease, Methods in Molecular Biology. Springer New York, New York, NY, pp. 97–120. https://doi.org/10.1007/978-1-4939-7825-0_6

Florez, J.C., 2017. The pharmacogenetics of metformin. Diabetologia 60, 1648–1655. https://doi.org/10.1007/s00125-017-4335-y

Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Ford, R.J., Fullerton, M.D., Pinkosky, S.L., Day, E.A., Scott, J.W., Oakhill, J.S., Bujak, A.L., Smith, B.K., Crane, J.D., Blümer, R.M., Marcinko, K., Kemp, B.E., Gerstein, H.C., Steinberg, G.R., 2015. Metformin and salicylate synergistically activate liver AMPK, inhibit lipogenesis and improve insulin sensitivity. Biochemical Journal 468, 125–132. https://doi.org/10.1042/BJ20150125

Forouzandeh, F., Salazar, G., Patrushev, N., Xiong, S., Hilenski, L., Fei, B., Alexander, R.W., 2014. Metformin Beyond Diabetes: Pleiotropic Benefits of Metformin in Attenuation of Atherosclerosis. JAHA 3. https://doi.org/10.1161/JAHA.114.001202

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Pedersen, H.K., Arumugam, M., Kristiansen, K., Voigt, A.Y., Vestergaard, H., Hercog, R., Costea, P.I., Kultima, J.R., Li, J., Jørgensen, T., Levenez, F., Dore, J., MetaHIT consortium, Nielsen, H.B., Brunak, S., Raes, J., Hansen, T., Wang, J., Ehrlich, S.D., Bork, P., Pedersen, O., 2015. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 528, 262–266. https://doi.org/10.1038/nature15766

García-Alfonso, P., Saiz-Rodríguez, M., Mondéjar, R., Salazar, J., Páez, D., Borobia, A.M., Safont, M.J., García-García, I., Colomer, R., García-González, X., Herrero, M.J., López-Fernández, L.A., Abad-Santos, F., 2021. Consensus of experts from the Spanish Pharmacogenetics and Pharmacogenomics Society and the Spanish Society of Medical Oncology for the genotyping of DPYD in cancer patients who are candidates for treatment with fluoropyrimidines. Clin Transl Oncol. https://doi.org/10.1007/s12094-021-02708-4

Geier, E.G., Chen, E.C., Webb, A., Papp, A.C., Yee, S.W., Sadee, W., Giacomini, K.M., 2013. Profiling Solute Carrier Transporters in the Human Blood-Brain Barrier. Clin Pharmacol Ther 94, 636–639. https://doi.org/10.1038/clpt.2013.175

Ghosh, R., Tabrizi, S.J., 2018. Clinical Features of Huntington's Disease, in: Nóbrega, C., Pereira de Almeida, L. (Eds.), Polyglutamine Disorders, Advances in Experimental Medicine and Biology. Springer International Publishing, Cham, pp. 1–28. https://doi.org/10.1007/978-3-319-71779-1_1

Gómez-Escribano, A., Bono-Yagüe, J., García-Gimeno, M., Sequedo, M., Hervás, D., Fornés-Ferrer, V., Torres-Sánchez, S., Millán, J., Sanz, P., Vázquez-Manrique, R., 2020. Synergistic activation of AMPK prevents from polyglutamine-induced toxicity in Caenorhabditis elegans. Pharmacological Research 161, 105105. https://doi.org/10.1016/j.phrs.2020.105105

Gong, L., Goswami, S., Giacomini, K.M., Altman, R.B., Klein, T.E., 2012. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 22, 820–827. https://doi.org/10.1097/FPC.0b013e3283559b22

Goodman, A.O.G., Murgatroyd, Peter.R., Medina-Gomez, G., Wood, N.I., Finer, N., Vidal-Puig, A.J., Morton, A.J., Barker, R.A., 2008. The metabolic profile of early Huntington's disease- a combined human and transgenic mouse study. Experimental Neurology 210, 691–698. https://doi.org/10.1016/j.expneurol.2007.12.026

Gormsen, L.C., Sundelin, E.I., Jensen, J.B., Vendelbo, M.H., Jakobsen, S., Munk, O.L., Christensen, M.M.H., Brøsen, K., Frøkiær, J., Jessen, N., 2016. In Vivo Imaging of Human 11C-Metformin in Peripheral Organs: Dosimetry, Biodistribution, and Kinetic Analyses. Journal of Nuclear Medicine 57, 1920–1926. https://doi.org/10.2967/jnumed.116.177774

Hackam, A.S., Hodgson, J.G., Singaraja, R., Zhang, T., Gan, L., Gutekunst, C.-A., Hersch, S.M., Hayden, M.R., 1999. Evidence for both nucleus and cytoplasm as subcellular sites of pathogenesis in Huntington'sdisease in cell culture and in transgenic mice expressing mutant huntingtin. Phil. Trans. R. Soc. Lond. B 354, 1047–1055. https://doi.org/10.1098/rstb.1999.0457

He, L., Wondisford, F.E., 2015. Metformin action: concentrations matter. Cell Metab 21, 159–162. https://doi.org/10.1016/j.cmet.2015.01.003

He, R., Zhang, D., Lu, W., Zheng, T., Wan, L., Liu, F., Jia, W., 2015. SLC47A1 gene rs2289669 G>A variants enhance the glucose-lowering effect of metformin via delaying its excretion in Chinese type 2 diabetes patients. Diabetes Res Clin Pract 109, 57–63. https://doi.org/10.1016/j.diabres.2015.05.003

Hervás, D., Fornés-Ferrer, V., Gómez-Escribano, A.P., Sequedo, M.D., Peiró, C., Millán, J.M., Vázquez-Manrique, R.P., 2017. Metformin intake associates with better cognitive function in patients with Huntington's disease. PLoS ONE 12, e0179283. https://doi.org/10.1371/journal.pone.0179283

Holmans, P.A., Massey, T.H., Jones, L., 2017. Genetic modifiers of Mendelian disease: Huntington's disease and the trinucleotide repeat disorders. Human Molecular Genetics 26, R83–R90. https://doi.org/10.1093/hmg/ddx261

Horiuchi, T., Sakata, N., Narumi, Y., Kimura, T., Hayashi, T., Nagano, K., Liu, K., Nishibori, M., Tsukita, S., Yamada, T., Katagiri, H., Shirakawa, R., Horiuchi, H., 2017. Metformin directly binds the alarmin HMGB1 and inhibits its proinflammatory activity. Journal of Biological Chemistry 292, 8436–8446. https://doi.org/10.1074/jbc.M116.769380

Howell, J.J., Hellberg, K., Turner, M., Talbott, G., Kolar, M.J., Ross, D.S., Hoxhaj, G., Saghatelian, A., Shaw, R.J., Manning, B.D., 2017. Metformin Inhibits Hepatic mTORC1 Signaling via Dose-Dependent Mechanisms Involving AMPK and the TSC Complex. Cell Metabolism 25, 463–471. https://doi.org/10.1016/j.cmet.2016.12.009

Jackson, M.A., Verdi, S., Maxan, M.-E., Shin, C.M., Zierer, J., Bowyer, R.C.E., Martin, T., Williams, F.M.K., Menni, C., Bell, J.T., Spector, T.D., Steves, C.J., 2018. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nat Commun 9, 2655. https://doi.org/10.1038/s41467-018-05184-7

Jimenez-Sanchez, M., Licitra, F., Underwood, B.R., Rubinsztein, D.C., 2017. Huntington's Disease: Mechanisms of Pathogenesis and Therapeutic

Strategies. Cold Spring Harb Perspect Med 7, a024240. https://doi.org/10.1101/cshperspect.a024240

Jin, J., Gu, H., Anders, N.M., Ren, T., Jiang, M., Tao, M., Peng, Q., Rudek, M.A., Duan, W., 2016. Metformin Protects Cells from Mutant Huntingtin Toxicity Through Activation of AMPK and Modulation of Mitochondrial Dynamics. Neuromol Med 18, 581–592. https://doi.org/10.1007/s12017-016-8412-z

Jones, U., Busse, M., Enright, S., Rosser, A.E., 2016. Respiratory decline is integral to disease progression in Huntington's disease. Eur Respir J 48, 585–588. https://doi.org/10.1183/13993003.02215-2015

