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Mouse models for inherited monoamine neurotransmitter disorders

B. Thöny[1] | J. Ng[2] | M. A. Kurian[3,4] | P. Mills[5] | A. Martinez[6,7]

[1]Division of Metabolism and Children's Research Center, University Children's Hospital Zurich, Zürich, Switzerland

[2]Genetic Therapy Accelerator Centre, University College London, Queen Square Institute of Neurology, London, UK

[3]Zayed Centre for Research into Rare Disease in Children, GOS Institute of Child Health, University College London, London, UK

[4]Department of Neurology, Great Ormond Street Hospital, London, UK

[5]Great Ormond Street Institute of Child Health, University College London, London, UK

[6]Department of Biomedicine and Center for Translational Research in Parkinson's Disease, University of Bergen, Bergen, Norway

[7]Neuro-SysMed, Department of Neurology, Haukeland University Hospital, Bergen, Norway

Correspondence
B. Thöny, Division of Metabolism, University Children's Hospital Zurich, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland.
Email: beat.thoeny@kispi.uzh.ch

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Abstract

Several mouse models have been developed to study human defects of primary and secondary inherited monoamine neurotransmitter disorders (iMND). As the field continues to expand, current defects in corresponding mouse models include enzymes and a molecular co-chaperone involved in monoamine synthesis and metabolism (PAH, TH, PITX3, AADC, DBH, MAOA, DNAJC6), tetrahydrobiopterin (BH_4) cofactor synthesis and recycling (adGTPCH1/DRD, arGTPCH1, PTPS, SR, DHPR), and vitamin B_6 cofactor deficiency (ALDH7A1), as well as defective monoamine neurotransmitter packaging (VMAT1, VMAT2) and reuptake (DAT). No mouse models are available for human DNAJC12 co-chaperone and PNPO-B_6 deficiencies, disorders associated with recessive variants that result in decreased stability and function of the aromatic amino acid hydroxylases and decreased neurotransmitter synthesis, respectively. More than one mutant mouse is available for some of these defects, which is invaluable as different variant-specific (knock-in) models may provide more insights into underlying mechanisms of disorders, while complete gene inactivation (knock-out) models often have limitations in terms of recapitulating complex human diseases. While these mouse models have common phenotypic traits also observed in patients, reflecting the defective homeostasis of the monoamine neurotransmitter pathways, they also present with disease-specific manifestations with toxic accumulation or deficiency of specific metabolites related to the specific gene affected. This review provides an overview of the currently available models and may give directions toward selecting existing

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models or generating new ones to investigate novel pathogenic mechanisms and precision therapies.

KEYWORDS

monoamine neurotransmitters, monogenetic defects, mouse models

1 | INTRODUCTION

The mouse has been studied genetically for over a century. It has been used as a primary model organism in combination with powerful tools for genetic manipulation to study, among others, human genetic variation and disease based on comparative studies of biology and physiology for several decades.[1] (For a technological overview of the potential for gene and genome engineering and elucidating human genetic variants using mouse models, we refer to more specialized literature.)[2] In particular, mouse models have been indispensable for research on disease mechanisms and for testing novel therapies for the group of inherited monoamine neurotransmitter disorders (iMNDs).

For pathways and details on these disorders, including information on enzymes and metabolites—under normal and disease conditions—we refer to excellent review articles[3–5] or overviews within this special issue. The first mouse models for neurotransmitter defects were reported more than three decades ago. Since then, the number of existing mouse models for these defects and more specifically for iMNDs, the focus of this review, have increased hugely. iMND are rare disorders caused by variants in genes involved in (a) biosynthesis and metabolism of monoamines, (b) chaperoning of enzymes, (c) cofactor (tetrahydrobiopterin [BH_4] and pyridoxal 5′-phosphate [vitamin B_6]) synthesis, homeostasis and recycling, (d) transport and uptake of monoamines into presynaptic vesicles, and (e) transport and reuptake of monoamines into presynaptic neurons (see also Table 1 for the list of diseases, associated genes, and relevant mice models). These iMNDs are rare disorders with a variable clinical spectrum, normally appearing in infancy or adolescence, with symptoms ranging from mild hypotonia, dystonia, and parkinsonian movement disorders to severe infantile encephalopathy. With the exception of vitamin B_6 deficiency, common to all of these disorders is that the defect in dopamine and downstream monoamine neurotransmitters is usually treated with levodopa (synthetic L-Dopa). However, for some patients this treatment is often not effective or, if a response is seen initially, this can often decline over time. Treatment can also lead to serious side effects leading to discontinuation. Thus, alternative, better precision therapies are necessary for many iMNDs. The mouse models discussed in this review will be important in these endeavors.

In this review we have included disorders, in addition to those listed in the registry of the "International Working Group on Neurotransmitter Related Disorders (iNTD)" (https://www.intd-registry.org/intd-registry-2-0), which are also known to also cause CSF neurotransmitter imbalance, that is, the monoamine deficiency disorders caused by variants in the two human HSP40/DNAJs co-chaperones, DNAJC6 and DNAJC12. DNAJC6 fulfills the criteria for a neurotransmitter disorder showing aberrant dopamine neurotransmission with childhood onset parkinsonism dystonia.[6,7] With respect to DNAJC12, a number of recent studies have clearly associated gene variants in this co-chaperon with dopa-responsive nonprogressive parkinsonism,[8,9] although to our knowledge, no mouse models are yet available (Table 1).

