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Medication effects on developmental sterol biosynthesis

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Abstract

Cholesterol is essential for normal brain function and development. Genetic disruptions of sterol biosynthesis result in intellectual and developmental disabilities. Developing neurons synthesize their own cholesterol, and disruption of this process can occur by both genetic and chemical mechanisms. Many commonly prescribed medications interfere with sterol biosynthesis, including haloperidol, aripiprazole, cariprazine, fluoxetine, trazodone and amiodarone. When used during pregnancy, these compounds might have detrimental effects on the developing brain of the offspring. In particular, inhibition of dehydrocholesterol-reductase 7 (DHCR7), the last enzyme in the biosynthesis pathway, results in accumulation of the immediate cholesterol precursor, 7-dehydrocholesterol (7-DHC). 7-DHC is highly unstable, giving rise to toxic oxysterols; this is particularly pronounced in a mouse model when both the mother and the offspring carry the *Dhcr7*$^{+/-}$ genotype. Studies of human dermal fibroblasts from individuals who carry *DCHR7*$^{+/-}$ single allele mutations suggest that the same *gene*medication* interaction also occurs in humans. The public health relevance of these findings is high, as DHCR7-inhibitors can be considered teratogens, and are commonly used by pregnant women. In addition, sterol biosynthesis inhibiting medications should be used with caution in individuals with mutations in sterol biosynthesis genes. In an age of precision medicine, further research in this area could open opportunities to improve patient and fetal/infant safety by tailoring medication prescriptions according to patient genotype and life stage.

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Keywords

Smith-Lemli Opitz syndrome (SLOS); DHCR7; 7-DHC; medication effect; sterol synthesis; toxicity; teratogen; aripiprazole; trazodone; cariprazine

Cholesterol and the brain

Cholesterol is indispensable for all mammalian cells (1). In particular, the brain requires an abundance of this lipid. Although the human brain only accounts for about 2% of total body weight, it contains as much as 25% of cholesterol and cholesterol derivatives (2, 3). As the blood-brain-barrier (BBB) prevents cholesterol from entering the central nervous system (4) from the rest of body, the brain fully relies on its own cholesterol biosynthesis. The developing brain starts synthesizing its own cholesterol during embryonic development (5, 6). All cholesterol in the brain is unesterified (7). Eighty percent of cholesterol is found in the myelin sheaths (8) (oligodendrocytes) and plasma membranes of the various brain cells (9).

Human brain cholesterol continues to increase from birth (~6 mg/g) until myelination is complete in young adults (23 mg/g) (2, 10). The estimate is that in the adult human brain cholesterol half-life is approximately 5 years (11). Cholesterol synthesis in the CNS exceeds the need for new cholesterol, so the excess is consistently cleared through excretory pathways as 24-hydroxycholesterol (24-OH-Chol) (12) at a rate of 6.4 mg/24 h (11, 13). While energetically unfavorable, this excess of cholesterol biosynthesis might play an important physiological role, and serve a neuroprotective role when CNS insults arise.

While all brain regions contain high amounts of cholesterol, rodent studies suggest that there are considerable differences in sterol content (and presumably synthesis) across the various brain regions (6, 14, 15). This is likely to be true also in humans, as different brain regions have distinct sterol levels, and express different levels of transcripts/proteins of sterol biosynthesis encoding enzymes (16). During development, CNS regions with the highest levels of sterol include the spinal cord, brainstem, cerebral white mater, and midbrain (17).

The function of cholesterol in the CNS goes beyond being a structural component of cellular membranes and lipid rafts: it is required for synapse and dendrite formation, axonal guidance, and it serves as a precursor for various biosynthetic pathways (Figure 1) (18). While endogenous cholesterol synthesis is essential for brain development, intact cholesterol metabolism is also critical for normal functioning of the adult brain (19). In the elderly, high brain cholesterol is associated with better memory function, while low cholesterol is associated with an increased risk for depression (20, 21). Dysfunction of the cholesterol biosynthesis pathways and/or metabolism might contribute to a number of psychiatric and neurodegenerative disorders including major depression, bipolar disorder, schizophrenia, Huntington’s disease, and Alzheimer’s disease (22–25).

Cholesterol biosynthesis pathway

Cholesterol is a membrane building molecule that is essential for life. In addition to ensuring membrane fluidity and playing a critical role in lipid raft assembly, the sterol biosynthesis pathway provides crucial precursors to many cellular processes, including steroid hormones and bile acids (26, 27). The formation of cholesterol molecule starts with acetate and involves a long sequence of enzymatic reactions coupled with a considerable energy investment of 36 ATP and 26 NADPH molecules (28, 29). Cholesterol synthesis takes place in the smooth endoplasmic reticulum, and cholesterol is required for efficient endoplasmic reticulum-to-Golgi transport of secretory membrane proteins (30, 31). Finally, cholesterol plays a critical role in vesicle fusion and motion (32).

The pre-squalene part of the pathway gives rise to farnesyl pyrophosphate, isoprenoids, and geranylgeranyl pyrophosphate which are important for anchoring multiple signaling proteins (RAS, phosphoinositide 3-kinase, AKT) to the membrane. The last, post-squalene phase of cholesterol biosynthesis, staring with lanosterol, is divided into the Bloch and Kandutsch-Russell branches (33–35) (Figure 2). This biosynthesis tree also gives rise to C4-methylated sterols (known as meiosis-activating sterols) and vitamin D (36).

The excess cholesterol is removed from the brain as 24S-hydroxycholesterol (24OHC) (37). 24OHC is the end product of cholesterol elimination by the neurons, and this conversion is mediated by neuron-specific cholesterol 24-hydroxylase (CYP46A1) (38). CSF from patients affected by neurodegenerative diseases show increased levels of 24OHC, including mild cognitive impairment and Alzheimer’s disease. In these patients, the CSF concentration of 24OHC is correlated with CSF ApoE, cholesterol and Tau, and it appears that 24OHC and cholesterol are sensitive biomarkers for evaluation of MCI and AD progression and severity (39).