Joseph, B.K., Liu, H.-Y., Francisco, J., Pandya, D., Donigan, M., Gallo-Ebert, C., Giordano, C., Bata, A., Nickels, J.T., 2015. Inhibition of AMP Kinase by the Protein Phosphatase 2A Heterotrimer, PP2APpp2r2d. Journal of Biological Chemistry 290, 10588–10598. https://doi.org/10.1074/jbc.M114.626259

Ju, T.-C., Chen, H.-M., Lin, J.-T., Chang, C.-P., Chang, W.-C., Kang, J.-J., Sun, C.-P., Tao, M.-H., Tu, P.-H., Chang, C., Dickson, D.W., Chern, Y., 2011. Nuclear translocation of AMPK-α1 potentiates striatal neurodegeneration in Huntington's disease. Journal of Cell Biology 194, 209–227. https://doi.org/10.1083/jcb.201105010

Kahn, S.E., Haffner, S.M., Heise, M.A., Herman, W.H., Holman, R.R., Jones, N.P., Kravitz, B.G., Lachin, J.M., O'Neill, M.C., Zinman, B., Viberti, G., ADOPT Study Group, 2006. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med 355, 2427–2443. https://doi.org/10.1056/NEJMoa066224

Kay, C., Hayden, M.R., Leavitt, B.R., 2017. Epidemiology of Huntington disease, in: Handbook of Clinical Neurology. Elsevier, pp. 31–46. https://doi.org/10.1016/B978-0-12-801893-4.00003-1

Kickstein, E., Krauss, S., Thornhill, P., Rutschow, D., Zeller, R., Sharkey, J., Williamson, R., Fuchs, M., Kohler, A., Glossmann, H., Schneider, R., Sutherland, C., Schweiger, S., 2010a. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. Proceedings of the National Academy of Sciences 107, 21830–21835. https://doi.org/10.1073/pnas.0912793107

Kickstein, E., Krauss, S., Thornhill, P., Rutschow, D., Zeller, R., Sharkey, J., Williamson, R., Fuchs, M., Kohler, A., Glossmann, H., Schneider, R., Sutherland, C., Schweiger, S., 2010b. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. Proceedings of the National Academy of Sciences 107, 21830–21835. https://doi.org/10.1073/pnas.0912793107

Kim, Joungmok, Yang, G., Kim, Y., Kim, Jin, Ha, J., 2016. AMPK activators: mechanisms of action and physiological activities. Exp Mol Med 48, e224–e224. https://doi.org/10.1038/emm.2016.16

Kingwell, K., 2021. Double setback for ASO trials in Huntington disease. Nat Rev Drug Discov 20, 412–413. https://doi.org/10.1038/d41573-021-00088-6

Klünemann, M., Andrejev, S., Blasche, S., Mateus, A., Phapale, P., Devendran, S., Vappiani, J., Simon, B., Scott, T.A., Kafkia, E., Konstantinidis, D., Zirngibl, K., Mastrorilli, E., Banzhaf, M., Mackmull, M.-T., Hövelmann, F., Nesme, L., Brochado, A.R., Maier, L., Bock, T., Periwal, V., Kumar, M., Kim, Y., Tramontano, M., Schultz, C., Beck, M., Hennig, J., Zimmermann, M., Sévin, D.C., Cabreiro, F., Savitski, M.M., Bork, P., Typas, A., Patil, K.R., 2021. Bioaccumulation of therapeutic drugs by human gut bacteria. Nature 597, 533–538. https://doi.org/10.1038/s41586-021-03891-8

Kobe, B., Gleichmann, T., Horne, J., Jennings, I.G., Scotney, P.D., Teh, T., 1999. Turn up the HEAT. Structure 7, R91–R97. https://doi.org/10.1016/S0969-2126(99)80060-4

Koepsell, H., 2013. The SLC22 family with transporters of organic cations, anions and zwitterions. Mol Aspects Med 34, 413–435. https://doi.org/10.1016/j.mam.2012.10.010

Kong, G., Cao, K.-A.L., Judd, L.M., Li, S., Renoir, T., Hannan, A.J., 2020. Microbiome profiling reveals gut dysbiosis in a transgenic mouse model of Huntington's disease. Neurobiol Dis 135, 104268. https://doi.org/10.1016/j.nbd.2018.09.001

Kopito, R.R., 2000. Aggresomes, inclusion bodies and protein aggregation. Trends in Cell Biology 10, 524–530. https://doi.org/10.1016/S0962-8924(00)01852-3

Krauß, S., Griesche, N., Jastrzebska, E., Chen, C., Rutschow, D., Achmüller, C., Dorn, S., Boesch, S.M., Lalowski, M., Wanker, E., Schneider, R., Schweiger, S., 2013. Translation of HTT mRNA with expanded CAG repeats is regulated by the MID1–PP2A protein complex. Nat Commun 4, 1511. https://doi.org/10.1038/ncomms2514

Lane, R.F., George-Hyslop, P.S., Hempstead, B.L., Small, S.A., Strittmatter, S.M., Gandy, S., 2012. Vps10 Family Proteins and the Retromer Complex in Aging-Related Neurodegeneration and Diabetes. J. Neurosci. 32, 14080–14086. https://doi.org/10.1523/JNEUROSCI.3359-12.2012

Lee, J.O., Lee, S.K., Kim, J.H., Kim, N., You, G.Y., Moon, J.W., Kim, S.J., Park, S.H., Kim, H.S., 2012. Metformin Regulates Glucose Transporter 4 (GLUT4) Translocation through AMP-activated Protein Kinase (AMPK)-mediated Cbl/CAP Signaling in 3T3-L1 Preadipocyte Cells. Journal of Biological Chemistry 287, 44121–44129. https://doi.org/10.1074/jbc.M112.361386

Leoni, V., Caccia, C., 2015. The impairment of cholesterol metabolism in Huntington disease. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, Brain Lipids 1851, 1095–1105. https://doi.org/10.1016/j.bbalip.2014.12.018

Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., Gordon, J.I., 2005. Obesity alters gut microbial ecology. PNAS 102, 11070–11075. https://doi.org/10.1073/pnas.0504978102

Lunkes, A., Lindenberg, K.S., Ben-Haïem, L., Weber, C., Devys, D., Landwehrmeyer, G.B., Mandel, J.-L., Trottier, Y., 2002. Proteases Acting on Mutant Huntingtin Generate Cleaved Products that Differentially Build Up Cytoplasmic and Nuclear Inclusions. Molecular Cell 10, 259–269. https://doi.org/10.1016/S1097-2765(02)00602-0

Luthi-Carter, R., 2000. Decreased expression of striatal signaling genes in a mouse model of Huntington's disease. Human Molecular Genetics 9, 1259–1271. https://doi.org/10.1093/hmg/9.9.1259

Ma, Buescher, J.L., Oatis, B., Funk, J.A., Nash, A.J., Carrier, R.L., Hoyt, K.R., 2007. Metformin therapy in a transgenic mouse model of Huntington's disease. Neuroscience Letters 411, 98–103. https://doi.org/10.1016/j.neulet.2006.10.039

Ma, W., Chen, J., Meng, Y., Yang, J., Cui, Q., Zhou, Y., 2018. Metformin Alters Gut Microbiota of Healthy Mice: Implication for Its Potential Role in Gut Microbiota Homeostasis. Front Microbiol 9, 1336. https://doi.org/10.3389/fmicb.2018.01336

Macdonald, M., 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72, 971–983. https://doi.org/10.1016/0092-8674(93)90585-E

Madsen, A., Bozickovic, O., Bjune, J.-I., Mellgren, G., Sagen, J.V., 2015. Metformin inhibits hepatocellular glucose, lipid and cholesterol biosynthetic pathways by transcriptionally suppressing steroid receptor coactivator 2 (SRC-2). Sci Rep 5, 16430. https://doi.org/10.1038/srep16430

Matthes, F., Hettich, M.M., Schilling, J., Flores-Dominguez, D., Blank, N., Wiglenda, T., Buntru, A., Wolf, H., Weber, S., Vorberg, I., Dagane, A., Dittmar, G., Wanker, E., Ehninger, D., Krauss, S., 2018. Inhibition of the MID1 protein complex: a novel approach targeting APP protein synthesis. Cell Death Discovery 4, 4. https://doi.org/10.1038/s41420-017-0003-8