While the models described in this review are invaluable, there is a need in the future for generating additional mouse models to enable better understanding of these disorders given the large spectrum of mutants, high degree of compound heterozygosity, and putative instability degree (upon breeding) associated with known variants. Models that represent specific disease variants, especially for certain alleles with high frequency in heterozygosity, would facilitate basic investigations and the search for novel treatments. Limitations could be overcome by extending murine models to test multiple genetic variants as homozygous or composite heterozygous mutants, generating conditional knock-outs (or knock-ins), and/or humanizing mouse models.

In this overview we have added an estimate of the "usefulness" of the available models (Table 1). Not included, due to lack of understanding, are secondary abnormalities of monoamine neurotransmitter metabolism such as those commonly observed in in cerebrospinal fluid in patients with neurological disorders of unknown origin(s). A peculiarity for brain neurotransmitter disorder studies is the fact that human metabolites are exclusively analyzed in cerebrospinal fluid and often compared to metabolites found in murine brain extracts. While cellular models, including patient-derived pluripotent stem cells (iPSCs), can give valuable insights into disease characteristics, murine models remain indispensable

TABLE 1 Overview of primary and secondary inherited monoamine neurotransmitter disorders (iMND) and corresponding murine models of human diseases based on monogenetic defects.

Disorder[a]	Gene[a] (human)	Corresponding mouse models[a,b]	Usefulness of model(s)[c]
Enzymes involved in biosynthesis and metabolism of monoamines			
PAH	*PAH*	*Enu1,2,3*; *Pah-ki* (R261Q, ΔE1, R408W)	+++
TH	*TH*	*Th-ko*, *Th-ki* (R203H, Q381K)	+ or +++
TH—PITX3	*PITX3*	*Pitx3-ko*, *Pitx3-Cre*, *Pitx3-Flox*	+++
AADC	*DDC*	*Ddc-ko*, *Ddc-ki* (IVS6/IVS6), *Ddc*-S250F	+++
DBH	*DBH*	*Dbh-ko*	++
MAOA/B	*MAOA/B*	*MaoA-ko*; *MaoB-ko*	+++
HSP40/DNJs co-chaperones			
DNAJC6	*DNAJC6*	*Aux-ko*, *Aux-ki* (R857G)	+ or ++
DNAJC12	*DNAJC12*	Not available	n.d.
Enzymes involved in cofactor synthesis, homeostasis, and recycling			
BH_4—adGTPCH1 (DRD)	*GCH1*	*hph-1*; DPS	++
BH_4—arGTPCH1	*GCH1*	*Gch1-ki* (L117R)	++
BH_4—PTPS	*PTS*	*Pts-ko*, *Pts-ki* (R15C), *Pts-ko/ki*	+++
BH_4—SR	*SPR*	*Spr-ko*	+
BH_4—DHPR	*QDPR*	*Dpr-ko*	+
B_6—ALDH7A1	*ALDH7A1*	*Aldh7a1-ko*	++
B_6—PNPO	*PNPO*	Not available	n.d.
B_6—PLPHP	*PLPHP*	Not available	n.d.
Proteins involved in transport and uptake of monoamines in presynaptic vesicles			
VMAT1	*SLC18A1*	*Vmat1-ko*, *Vmat1-ki* (N136I)	+++
VMAT2	*SLC18A2*	*Vmat2-ko*, *Vmat2*-LO	+ or ++
Protein involved in transport and re-uptake of dopamine			
DAT	*SLC6A3*	*Dat-ko*, *Dat-ki* (A559V, T356M)	+++

[a]For abbreviations and references see text.
[b]*ki*, knock-in; *ko*, knock-out.
[c]+++ replicates the human phenotype; ++ partially replicates the human phenotype; + poor replication of human phenotype. n.d., not defined (see text for details).

"tools" for studying disease mechanism and therapy approaches.

2 | PHENYLALANINE HYDROXYLASE DEFICIENCY

Phenylalanine (L-Phe) is an essential amino acid in humans. Phenylalanine hydroxylase (PAH) is a BH_4-dependent aromatic amino acid hydroxylase that catalyzes the hydroxylation of L-Phe to L-tyrosine (L-Tyr), the precursor of catecholamine neurotransmitters and hormones dopamine, noradrenaline and adrenaline. PAH is mainly expressed in liver and is regulated by the concentration of L-Phe, that activates homo-tetrameric PAH in an allosteric positive cooperative manner.[10–13]

PAH deficiency is associated with variants in *PAH*, that decrease total enzyme activity and lead to increased (systemic) blood L-Phe levels, that is, hyperphenylalaninemia (HPA), which is associated with (secondary) hypopigmentation, and in severe cases to phenylketonuria (PKU), where L-Phe levels become neurotoxic, resulting in abnormally low catecholamine and serotonin metabolites in CSF.[14,15] According to the European Guidelines PKU is presently classified into mild HPA (L-Phe 120–360 μmol/L), which does not require treatment, and PKU (L-Phe >360 μmol/L), which requires treatment. PKU patients are further divided into BH_4 (referred to as sapropterin)-responsive and non-BH_4-responsive.[15]