Technological advances in sterol measurements

Sterol biosynthesis assessment was revolutionized by advances in liquid chromatography-mass spectrometry (LC-MS/MS) technology (17, 35). In particular, about five years ago 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) derivatization finally permitted the assessment of five post-lanosterol sterols in a 1-minute reaction from fewer than 5,000 cells (40). This allowed high-throughput testing of the NIH Clinical Collection of biologically active compounds (Molecular Libraries Roadmap Initiative) for their ability to interfere with sterol biosynthesis. Assessment of 727 compounds, tested in Neuro2a cells at 1 μM concentration, revealed that 37 compounds were DHCR7 inhibitors, 20 chemicals increased desmosterol, and 24 compounds elevated lanosterol levels (41, 42). Several of the tested compounds inhibited more than one of the sterol synthesis enzymes. Using the same technology and methodology, these findings were quickly followed up by screening of an FDA-approved drug library (a unique collection of 2697 approved medications) from Selleck Chemicals (43). The outcome of these studies was astonishing: of the FDA-approved medications showing sterol biosynthesis inhibition, 27 made the list of the 200 most prescribed pharmaceuticals in the US. These studies provided a chemical tool set to begin exploring the mechanistic involvement of the entire cholesterol biosynthetic pathway in a number

of disorders, and set the stage for cataloguing commonly used medications with sterol-inhibiting side effects.

The latest major breakthrough in sterol measurement is the most recently published N,N-dimethylglycine (DMG) derivatization based LS-MS/MS method (44). The DMG-derivatization, coupled with LC-MS/MS allows unparalleled resolution of previously hard to measure sterols, with a capability of separating molecules with almost identical chemical properties (e.g. 7-DHD from 8-DHC). This new method also allows simultaneous analysis of 14 sterols and 7 oxysterols in a single sample in about 15 minutes using electrospray ionization (ESI) method (compared to atmospheric pressure chemical ionization [APCI] used in the previous PTAD studies). Further advancements are expected to come from ion-mobility spectrometry (iMS) technology, which adds ‘just in place’ assessments, allowing important sterol measurements in individual brain regions (45, 46).

Neuronal and glial cholesterol biosynthesis

As the brain synthesizes its own sterols, it expresses the genes that are necessary for sterol synthesis. The expression of these genes is complex at the subcellular, cellular and regional level. For example, hippocampal neurons and midbrain neurons express very high levels of multiple sterol synthesis enzymes (including β-Hydroxy β-methylglutaryl-CoA [HMG-CoA] and 7-dehydrocholesterol reductase [DHCR7], the first and the last enzymes in the pathway) (47). However, until recently neuronal sterol synthesis was considered small and irrelevant, as it was believed that the main source of cholesterol for the neurons is provided by glial cells in an Apolipoprotein E (APOE) dependent fashion (48, 49). Based on recently published findings, we can confidently state that this is not the case during development.

In recent studies we and others established that embryonic cortical neurons are a site of an active *de novo* cholesterol synthesis (50). In addition, we found that neurons have higher levels of most cholesterol intermediates than astrocytes. Furthermore, 93% and 98% of the sterol precursors found in neurons and astrocytes, respectively, were part of the Bloch pathway. More specifically, the most abundant sterols in neurons (in order of abundance) are cholesterol >> desmosterol >> 7-DHD = zymosterol = zymostenol > all other sterols. In astrocytes, a similar profile is observed where the most abundant sterols are cholesterol >> desmosterol >> 7-DHD > all other sterols.

Although either the Kandutsch-Russell or Bloch biosynthetic pathways can theoretically synthesize cholesterol, our findings show that the two pathways are not equally utilized in the developing brain cells. In all cell lines investigated to date (e.g. fibroblasts, Neuro2a cells, neurons, astrocytes) cholesterol is the most abundant sterol followed by desmosterol, which indicates that all the investigated cell types in the brain and peripheral tissues preferentially use the Bloch pathway, where desmosterol is the immediate precursor to cholesterol (41, 51). While the 24-dehydrocholesterol reductase (DHCR24) enzyme can theoretically reduce the C24 double bond at any stage in the post-squalene synthesis, our findings suggest that over 90% of DHCR24 activity serves the conversion of desmosterol into cholesterol. The preferential use of the Bloch pathway by developing neurons is

confirmed by assessment of *de novo* synthesis in the presence of $^{13}C_6$-glucose: the levels of ^{13}C-desmosterol (last intermediate in the Bloch pathway) are ~ 60 times higher than ^{13}C-7-DHC (last intermediate in the Kandutsch-Russell pathway) (50). However, data in the literature suggests that the balance of these two pathways is dynamic and may change under pathophysiological conditions (52). For instance, detection of novel sterol intermediates in a cancer cell line led to the initial discovery of the Kandutsch-Russell pathway (33, 34). To date, such data are not available for CNS disorders, and this shift in the balance between pathways should be a further topic of investigation.

Developing neurons and astrocytes both synthesize, release and take up cholesterol depending on their homeostatic needs under *in vitro* conditions (50). However, strong neuronal cholesterol biosynthesis does not appear to be the result of *in vitro* conditions. Developing neurons express the key cholesterol biosynthesis enzymes HMG-CoA and DHCR7 in the mouse brain, and cortical projection neurons synthesize cholesterol during their entire lifetime. Furthermore, during the phase of maximal membrane growth and greatest cholesterol demand, neuronal cholesterol biosynthesis is indispensable in the mouse brain (53).

Our results show that during early postnatal development, the steady-state levels of cholesterol are higher in neurons than in astrocytes, coupled with a significantly higher amount of cholesterol produced in neurons. While exogenous sterol uptake in neurons depends on sterol binding to ApoE, astrocytes can take up free sterols from the extracellular milieu (50). This complex interplay between neuronal and astroglial cholesterol trafficking suggest that the early developmental control of sterol levels is a tightly regulated homeostatic process, where glial cells might act as a “cholesterol sink” or “cholesterol source” depending on the developmental needs of neurons. Furthermore, recent studies of Berghoff *et al.* (54) highlighted the critical interplay between microglia and demyelination, showing that microglia facilitate repair of demyelinated lesions via post-squalene sterol synthesis. Very little is known about oligodendrocyte (55) and microglia (56) sterol synthesis or these cells’ possible roles in developmental sterol homeostasis, and this promises to be a rewarding research area.