Mazzetti, A.P., Fiorile, M.C., Primavera, A., Lo Bello, M., 2015. Glutathione transferases and neurodegenerative diseases. Neurochemistry International 82, 10–18. https://doi.org/10.1016/j.neuint.2015.01.008

Melkani, G., 2016. Huntington's Disease-Induced Cardiac Disorders Affect Multiple Cellular Pathways. ROS. https://doi.org/10.20455/ros.2016.859

Menalled, L.B., Kudwa, A.E., Miller, S., Fitzpatrick, J., Watson-Johnson, J., Keating, N., Ruiz, M., Mushlin, R., Alosio, W., McConnell, K., Connor, D., Murphy, C., Oakeshott, S., Kwan, M., Beltran, J., Ghavami, A., Brunner, D., Park, L.C., Ramboz, S., Howland, D., 2012. Comprehensive Behavioral and Molecular Characterization of a New Knock-In Mouse

Model of Huntington's Disease: zQ175. PLoS ONE 7, e49838. https://doi.org/10.1371/journal.pone.0049838
Mielcarek, M., 2015. Huntington's disease is a multi-system disorder. Rare Diseases 3, e1058464. https://doi.org/10.1080/21675511.2015.1058464
Mihaylova, M.M., Shaw, R.J., 2011. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol 13, 1016–1023. https://doi.org/10.1038/ncb2329
Miller, R.A., Chu, Q., Xie, J., Foretz, M., Viollet, B., Birnbaum, M.J., 2013a. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494, 256–260. https://doi.org/10.1038/nature11808
Miller, R.A., Chu, Q., Xie, J., Foretz, M., Viollet, B., Birnbaum, M.J., 2013b. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494, 256–260. https://doi.org/10.1038/nature11808
Montoya, A., Price, B.H., Menear, M., Lepage, M., 2006. Brain imaging and cognitive dysfunctions in Huntington's disease. J Psychiatry Neurosci 31, 21–29.
Moon, S.J., Oh, J., Lee, S.H., Choi, Y., Yu, K.-S., Chung, J.-Y., 2018. Effect of plasma membrane monoamine transporter genetic variants on pharmacokinetics of metformin in humans. Transl Clin Pharmacol 26, 79–85. https://doi.org/10.12793/tcp.2018.26.2.79
Mousavi, S., Kohan, L., Yavarian, M., Habib, A., 2017. Pharmacogenetic variation of SLC47A1 gene and metformin response in type2 diabetes patients. Mol Biol Res Commun 6, 91–94.
Nasri, H., Rafieian-Kopaei, M., 2014. Metformin: Current knowledge. J Res Med Sci 19, 658–664.
Novelle, M.G., Ali, A., Diéguez, C., Bernier, M., de Cabo, R., 2016. Metformin: A Hopeful Promise in Aging Research. Cold Spring Harb Perspect Med 6, a025932. https://doi.org/10.1101/cshperspect.a025932
O'Brien, A.J., Villani, L.A., Broadfield, L.A., Houde, V.P., Galic, S., Blandino, G., Kemp, B.E., Tsakiridis, T., Muti, P., Steinberg, G.R., 2015. Salicylate activates AMPK and synergizes with metformin to reduce the survival of prostate and lung cancer cells ex vivo through inhibition of de novo lipogenesis. Biochemical Journal 469, 177–187. https://doi.org/10.1042/BJ20150122
Onken, B., Driscoll, M., 2010a. Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1. PLoS One 5, e8758. https://doi.org/10.1371/journal.pone.0008758
Onken, B., Driscoll, M., 2010b. Metformin Induces a Dietary Restriction–Like State and the Oxidative Stress Response to Extend C. elegans Healthspan

via AMPK, LKB1, and SKN-1. PLoS ONE 5, e8758. https://doi.org/10.1371/journal.pone.0008758

Owen, M.R., Doran, E., Halestrap, A.P., 2000. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochemical Journal 348, 607–614. https://doi.org/10.1042/bj3480607

Patassini, S., Begley, P., Xu, J., Church, S.J., Reid, S.J., Kim, E.H., Curtis, M.A., Dragunow, M., Waldvogel, H.J., Snell, R.G., Unwin, R.D., Faull, R.L.M., Cooper, G.J.S., 2016. Metabolite mapping reveals severe widespread perturbation of multiple metabolic processes in Huntington's disease human brain. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1862, 1650–1662. https://doi.org/10.1016/j.bbadis.2016.06.002

Pattison, J.S., Robbins, J., 2008. Protein misfolding and cardiac disease: Establishing cause and effect. Autophagy 4, 821–823. https://doi.org/10.4161/auto.6502

Pattison, J.S., Sanbe, A., Maloyan, A., Osinska, H., Klevitsky, R., Robbins, J., 2008. Cardiomyocyte Expression of a Polyglutamine Preamyloid Oligomer Causes Heart Failure. Circulation 117, 2743–2751. https://doi.org/10.1161/CIRCULATIONAHA.107.750232

Pedersen, A.J.T., Stage, T.B., Glintborg, D., Andersen, M., Christensen, M.M.H., 2018. The Pharmacogenetics of Metformin in Women with Polycystic Ovary Syndrome: A Randomized Trial. Basic & Clinical Pharmacology & Toxicology 122, 239–244. https://doi.org/10.1111/bcpt.12874

Provost, J.-S., Hanganu, A., Monchi, O., 2015. Neuroimaging studies of the striatum in cognition Part I: healthy individuals. Front. Syst. Neurosci. 9. https://doi.org/10.3389/fnsys.2015.00140

Pryor, R., Norvaisas, P., Marinos, G., Best, L., Thingholm, L.B., Quintaneiro, L.M., De Haes, W., Esser, D., Waschina, S., Lujan, C., Smith, R.L., Scott, T.A., Martinez-Martinez, D., Woodward, O., Bryson, K., Laudes, M., Lieb, W., Houtkooper, R.H., Franke, A., Temmerman, L., Bjedov, I., Cochemé, H.M., Kaleta, C., Cabreiro, F., 2019. Host-Microbe-Drug-Nutrient Screen Identifies Bacterial Effectors of Metformin Therapy. Cell 178, 1299-1312.e29. https://doi.org/10.1016/j.cell.2019.08.003

Ramamurthy, S., Ronnett, G., 2012. AMP-Activated Protein Kinase (AMPK) and Energy-Sensing in the Brain. Exp Neurobiol 21, 52–60. https://doi.org/10.5607/en.2012.21.2.52

Reiner, A., Dragatsis, I., Zeitlin, S., Goldowitz, D., 2003. Wild-Type Huntingtin Plays a Role in Brain Development and Neuronal Survival. MN 28, 259–276. https://doi.org/10.1385/MN:28:3:259

Rojas, L.B.A., Gomes, M.B., 2013. Metformin: an old but still the best treatment for type 2 diabetes. Diabetology & Metabolic Syndrome 5, 6. https://doi.org/10.1186/1758-5996-5-6

Rui, Y.-N., Xu, Z., Patel, B., Chen, Z., Chen, D., Tito, A., David, G., Sun, Y., Stimming, E.F., Bellen, H.J., Cuervo, A.M., Zhang, S., 2015. Huntingtin functions as a scaffold for selective macroautophagy. Nat Cell Biol 17, 262–275. https://doi.org/10.1038/ncb3101

Sanchis, A., García-Gimeno, M.A., Cañada-Martínez, A.J., Sequedo, M.D., Millán, J.M., Sanz, P., Vázquez-Manrique, R.P., 2019. Metformin treatment reduces motor and neuropsychiatric phenotypes in the zQ175 mouse model of Huntington disease. Exp Mol Med 51, 1–16. https://doi.org/10.1038/s12276-019-0264-9

Santoro, A.B., Botton, M.R., Struchiner, C.J., Suarez-Kurtz, G., 2018. Influence of pharmacogenetic polymorphisms and demographic variables on metformin pharmacokinetics in an admixed Brazilian cohort. Br J Clin Pharmacol 84, 987–996. https://doi.org/10.1111/bcp.13522

Sanz, P., Rubio, T., Garcia-Gimeno, M.A., 2013. AMPKbeta subunits: more than just a scaffold in the formation of AMPK complex. FEBS J 280, 3723–3733. https://doi.org/10.1111/febs.12364