Mouse models of PKU have been available since the 90s, when models *enu1-3* were generated by *N*-ethyl-*N*-nitrosourea (*enu*) germline mutagenesis followed by

selection based on HPA measurement.[16] *Enu1* and *Enu2* have been extensively used in PKU research and development of treatments. *Enu1* harbors the variant p.V106A-PAH, which is very rare in patients,[17] causes PAH destabilization, and results in BH_4-responsive mild HPA.[16,18] *Enu2* carries the variant p.F263S-PAH, which is also very rare and does not largely affect PAH stability but is mainly devoid of catalytic activity, resulting in severe BH_4-non-responsive PKU.[16,18] This *Enu2* (or PKU) mouse has low concentrations of catecholamine and serotonin metabolites in brain extracts.[19,20] *Enu3* has a splice-site mutation (junctions of exons 11 and 12), generating a sequence frameshift and a premature termination codon that leads to total absence of the PAH protein.[21] Similar to untreated PKU patients with a severe phenotype, *Enu2* and *Enu3* mice have pronounced hypopigmentation. In addition to enabling the investigation of pathogenic mechanisms behind HPA and PKU, these models have been valuable for testing novel therapeutic approaches. *Enu1*[22] and the compound heterozygous *Enu1/2*[19,23] have revealed the mechanisms involved in BH_4-responsive PKU, and the effect of the treatment in liver and brain. *Enu2* has been used to test therapies at the preclinical stage, such as neutral amino acid supplementation,[24] different approaches for gene therapy including gene addition, genome base editing and mRNA therapy (for an overview see[25]), and increased L-Phe elimination by phenylalanine ammonia lyase (pegvaliase), both when injected intraperitoneally[26,27] and in engineered *Escherichia coli* Nissle.[28]

A limitation of the *Enu2* mouse is the stable hepatic expression of non-functional PAH protein that is commonly recognized by any anti-PAH antibody and its pseudo-dominant behavior regarding tetramer formation. A *Pah*-exon 1 deletion (ΔE1) murine model has been developed subsequently that replicates human classical PKU and lacks detectable liver PAH.[29]

More recently, transgenic *Pah-knock-in* (*Pah-ki*) models have been generated such as *Pah-R261Q*,[30] harboring a frequent PKU variant in humans[14] (www.biopkuorg). This mouse has the expected BH_4-responsive mild HPA, but also unanticipated traits such as altered lipid metabolism, reduction of liver BH_4 content, and a metabolic profile indicative of oxidative stress.[30] Furthermore, the characterization revealed novel pathogenic mechanisms of the p.R261Q-PAH protein variant, such as a reduced stability, misfolding, and aggregation, explaining the large ubiquitin-positive, amyloid-like oligomeric aggregates of PAH variants in the liver, where they appear to be processed by autophagy. Instability and tendency to aggregate have also been reported for mutant p.V106A-PAH, in the *Enu1* mouse,[31] but in this case the aggregates are smaller and most probably processed by the ubiquitin-dependent proteasome system.[30] An additional knock-in mouse model with the variant *Pah*-R408W has been generated by two research groups[32,33] (note that also a porcine model of PKU with a humanized p.R408W variant is available[34]). The p.R408W-PAH variant is very frequent in PKU patients, associated with classical, non-BH_4-responsive phenotype, predicted to be caused by a highly unstable and misfolded PAH (www.biopku.org). As expected, these mice have decreased liver PAH and high blood L-Phe levels (≥1500 μM). The *Pah*-R408W mouse has been used to demonstrate the L-Phe-reducing effect of a stabilizing long noncoding-RNA[32] and the benefit of genome editing through adeno-associated viral (AAV) vectors in PKU gene therapy.[33]

Summing up, the existing mouse models of PAH deficiency have been very useful, as they strongly replicate the human phenotypes (Table 1). The earlier *Enu*-type mouse models have largely contributed to explain the relation between the wide spectrum of blood L-Phe values with the classification of the phenotypes, ranging from mild HPA to classical, severe PKU, and their association to other relevant metabolites, notably neurotransmitter levels in brain. Furthermore, the *Enu* models have also been fundamental in in vivo proof-of-concept and preclinical studies of novel therapies, such as *Enu1* and *Enu1/2* for BH_4 (sapropterin)-responsiveness, and *Enu2* for pegvaliase. However, the more recent *Pah-ki* models, which represent patients not only by the remaining L-Phe value, but also by the pathogenic mechanism of their specific variant appear superior, notably for the development of patient-tailored therapeutics.