Sterol intermediates and toxic oxysterols

The immediate precursor of cholesterol is 7-DHC, which is converted to cholesterol by the DHCR7 enzyme (57). Thus, any disruption of DHCR7 leads to accumulation of the precursor, 7-DHC, (57) with yet unexplored effects on intracellular lipid sorting (58). Importantly, 7-DHC is the most oxidizable lipid known to date, with a propagation rate constant of 2,160 (59). This is an extraordinary 200 times greater propagation rate that of cholesterol and 10 times greater that of arachidonic acid. Simply put, 7-DHC is highly unstable and spontaneously oxidizes (60, 61). The result is formation of highly reactive autoxidation sterols, called 7-DHC derived oxysterols (62, 63). 7-DHC derived oxysterols are toxic, affecting cell viability, differentiation and growth (64). Others and we have shown that DHCEO, the most stable and best-studied 7-DHC derived oxysterol has a profound effect on neuronal morphology, neurite outgrowth and fasciculation (65), potentially through *Sonic hedgehog* signaling (66). At least one study also suggests that elevated 7-DHC alters

raft sterol composition and perturbs raft protein content (67), although other studies found that cholesterol and 7-DHC possess virtually the same ability to condense and order cellular membranes (68).

However, not only 7-DHC derived oxysterols have biological effects. All sterols can be modified by enzymatic and non-enzymatic processes through oxidation of the sterol backbone and/or oxidation of the side chains (69). Through action of several cytochrome P450 enzymes (CYPs), cholesterol itself is modified to give rise to about a dozen oxysterols (28, 70). In healthy tissue these oxysterols have complex biological functions: they are ligands for LXR and ROR nuclear receptors with roles in regulation of cholesterol biosynthesis, and inflammation (71, 72). Notably, some oxysterols are pro-inflammatory while others have anti-inflammatory effects. These biological effects are very complex, multifaceted, and not understood in sufficient detail. For example, 24SOHC is the main mechanism for excretion of excess cholesterol from the brain (73), yet it is also considered a positive allosteric modulator of NMDA receptors (74, 75). Furthermore, together with 24SOHC, 25-epoxycholesterol serves as a ligand for LXRs, and through interaction with frizzled (Class F) G protein-coupled receptor *smoothened* (SMO) activates *hedgehog* signaling (76).

Genetic disruption of cholesterol biosynthesis

A complete absence of cholesterol biosynthesis is incompatible with life. Mutations in the pre-squalene steps during normal development are lethal in all eukaryotes due to the disruption of critical membrane-based signaling (77). Mutations in the post-squalene pathway can be viable if partial cholesterol synthesis is preserved, but give rise to severe developmental disorders (26). Mutations in the enzymes serving post-lanosterol biosynthesis are associated with Smith-Lemli-Opitz syndrome (SLOS) (mutations in *DHCR7*) (78), desmosterolosis (mutations in *DHCR24*) (79), chondrodysplasia punctata (mutations in *EBP*) (80), lathosterolosis (mutations in *SC5D*) (81, 82), and CHILD syndrome (mutations in *NSDHL*) (83) (Figure 2). All of these syndromes affect brain and craniofacial development, and lead to intellectual and developmental disabilities.

Smith-Lemli Opitz syndrome (SLOS).

In the human population, SLOS is the most commonly diagnosed genetic disorder of sterol biosynthesis, with a frequency of 1:50,000 live births (84). It has a recessive inheritance pattern most commonly associated with compound heterozygosity, where different mutant alleles are inherited from each parent (85, 86). Biochemically it is characterized by highly elevated 7-DHC and oxysterol levels, reduced cholesterol, and decreased desmosterol (87–91). The phenotype of these patients is complex, and it depends on the amount of residual cholesterol biosynthesis (88, 92–95). Brain magnetic resonance imaging findings in SLOS point to myelination deficits, and found significant correlations between MRI findings with sterol levels and somatic malformations (96). Clinical manifestations can include craniofacial dysmorphic features, and 50–70% of patients meet the diagnostic features for autism spectrum disorders (97). Treatment of these patients is mainly symptomatic, as dietary cholesterol supplementation does not appear to improve brain function (98–

100). It is important to point out that reduced cholesterol biosynthesis is only part of the pathophysiology of SLOS – it is likely that the toxic levels of 7-DHC and 7-DHC-dervied oxysterols play a critical part in the disrupted development that lead to the SLOS phenotype (101–103).

DHCR7 heterozygosity.

The carrier frequency (the proportion of individuals with one copy of a known SLOS-inducing DHCR7 mutation) is approximately 3% among persons of European ancestry (84, 104). At this time, over 200 mutations have been described in the *DHCR7* gene (105). Humans with single allele *DHCR7* mutations appear to be healthy, without a distinct phenotype. However, human dermal fibroblasts with a single mutant allele in the *DHCR7* gene have elevated 7-DHC levels (51, 106). In addition, mouse studies suggest that even a single mutant allele might modulate biochemistry and behavior (107, 108). In addition to elevated 7-DHC levels these mice displayed a mild behavioral phenotype, including social dominance changes and differential response to a pharmacological challenge with a 5-HT2a agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-amionopropane. This raises the question whether single-allele human *DHCR7* mutation carriers might be more vulnerable to certain life circumstances that expose them to additional interference with sterol biosynthesis, or to oxidative stress (see below).

Chemical modulation of cholesterol biosynthesis

Hall et al. made a critical observation of cholesterol biosynthesis interference by medications, noting that some patients who used aripiprazole and trazodone were misidentified as SLOS patients based on their blood 7-DHC levels (109). Following up on these observations, over the last seven years we validated that haloperidol, aripiprazole, trazodone, and cariprazine are all strong inhibitors of sterol biosynthesis, and that they have profound biochemical effects on the fetal brain in rodent models (110–113). It is also important to point out that the above-mentioned compounds appears to inhibit directly the DHCR7 enzyme, as it occurs in the cell systems without the receptors targeted by the drugs.

