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From Myo-inositol to D-chiro-inositol molecular pathways

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Abstract. – OBJECTIVE: Inositol is a carbocyclic sugar polyalcohol. By epimerization of its hydroxyl groups, nine possible stereoisomers can be generated, two of major physiological and clinical relevance: myo-inositol and D-chiro-inositol. Myo-inositol and D-chiro-inositol are normally stored in kidney, brain and liver and are necessary for functions, such as signal transduction, metabolic flux, insulin signaling, regulation of ion-channel permeability, stress response and embryo development. In this narrative review, we summarize the mechanisms by which myo-inositol and D-chiro-inositol can be synthesized and absorbed and their possible role in the etiopathogenesis of neural tube defects.

MATERIALS AND METHODS: We performed an online search in the PubMed database using the following keywords: "inositol", "D-chiro-inositol", "myo-inositol", "neural tube defects and inositol".

RESULTS: Inositol requirements are partly met by dietary intake, while the rest is synthesized endogenously. Inositol deficiency may be involved in the pathogenesis of diseases, such as metabolic syndrome, spina bifida (a neural tube defect), polycystic ovary syndrome and diabetes. Supplementation of the two inositol stereoisomers, D-chiro-inositol and myo-inositol is important to prevent these conditions.

CONCLUSIONS: Inositol is fundamental for signal transduction in the brain, kidneys, reproductive organs and other tissues in response to neurotransmitters, hormones and growth factors. Various genes are involved in inositol metabolism and associated pathways. Altered inositol concentrations are observed in several diseases. Analysis of the genes involved in inositol metabolism may provide important information for the clinical management of these conditions.

Key Words:
Myo-inositol, D-chiro-inositol, Epimerase, Polymorphisms, Neural tube defects.

Introduction

Inositol, a cyclohexane-1,2,3,4,5,6-hexol, is a carbocyclic sugar polyalcohol (Figure 1) which accumulates in kidney, brain, liver and other tissues. It mediates cell signal transduction in response to various neurotransmitters, hormones, and growth factors. Inositol can be converted in-

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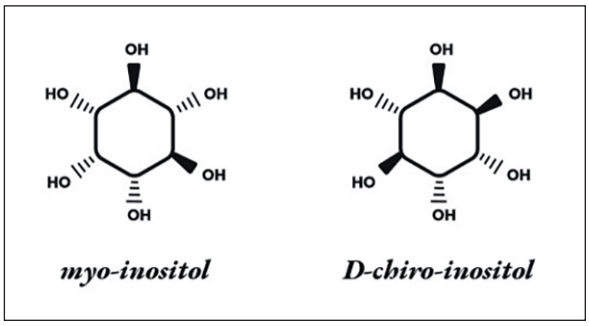

Figure 1. Chemical structure of myo- and D-chiro-inositol.

to nine different stereoisomers by epimerization of its hydroxyl groups: myo-inositol, muco-inositol, scyllo-inositol, epi-inositol, L-chiro-inositol, D-chiro-inositol, neo-inositol, cis-inositol, allo-inositol. Myo-inositol (cis-1,2,3,5-trans-4,6 cyclohexanehexol) and D-chiro-inositol (cis-1,2,4-trans-3,5,6-cyclohexanehexol) are the most clinically relevant[1].

Myo-inositol and its derivatives have many functions in different taxonomic groups, including regulation of ion-channel permeability, metabolic flux, phosphate levels, insulin signaling, mRNA transcription and export, translation, stress response and embryo development. Myo-inositol is also a significant constituent of membrane-incorporated phosphatidylinositols[2].

Myo-inositol was considered a member of the vitamin B complex family, although the myo-inositol requirements of human newborns are generally met by endogenous synthesis. Dietary myo-inositol intake is important to reach concentrations that can effectively improve endocrine disorders like diabetes and insulin resistance, in which myo-inositol plays a role. Myo-inositol may therefore be considered a semi-essential compound that may be deficient in certain physiological and pathological conditions[3]. It is synthesized *de novo* from glucose and is a product of phosphatidylinositol (PI), inositol phosphate (InsP) and phosphoinositide (PIP) catabolism. It enters the diacylglycerol pathway where it generates new PIP. In mammals, myo-inositol is broken down in the kidneys. Exogenous myo-inositol is absorbed by the gut epithelium after gastrointestinal degradation of $InsP_6$[4]. Myo-inositol has a significant role in many physiological processes: it is the precursor of InsP and PIP, two second messengers in many cell signaling pathways. It also acts as an osmolyte in tissues like the renal medulla and brain that have specific osmolarity due to their biological functions. It is also considered a modulator of glucose homeostasis and a regulator of insulin. Finally, some studies have explored the effects of dietary supplementation of myo-inositol, finding an improvement in bone stability and general performance in animal models[5-7]. Foods rich in inositol include vegetables, fresh fruit, milk, grains, fish, meat and eggs[8].