3 | TYROSINE HYDROXYLASE DEFICIENCY

Dopamine is a neurotransmitter that modulates motor activity, pain, motivation, reward, and cognition through action via dopamine receptors. The rate-limiting step in dopamine synthesis is catalyzed by tyrosine hydroxylase (TH), which is like PAH a member of the BH_4-dependent aromatic amino acid hydroxylases. TH hydroxylates L-Tyr to L-Dopa,[35] and aromatic L-amino acid decarboxylase (AADC) converts L-Dopa to dopamine, which is then taken up by vesicular monoamine transporter 2 (VMAT2; SLC18A family) into vesicles that are transported to presynaptic terminals for neurotransmitter release.[36,37] Dopamine is the precursor of the catecholamine neurotransmitters and neurohormones noradrenaline and adrenaline. Dopamine homeostasis is highly controlled to avoid accumulation of toxic dopamine metabolites, particularly in neurons.[38,39] AADC is not normally rate-limiting whereas TH catalyzes the committed step and is therefore very regulated, notably by feedback inhibition by dopamine.[40] In the brain TH is

expressed at high levels in dopaminergic neurons of the substantia nigra and is also expressed in the ventral tegmental area that projects to the ventral striatum.

TH deficiency is a rare neurometabolic genetic disorder caused by variants in the *TH* gene. This leads to significant decrease in striatal dopamine, with phenotypes ranging from dopa responsive dystonia (DRD) and infantile parkinsonism to progressive and severe infantile encephalopathy, which is often less responsive to L-Dopa.[5,41,42] Unlike Parkinson's disease, degeneration of dopaminergic neurons in the midbrain is not observed in THD. Reliable genotype–phenotype correlations have yet to be identified, but recombinant expression of THD mutants indicates that most variants cause TH instability and misfolding, resulting in reduced levels of TH and dopamine.[43,44] The most frequent THD variant in Europe is *TH*-R233H,[42] equivalent to p.R202H in the numeration for the most abundant splicing isoform TH1. This *TH* variant is mostly associated with DRD when in homozygosity, although 20%–30% of these patients present the most severe, non-Dopa responsive form.[42,45]

Various transgenic mice with TH deficiency have been described, initially independently by two laboratories, by complete knock-outs (*Th-ko*) which presented mid-gestational or perinatal lethality.[46,47] Rescue mutants did not fully reproduce the clinical and pathological features of TH deficiency either but they were fundamental to demonstrate the crucial role of dopamine for movement and feeding. A constitutive *Th-knock-in* (*Th-ki*) mouse homozygous for the variant *Th*-R203H, equivalent to human TH-p.R202H, was therefore generated in order to provide more insights into catecholamine function and pathophysiological mechanisms involved in TH deficiency.[44] The *Th-ki* mouse shows hypotension, hypokinesia, and motor dysfunction due to progressively reduced level of TH protein and activity, notably in the striatum, and gradual loss of brain dopamine and catecholamine metabolites without affecting the serotonin system.[44] Unlike most homozygous TH deficient patients with this variant, the motor dysfunction of the *Th-ki* mouse was not corrected by standard L-Dopa treatment (at least under the conditions tested). Detailed histological studies revealed lack of neurodegeneration in the dopaminergic projections and a greater decrease of TH in the striatum than in the substantia nigra, indicating a variant-associated mis-localization that might be related to the conformational instability of the mutant TH protein. Independently, a homozygous knock-in mouse with the less common human variant TH-p.Q381K has been generated.[48] These mice recapitulate the phenotype of DRD, displaying the core features of the human disorder, including reduced TH activity, dystonia that worsened throughout the course of the active phase, and improvement in response to both L-Dopa and trihexyphenidyl. The mice exhibited age-related reduction in movement although there was no evidence for degeneration of (midbrain) dopamine neurons. Histological assays and treatment experiments supported the hypothesis that the development of dystonia may depend on a reduction in dopamine in combination with specific abnormal dopamine receptor responses.[49] A broader discussion on the utility of this mouse model has been presented by Rose and Hess.[50]

To summarize, whereas the earlier non-viable *Th-ko* mice do not serve as models of THD they have clearly demonstrated the crucial, vital function of TH and dopamine. On the other hand the two mice models harboring THD-associated variants nicely reproduce the human phenotypes observed in patients (Table 1), both the milder L-Dopa responsive (*Th*-Q381K mice) and the more severe non-L-Dopa-responsive (TH-p.R202H) THD. These models appear very useful for the development of both biomarkers and mechanistic, disease-modifying therapies, alternative to L-Dopa.

4 | TH—PITX3 DEFICIENCY

PITX3, pituitary homeobox 3, is a transcription factor involved in *TH*-transcription. It is expressed in the developing lens, skeletal muscle, dopaminergic neurons of the substantia nigra, and in the ventral tegmental area that projects to the ventral striatum.[51] In humans, variants in *PITX3* are associated with anterior segment mesenchymal dysgenesis and congenital cataract.[52] However, a *PITX3* homozygous C-terminal deletion has been identified in two affected brothers from a highly consanguineous kindred who presented microphthalmia and a neurodevelopmental disorder associated with increased muscle tone and a choreiform movement disorder.[53] A larger deletion at chromosome 10q24.32, encompassing *PITX3*, was identified in an adolescent male with mild learning difficulties, hyperactivity, behavioral problems, and sleep disturbance. He had a high forehead, open mouth, synophrys, short broad nose, and hypoplastic middle phalanges of fifth digits. His CSF levels of homovanillic acid (HVA), 5-hydroxy acetic acid (5-HIAA) and biopterin were low, and levodopa was absent. Treatment with levodopa improved behavior, attention, and sleep.[54]

Murine *Pitx3−/−* (*Pitx3-ko*) resulted from spontaneous gene deletion. The mice exhibit loss of dopaminergic neurons in the substantia nigra and ventral tegmental area, leading to markedly reduced dopamine levels in the nigrostriatal pathway and dorsal striatum.[55] They exhibit aberrant striatum-dependent cognition including rotarod learning, T-maze and inhibitory avoidance tasks and show nigrostriatal pathway sensorimotor deficits in climbing and lower motor behavior.[56] Treatment of the

knock-out mice with levodopa, or dopamine agonists normalized sensorimotor function.[51,55] The *Pitx3-ko* mouse model thus appears to recapitulate some human disease characteristics and also responds to treatment.