Furthermore, our high-throughput screening data in cell culture systems revealed that many commonly used medications are sterol biosynthesis inhibitors (41–43, 114). Based on these data and literature review we estimate that the combined volume of sterol inhibiting medications, in the US alone, exceeded 300 million prescriptions in 2017. Commonly used medications that inhibited sterol biosynthesis in our screenings include haloperidol, aripiprazole, cariprazine, trazodone, amitriptyline, bupropion, sertraline, buspirone, risperidone, nortriptyline, fluoxetine, doxepin, metoprolol, nebivolol, atenolol, propranolol, hydralazine, and hydroxyzine. Importantly, many of these medications are prescribed to pregnant women, cross the placenta, and reach the brain of the developing child – with mostly unknown long-term outcomes in the maternally exposed offspring. Most of these drugs carry an FDA warning, stating that *“Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.”*

Interaction of genetic and chemical inhibition

As described above, both genetic mutations and medication use can inhibit DHCR7 function, resulting in 7-DHC elevation and toxic oxysterol production. This raised the question of whether genetic and environmental factors can interact, especially during development (Figure 3). To test this, we assessed the roles of medication, maternal *Dhcr7* genotype, offspring *Dhcr7* genotype, and their interaction (110, 111). Our work focused on three commonly prescribed medications: aripiprazole, trazodone and cariprazine. We used cultured mouse cortical neurons and astrocytes, *Dchr7*$^{+/-}$ transgenic mouse models, and human dermal fibroblasts from individuals with single allele *DHCR7* mutations (parents of children with SLOS) (51). Furthermore, we validated our findings in patients taking these medications (111, 112).

Cortical neurons and astrocytes.

In the latest study using DMG-derivatized LC-MS-MS ESI we examined the effects of three antipsychotics (haloperidol, aripiprazole, and cariprazine), two antidepressants (trazodone and sertraline), and an anti-arrhythmic (amiodarone) on sterol synthesis of developing cortical neurons and astrocytes (44). The method allowed us to simultaneously assess 14 sterols and 7 oxysterols in a single sample run. Both neurons and astrocytes showed altered sterol composition in response to exposure to all six investigated drugs. ARI, HAL, TRZ and CAR strongly increased 7-DHC, coupled with reductions in desmosterol levels in both cell types.

The medications we tested are likely to affect all sterol-synthesizing cells in the brain, and perhaps the whole body. However, the physiological relevance of the different patterns of sterol biosynthesis inhibition by the various medications is currently unknown. The possibility of multi-system 7-DHC/oxysterol toxicity is consistent with the complex, characteristic dysmorphology phenotype seen in patients with SLOS.

Maternal drug exposure model.

We tested if medications given to pregnant mice affect cholesterol biosynthesis in developing brain of WT and *Dhcr7*$^{+/-}$ embryos. Embryos were maternally exposed to aripiprazole, cariprazine, trazodone or vehicle from E12 to E19 (110, 111, 113). This exposure time was chosen based on developmental closure of blood-brain-barrier, and allowed us to examine endogenous brain sterol synthesis. Levels of cholesterol, its precursors, medications and their metabolites were measured in the brain of pups at birth. We found that all three medications and their metabolites reached the brain of fetuses and inhibited the DHCR7 enzyme in the brain of all embryos, regardless of maternal or offspring *Dhcr7* genotypes. These medications increased 7-DHC levels in WT pups to levels higher than those observed in *Dhcr7*$^{+/-}$ pups under vehicle-treated conditions, suggesting that the DHCR7 inhibiting drug effect is stronger than the *Dhcr7*$^{+/-}$ heterozygosity effect. Finally, we observed a summation between the genetic inhibition and chemical inhibition on 7-DHC levels, suggesting that *Dhcr7*$^{+/-}$ pups from *Dhcr7*$^{+/-}$ mothers are the most vulnerable to chemical DHCR7 inhibitors.

In summary, maternally administered aripiprazole, cariprazine and trazodone all inhibited DHCR7 activity, increased 7-DHC, elevated resulting oxysterols, and decreased desmosterol in the exposed pups' brains. Due to its long half-life, detectable levels of CAR, and a corresponding elevation in 7-DHC levels were observed in the brain of newborn pups up to 14 days after drug exposure (111). Furthermore, three-way ANOVA analyses revealed that maternal *Dhcr7*$^{+/-}$ genotype significantly contributed to the observed 7-DHC elevation for CAR and ARI, but the TRZ-driven changes appeared to be driven only by drug exposure and pup *Dhcr7*$^{+/-}$ genotype.

The magnitude of the 7-DHC elevation we observed as result of drug treatment is quite remarkable (Figure 4). Namely, CAR-induced 7-DHC levels and the increased DHCEO levels detected in *Dhcr7*$^{+/-}$ pups are in the range of that seen in SLOS mouse models. It is also noteworthy that actual cholesterol changes we observed were always much milder than the changes in the sterol precursor profiles, suggesting that alterations in cholesterol levels are perhaps not the best readouts of disrupted sterol biosynthesis. As cholesterol is a very stable molecule with a long half-life (five years in the human brain), *precursor/cholesterol* ratios and oxysterol assessments might be much better indicators of the state of sterol biosynthesis in patients.

Human dermal fibroblast model.