D-chiro-inositol has been detected in mammals, protozoa, and bacteria. In mammals, D-chiro-inositol is important in the insulin pathway and can restore insulin sensitivity and reduce hyperglycemia[2]. D-chiro-inositol is synthesized from myo-inositol by a specific epimerase during metabolic stress, in response to increased insulin release[9,10]. Myo-inositol epimerization is severely impaired when insulin-sensitive tissues (muscle, fat and liver) become insulin resistant, and a reduced D-chiro-inositol/myo-inositol ratio may be a measure of insulin resistance[11]. D-chiro-inositol has a role in a number of pathways. Since it is able to reduce hepatic diacylglycerol generation and prevent ectopic protein kinase Cε translocation to the membrane, it has a role in suppression of PKCε activation and inhibition of intracellular diglyceride increase in the liver. In fact, diacylglycerol/protein kinase Cε signaling pathway abnormalities in liver can cause lipid metabolic disorder and insulin resistance[12]. Expression levels of insulin receptor β and PI3Kp85 (necessary for insulin-stimulated increase in glucose uptake and glycogen synthesis in insulin-sensitive tissues) are downregulated in mice on a high-fat diet, and D-chiro-inositol exacerbates their low levels. In addition, the phosphorylation levels of IRS2 and AKT are reduced, and this downregulation can be reversed by D-chiro-inositol treatment. D-chiro-inositol therefore mediates activation of PI3K/AKT signaling[12]. FOXO1 is a transcription factor that plays an essential role in the regulation of gluconeogenic enzymes like PEPCK (an enzyme that catalyzes conversion of oxaloacetate to phosphoenolpyruvate, the rate-limiting step in the pathway of glucose synthesis from precursors derived from the citric acid cycle) and G6Pase (an enzyme that hydrolyzes glucose-6-phosphate to glucose in the endoplasmic reticulum). Insulin and D-chiro-inositol suppress FOXO1 activity by inducing the PI3K/AKT signaling pathway[12].

Moreover, recent literature[13,14] indicates that D-chiro-inositol influences steroidogenesis affecting the activity of aromatase, an enzyme detected for example in fatty tissue, ovaries,

testicles, placenta, brain and bone. In particular, in ovarian granulosa cells, D-chiro-inositol dose-dependently reduces expression of the aromatase gene *CYP19A1*[15]. As a consequence, it also reduces the conversion of testosterone to estrogen, leading to a systemic increase in testosterone levels. Furthermore, in theca cells, D-chiro-inositol, like insulin, is able to directly stimulate testosterone biosynthesis[16].

In view of its effect on aromatase, clinical use of D-chiro-inositol requires careful assessment. Specifically, raising levels of D-chiro-inositol may be considered as a potential therapeutic and/or adjuvant strategy when reduction of estrogens is the outcome; on the other hand, D-chiro-inositol could worsen clinical conditions, such as PCOS, especially if used at high doses for extended periods[17].

In its methylated form, 3-O-methyl-D-chiro-inositol or pinitol, D-chiro-inositol is obtained from the diet. Plants containing D-chiro-inositol are the soybean, white clover, red clover, bush clover, locust tree, wisteria and kudzu[18].

Myo- and D-Chiro-Inositol Metabolic Pathways

Absorption and Transport of Myo-Inositol and Conversion to D-Chiro-Inositol

Partial degradation of dietary InsP is performed by brush border membrane-associated endogenous phosphatases, phytases, microbial phytases and pancreatic phospholipases that increase free myo-inositol in the digestive tract. In humans 99.8% of myo-inositol is absorbed by the gastrointestinal tract[19].

Myo-inositol is transported across the apical membrane of enterocytes by sodium ion- and proton-dependent transport in rats, while in rabbits it is absorbed by renal epithelial cells. In chickens, myo-inositol absorption takes place in the intestine and involves transporters encoded by the *SLC5A11, SLC5A3* and *HMIT* genes that are highly expressed in the ileum and jejunum. Myo-inositol enters the bloodstream through a sodium/potassium ATPase channel in the basolateral membrane of enterocytes[19]. Once absorbed, myo-inositol reaches other tissues through the bloodstream. A portion of this myo-inositol is broken down in the liver. Myo-inositol concentrations vary according to the tissue[20].

In mouse kidney and liver, myo-inositol concentrations are reported to be almost 3.5 and 0.5 mmol/g wet weight, respectively. In male rats, radioactively labeled intraperitoneal injection of myo-inositol revealed that organs like the brain, kidneys, thyroid, liver, reproductive tract, spleen, prostate and pituitary gland actively accumulate myo-inositol, whereas muscle and adipose tissue store less myo-inositol due to their partial *de novo* synthesis capacity[3].