Further studies have explored co-morbid depressive disorders associated with Parkinson's disease in the *Pitx3-ko* mouse model revealing anhedonia in a sucrose preference test that was reversed by antidepressant treatment.[57] To assess response to stress, c-Fos levels were measured in wildtype and *Pitx3-ko* mice following restraint test. While both groups showed increased c-Fos in various brain regions, increases in the *Pitx3-ko* compared to wildtype in the prefrontal cortex, dorsal striatum and paraventricular nucleus of the hypothalamus (PVN) regions were significantly greater but was reduced in nucleus accumbens. These studies further affirm the role of dopamine in neuropsychiatric disorders and potential clinical features of individuals identified with PITX3 deletions.

A *Pitx3-Cre* model was generated to gain more insight into its role in the programming of midbrain dopaminergic neurons.[58] The *Pitx3-Cre* were crossed with Lox-P-stop-Lox-eYFP mice in order to delineate the expression of *Pitx3* during development. Expression was observed in the lens, cortex, and hindbrain and predominantly in the mesodiencephalon.[58] In addition it was shown that *Pitx3* is expressed at the start of midbrain dopaminergic neuron terminal differentiation in mice at E13.5. There was transient expression in medial non-dopaminergic neurons in the mesodiencephalon which are not part of serotonergic system. Pitx3 has also been reported to be expressed in embryonic striated muscle through an alternative exon1 and this was reaffirmed in this model in E13.5 and E14.5 embryos.[58]

A further conditional knock-out was developed to understand the role of *Pitx3* in maintenance of mature midbrain dopaminergic neurons by generating a *Pitx3*-fl/fl/DATCreERT2 (*Pitx3-Flox*) that knocks-out *Pitx3* in cells expressing the dopamine transporter (DAT) with Tamoxifen treatment.[59] Following *Pitx3-Flox* induced knock-out at 4 months, midbrain dopaminergic neuronal loss in the substantia nigra was observed at 11 months with progressive decline to 15 months, with lesser effects in the ventral tegmental area. There was reduction in TH striatal neurons alongside reduced dopamine observed from 6 months onwards. Motor effects were observed at 11 months on open field and rotarod tests with progressive α-synuclein accumulation, microglyosis, and astrocytosis in midbrain at 15 months. This model supports that the loss of PITX3 transcription factor results in progressive neurodegeneration of midbrain dopaminergic neurons recapitulating features of Parkinson's disease.[59]

In summary, three mouse models exist for the study of PITX3 transcription factor. The deletion model demonstrates its role in eye defects and impaired dopamine synthesis with associated motor and motivation deficits present in patients recapitulated. The two inducible models highlight the wide-ranging role of PITX3 in neurodevelopment and dopamine neuronal survival. These models may indicate potential progressive neurodegenerative aspects in human disease.

5 | AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) DEFICIENCY

AADC deficiency is an autosomal recessive neurodevelopmental disorder characterized by impaired monoamine synthesis of dopamine, noradrenaline, adrenaline, and serotonin. It is caused by biallelic loss of function variants in the *DDC* gene and is estimated to affect close to 350 patients worldwide.[60] AADC deficiency patients have absent or non-functional DDC enzyme and cannot convert L-Dopa to dopamine or 5-hydroxytryptophan to serotonin. Clinical manifestations include hypotonia, dystonia, hypokinesia and parkinsonism, oculogyric crises, and autonomic dysfunction. Knock-out of the mouse *Ddc* gene results in death in utero.[61] There is a common splice-site mutation observed in the Taiwanese population (IVS6 + 4A > T)[62] that results in severe disease. A knock-in model, *Ddc-ki*, was generated using a vector containing a variant at the donor site of intron 6 and a *Neo* gene cassette flanked by a *loxP* site.[63] Only homozygous *Ddc-ki* (IVS6/IVS6) mice with a *Neo* selection cassette display a phenotype.