Mouse sterol biosynthesis closely mimics human sterol biosynthesis, thus we hypothesized that our findings are very likely to be translatable to the human population. To test the relevance for human pathophysiology, we also performed several investigations on *DCHR7*$^{+/+}$ and *DCHR7*$^{+/-}$ human dermal fibroblasts (51). To ensure that the *DHCR7*$^{+/-}$ mutations were representative of the carriers in the human population, we used biomaterial donated from parents of SLOS patients. Six matched pairs of fibroblast cultures were treated with ARI and TRZ, and their sterol profile was analyzed by LC-MS-MS. Upon treatment with ARI and TRZ, both *DHCR7*$^{+/-}$ and *DHCR7*$^{+/-}$ fibroblasts increased their baseline 7-DHC levels by 10- to 60-fold (Figure 5). Notably, the total accumulation of 7-DHC was the highest in drug-treated *DHCR7*$^{+/-}$ cells. Repeating the same set of experiments in the presence of ^{13}C-lanosterol revealed that ARI and TRZ strongly inhibited *de novo* sterol biosynthesis. The results suggest that *DHCR7* mutation carriers have increased vulnerability to both ARI and TRZ exposure, and perhaps many other commonly used medications. Thus, this segment 1–4% of the population may be more likely to sustain deleterious health consequences when exposed to medications that increase levels of 7-DHC.

Human biobank findings.

To further investigate the human relevance of our findings, we assessed the blood levels of five sterols from patients who received psychotropic medications (112). We examined cholesterol, desmosterol, lanosterol, 7DHC and 8DHC levels in blood samples of 123 psychiatric patients on various antipsychotic and antidepressant drugs, and 85 healthy controls. Three drugs, aripiprazole, haloperidol and trazodone were associated with increased circulating 7DHC and 8DHC levels, while five other drugs, clozapine, escitalopram/citalopram, lamotrigine, olanzapine, and risperidone, were not. In separate biobank sample studies, we found that 7-DHC levels were also higher in patients with

detectable levels of CAR and TRZ in their blood (111, 113). While these findings are only minimally informative about the sterol synthesis events in the brain (as brain and body sterol synthesis are distinct and separated by the blood-brain barrier), they lead us to the conclusion that drugs can possess sterol-inhibiting function both in the brain and the body. The long-term consequences of developmental sterol inhibition by medications remain unknown.

Public health considerations

Population studies have suggested that DHCR7 inhibitors act as teratogens for a developing child. A 2016 review by Boland and Tatonetti demonstrated that first-trimester exposure to DHCR7 inhibiting medications results in outcomes similar to those of known teratogens, and that DHCR7 activity should be considered during drug development and prenatal toxicity assessment (105). As a result, in the age of personalized/precision medicine, the implications of the current findings are quite significant.

First, heterozygous DHCR7 mutation carriers in the human population are quite frequent, and range from 1–4% depending on ethnicity and geographic region (84, 104). Emerging data suggest they are more vulnerable to side effects of treatment with sterol inhibitors.

Second, SLOS patients are often treated with psychotropic medications. The precise choice of medication should be an important consideration, as sterol biosynthesis inhibiting medications (such as aripiprazole, cariprazine, trazodone) might further exacerbate the already high 7-DHC levels in these patients.

Third, polypharmacy in the US is extremely high. An average person at age of 50 takes 2.5 prescription medications (115). Many sterol biosynthesis inhibiting medications are highly prescribed (we estimate >300 million prescriptions/year in the US), and often taken simultaneously. The summation of these effects are unknown, but it would be reasonable to expect that that simultaneously prescribed drugs in this category likely have additive effects.

Fourth, it appears that the effects of 7-DHC elevation are most pronounced in the developing brain. Yet, pregnant women frequently use medications that inhibit sterol biosynthesis (116–120). The long-term consequences of such drug use in the human population are not known, especially when the mother and/or unborn child are both single-allele *DHCR7*$^{+/-}$ mutation carriers. For this population, sterol biosynthesis inhibiting medications should be replaced by other drugs with similar activity that do not give rise to such side effects.

Finally, we need to educate the public and prescribing physicians that sterol-inhibiting side effect of frequently used medications are quite common, and that they represent a potential harm for the brain of offspring.

Further studies

The literature data reveals that our knowledge of sterol biosynthesis and its homeostatic mechanisms in the brain are greatly understudied.

We can only partially predict which chemical structures (and new medications) will incur side effects of sterol biosynthesis inhibition. We know that medications containing the 2,3-dichlorophenylpiperazine ring (like aripiprazole and cariprazine) are likely to be sterol biosynthesis inhibitors. However, many other medications elevate 7-DHC, but do not share an apparent chemical structure.

To date, we have no validated and approved medications that would decrease 7-DHC and oxysterol levels. While our HTS studies revealed a number of compounds that could potentially counteract 7-DHC elevation effects, and vitamin E counteracts peroxidation in a rodent model (101), this area remains greatly understudied.

Medication effects on sterol biosynthesis in the developing brain are undisputable. However, do these medications equally affect all brain and somatic cells during development? The various developing neuronal subpopulations might rely on sterol biosynthesis in specific ways, and could be differentially affected by DHCR7 (and other sterol) inhibitors. For example, heterozygous *Dhcr7*$^{+/-}$ transgenic animal model studies suggest that serotonergic neurons might be preferentially affected by sterol-inhibiting drug exposure (107, 121, 122), as these animals show differential response to 5HT agonists. Similarly, *Dhcr7* is a negative regulator of sonic hedgehog signaling (66, 123, 124), which is essential for development for somatostatin-containing interneurons (125, 126). In addition, the sensitivity of peripheral neurons of the autonomic nervous system to disrupted sterol biosynthesis is unknown. As they are located outside the blood brain barrier and have access to the peripheral sterol pool, disturbances in their sterol synthesis might be governed by a set of different regulatory mechanisms.

The pathophysiology studies related to 7-DHC elevation have already yielded highly interesting and impactful results. Yet, the biological functions of at least two other, potentially very important sterol intermediates are not understood at all. 7-DHD (a precursor of desmosterol) and 8-DHC (an EBP-created isomer of 7-DHC) can also accumulate as result of treatment with approved medications. They are also highly oxidizable and give rise to oxysterols in the brain tissue. The recent advances in technology now allow to separate and measure these two compounds using the DMG-derivatized LC-MS-MS technology, helping us to further decipher this very complex process.