Saudou, F., Humbert, S., 2016a. The Biology of Huntingtin. Neuron 89, 910–926. https://doi.org/10.1016/j.neuron.2016.02.003

Saudou, F., Humbert, S., 2016b. The Biology of Huntingtin. Neuron 89, 910–926. https://doi.org/10.1016/j.neuron.2016.02.003

Savas, J.N., Ribeiro, L.F., Wierda, K.D., Wright, R., DeNardo, L.A., Rice, H.C., Chamma, I., Wang, Y.-Z., Zemla, R., Lavallée-Adam, M., Vennekens, K.M., O'Sullivan, M.L., Antonios, J.K., Hall, E.A., Thoumine, O., Attie, A.D., Ghosh, A., Yates, J.R., de Wit, J., 2015. The Sorting Receptor SorCS1 Regulates Trafficking of Neurexin and AMPA Receptors. Neuron 87, 764–780. https://doi.org/10.1016/j.neuron.2015.08.007

Scheperjans, F., Aho, V., Pereira, P.A.B., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E., Kaakkola, S., Eerola-Rautio, J., Pohja, M., Kinnunen, E., Murros, K., Auvinen, P., 2015. Gut microbiota are related to Parkinson's disease and clinical phenotype. Mov Disord 30, 350–358. https://doi.org/10.1002/mds.26069

Seo, H., Sonntag, K.-C., Isacson, O., 2004. Generalized brain and skin proteasome inhibition in Huntington's disease. Ann Neurol. 56, 319–328. https://doi.org/10.1002/ana.20207

Settanni, C.R., Ianiro, G., Bibbò, S., Cammarota, G., Gasbarrini, A., 2021. Gut microbiota alteration and modulation in psychiatric disorders: Current evidence on fecal microbiota transplantation. Progress in Neuro-Psychopharmacology and Biological Psychiatry 109, 110258. https://doi.org/10.1016/j.pnpbp.2021.110258

Shikata, E., Yamamoto, R., Takane, H., Shigemasa, C., Ikeda, T., Otsubo, K., Ieiri, I., 2007. Human organic cation transporter (OCT1 and OCT2) gene polymorphisms and therapeutic effects of metformin. J Hum Genet 52, 117–122. https://doi.org/10.1007/s10038-006-0087-0

Shin, N.-R., Lee, J.-C., Lee, H.-Y., Kim, M.-S., Whon, T.W., Lee, M.-S., Bae, J.-W., 2014. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut 63, 727–735. https://doi.org/10.1136/gutjnl-2012-303839

Shoulson, I., Fahn, S., 1979. Huntington disease: Clinical care and evaluation. Neurology 29, 1–1. https://doi.org/10.1212/WNL.29.1.1

Shu, Y., Brown, C., Castro, R., Shi, R., Lin, E., Owen, R., Sheardown, S., Yue, L., Burchard, E., Brett, C., Giacomini, K., 2008. Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. Clin Pharmacol Ther 83, 273–280. https://doi.org/10.1038/sj.clpt.6100275

Shu, Y., Sheardown, S.A., Brown, C., Owen, R.P., Zhang, S., Castro, R.A., Ianculescu, A.G., Yue, L., Lo, J.C., Burchard, E.G., Brett, C.M., Giacomini, K.M., 2007. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 117, 1422–1431. https://doi.org/10.1172/JCI30558

Sidik, H., Ang, C.J., Pouladi, M.A., 2020. Huntingtin confers fitness but is not embryonically essential in zebrafish development. Developmental Biology 458, 98–105. https://doi.org/10.1016/j.ydbio.2019.10.037

Solís-Maldonado, M., Miró, M.P., Acuña, A.I., Covarrubias-Pinto, A., Loaiza, A., Mayorga, G., Beltrán, F.A., Cepeda, C., Levine, M.S., Concha, I.I., Bátiz, L.F., Carrasco, M.A., Castro, M.A., 2018. Altered lactate metabolism in Huntington's disease is dependent on GLUT3 expression. CNS Neurosci Ther 24, 343–352. https://doi.org/10.1111/cns.12837

Song, I.S., Shin, H.J., Shim, E.J., Jung, I.S., Kim, W.Y., Shon, J.H., Shin, J.G., 2008. Genetic variants of the organic cation transporter 2 influence the disposition of metformin. Clin Pharmacol Ther 84, 559–562. https://doi.org/10.1038/clpt.2008.61

Stan, T.L., Soylu-Kucharz, R., Burleigh, S., Prykhodko, O., Cao, L., Franke, N., Sjögren, M., Haikal, C., Hållenius, F., Björkqvist, M., 2020. Increased intestinal permeability and gut dysbiosis in the R6/2 mouse model of Huntington's disease. Sci Rep 10, 18270. https://doi.org/10.1038/s41598-020-75229-9

Stepensky, D., Friedman, M., Raz, I., Hoffman, A., 2002. Pharmacokinetic-pharmacodynamic analysis of the glucose-lowering effect of metformin in diabetic rats reveals first-pass pharmacodynamic effect. Drug Metab Dispos 30, 861–868. https://doi.org/10.1124/dmd.30.8.861

Sundelin, E., Jensen, J.B., Jakobsen, S., Gormsen, L.C., Jessen, N., 2020. Metformin Biodistribution: A Key to Mechanisms of Action? J Clin Endocrinol Metab 105, dgaa332. https://doi.org/10.1210/clinem/dgaa332

Tabrizi, S.J., Leavitt, B.R., Landwehrmeyer, G.B., Wild, E.J., Saft, C., Barker, R.A., Blair, N.F., Craufurd, D., Priller, J., Rickards, H., Rosser, A., Kordasiewicz, H.B., Czech, C., Swayze, E.E., Norris, D.A., Baumann, T.,

Gerlach, I., Schobel, S.A., Paz, E., Smith, A.V., Bennett, C.F., Lane, R.M., 2019. Targeting Huntingtin Expression in Patients with Huntington's Disease. N Engl J Med 380, 2307–2316. https://doi.org/10.1056/NEJMoa1900907

Takahashi, T., Katada, S., Onodera, O., 2010. Polyglutamine Diseases: Where does Toxicity Come from? What is Toxicity? Where are We Going? Journal of Molecular Cell Biology 2, 180–191. https://doi.org/10.1093/jmcb/mjq005

Takano, H., Gusella, J.F., 2002. The predominantly HEAT-like motif structure of huntingtin and its association and coincident nuclear entry with dorsal, an NF-kB/Rel/dorsal family transcription factor. BMC Neurosci 3, 15. https://doi.org/10.1186/1471-2202-3-15

Talarico, G., Orecchioni, S., Dallaglio, K., Reggiani, F., Mancuso, P., Calleri, A., Gregato, G., Labanca, V., Rossi, T., Noonan, D.M., Albini, A., Bertolini, F., 2016. Aspirin and atenolol enhance metformin activity against breast cancer by targeting both neoplastic and microenvironment cells. Sci Rep 6, 18673. https://doi.org/10.1038/srep18673

Talukder, P., Jana, A., Dhar, S., Ghosh, S., 2021. Huntington's Chorea—a Rare Neurodegenerative Autosomal Dominant Disease: Insight into Molecular Genetics, Prognosis and Diagnosis. Appl Biochem Biotechnol 193, 2634–2648. https://doi.org/10.1007/s12010-021-03523-x

Tang, B.L., 2020. Could metformin be therapeutically useful in Huntington's disease? Reviews in the Neurosciences 31, 297–317. https://doi.org/10.1515/revneuro-2019-0072

Thakur, S., Daley, B., Gaskins, K., Vasko, V.V., Boufraqech, M., Patel, D., Sourbier, C., Reece, J., Cheng, S.-Y., Kebebew, E., Agarwal, S., Klubo-Gwiezdzinska, J., 2018. Metformin Targets Mitochondrial Glycerophosphate Dehydrogenase to Control Rate of Oxidative Phosphorylation and Growth of Thyroid Cancer *In Vitro* and *In Vivo*. Clin Cancer Res 24, 4030–4043. https://doi.org/10.1158/1078-0432.CCR-17-3167

The Human Protein Atlas https://www.proteinatlas.org/ (accessed 1.31.22).

