CANDY

Comorbid Analysis of Neurodevelopmental Disorders and epilepsY

Deliverable D1.2
Report on developmental trajectories in PTCHD1 genetic mouse model

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1 Executive summary

A key element of the research in CANDY is to focus on rare genetic variants that increase the risk of NDDs and converge on common signaling pathways. Moreover, the CANDY project is testing whether NDD and common mental and somatic multimorbidity is related to specific "sensitive time points" in development. We focused on the PTCHD1 mouse model – a model carrying a rare high-impact mutation that in the human population is associated with increased risk of ID and ASD. PTCHD1 was hypothesized to regulate lipid homeostasis. Notably, alterations in cholesterol have been linked to NDD phenotypes and neuroinflammation. Thus, we probed lipid content in young adolescent and adult mice. Moreover, we assessed hyperactivity and cognitive performance in PTCHD1 model.

2 Introduction

Neurodevelopmental conditions, including autism, are highly heterogeneous in their severity, phenotypic characteristics, and underlying mechanisms. Previous work identified alterations in specific synaptic neurotransmitter receptor systems, neuromodulators, intracellular signaling pathways, and transcriptional regulators that arise from autism-associated mutations and that resemble candidate targets for interventions [1, 4-9]. Lipid homeostasis is a comparably less deeply explored process that – when perturbed – increases the likelihood of neurodevelopmental conditions, including autism. There is a high incidence of autistic spectrum features in individuals with Smith-Lemli-Opitz syndrome (SLOS) with more than half of individuals meeting diagnostic criteria for autism [10, 11]. SLOS arises from loss-of-function mutations in the 7-dehydrocholesterol reductase (DHCR7), an enzyme that catalyzes the conversion of 7-dehydrocholesterol to cholesterol. Metabolically, SLOS is characterized by cholesterol insufficiency and accumulation of its dehydrocholesterol precursors. Notably, alterations in cholesterol homeostasis are more widely associated with neurodevelopmental disorders (NDDs). Population-level differences in blood lipid profiles (LDL, total cholesterol, and triglycerides) were reported between individuals with ASD and matched controls [12, 13]. Moreover, cholesterol alterations have been implicated in Fragile X Syndrome [14] and Rett's Syndrome [15], two monogenic syndromes where a substantial fraction of individuals meets diagnostic criteria for autism. Overall, defects in cholesterol homeostasis were hypothesized to play an important role in contributing to neuroinflammation and increasing risk and/or severity of neurodevelopmental conditions including ADHD, ID and/or epilepsy [18].

PTCHD1 contains sterol-sensing domains, a key domain of proteins involved in the regulation of cholesterol homeostasis. Cholesterol homeostasis was hypothesized to play an important role in a sub-group of neurodevelopmental conditions and to contribute to the overlap with ADHD, ID and/or epilepsy [18]. Thus, we hypothesised that phenotypes of PTCHD1 mutant mice might arise from alterations in cholesterol homeostasis. To test this, we employed mass-spectrometric assays to probe lipid composition of PTCHD1 mutant mice in early postnatal development and in adolescent animals. We further complemented this analysis with examinations of neuronal morphology and behavioral parameters in the mice.

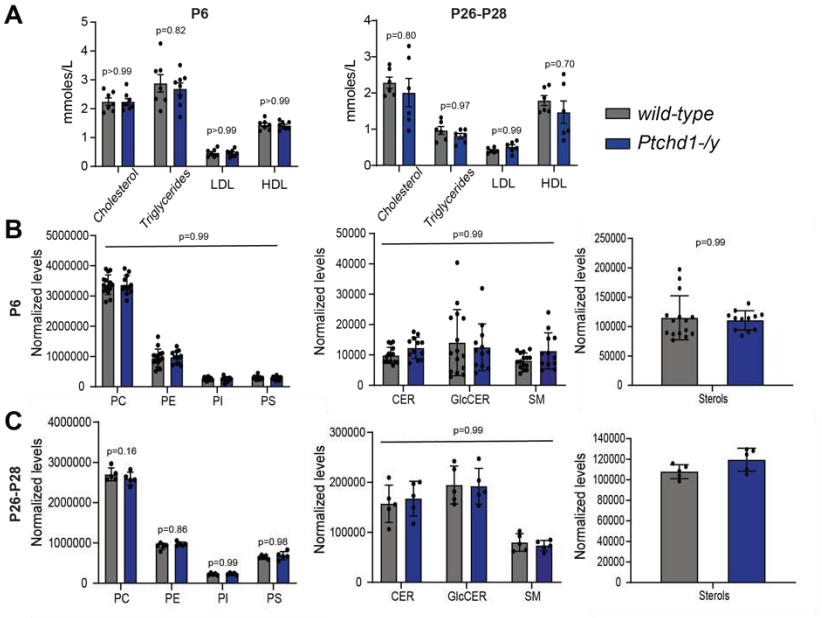
3 Main section

3.1 Alterations in regulators of sterol metabolism are associated with neurodevelopmental conditions

We first collected evidence supporting alterations in lipid homeostasis in NDDs. Genes involved in lipid regulation are enriched amongst deleterious variants that segregate with ASD in the population [12, 21]. To extend these observations specifically to cholesterol homeostasis, we used the GeneTrek tool [22] to search for NDDs associated with mutations in genes controlling cholesterol metabolism (89 genes, KEGG pathways hsa04979 and hsa00900). We also searched for NDD-associated genes predicted to contain sterol-sensing domains (SSD) (13 genes) [23, 24], a membrane domain contained in proteins involved in cholesterol biosynthesis (e.g. HMG-CoA reductase and 7-dehydrocholesterol reductase enzymes), intracellular cholesterol transport (NPC1), and cholesterol efflux involved in cell signaling (Patched-1, PTCH1). Notably, 20 of the 89 genes involved in sterol metabolism were classified as associated with epilepsy or NDDs. When focusing on SSD proteins, four of the 13 genes are defined as risk genes: NPC1, NPC1L1, PTCH1, and PTCHD1. NPC1, NPC1L1 and PTCH1 encode proteins with cholesterol transporter functions. For PTCHD1, knock-out in mice results in behavioral and electrophysiological alterations [25-27]. Moreover, loss of PTCHD1 impairs cholesterol-dependent μ-opioid receptor trafficking and function [28]. However, it is unknown whether loss of Ptchd1 affects cholesterol homeostasis *in vivo*.