As mentioned above, in kidney cell cultures myo-inositol enters cells with the help of SLC5A3 and SLC5A11 transporters, whose activity depends on extracellular hyper-osmolarity. Although both transporters are specifically expressed in the kidneys, brain and intestine, SLC5A11 is expressed predominantly in the renal cortex where its main role is reabsorption of myo-inositol from glomerular filtrate (Figure 2). Other transporters include HMIT, which is mainly involved in brain inositol metabolism, and SLC2A13, expressed in rodent kidney and adipose tissue and in chicken liver, kidney and intestine[21,22]. Myo-inositol can

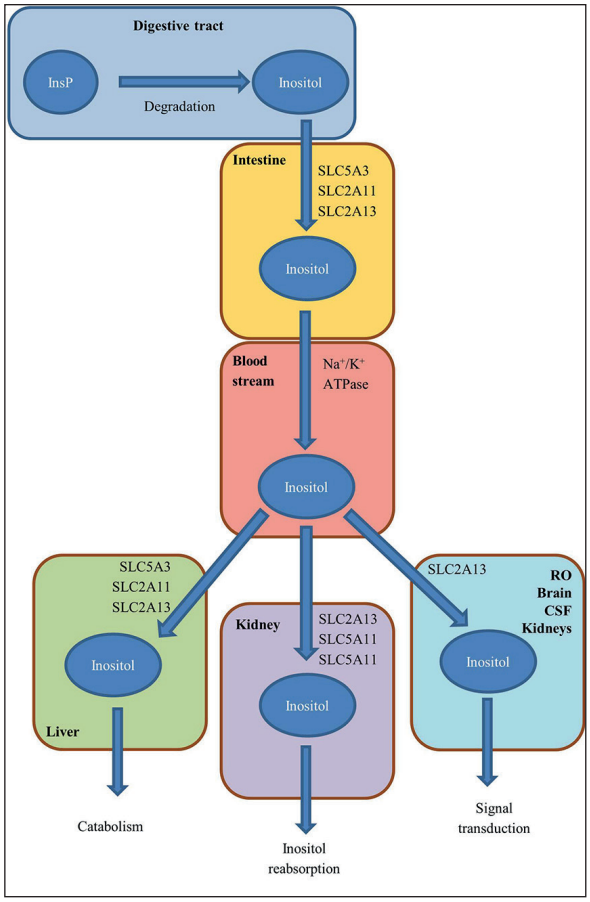

Figure 2. Representation of inositol absorption from diet. CSF = Cerebrospinal fluid; InsP = Inositol-phosphate; RO = Reproductive organs.

eventually be converted into D-chiro-inositol by a NADPH-dependent epimerase through an insulin-mediated pathway[9].

Cell Synthesis of Myo-Inositol

In humans, myo-inositol, but not D-chiro-inositol, is synthesized *de novo*, according to the pathways reported in the Kyoto Encyclopedia of Genes and Genomes database, myo-inositol is generated using glucose, PIP and InsP as substrates (https://www.genome.jp/kegg/pathway.html). Cells must maintain sufficient myo-inositol levels for PIP re-synthesis, important for signal transduction efficiency and maintenance. Likewise, in rats, myo-inositol *de novo* synthesis occurs in brain, testis, liver and kidney[23].

Myo-inositol synthesis from glucose mainly involves three biochemical reactions. In the first, a hexokinase enzyme phosphorylates glucose and generates glucose-6-phosphate; the second involves isomerization of glucose-6-phosphate to inositol-3-phosphate catalyzed by NADH-dependent cytosolic inositol-3-phosphate synthase; in the final step, inositol-3-phosphate is dephosphorylated by inositol-monophosphatase to produce free myo-inositol. In turn, myo-inositol is the substrate of phosphatidylinositol synthesis. Clements and Diethelm established that *de novo* synthesis of myo-inositol from glucose is a quantitative process, 4 g of myo-inositol are synthesized in human kidneys every day[24-26] (Figure 2).

Another significant pathway for the generation of cellular myo-inositol is the dephosphorylation of InsP. The two isoforms of $InsP_3$, namely $Ins(1,3,4)P_3$ and $Ins(1,4,5)P_3$, are the critical intermediates in this pathway. Ultimately, both $InsP_3$ isoforms are dephosphorylated by inositol-1,4,5-trisphosphate 5-phosphatase to $Ins(1,4)P_2$. $Ins(1,4)P_2$ is further dephosphorylated to $Ins(4)P_1$ by inositol polyphosphate 1-phosphatase and ultimately this $InsP_1$ is converted to free myo-inositol by the action of inositol monophosphatase (IMPase). Free MI enhances the synthesis of phosphatidylinositol in endoplasmic reticulum by phosphatidylinositol synthase (Figure 3)[25,27].