Theken, K.N., Lee, C.R., Gong, L., Caudle, K.E., Formea, C.M., Gaedigk, A., Klein, T.E., Agúndez, J.A.G., Grosser, T., 2020. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and Nonsteroidal Anti-Inflammatory Drugs. Clinical Pharmacology & Therapeutics 108, 191–200. https://doi.org/10.1002/cpt.1830

Thul, P.J., Åkesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Ait Blal, H., Alm, T., Asplund, A., Björk, L., Breckels, L.M., Bäckström, A., Danielsson, F., Fagerberg, L., Fall, J., Gatto, L., Gnann, C., Hober, S., Hjelmare, M., Johansson, F., Lee, S., Lindskog, C., Mulder, J., Mulvey, C.M., Nilsson, P., Oksvold, P., Rockberg, J., Schutten, R., Schwenk, J.M., Sivertsson, Å., Sjöstedt, E., Skogs, M., Stadler, C., Sullivan, D.P., Tegel,

H., Winsnes, C., Zhang, C., Zwahlen, M., Mardinoglu, A., Pontén, F., von Feilitzen, K., Lilley, K.S., Uhlén, M., Lundberg, E., 2017. A subcellular map of the human proteome. Science 356, eaal3321. https://doi.org/10.1126/science.aal3321

Tissue expression of HTT - Summary - The Human Protein Atlas [WWW Document], n.d. URL https://www.proteinatlas.org/ENSG00000197386-HTT/tissue (accessed 1.31.22).

Tkáč, I., Klimčáková, L., Javorský, M., Fabianová, M., Schroner, Z., Hermanová, H., Babjaková, E., Tkáčová, R., 2013. Pharmacogenomic association between a variant in SLC47A1 gene and therapeutic response to metformin in type 2 diabetes. Diabetes, Obesity and Metabolism 15, 189–191. https://doi.org/10.1111/j.1463-1326.2012.01691.x

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027–1031. https://doi.org/10.1038/nature05414

Turner, R.C., Cull, C.A., Frighi, V., Holman, R.R., 1999. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. JAMA 281, 2005–2012. https://doi.org/10.1001/jama.281.21.2005

Tzvetkov, M.V., Vormfelde, S.V., Balen, D., Meineke, I., Schmidt, T., Sehrt, D., Sabolić, I., Koepsell, H., Brockmöller, J., 2009. The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther 86, 299–306. https://doi.org/10.1038/clpt.2009.92

Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, A., Kampf, C., Sjostedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C.A.-K., Odeberg, J., Djureinovic, D., Takanen, J.O., Hober, S., Alm, T., Edqvist, P.-H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J.M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., Ponten, F., 2015. Tissue-based map of the human proteome. Science 347, 1260419–1260419. https://doi.org/10.1126/science.1260419

Van Raamsdonk, J.M., Murphy, Z., Selva, D.M., Hamidizadeh, R., Pearson, J., Petersén, Åsa, Björkqvist, M., Muir, C., Mackenzie, I.R., Hammond, G.L., Vogl, A.W., Hayden, M.R., Leavitt, B.R., 2007. Testicular degeneration in Huntington disease. Neurobiology of Disease 26, 512–520. https://doi.org/10.1016/j.nbd.2007.01.006

Vázquez-Manrique, R.P., Farina, F., Cambon, K., Dolores Sequedo, M., Parker, A.J., Millán, J.M., Weiss, A., Déglon, N., Neri, C., 2016. AMPK activation protects from neuronal dysfunction and vulnerability across nematode,

cellular and mouse models of Huntington's disease. Human Molecular Genetics 25, 1043–1058. https://doi.org/10.1093/hmg/ddv513

Vazquez-Manrique, R.P., Farina, F., Cambon, K., Dolores Sequedo, M., Parker, A.J., Mill?n, J.M., Weiss, A., D?glon, N., Neri, C., 2016. AMPK activation protects from neuronal dysfunction and vulnerability across nematode, cellular and mouse models of Huntington's disease. Human Molecular Genetics 25, 1043–1058. https://doi.org/10.1093/hmg/ddv513

Velier, J., Kim, M., Schwarz, C., Kim, T.W., Sapp, E., Chase, K., Aronin, N., DiFiglia, M., 1998. Wild-Type and Mutant Huntingtins Function in Vesicle Trafficking in the Secretory and Endocytic Pathways. Experimental Neurology 152, 34–40. https://doi.org/10.1006/exnr.1998.6832

Vich Vila, A., Collij, V., Sanna, S., Sinha, T., Imhann, F., Bourgonje, A.R., Mujagic, Z., Jonkers, D.M.A.E., Masclee, A.A.M., Fu, J., Kurilshikov, A., Wijmenga, C., Zhernakova, A., Weersma, R.K., 2020. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Nat Commun 11, 362. https://doi.org/10.1038/s41467-019-14177-z

Virtanen, K.A., Hällsten, K., Parkkola, R., Janatuinen, T., Lönnqvist, F., Viljanen, T., Rönnemaa, T., Knuuti, J., Huupponen, R., Lönnroth, P., Nuutila, P., 2003. Differential effects of rosiglitazone and metformin on adipose tissue distribution and glucose uptake in type 2 diabetic subjects. Diabetes 52, 283–290. https://doi.org/10.2337/diabetes.52.2.283

Vitet, H., Brandt, V., Saudou, F., 2020. Traffic signaling: new functions of huntingtin and axonal transport in neurological disease. Current Opinion in Neurobiology 63, 122–130. https://doi.org/10.1016/j.conb.2020.04.001

Vogt, N.M., Kerby, R.L., Dill-McFarland, K.A., Harding, S.J., Merluzzi, A.P., Johnson, S.C., Carlsson, C.M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B.B., Rey, F.E., 2017. Gut microbiome alterations in Alzheimer's disease. Sci Rep 7, 13537. https://doi.org/10.1038/s41598-017-13601-y

Walter, C., Clemens, L.E., Müller, A.J., Fallier-Becker, P., Proikas-Cezanne, T., Riess, O., Metzger, S., Nguyen, H.P., 2016. Activation of AMPK-induced autophagy ameliorates Huntington disease pathology in vitro. Neuropharmacology 108, 24–38. https://doi.org/10.1016/j.neuropharm.2016.04.041

Wang, C., Liu, F., Yuan, Y., Wu, J., Wang, H., Zhang, L., Hu, P., Li, Z., Li, Q., Ye, J., 2014. Metformin suppresses lipid accumulation in skeletal muscle by promoting fatty acid oxidation. Clin Lab 60, 887–896. https://doi.org/10.7754/clin.lab.2013.130531

Wang, H.-X., Wang, Y.-P., 2016. Gut Microbiota-brain Axis. Chin Med J (Engl) 129, 2373–2380. https://doi.org/10.4103/0366-6999.190667

Wang, L.-C., Chen, K.-Y., Pan, H., Wu, C.-C., Chen, P.-H., Liao, Y.-T., Li, C., Huang, M.-L., Hsiao, K.-M., 2011. Muscleblind participates in RNA toxicity of expanded CAG and CUG repeats in Caenorhabditis elegans. Cell. Mol. Life Sci. 68, 1255–1267. https://doi.org/10.1007/s00018-010-0522-4

Wasser, C.I., Mercieca, E.-C., Kong, G., Hannan, A.J., McKeown, S.J., Glikmann-Johnston, Y., Stout, J.C., 2020. Gut dysbiosis in Huntington's disease: associations among gut microbiota, cognitive performance and clinical outcomes. Brain Commun 2, fcaa110. https://doi.org/10.1093/braincomms/fcaa110

Weersma, Rinse K, Zhernakova, A., Fu, J., 2020. Interaction between drugs and the gut microbiome. Gut 69, 1510–1519. https://doi.org/10.1136/gutjnl-2019-320204

Weersma, Rinse K., Zhernakova, A., Fu, J., 2020. Interaction between drugs and the gut microbiome. Gut 69, 1510–1519. https://doi.org/10.1136/gutjnl-2019-320204

Whirl-Carrillo, M., Huddart, R., Gong, L., Sangkuhl, K., Thorn, C.F., Whaley, R., Klein, T.E., 2021. An Evidence-Based Framework for Evaluating Pharmacogenomics Knowledge for Personalized Medicine. Clinical Pharmacology & Therapeutics 110, 563–572. https://doi.org/10.1002/cpt.2350