Considering that Ptchd1 is also expressed in non-neuronal tissues [29] and that alterations in blood cholesterol levels were reported in ASD patients [12, 13], we performed blood sampling from 6 day and 26-28 day old Ptchd1-/y and littermate control animals and quantified cholesterol, LDL, HDL, and triglycerides levels. Notably, we did not observe significant genotype-related differences in any of these parameters at both ages (**Fig. 1A**). We then performed a detailed lipid analysis on overall lipid composition in brain tissue of Ptchd1-/y mice, including sterol levels and fatty acid chain length saturation. We observed no genotype-dependent differences between wild-type and Ptchd -/y mutant brain samples at either age (P6 and P26-28, **Fig.1B,C**).

Figure 1. ***A,*** Plasma concentration of cholesterol-related metabolites from P6 or P30 wild-type and Ptchd1^{KO} mice. LDL: low density lipoprotein, HDL: high density lipoprotein. ***B,*** Lipids levels (picomoles, normalized) in cerebellar tissue of P6 and ***C,*** P26-28 Ptchd1 wild-type or -/y mice (n=5) by lipidomics analysis. Note that cerebellar tissue was used in these experiments as it is a site of high PTCHD1 mRNA expression [27]. PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, Cer: ceramide, GlcCer: glucosylceramide, SM: sphingomyelin. Mean ± SD, Ordinary two-way ANOVA with Tukey's multiple comparisons tests.



Finally, we attempted to relate our lipid analysis to behavioral analysis in Ptchd1 mutant mice. We focused on hyperactivity – as a read-out for a frequent co-morbidity in neurodevelopmental disorders. Moreover, we assessed cognition in object recognition tests. Adolescent mice were tested in an open field test and tracked (**Fig.2A**). Analysis revealed a genotype-specific increase in jumping behavior (**Fig.2B**), a form of hyperactivity. In addition, we performed object recognition tests to assess cognition (Fig.3C). We found that Ptchd1 knock-out mice were impaired in object discrimination at 1 hr and 24 hrs. Interestingly, this lack of object discrimination arose from a lack of habituation to the familiar objects (**Fig.2C**).

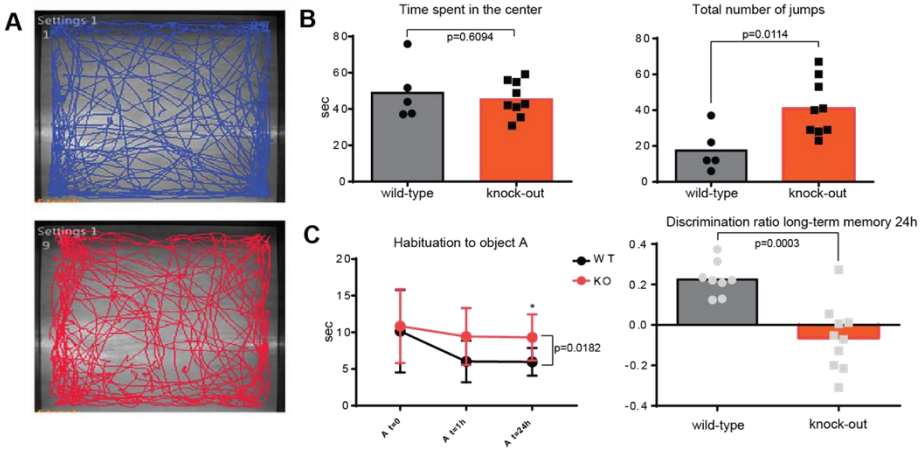


Figure 2. A, Behavioral tracking of a WT (blue) and KO (red) mouse. **B,** Time in center and number of jumps (n = 5-9 mice), unpaired t-test. **C,** Habituation and discrimination of object in recognition task (n-8-10 mice), ANOVA.

4 Conclusions

Taken together, our results suggest that deletion of the sterol-sensing domain protein PTCHD1 does not significantly alter overall cholesterol level or several other major lipid species in the mouse brain, neither early in postnatal development nor in adolescence. However, our behavioral analysis confirms hyperactivity and cognitive alterations in this model.

The lack of cholesterol alterations was unexpected. However, it is possible that brain cholesterol distribution is altered rather than overall levels of cholesterol (which are assessed by biochemical / mass-spectrometric methods). This highlights the need of developing probes for spatial assessment of cholesterol with cell-type-specific resolution.

Initial trials in individuals with Fragile X have not supported therapeutic benefits from treatment with cholesterol lowering drugs. The approach developed in our work should enable probing brain cholesterol distribution on a cellular level in models of brain disorders or in patient-derived cell preparations. Such an analysis should provide valuable information on forms of ASD, where a modification of cholesterol levels may hold promise for therapeutic intervention.

5 Glossary

Abbreviation / acronym	Description
SSD	Sterol-sensing domain
DHCR7	7-dehydrocholesterol reductase
SLOS	Smith-Lemli-Opitz syndrome
NDD	Neurodevelopmental disorder
ID	Intellectual disability

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7 Annexes

Manuscript - Cell type-specific assessment of cholesterol distribution in models of neurodevelopmental disorders

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