Half of the homozygous *Ddc-ki* mice (IVS6/IVS6) are born alive and pups show poor suckling and from 10 days are significantly smaller with 67.7% survival. Infant *Ddc-ki* mice display delayed eye-opening and ptosis with motor deficits.[63] The pups are hypoactive and dyskinetic, with resting tremor, hindlimb clasping and fall earlier from the rotarod aged 2 weeks. Those that survive post-weaning show normal longevity and growth and motor performance improves. Adult *Ddc-ki* mice show both lower systolic blood pressure and heart rates reflecting autonomic dysfunction. Biochemical analysis shows the mice recapitulate patient CSF neurotransmitter profiles with lower 3,4-Dihydroxyphenylacetic acid (DOPAC), dopamine, HVA, norepinephrine, serotonin, and 5-HIAA. The AADC protein is absent in the substantia nigra on western blot and immunohistochemistry, with normal TH staining in the substantia nigra.[63] This model has been used to validate proof of concept viral gene therapy approaches delivered by neonatal intracerebroventricular delivery using adeno-associated viral vector serotype 9. This non-targeted approach resulted in therapeutic efficacy with higher body weights, increased survival (90%) and supraphysiological levels of AADC.[64]

However, the animals were hyperactive and this was attributed to broad intracranial expression with mixed neuronal and astrocytic expression. A preclinical study evaluating intraperitoneal delivery into 7-day-old *Ddc-ki* mice of a neuronal targeted vector using a AAV9/3 capsid with a human synapsin promoter showed improved body weight and survival with an increase in brain dopamine and serotonin.[65] The hyperactive behavior was not observed in treated mice. However, while this new vector was designed to restrict expression to neurons, systemic delivery resulted in off-target liver expression, although no hepatoxicity or immune response was observed. These studies provide important proof-of-concept that wider brain expression of AADC increases dopamine and serotonin levels. Serotonin levels remain low in targeted delivery of eladocagene exuparvovec (Upstaza) to the putamen[66] and AAV2-hAADC clinical gene therapy to the midbrain.[67]

Another knock-in mouse model was generated harboring a milder disease due to the missense variant p.S250F (*Ddc*-S250F).[68] This model, when compared to that of wildtype mice, shows modestly reduced brain dopamine while serotonin was markedly reduced, with 4% residual enzyme activity. Survival is severely reduced with only 8% homozygotes born alive and a subset of non-viable homozygotes were poorly developed and dysmorphic at E15.5. The homozygotes that survived to adult mice showed increased body weight compared to wildtype from 11 weeks onwards. The dopaminergic systems did not show significantly abnormal histological features but there was significant motor impairment on the rotarod at 11 and 12 weeks, and autonomic dysfunction with impaired thermoregulation.[68]

In summary, all of the AADC models recapitulate some features of the human disease biochemically but severity of motor dysfunction as observed in the patients is not fully represented in the mouse models. The models reveal a mixed phenotype with reduced survival at fetal and post-natal stages in some. Further studies on the impact of absent dopamine and serotonin during fetal neurodevelopment could be another avenue of study alongside human iPSC derived organoids. The models have been used to evaluate AAV gene therapy approaches but further therapeutic strategies to address serotonin deficiency and cognitive profile may be future translational areas these models may be utilized to address.

6 | DOPAMINE B-HYDROXYLASE DEFICIENCY

Dopamine is converted to norepinephrine by dopamine β-hydroxylase (DBH). DBH deficiency is an ultrarare autosomal recessive condition that is caused by homozygous or compound heterozygous variants in *DBH*, affecting autonomic function. The condition presents in childhood, typically with ptosis, hypotension, and fatigability and is very rare with fewer than 25 patients diagnosed.[69] By early adulthood, symptoms include profound orthostatic hypotension, reduced exercise tolerance, ptosis, nasal congestion, and pre-syncopal symptoms (dizziness, blurred vision, dyspnoea, nuchal discomfort, and chest pain).[70] Biochemical hallmarks include minimal or absent levels of norepinephrine and epinephrine in the plasma, and 5- to 10-fold elevated plasma dopamine.[71] A *Dbh* knock-out mouse model was generated but only 5% homozygous *Dbh-ko* survived into adulthood. The high mortality resulted from abnormal cardiac development and established an essential role for norepinephrine in fetal development.[72] Administration of L-dihydroxyphenylserine (L-DOPS), a norepinephrine pro-drug that bypasses DBH and is converted by DDC directly to norepinephrine, improved mouse embryo viability and survival.[73] This is the drug used in the clinical treatment for DBH deficiency as it alleviates orthostatic hypotension and other symptoms.[74] Affected individuals do not respond as well to standard therapeutic approaches for autonomic failure and surgery is used to correct ptosis.

Overall the study of the mouse model has helped delineate the key role of DBH in norepinephrine synthesis and a role of norepinephrine and epinephrine in cardiac development. The study of this mouse model has been key in development of drug treatment for this ultrarare disorder.

7 | MONOAMINE OXIDASE A AND B DEFICIENCIES

Monoamine oxidase (MAO) A and B catalyze the oxidative deamination of dopamine to HVA and serotonin to 5-HIAA. MAOA deficiency was first identified in a family of 14 affected males and was referred to as Brunner Syndrome.[75] The clinical characteristics include episodic impulsive aggression with mild cognitive impairment, stereotyped hand movements and parasomnias. Levels of HVA and 5-HIAA in CSF are often reduced.[76] Since then the clinical phenotype has broadened to include autism spectrum disorder symptoms encompassing repetitive behavior, stereotypies, and social conduct disorder. In contrast, individuals with MAOB deficiency are asymptomatic. The *NBD* gene, deletion of which causes Norrie disease, is adjacent to *MAOA* and *MAOB*, and reports of *dual MAO* deletion often include *NBD* deletion.[77] Patients with combined *NBD* and *MAO* deletion often exhibit features of Norrie disease, such as retinal

dysplasia and congenital visual impairment. Boys with a deletion encompassing *MAOA* and *MAOB* showed severe developmental delay, intellectual disability, seizures, and stereotypies. They develop worsening episodic hypotonia that resembled seizures and mild facial dysmorphism, similar to Rett Syndrome patients.[78–80]

The MAOA knock-out mouse (*MaoA-ko*) has been extensively evaluated and shows overt increase in aggression toward familiar and unfamiliar social counterparts, and reduced anxiety with maladaptive response to stress.[81] The mice also show perseverative behaviors on marble burying and T-maze alluding to social communication and cognitive impairment (for more details see[82]).