Wu, H., Esteve, E., Tremaroli, V., Khan, M.T., Caesar, R., Mannerås-Holm, L., Ståhlman, M., Olsson, L.M., Serino, M., Planas-Fèlix, M., Xifra, G., Mercader, J.M., Torrents, D., Burcelin, R., Ricart, W., Perkins, R., Fernàndez-Real, J.M., Bäckhed, F., 2017. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 23, 850–858. https://doi.org/10.1038/nm.4345

Wu, L., Zhou, B., Oshiro-Rapley, N., Li, M., Paulo, J.A., Webster, C.M., Mou, F., Kacergis, M.C., Talkowski, M.E., Carr, C.E., Gygi, S.P., Zheng, B., Soukas, A.A., 2016. An Ancient, Unified Mechanism for Metformin Growth Inhibition in C. elegans and Cancer. Cell 167, 1705-1718.e13. https://doi.org/10.1016/j.cell.2016.11.055

Ye, W., Ramos, E.H., Wong, B.C., Belsham, D.D., 2016. Beneficial Effects of Metformin and/or Salicylate on Palmitate- or TNFα-Induced Neuroinflammatory Marker and Neuropeptide Gene Regulation in Immortalized NPY/AgRP Neurons. PLoS ONE 11, e0166973. https://doi.org/10.1371/journal.pone.0166973

Zake, D.M., Kurlovics, J., Zaharenko, L., Komasilovs, V., Klovins, J., Stalidzans, E., 2021. Physiologically based metformin pharmacokinetics model of mice and scale-up to humans for the estimation of concentrations in various tissues. PLOS ONE 16, e0249594. https://doi.org/10.1371/journal.pone.0249594

Zečić, A., Dhondt, I., Braeckman, B.P., 2019. The nutritional requirements of Caenorhabditis elegans. Genes Nutr 14, 15. https://doi.org/10.1186/s12263-019-0637-7

Zhang, F., Sun, W., Chen, J., Jiang, L., Yang, P., Huang, Y., Gong, A., Liu, S., Ma, S., 2018. SREBP-2, a new target of metformin? DDDT Volume 12, 4163–4170. https://doi.org/10.2147/DDDT.S190094

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., Wang, J., Imhann, F., Brandsma, E., Jankipersadsing, S.A., Joossens, M., Cenit, M.C., Deelen, P., Swertz, M.A., LifeLines cohort study, Weersma, R.K., Feskens, E.J.M., Netea, M.G., Gevers, D., Jonkers, D., Franke, L., Aulchenko, Y.S., Huttenhower, C., Raes, J., Hofker, M.H., Xavier, R.J., Wijmenga, C., Fu, J., 2016. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352, 565–569. https://doi.org/10.1126/science.aad3369

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M.F., Goodyear, L.J., Moller, D.E., 2001. Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108, 1167–1174. https://doi.org/10.1172/JCI13505

Zhou, X., Chen, J., Yi, G., Deng, M., Liu, H., Liang, M., Shi, B., Fu, X., Chen, Y., Chen, L., He, Z., Wang, J., Liu, J., 2016. Metformin suppresses hypoxia-induced stabilization of HIF-1α through reprogramming of oxygen metabolism in hepatocellular carcinoma. Oncotarget 7, 873–884. https://doi.org/10.18632/oncotarget.6418

Zhou, Z.-Y., Ren, L.-W., Zhan, P., Yang, H.-Y., Chai, D.-D., Yu, Z.-W., 2016. Metformin exerts glucose-lowering action in high-fat fed mice via attenuating endotoxemia and enhancing insulin signaling. Acta Pharmacol Sin 37, 1063–1075. https://doi.org/10.1038/aps.2016.21

Zhu, X., Shen, W., Liu, Z., Sheng, S., Xiong, W., He, R., Zhang, X., Ma, L., Ju, Z., 2021. Effect of Metformin on Cardiac Metabolism and Longevity in Aged Female Mice. Front. Cell Dev. Biol. 8, 626011. https://doi.org/10.3389/fcell.2020.626011

Zimmermann, M., Patil, K.R., Typas, A., Maier, L., 2021. Towards a mechanistic understanding of reciprocal drug–microbiome interactions. Mol Syst Biol 17, e10116. https://doi.org/10.15252/msb.202010116

Zu, T., Guo, S., Bardhi, O., Ryskamp, D.A., Li, J., Khoramian Tusi, S., Engelbrecht, A., Klippel, K., Chakrabarty, P., Nguyen, L., Golde, T.E., Sonenberg, N., Ranum, L.P.W., 2020a. Metformin inhibits RAN translation through PKR pathway and mitigates disease in *C9orf72* ALS/FTD mice. Proc Natl Acad Sci USA 202005748. https://doi.org/10.1073/pnas.2005748117

Zu, T., Guo, S., Bardhi, O., Ryskamp, D.A., Li, J., Khoramian Tusi, S., Engelbrecht, A., Klippel, K., Chakrabarty, P., Nguyen, L., Golde, T.E., Sonenberg, N., Ranum, L.P.W., 2020b. Metformin inhibits RAN translation through PKR pathway and mitigates disease in *C9orf72* ALS/FTD mice. Proc Natl Acad Sci USA 117, 18591–18599. https://doi.org/10.1073/pnas.2005748117

Zuccato, C., 2001. Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease. Science 293, 493–498. https://doi.org/10.1126/science.1059581

Table 1. Targets of metformin and mechanisms of action

Targets[1]	Mode of action[2]	Model organism/cell	Effect upon treatment	References[3]
Mitochondrial respiratory chain (complex I)	Direct interaction, inhibition. Reduction of ATP production, and increase in AMP	Cultured rat hepatocytes Cultured rat hepatocytes	Metabolic changes	(El-Mir et al., 2000; Owen et al., 2000)
AMPK	Indirect activation by drops in ATP after inhibition of Complex I	Cultured rat hepatocytes *C. elegans*	Metabolic changes Lifespan and healthspan increase	(Onken & Driscoll, 2010; G. Zhou et al., 2001)
GLUT4	Indirect, AMPK-dependent	3T3-L1 preadipocytes	GLUT4 translocation from vesicles to plasma membrane	(Lee et al., 2012)
SREBP-2	Indirect, inhibition of transcription, partially AMPK-dependent	HepG2, human liver cancer cell line	Reduction of glucose and fat production in hepatocytes	(Madsen et al., 2015; Zhang et al., 2018)
Adenylate cyclase	Indirect inhibition	Mouse liver and mouse and human hepatocytes	TSC complex-mediated attenuation of protein synthesis	(Miller et al., 2013)
Protein kinase A	Indirect inhibition		Reduction of gluconeogenesis	(Miller et al., 2013)
Mitochondrial glycerophosphate dehydrogenase	Downregulation of expression	Human thyroid cancer cell lines	Attenuated tumour growth	(Thakur et al., 2018)
Alarmin HMGB1	Direct binding	Rat liver	Reduction of inflammation	(Horiuchi et al., 2017)

KDM6A/UTX, H3K27me3 demethylase[4]	Direct binding	Immortalized mouse embryonic fibroblasts	Reversion of aging-related epigenetics	(Cuyàs et al., 2018b)
SIRT1	Direct binding, activation	*In silico* and *in vitro* analysis	Activation of enzymatic activity	(Cuyàs et al., 2018a)
PP2A	Direct binding	Primary neurons	Reduction of TAU phosphorylation	(Kickstein et al., 2010b)
MID1 complex	Disrupts MID1 association to other enzymes	APP/PS1 mice (*in vivo*) and cultured murine primary cortical neurons	Reduction of expression of the amyloid precursor protein	(Matthes et al., 2018)
Double-stranded RNA-dependent protein kinase	Indirect inhibition (?)	BAC-HD mouse model of HD	Reduction of RAN translation	(Zu et al., 2020)
nuclear pore complex-mTORC1-acyl-CoA dehydrogenase	Indirect inhibition, due to inhibition of Mitochondrial Complex I	*C. elegans*	Inhibition of organismal growth	(Wu et al., 2016)
NF-κB	Indirect inhibition, AMPK-mediated	Cultured human umbilical vein endothelial cells	Anti-atherogenic effects	(Cacicedo et al., 2004)
mTORC1	Indirect inhibition, partially AMPK-dependent	Mouse liver (*in vivo*) and mouse and human hepatocytes	Strong inhibition of protein synthesis in liver	(Howell et al., 2017)

[1]This table is not be exhaustive as many targets are indirectly activated/inhibited as a result of the activation of other targets, as it happens, for example, with downstream effectors of AMPK.