Catabolism of Myo-Inositol and D-Chiro-Inositol

Catabolism of myo-inositol is an essential process for the regulation and maintenance of inositol homeostasis. It takes place in the kidney with the help of myo-inositol oxygenase

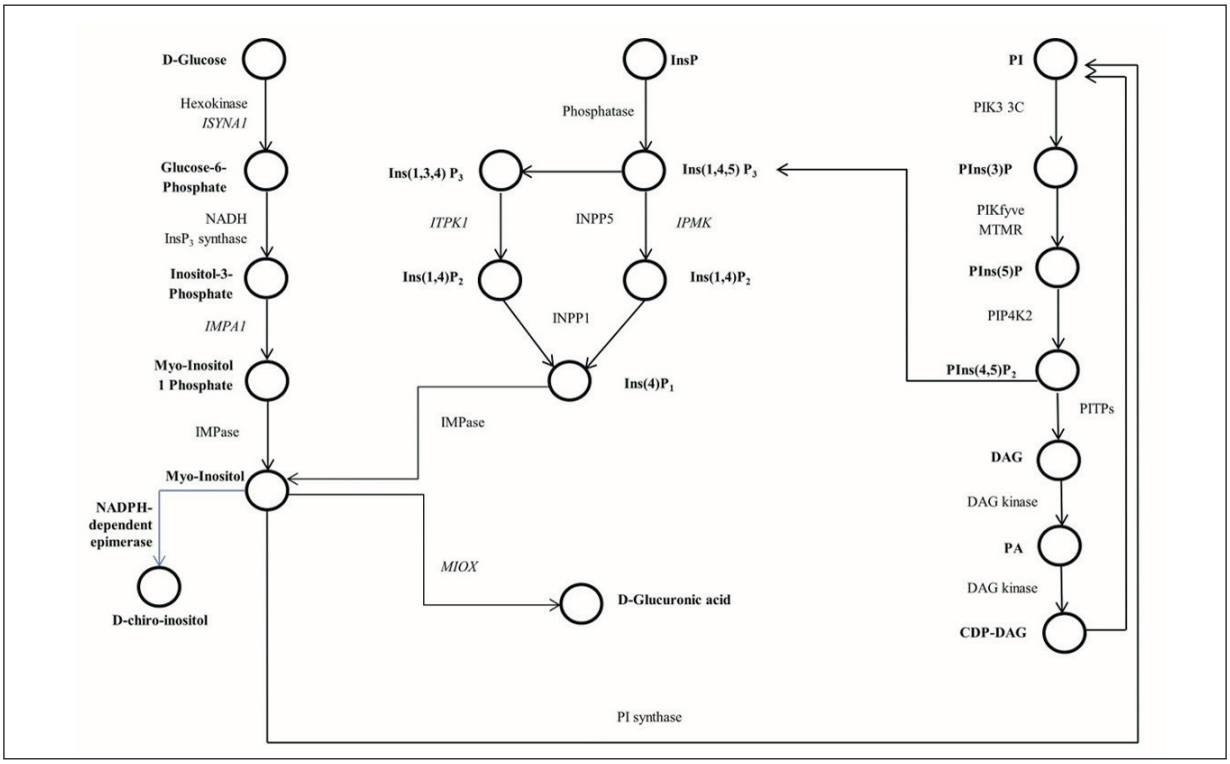

Figure 3. Molecular pathways involved in the synthesis of myo-inositol and D-chiro-inositol.

(MIOX). MIOX is a non-heme iron enzyme that converts myo-inositol to D-glucuronic acid. D-glucuronic acid is subsequently converted to D-xylulose-5-phosphate which ultimately enters the pentose phosphate pathway, a major pathway of oxidative energy production. In kidneys, primary urine contains significant amounts of myo-inositol, 98% of which is reabsorbed into the blood[28]. Thus, the kidney is the most significant organ regulating plasma concentrations of inositol in humans and animals. Increased glucose concentrations cause upregulation of MIOX activity. MIOX upregulation is also influenced by polymorphisms in the promoter region of the *MIOX* gene, hyperglycemia-induced activation of transcription factors and MIOX post-translational modifications. The latter are based on MIOX phosphorylation by protein kinase A, protein kinase C and 3-phosphoinositide-dependent protein kinase 1. A significant effect of MIOX activity, induced by glucose in human and porcine kidney cell cultures, is damage to mitochondrial integrity due to an increase in reactive oxygen species, a decrease in autophagy and a higher apoptosis rate[29].

MIOX is also able to catabolize D-chiro-inositol through oxidation but is less efficient. In fact, the K_m for myo-inositol is 5.9 mM and k_{cat} is 11min^{-1}, whereas the K_m and k_{cat} for D-chiro-inositol are estimated to be 33.5 mM and 2.3 min^{-1}, respectively[30].

Intracellular and Dietary Myo-Inositol and D-Chiro-Inositol Depletion

Intracellular myo-inositol depletion is regulated by intestinal absorption, *de novo* synthesis, increase in renal excretion and efflux from organs. Cellular myo-inositol depletion leads to a reduction in the activity of enzymes like myo-inositol-3-phosphate synthase, IMPase and glycogen synthase kinase 3. Cellular depletion of myo-inositol causes a reduction in PIP and diacylglycerol concentrations, lowering of Na^+/K^+–ATPase activity and decline in cell differentiation and development. Intracellular myo-inositol depletion is also linked to intracellular osmotic stress. An increase in intracellular osmolarity leads to release of myo-inositol, and subsequently to intracellular depletion of myo-inositol under chronic conditions. Other chemical compounds, such as valproic acid and lithium cause intracellular depletion of myo-inositol[31].