A spontaneous combined deletion was observed in a colony of *MaoB-ko* mice.[83] The *MaoA/B-ko* mice show increased brain levels of 5-hydroxytryptophan (850%), norepinephrine (220%), and dopamine (170%). *MaoA/B-ko* mice show significantly reduced body weight, elevated intermale aggression, over-generalized fear conditioning, socio-communicative deficits, and perseverative behaviors.[83] There is loss of Purkinje cells in the cerebellum and reduced thickness of the rostral corpus callosum[82] and these effects may be due to high levels of serotonin, particularly in early development.[84] The role of early-life serotonin in *MaoA/B-ko* mice is indirectly confirmed by other findings on the abnormal neurogenesis in these mice.[84] There have been only a few clinical reports regarding a combined deletion of MAOA/B and no report on brain imaging findings to affirm this in patients or its significance in the mouse model.

Disorders in oxidative deamination dopamine and serotonin are reported in clinical spectrum of intellectual impairment, social communication disorders and associated perseverative and stereotypy behaviors. With increasing genomic diagnosis being made more individuals with MAOA/B defects will be identified hence further need to use these mouse models to understand disease mechanism(s) and to develop therapeutic approaches are required. These models recapitulate the neurocognitive and neuropsychiatric features with both cerebellar and neurotransmitter abnormalities to support such study.

8 | DNAJC6 CO-CHAPERONE DEFICIENCY

DNAJC6 encodes for a neural specific Hsc70 co-chaperone called *auxilin* that is required for clathrin mediated endocytosis for recycling of synaptic vesicles.[85] Neurotransmission requires rapid and continuous recycling of synaptic vesicles through clathrin mediated endocytosis, and with auxilin deficiency there is failure to replenish synaptic vesicles to maintain normal neurotransmission. Loss-of-function variants are associated with juvenile and early onset parkinsonism (Park 19).[86] Following fusion, clathrin assembles into a lattice on the presynaptic plasma membrane and deforms the plasma membrane into a clathrin coated vesicle that is severed from the membrane. After internalization auxilin binds clathrin and Heat shock protein 70 (Hsc70) and, through a J domain-dependent process, un-coats clathrin to replenish the synaptic vesicles. The clathrin binding motif and J domain are located at the C terminus.[85] Patients with childhood-onset DNAJC6 deficiency show progressive parkinsonism in the first decade with neurodevelopmental delay, seizures and significant neurological regression thereafter leading to loss of ambulation in mid-adolescence. These children do not respond to currently available treatments. Early onset patients harbor splice-site variants with large multi-exonic deletions, truncating variants and nonsense variants that would be most closely modeled with a deletion model.[87]

The auxilin (or *Dnajc6*) knock-out mouse (*Aux-ko*) has a high rate of postnatal mortality and surviving pups show impaired neuronal clathrin mediated endocytosis.[88] Surviving *Aux-ko* mutants display motor deficits with progressive diminishment of locomotion from 9 to 15 months. There is progressive deterioration in balance beam performance from 6 months and 40% loss of dopaminergic neurons and gliosis from 9 months old.[89] Aging *Aux-ko* mice demonstrated that impaired clathrin mediated endocytosis resulted in toxic accumulation of dopamine due to imbalance of clathrin coated vesicles and synaptic vesicles and mis-trafficking of DAT leading to impaired dopamine homeostasis and recycling. Moreover, the *Aux-ko* animals respond to L-Dopa treatment,[89] while juvenile parkinsonism patients do not respond to L-Dopa or other pharmacological treatments.

The later onset DNAJC6 phenotype presenting in adults as early onset PD, has been associated with biallelic missense variants. Adults patients may respond clinically to medical therapies and deep brain stimulation.[86] A missense knock-in model representing the human p.R927G has been generated with the equivalent p.R857G missense variant in mice (*Aux-ki*). Homozygous *Aux-ki* mice showed reduced Mendelian ratio. Surviving homozygotes were found to develop neurological phenotypes as early as 6 months old including motor impairment, seizures, impaired memory and anxiety. An increased time to turn and to descend was observed in *Aux-ki* mice compared to wildtype at 12 months suggesting decreased agility and bradykinesia. Electron microscopy revealed increased synaptic vesicles with swollen Golgi and accumulation of lipids in dopaminergic neurons was also observed.[90]

DNAJC6 co-chaperone deficiency causes a spectrum of early childhood and juvenile presentations to adult early onset PD that is recapitulated by a more severe *Aux-ko* mouse model and *Aux-ki* that shows parkinsonian features with aging. The models show the pathological consequences of loss of function of auxilin with impaired synaptic recycling revealing mechanisms toward dopaminergic neuronal loss. These models have helped understand auxilin function and may be used to identify therapeutic targets for Park 19 or broader PD mechanisms.