[2]Many mechanisms of action of metformin are linked. For example, inhibition of complex I of the mitochondrial respiratory chain induces indirect activation of AMPK, but also induces activation of the adenyl cyclase, which in turns inhibits protein kinase A.

[3]Literature not exhaustive. We do not intent to report every target in every organism reported to date.

[4]From 41 *in silico*-predicted targets of metformin, several methylation marks were studied and this demethylase was confirmed as a target of metformin.

Table 2. Allelic frequencies of different alleles implicated in the performance of metformin

		gnomAD (Exome/Genome)[1]				
Associated genes	Variant	Alleles	Frequency All populations(%)	Major Allele Range of freq. in populations[4]	Allelic implications	Evidence[2]
ATM or C11orf65	rs11212617	C>A	51.27/48.73	35.85 ASJ-72.74 AFR	AC or CC carriers may have better response to metformin	Level 4[3]
SLC47A1 or *SNORA59B*	rs2289669	G>A	68.10/31.90	48.53 ASJ-91.27 AFR	AA carriers may have better response to metformin	Level 3[3]
SLC22A1	rs628031	G>A/C	63.26/36.74/0.01	55.18 FIN-73.36 AMR	GG carriers may have increased response to metformin response and decreased risk for gastrointestinal side effects	Level 3
SLC22A1	rs12208357	C>T	94.83/5.18	90.46 ASJ-99.98 EAS	Patients with the CC or CT genotypes may have increased bioavailability of metformin	Level 3
SLC22A1	rs72552763	GAT>del	87.90/12.10	78.32 AMR-99.93 EAS	Patients with the GAT/GAT genotype who	Level 3

					are treated with metformin may have an increased trough metformin steady-state concentration	
SLC22A1	rs2282143	C>T	96.24/3.76	86.83 EAS-99.98 ASJ	Patients with the CC genotype may have increased clearance of metformin	Level 3
SLC22A1	rs594709	A>G	64.09/35.91	56.23 FIN-73.10 AMR	Patients with AA or AG genotypes may have improved response to metformin	Level 3
SLC22A1	rs36056065	GTAAGTTG > del	No data available		Patients with the GTAAGTTG/GTAAGTTG genotype may have increased risk for gastrointestinal side effects	Level 3
SLC22A1	rs622342	A>C	70.01/29.99	63.01 NFE-83.89 EAS	Patients with the AA genotype and the GG genotype at rs2289669 may have a better response to metformin	Level 3
SLC22A2	rs316019	C>A	89.85/10.15	84.69 AFR-94.84 AMR	Patients with the CC genotype may have	Level 3

					increased clearance of metformin	
SLC22A3	rs8187725	C>T	100/0	99.9-0.01 NFE	Cells with the TT genotype may have decreased uptake of metformin	Level 3
SLC22A3	rs2076828	C>G	56.79/43.21	38.65 SAS-67.63 AMR	Patients with GG genotype may have increased response to metformin	Level 3
SLC2A2	rs8192675	T>C	58.24-41.76	29.13 AFR-76.57 EAS	Patients with the CC or CT genotypes may have increased response to metformin	Level 3
SLC47A1	rs2252281	T>C	67.90-32.10	59.96 ASJ-82.83 SAS	Patients with the CC genotype treated with metformin may have an increased response	Level 3
SLC47A1	rs2289669	G>A	68.10-31.90	48.53 ASJ-91.27 AFR	Patients with the genotype AA may have better response to metformin	Level 3
SLC47A2	rs34834489	G>A	72.58-27.42	60.83 FIN-90.75 AFR	Patients with the GG genotype may have decreased renal clearance	Level 3

					and secretion clearance of metformin	
SLC47A2	rs12943590	G>A	72.63-27.37	54.86 EAS-78.12 AFR	Individuals with the GG genotype may have decreased renal and secretory clearance and increased response to metformin	Level 3
AMHR2	rs784892	G>A	91.10-8.90	71.21 AFR-100 AMJ, EAS, FIN	AA carriers may have decreased efficacy	Level 3
CAPN10	rs3792269	A>G	85.31-14.69	80.16 SAS-95.42 AFR	Patients with AA genotype may have an increased response	Level 3
CPA6	rs2162145	C>T	60.15-39.85	24.69 AFR-75.29 NFE	Patients with the CC genotype may have decreased response to metformin	Level 3
FMO5	rs7541245	C>A	96.32-3.68	90.57 SAS-99.81 EAS	Patients with the AA genotype may have a decreased response to metformin	Level 3
KCNJ11	rs5219	C>T	64.02-35.98	52.32 FIN-93.79 AFR	Patients with the TT genotype may have an	Level 3

					increased likelihood of treatment failure	
NBEA	rs57081354	T>C	91.91-8.09	88.42 AFR-96.34 AMR	Patients with the TT genotype may have increased response to metformin	Level 3
PPARA	rs149711321	T>C	94.09-5.91	81.29 AFR-100 EAS	CC and TC genotypes may be associated with an increased secretory clearance of metformin, leading to reduced exposure and decreased metformin efficacy	Level 3
PRPF31	rs254271	G>C/A	68.17-31.81-0.02	57.34 EAS-76.64 AFR	Patients with the GG genotype may have increased response to metformin	Level 3
SP1	rs2683511	C>T	91.47-8.53	72.37 AFR-100 AMI,EAS,FIN	CC genotype may be associated with a decreased secretory clearance of metformin, leading to increased exposure and improved metformin efficacy	Level 3

SP1	rs784888	G>C	89.52-10.48	66.29 AFR-100 AMI, EAS	Patients with the GG genotype may have decreased clearance of metformin leading to improved response to metformin	Level 3

[1]The gnomAD database considered has been "Exome, v2" for exonic variants and "Genome, v3" for intronic. Prevalence can slightly change depending on database.

[2]PharmGKB ranks the variants, attending at the Clinical Annotation Levels of Evidence (https://www.pharmgkb.org/page/clinAnnLevels), from Level 1 (the highest level of evidence) to Level 4 (unsupported evidence).

[3]This variant is ranked Level 3, from recently, due to a change in assessment. This variant used to be Level 2.

[4]Abreviations of populations: AFR (African people), AMI (Amish), AMR (Amerindian), ASJ (Ashkenazi Jewish), EAS (East Asian), FIN (Finnish), NFE (Non-Finnish European), SAS (south Asian)

FIGURE LEGENDS

Figure 1. Huntingtin and AMPK are expressed ubiquitously in humans. mHtt is ubiquitously expressed through the human body. In agreement with this, expression of mHtt causes phenotypes in many tissues, including bones, skin, heart tissue and many other organs and tissues, aside of the well-known effects on the brain of patients. These tissues affected, are pointed out in the diagram with a cartoon. One of the better-known targets of metformin is AMPK. This enzyme has shown to be a potential target to treat HD. Many isoforms of the three components of the enzyme exist in humans: two AMPKα, two AMPKβ and AMPKγ subunit isoforms. Combinations of one or other of these subunits are expressed in all tissues, making them a good target, to treat a ubiquitously expressed toxic molecule, such as mHtt. In this diagram, it is represented the expression of only one of the subunits (AMPKα2). Blue color means mRNA expression, while yellow shows the expression of the protein of this subunit. Diagram made using Biorender. Expression data obtained from The Protein Atlas (http://www.proteinatlas.org) (Thul et al., 2017). This figure was created using Bioredender.