Dietary myo-inositol deficiency is related to inflammation of the intestinal mucosa, increased apoptosis, reduced antioxidant capacity, cell proliferation and intestinal bacterial activity. Importantly, dietary myo-inositol is considered to be vital for lipid metabolism, skeletal muscle metabolism, bone formation, nervous system development and reproduction in humans and animals[32].

Decreased availability of inositol or inositol phosphoglycans, as found in animals and humans with insulin resistance, is caused by increased urinary loss of myo-inositol due to glucose-mediated inhibition of myo-inositol reabsorption by the kidney. As a result, reduction of myo-inositol availability has a direct negative impact on levels of D-chiro-inositol. This is observed in diabetes, insulin resistance and metabolic syndrome[33].

Genes Involved in the Metabolism of Myo- and D-Chiro-Inositol

In the following sections, we list the major proteins involved in myo-inositol and D-chiro-inositol metabolism, along with the corresponding polymorphisms (Table I).

SLC5A3

The *SLC5A3* gene previously referred as SMIT encodes SLC5A3 protein, a Na^+/myo-inositol cotransporter. In humans, *SLC5A3* is located at 21q22 and is principally expressed in the brain. In cells with three chromosomes 21, the flux of myo-inositol and Na^+ across the plasma membrane is increased, due to the incapacity of cells to reduce expression of the additional copy of *SLC5A3*. Effects of this imbalance may include dysregulation of cell membrane potential and tissue osmolyte levels. *SLC5A3* may therefore play a significant role in the pathogenesis of Down syndrome[34,35].

Myo-inositol also plays a significant role in osmoregulation. In mammalian brain and renal cells, its transport and levels are regulated by extracellular osmolality and tonicity. In most mammalian cells, myo-inositol concentrations in the cell are 5- to 500-fold greater than in the extracellular fluid. The highest concentrations of myo-inositol are observed in specific regions of the brain. Due to a combination of factors (*de novo* synthesis of inositol in brain capillary pericytes, restricted transport across the blood brain barrier and concentrative uptake of inositol at the choroid plexus), inositol concentrations are much higher in cerebrospinal fluid than in blood[34].

Table I. Genes involved in inositol metabolism and associated phenotypes (https://www.ebi.ac.uk/gwas/home).

Gene (OMIM ID)	OMIM phenotype (ID)	Polymorphism (nucleotide change)	Phenotype (associated-allele)	Tissue of expression	Ref.
SLC5A3 (*600444)	/	rs9982601 (C>T)	Early onset myocardial infarction (T), coronary heart disease (T)	Kidney, brain	29
IMPA1 (*602064)	Autosomal recessive mental retardation 59 (#617323)	rs7017336 (G>T)	Decrease in proportion of saturated free fatty acids in glucose-insulin-potassium-treated patients (T)	Brain	31
IMPA2 (*605922)	/	rs1489002707 (C>T) rs589247 (C>T)	Bipolar disorder (T) Ischemic stroke (T)	Brain	35 77
MIOX (*606774)	/	rs761745 (C>T)	Type 1 diabetes mellitus (T)	Kidney	42
ISYNA1 (*611670)	/	rs2303697 (A>G) rs4808136 (G>A)	Lower risk of spina bifida (G) Increased blood levels of myo-inositol (A)	Testis, ovary, heart, placenta, pancreas	47,78
SLC5A11 (*610238)	/	rs4787294 (C>T)	Significantly lower MI concentrations (C)	Kidney, heart, placenta, liver, skeletal muscle	47
SLC2A13 (*611036)	/	rs515291 (A>G)	Carotid intima-media thickness and plaque in patients with rheumatoid arthritis (G)	Brain (cortex, hypothalamus, hippocampus, brainstem, cerebellum)	79
IMPAD1 (*614010)	Chondrodysplasia with joint dislocations (#614078)	rs16921695 (G>T)	Openness (T)	Spinal cord, brain, kidney, costal cartilage, lung	53
HDAC3 (*605166)	/	rs2530223 (T>C) rs976552 (A>C)	Increased risk of type 2 diabetes mellitus (C) Increased risk of schizophrenia (C)	All tissues	76 80
GRHL3 (*608317)	Van der Woude syndrome 2 (#606713)	c.48dupC c.1047+2T>C rs879255245 (C>T) rs41268753 (C>T)	Spina bifida Spina bifida Van der Woude syndrome 2 (T) Nonsyndromic cleft palate (T)	Brain, placenta, thymus, kidney, tonsil, pancreas, prostate, testis, colon	63,81
ITPK1 (*601838)	/	rs3783903 (A<G)	Spina bifida (G)	All tissues	67
IPMK (*609851)	/	rs6481383 (C>T) rs2790234 (C>G)	Female longevity (T) Female longevity (G)	All tissues	69