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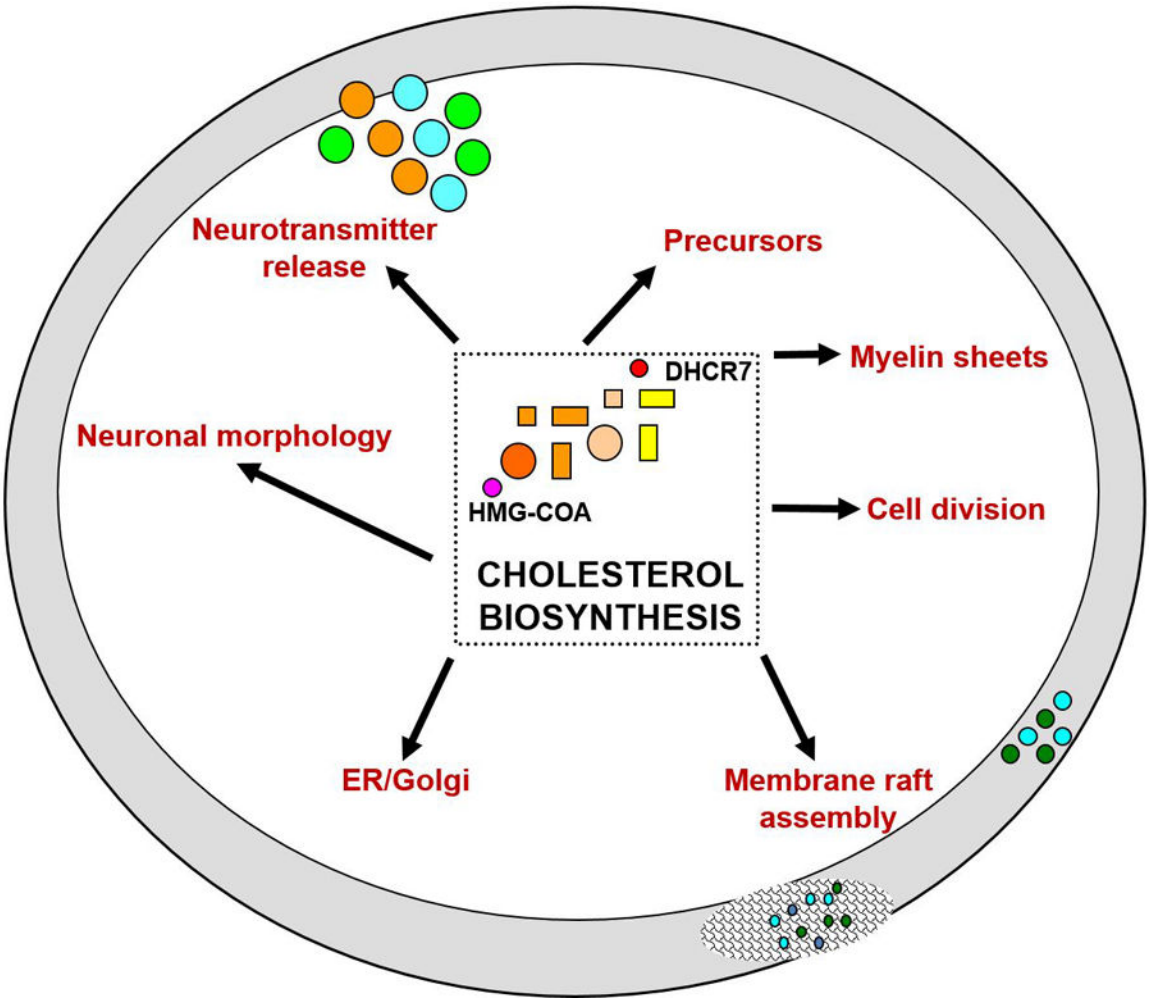

Figure 1. Healthy cholesterol biosynthesis is essential for neuronal homeostasis.

Cholesterol is an essential structural component of membranes, critical for assembly and proper maintenance of lipid rafts, required for synapse and dendrite formation, necessary for axonal guidance, and it serves as a precursor for various biosynthetic pathways. Brain cells synthesize their own cholesterol, independent of the systemic cholesterol pool.

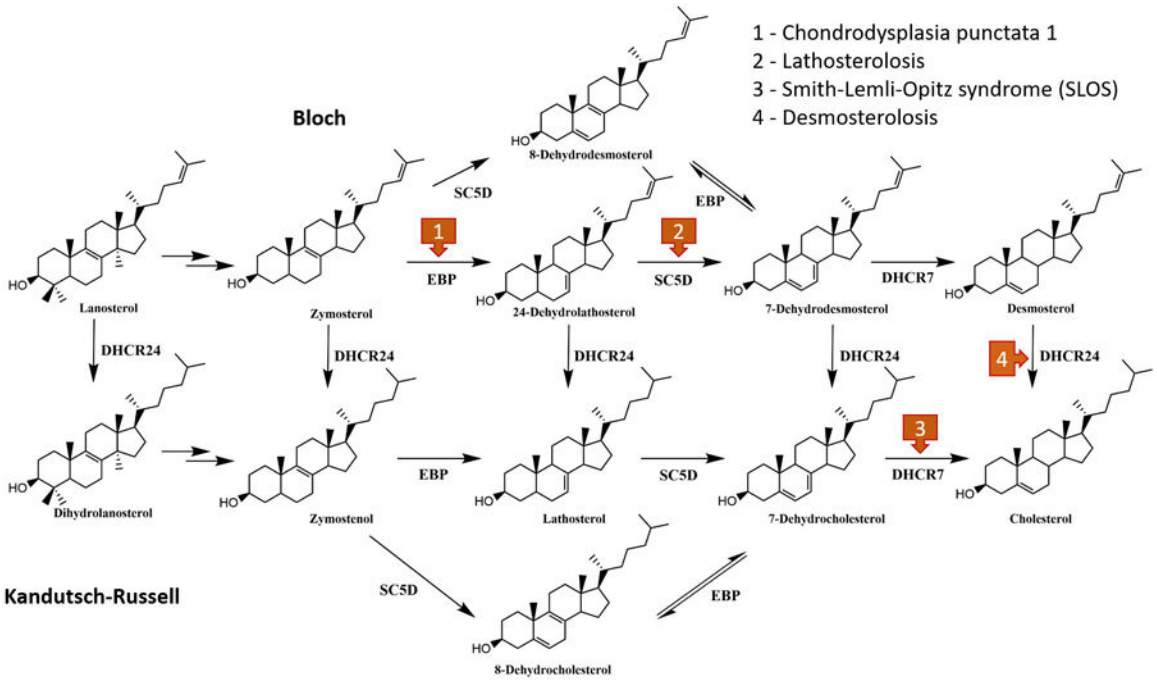

Figure 2. Cholesterol biosynthesis and inborn errors of post-lanosterol biosynthesis.