Figure 2. Metformin is a pleiotropic drug. Diagram showing some of the known targets of metformin. (1) Inhibits the complex I of the mitochondrial respiratory chain, by direct binding. This in turn induces the rise of the AMP ratio, therefore inhibiting the adenylate cyclase (2). This is translated in reduced cAMP concentration, which reduces gluconeogenesis. This results in better glucemic indexes and better healthspan. (3) The rise in AMP also activates the heterotrimer enzyme AMPK. AMPK is able to activate autophagy, which digest mHtt, but also is able to induce oxidative stress scavenging resources, which also impacts in better DNA stability. Moreover, activation of AMPK results in reduced inflammation. (4) Metformin is also able to directly activate SIRT1, a well-known pro-healthspan molecule. (5) This drug also directly activates the MID1/PP2A/mTOR complex which in turns inhibits translation of mHtt. (6) Metformin inhibits demethylases, which reverts aging-related epigenetic marks. (7) This compound also inhibits mitochondrial glycerophosphate dehydrogenase, which results in reduced

gluconeogenesis. (8) Metformin binds alarmin, and inhibits its binding to the toll-like receptor 4, which further reduces inflammation. This figure was created using Bioredender.

FIGURES

Figure 1

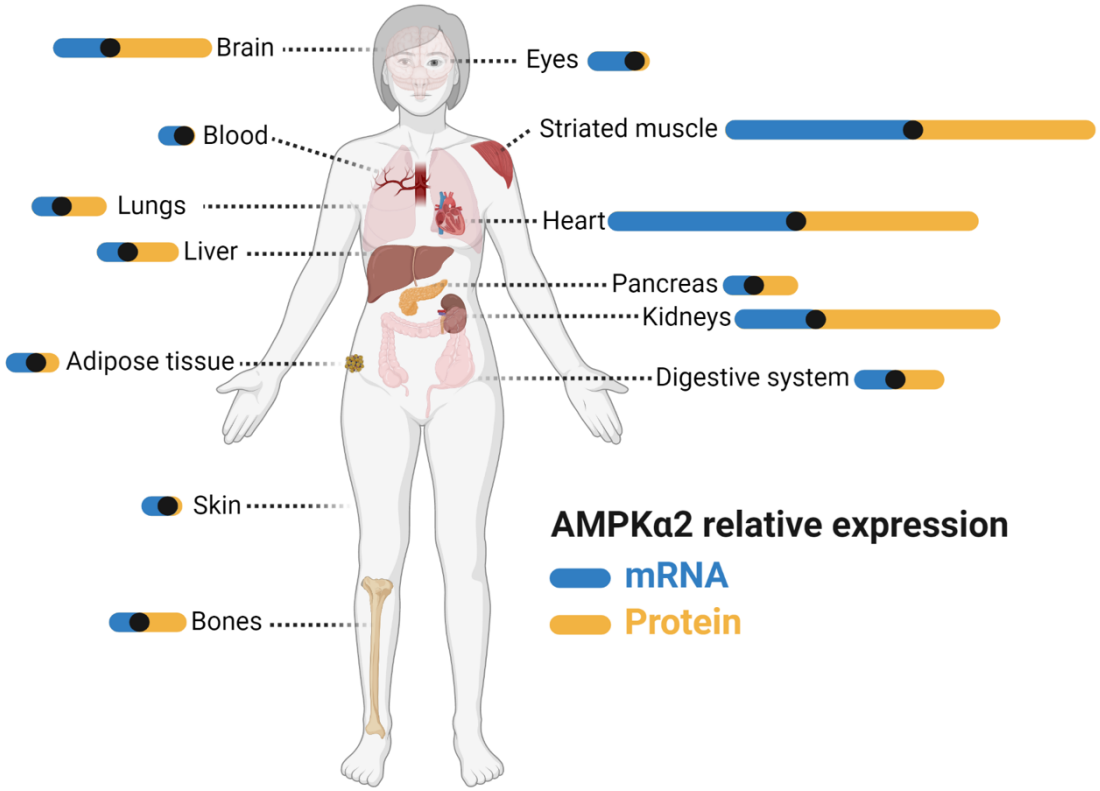
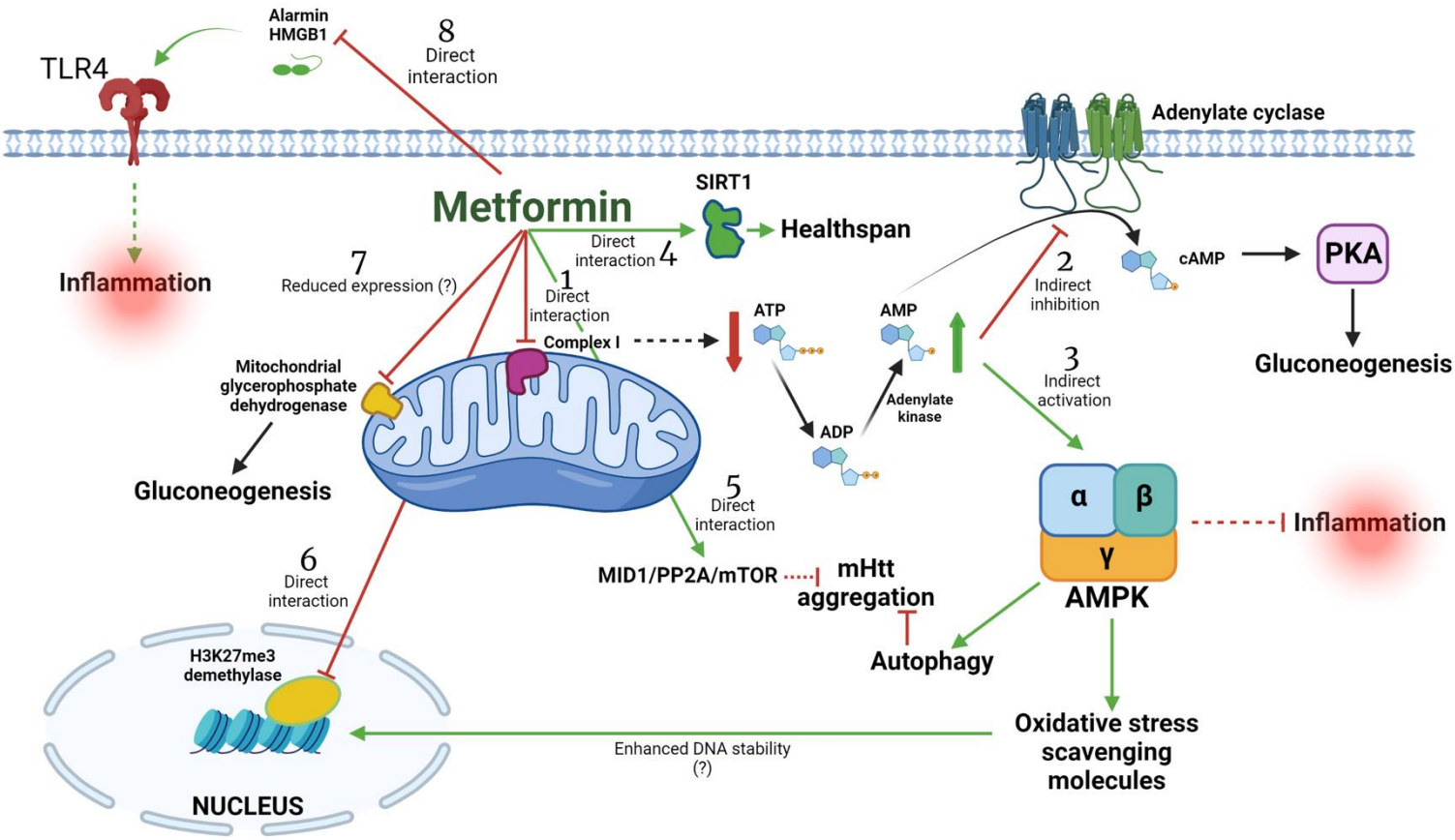


Figure 2


Alarmin
HMGB1
8
Direct
interaction
TLR4
Adenylate cyclase
Metformin
SIRT1
Healthspan
Direct
interaction
4
7
Reduced expression (?)
1
Direct
interaction
Inflammation
Complex I
ATP
AMP
2
Indirect
inhibition
cAMP
PKA
Gluconeogenesis
Mitochondrial
glycerophosphate
dehydrogenase
3
Indirect
activation
Adenylate
kinase
ADP
Gluconeogenesis
5
Direct
interaction
α
β
γ
Inflammation
6
Direct
interaction
MID1/PP2A/mTOR
mHtt
aggregation
AMPK
H3K27me3
demethylase
Autophagy
Oxidative stress
scavenging
molecules
Enhanced DNA stability
(?)
NUCLEUS