IMPA1

The *IMPA1* gene encodes inositol monophosphatase-1, an enzyme vital for inositol cycle recovery. In humans, it plays a significant role in inositol *de novo* synthesis and inositol polyphosphate recycling[36]. The activity of the IMPA enzyme is Mg^{2+}-dependent. IMPA1 is involved in the final steps of inositol triphosphate synthesis and in the synthesis of diacylglycerol. Inorganic phosphate inhibits the activity of IMPA enzyme competitively, while lithium (the most widely used medication for bipolar disorder) inhibits IMPA enzyme activity noncompetitively[35]. *Xenopus* studies have shown that lithium indirectly blocks

IMPase activity by inhibiting glycogen synthase kinase beta. These studies provide an insight into the pathogenesis and treatment options for bipolar disorder[37].

Homozygous loss-of-function variants in *IMPA1* cause severe intellectual disability. This was observed in a consanguineous Brazilian family in which nine adult family members showed severe intellectual disability and disruptive behavior[36].

Animal studies have shown that reduction of IMPA1 activity in the brain of *Impa1* -/- mice does not affect inositol levels. *Impa1* -/- mice exhibit enhanced motor activity in open-field and forced-swim tests, increased susceptibility to pilocarpine-induced seizures and stereotypy, and hyperactivity in the home cage. Complete prevention of Impa1-deletion-associated embryo lethality was obtained by dietary intake of inositol by the mother, however these treated Impa1 -/- mice still exhibited signs of stereotypy and hyperactivity in the home cage[38].

The SNP rs7017336 is located near the *IMPA1* gene and is associated with decreased levels of saturated free fatty acids, 6 and 12 hours after administration of glucose-insulin-potassium in patients with acute coronary syndrome. This inositol monophosphatase is involved in the pathogenesis of bipolar disorder through upregulation of mitochondrial-related genes that may cause mitochondrial dysfunction. The prolonged decrease in plasma free fatty acids increases mitochondrial activity together with insulin sensitivity in type 2 diabetics and obese individuals[39].

IMPA2

IMPA2 encodes inositol monophosphatase 2[40]. The IMPA2 protein has two motifs common to other inositol phosphatases important for metal binding and catalytic activity. The *IMPA2* gene is uniformly expressed in adults and fetuses[41].

IMPA2 is associated with predisposition for bipolar disorder. This association was found in a patient who carried a missense variant (p.His76Tyr) affecting a conserved amino acid sequence that introduced a putative new phosphorylation site. The phosphatidylinositol pathway is significantly affected by administration of lithium that has IMPA-inhibitory effects[40].

MIOX

The *MIOX* gene encodes myo-inositol oxygenase, an enzyme involved in catalysis of the first enzyme reaction in the myo-inositol catabolism pathway, which mostly takes place in the kidney. The enzyme is a specific non-heme iron enzyme involved in catalysis of cleavage of the myo-inositol ring and addition of an oxygen atom. Myo-inositol, and its isomer D-chiro-inositol, are substrates of the MIOX enzyme. Both are involved in the pathogenesis of diabetes. MIOX activity is directly proportional to serum concentrations of glucose, which may be the cause of the D-chiro- and myo-inositol depletion observed in diabetic complications. An understanding of how *MIOX* gene expression is regulated could therefore provide significant insights into the etiology of diabetes and the regulation of pathways involving intracellular phosphoinositol signaling[30]. Moreover, the rs761745 polymorphism in the *MIOX* promoter region is associated with the pathogenesis and development of type 1 diabetes in the Caucasian population[42]. Consistently, the *MIOX* gene is continuously expressed in the cortex of mouse kidney proximal tubules, and its expression increases in diabetic kidneys[43].

ISYNA1

The *ISYNA1* gene encodes inositol-3-phosphate synthase 1, an enzyme involved in the catalysis of *de novo* inositol 1-phosphate from glucose 6-phosphate[44]. The ISYNA1 enzyme acts as a target of drugs like valproate and lithium, both linked to mood-stabilization. Lovastatin, a G protein activation inhibitor drug, enhances myo-inositol 1-phosphate synthase transcription two- to three-fold in a concentration- and time-dependent manner, suggesting G protein-mediated signal transduction[45].

In cell models, expression of *ISYNA1* is enhanced by the E2F1 transcription factor in a dose-dependent manner, while expression of *ISYNA1* is repressed by the oncosuppressor retinoblastoma protein 1, which complexes with E2F1[44].

ISYNA1 polymorphisms are linked to risk of spina bifida, one of the most prevalent neural tube defects in humans. Interestingly, myo-inositol and D-chiro-inositol supplementation minimizes the risk of spina bifida in mouse model offspring with a genetic predisposition for spina bifida (curly tail mouse)[46]. Human studies concord with these findings. Reduced concentrations of maternal inositol and slightly elevated glucose concentrations are considered risk factors for spina bifida. Moreover, *ISYNA1* defects in adult mice may result in reduced intracellular concentrations of inositol in mother and fetus, disturbing inositol-dependent signal transduction and possibly leading to spina bifida. Some research studies

have shown that lower maternal serum concentrations of glucose and the *ISYNA1* gene c.1029A>G polymorphism (rs2303697) reduce the risk of spina bifida, but these results need to be confirmed in larger series[47].