The last, post-squalene phase of cholesterol biosynthesis, starting with lanosterol, is divided into the Bloch and Kandutsch-Russell branches. Mutations in the genes encoding the enzymes of post-lanosterol biosynthesis result in *Smith-Lemli-Opitz syndrome* (SLOS) (mutations in DHCR7), *desmosterolosis* (mutations in DHCR24), *chondrodysplasia punctata 1* (mutations in EBP) and *lathosterolosis* (mutations in SC5D). All of these syndromes affect brain and craniofacial development, and lead to intellectual and developmental disabilities. Note that these sterol biosynthesis enzymes participate in multiple conversion processes, but for simplicity, the arrows denote only one of their main actions.

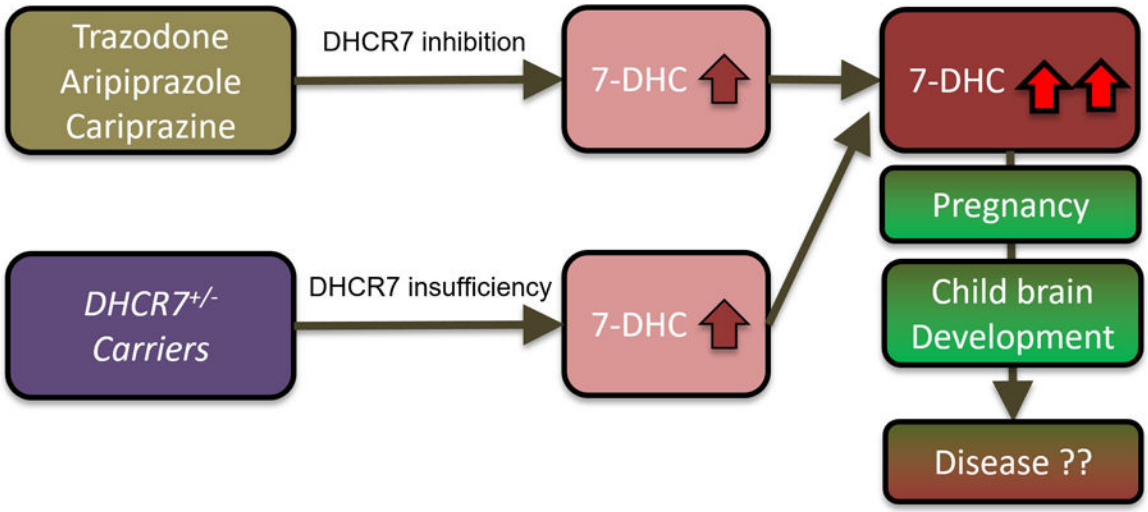

Figure 3. Genetic and medication effects on 7-DHC levels.

Both medications and genetic *Dhcr7* heterozygosity elevate 7-DHC levels. In mouse models, when these two factors (with a similar biochemical consequence) are combined, their biochemical effects are greatly exacerbated during pregnancy. Many sterol biosynthesis inhibiting medications are highly prescribed (we estimate >300 million prescriptions/year in the US), are often used by pregnant women, and 1–4% of the human population carry single-copy mutations in the *DHCR7* gene. The *sterol inhibiting medication* DHCR7 genotype* interactions, and the long-term effects on the offspring brain are unknown in the human population.

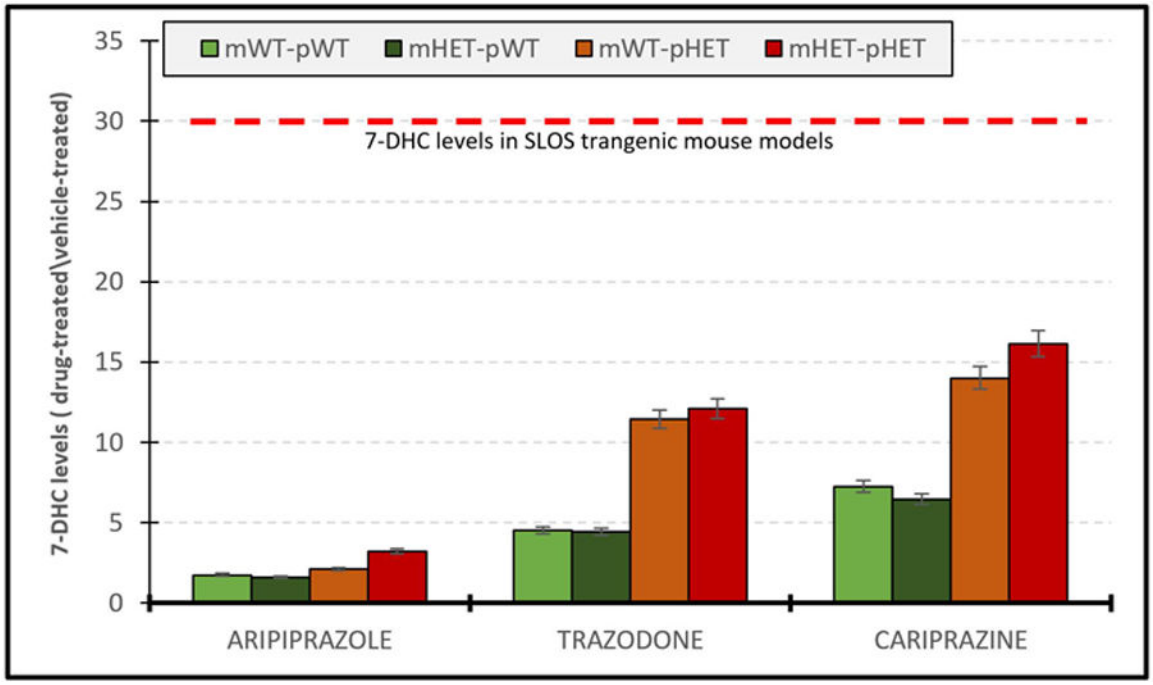

Figure 4. 7-DHC levels in the brain of newborn pups in response to maternal drug treatment and *Dhcr7* genotype.