SLC5A11

The *SLC5A11* gene encodes a protein that acts as a sodium/glucose and a sodium/inositol cotransporter. It is highly expressed in kidney, heart, placenta, liver and skeletal muscle, and at lower levels in brain, leukocytes, lungs and spleen[48]. SLC5A11 protein is stereospecific and acts as a cotransporter for D-glucose, D-chiro-inositol and D-xylose. *SLC5A11* has a distribution in tissues which is different from that of *SLC5A3,* and these differences may explain inositol transport heterogeneity. Several studies have recognized an association between *SLC5A11* polymorphisms and risk of spina bifida and have established that the polymorphism c.544C>T is involved in the regulation of serum levels of inositol[47].

SLC2A13

The *SLC2A13* gene encodes an H^+-inositol symporter (HMIT) highly expressed in the brain, especially in the cortex, hypothalamus, hippocampus, brainstem and cerebellum, thus suggesting the vital role of the regulation of inositol metabolism in the brain[49].

SLC2A13 protein is specifically involved in inositol transport. Since the latter depends on the concentration gradient of H^+, a reduction in extracellular pH from 7.0 to 5.0 significantly increases inositol transport. However, changes in brain levels of inositol are observed in central nervous system disorders. As already mentioned, bipolar disorder is treated with lithium salt because of its effect on inositol metabolism. The typical neurological dysfunctions of Down syndrome are also associated with an increase in brain levels of inositol, due to three copies of the Na^+/inositol transporter gene on chromosome 21[50].

IMPAD1

The *IMPAD1* gene encodes an inositol monophosphatase with 3'-nucleotidase activity for 3'-phosphoadenosine 5'-phosphate. *IMPAD1* is predominantly expressed in the spinal cord, brain, kidney, costal cartilage and lungs[51].

IMPAD1 knockout mice cannot survive because they suffer extreme respiratory distress shortly after birth. In addition, the *IMPAD1* -/- mouse model shows dwarfism, abnormal cartilage morphology, undersized and unorganized growth plates, wide metaphyses, thick bone collars and reduced bone length due to endochondral ossification. Biochemical analyses revealed that *IMPAD1* -/- mice cartilage lacks chondroitin 4-sulfate and has more non-sulfated chondroitin than wild-type cartilage. In the same way, the lungs show alterations in levels of chondroitin sulfate and heparin sulfate. IMPAD1 may therefore be needed for production of bioactive metabolites, like 5'-AMP, for chondroitin or heparin sulfation[51].

In humans, *IMPAD1* homozygous variants have been linked to a short stature syndrome, chondrodysplasia with micrognathia, brachydactyly, congenital joint dislocations, facial dysmorphism and cleft palate[52,53].

NCOR2

The *NCOR2* gene encodes the nuclear corepressor receptor 2[54]. NCOR2 protein is a transcriptional corepressor/co-regulator that turns off non-ligated nuclear receptors. NCOR2 regulates transcription by acting as a scaffold protein for the recruitment of histone deacetylase complexes and other factors involved in chromatin remodeling[55].

NCOR2 and histone deacetylase complexes are formed and stabilized with the help of inositol tetraphosphate ($Ins(1,4,5,6)P_4$) which acts as "intermolecular glue" to hold the two proteins together. $Ins(1,4,5,6)P_4$ therefore plays a regulatory role which explains the transcriptional regulatory activity of inositol phosphates and their kinases[56].

HDAC3

The *HDAC3* gene encodes histone deacetylase 3, an enzyme expressed in all human tissues[57,58]. HDAC3 is a part of the nuclear co-receptor repressor complex, which for catalysis of the deacetylation reaction depends on the NCOR1 and 2 deacetylase activation domain, and on the presence of $Ins(1,4,5,6)P_4$ at the protein interface[59].

Alenghat et al[60] established that HDAC3 activation through NCOR1 is a vital step in circadian epigenetic regulation. In the mice model, Hdac3 knockout reduces expression of genes related to mitochondrial bioenergetics and lipid metabolism and upregulates expression of genes involved in the immune response[61].

GRHL3

The *GRHL3* gene encodes a member of the grainyhead family of transcription factors asso-

ciated with Drosophila grainyhead protein. In Drosophila, grainyhead protein is involved in the early establishment of dorsal/ventral pattern. *GRHL3* is mostly expressed in brain, placenta, thymus, kidney, tonsil, pancreas, prostate, testis and colon[62].

During mouse embryogenesis Grhl3 is expressed in the non-neural ectoderm adjacent the neural plate, which is involved in neural tube formation. GRHL3 expression later becomes widespread in other tissues[62].