The X-axis denotes the three drug treatments (aripiprazole, trazodone and cariprazine), and the Y-axis denotes fold increase over vehicle-treated control. mWT and mHET denotes maternal *Dhcr7*$^{+/+}$ and *Dhcr7*$^{+/-}$ genotypes, respectively; pWT and pHET denote pup *Dhcr7*$^{+/+}$ and *Dhcr7*$^{+/-}$ genotypes, respectively. Note that all maternal treatments strongly elevate 7-DHC levels in the brain of newborn pups, and that the most affected pups are those with the *Dhcr7*$^{+/-}$ genotype born to *Dhcr7*$^{+/-}$ mothers (3–16 fold increase in brain 7-DHC levels, depending on medication). These 7-DHC levels reach up to 55% of the 7-DHC levels seen in the brains of untreated *Dhcr7*$^{T93M/T93M}$ transgenic SLOS mouse model pups (red dashed line). The long-term consequences of this 7-DHC elevation are currently not known. Data are compiled from these publications: (110, 111).

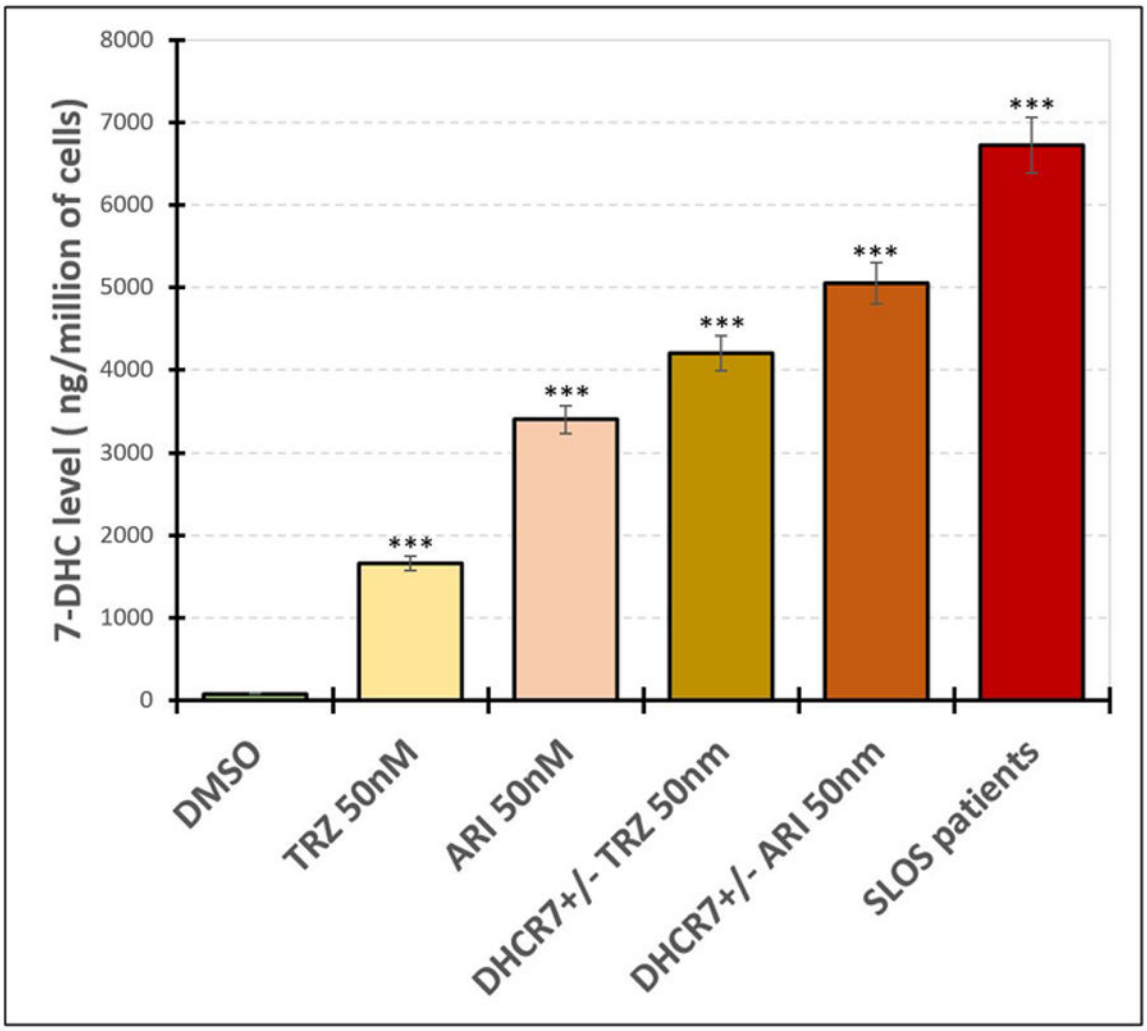

Figure 5. Human fibroblasts strongly elevate 7-DHC levels in response to TRZ and ARI, in a DHCR7 genotype-dependent manner.

Note that 50nm ARI-treated *DHCR7*$^{+/-}$ human patient fibroblasts increase their 7-DHC levels up to 62 fold, reaching approximately 75% of 7-DHC levels seen in SLOS patient fibroblasts. Notably, similar results have been obtained for CAR (data not shown). Adapted from Korade at al, 2017 (51). ***p<0.001 vs control (DMSO).