Dominant negative mutations in *GRHL3* cause spina bifida, cleft palate/lip, and lip pits. Neural tube defects present in mouse models like the curly tail strain, may be caused by *GRHL3* hypomorphic alleles. Similar studies have highlighted the possible presence of a modifier gene that could modulate phenotypic expression of *GRHL3* variants[63].

ITPK1

The *ITPK1* gene encodes inositol 1,3,4-trisphosphate 5/6-kinase, an enzyme expressed in nearly all tissues, especially the brain. The enzyme is involved in catalyzing the first steps of inositol phosphate synthesis by phosphorylating inositol in D5 and D6 positions[64]. When mammalian cells are depleted of ITPK1 enzyme, they show extreme reduction of inositol phosphate[65]. ITPK1 is a fundamental enzyme for Ins(3,4,5,6) P_4 synthesis, and is involved in the regulation of neurotransmission, fluid and salt secretion, and pancreatic insulin secretion by β-cells. Several studies have demonstrated that reduced ITPK1 levels in mice increase the risk of neural tube defects[66]. Thus, genetic variants influencing ITPK1 enzyme activity may affect cell differentiation, proliferation and apoptosis, thereby disturbing normal neural tube development. In China, a case-control study of pregnant women with neural tube defects supported these findings and suggested an association of maternal SNP rs3783903 with spina bifida. The G allele may affect AP-1 binding and decrease in concentrations of inositol hexakisphosphate in maternal plasma of that population[67]. It was recently found that the severity of cystic fibrosis may be modulated by levels of Ins(3,4,5,6)P_4, which are in turn influenced by changes in ITPK1 expression[68].

IPMK

The *IPMK* gene encodes inositol polyphosphate multikinase, an enzyme involved several functions. IPMK protein is a key enzyme for the synthesis of inositol phosphates, but it is also involved in several other metabolic pathways related to aging. IPMK enzyme takes part in the regulation of basic transcription, nutrient-sensing, telomere homeostasis, oxidative stress and metabolism. Female longevity is supposed to be correlated with two polymorphisms rs6481383and rs2790234[69].

Effects of Myo-Inositol and D-Chiro-Inositol on Neural Tube Defects

Inositol deficiency can be caused by insufficient dietary intake, insufficient intracellular uptake, insufficient endogenous synthesis or an excessive elimination rate. Deficiency may lead to defects like spina bifida and neural tube defects in fetuses, metabolic diseases, respiratory distress syndrome, reduced motor nerve conduction and polycystic ovary syndrome[70]. Here we concentrate on neural tube defects caused by deficiency of inositol.

The curly tail mouse model of neural tube defects is a strain predisposed for onset of defects in the neural tube closure not helped by folic acid supplementation. This strain arises from reduced expression of Grhl3 transcription factor due to a hypomorphic allele homozygosity, with a strong influence of genetic modifiers, such as a polymorphism in the *Lmnb1* gene[62,63]. Normally, curly tail embryos have an incidence of 3-7% of cranial neural tube defects[71]. However, when there is inositol-deficiency this incidence may be as high as 70%. The defects can be corrected by increasing the concentration of inositol[46]. This protective effect was also confirmed by *in vivo* supplementation to pregnant mothers[72].

Inositol may also improve diabetes-induced and folate deficiency-induced neural tube defects. Neural tube defects may occur in cultured rat and mouse embryos exposed to hyperglycemia[73] and supplementation with inositol may correct those defects[72].

Embryos genetically similar to the curly tail mice, but with two wild-type copies of *Grhl3*, do not develop neural tube defects on a normal diet but only with folate-deficient diets. Interestingly, in this model, neural tube defects are prevented by maternal administration of either folic acid or inositol[74].

Although results are promising in mice, in humans there is no clear association between serum inositol concentrations and risk of neural

tube defects. Further studies on this correlation are needed[75]. In adult humans, inositol administration has been tested in cases of psoriasis, panic disorder, depression, eating disorders, polycystic ovary syndrome and neural tube defects. There were no major side effects in any case, even when administered to fetus and mother in the periconceptional period[76]. Both isomers enter the phosphoinositide cycle, eventually activating protein kinase C, which is fundamental for avoiding neural tube defects. The different effectiveness of the two isomers may be related to the readiness with which they enter the phosphoinisotide pathway, especially after insulin stimulation[10].

To date, inositol supplementation has only been studied in women with a higher risk of offspring with neural tube defects (women with history of at least one pregnancy with neural tube defects have an estimated recurrence risk of 3%). The results of these studies seem promising[71].

Conclusions

Inositol is the precursor of many second messengers fundamental for signal transduction in brain, kidney, reproductive organs and other tissues, in response to neurotransmitters, hormones and growth factors. Various genes are involved in inositol metabolism and associated pathways. Altered inositol concentrations are observed in diseases such as neural tube defects, metabolic disorders, polycystic ovary syndrome, and diabetes. Analysis of polymorphisms in the aforementioned genes may provide additional information useful for the clinical management of these conditions.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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