9 | TETRAHYDROBIOPTERIN (BH_4) DEFICIENCIES

BH_4-deficiencies includes a group of individual disorders with abnormalities of BH_4-cofactor biosynthesis or regeneration that cause low levels of brain monoamine neurotransmitters. The corresponding genes (for metabolic enzymes) include GTP cyclohydrolase I (*GCH1*), 6-pyruvoyltetrahydropterin synthase (*PTS*), sepiapterin reductase (*SPR*), and dihydropteridine reductase (*QDPR*; see Table 1). With the exception of *GCH1* where autosomal dominant (ad) and autosomal recessive (ar) variants lead to distinct phenotypes in man, all other gene variants are recessively inherited.[3,91] There are several mouse models of BH_4 deficiency that have been developed to study primary and secondary monoamine neurotransmitter disorders. An overview of available murine models for these defects was presented in a review article in 2011.[92] To our knowledge, while no additional studies on mouse models for *PTS*, *SPR*, and *QDPR* have been published since then, there has been a few more recent studies on the *hph-1* mouse and a novel *GCH1* knock-in mouse expanding the number of models available for GCH1 deficiency. *Gch1-ko* mice are embryonically lethal and are thus considered not to be viable.[93] The *hph-1* mouse, developed by chemical mutagenesis and with a hitherto not defined variant within the mouse *Gch1* gene,[94] was proposed to be a model for human DRD, as it exhibits mild neonatal (and transient) HPA due to low GTPCH activity (approx. 10% of normal), reduced BH_4 in the liver, and low levels of brain monoamine neurotransmitter metabolites, including dopamine and serotonin.[95,96] Nevertheless, *hph-1* mice do neither show signs of motor impairment nor dystonia-like symptoms while behavioral studies identified increased anxiety-like and depression-like reactions, and pain-like hypersensitivity. A so-called "refinement model" for DRD, the "DPS" mouse, was generated by introducing a *Pts-ko* allele into the *hph-1* background (see below) with expression under the control of the dopamine β-hydroxylase promoter. This mutant mouse exhibits severely reduced striatal BH_4 and TH.[97,98] The *Gch1-ki* mouse, containing a variant (p.L117R) that was identified in a human subject, was described as the first "viable" arGTPCH1 deficiency model as it exhibits reduced BH_4 and elevated L-Phe (in liver and brain), concomitant with progressive brain monoamine neurotransmitter metabolites reduction and early death (if untreated).[99]

Mouse models for PTPS, SR, and DHPR deficiencies were generated by complete knock-outs (*Pts-ko*, *Spr-ko*, and *Dpr-ko*) and, in the case of PTPS deficiency, *Pts-ki* (p.R15C) and *Pts-ki/ko* compound heterozygous animals.[100-104] *Pts-ko* mice exhibit perinatal lethality and can only be partially rescued to become dwarf mice (due to low IGF-1) by replacement therapy, that is, oral administration of BH_4 in combination with neurotransmitter precursors L-Dopa/carbidopa and 5-hydroxytryptophan. A much milder form of PTPS deficiency with no HPA was generated by the *Pts*-p.R15C knock-in mouse, equivalent to the mild human variant p.R16C. Finally, compound heterozygous *Pts-ko/ki* mice showed reduced BH_4 and PTPS activity in liver and brain, elevated neopterin and monoamine neurotransmitter metabolite deficiency in brain, and mild HPA, and thus represent a model for human PTPS deficiency. Homozygous *Spr-ko* mice exhibited high HPA/PKU which is not the case in human patients, thus not representing a model for SR deficiency. A *Qdpr-ko* mouse was viable with HPA and low brain monoamine neurotransmitter metabolites, as expected from human DHPR deficiency.

In summary, the various mouse models for the four genes responsible for BH_4 deficiency are very heterogenous regarding replication of human phenotypes (see Table 1). While some mouse mutants are not viable or die at an early stage (*Gch1-ko*, *Gch1-ki*, *Pts-ko*), others replicate the human phenotype poorly and/or have an unexpected phenotype (*hph-1*, *Spr-ko*). Going forward, the *Pts-ko/ki* mouse will be very useful for further studies, while the DPS and the *Gch1-k* mutants may be of use in helping to define this disorder further.

10 | VITAMIN B_6 DEFICIENCY

Humans are not capable of synthesizing vitamin B_6 and must therefore obtain it from their diet. The only active form of vitamin B_6, pyridoxal 5′-phosphate (PLP), is an essential cofactor required by more than 70 enzymes and is produced by conversion of dietary B_6 forms or those produced by the gut microbiota.[105] Many biological pathways are reliant on PLP including those involved in neurotransmitter and monoamine metabolism, e.g. for the activity of AADC and therefore synthesis of dopamine

and serotonin. Because of the vital role that PLP plays in neurotransmitter metabolism, it is not surprising that inborn errors leading to PLP deficiency, typically present clinically as a B_6-responsive epilepsy, usually of early onset.

Several inherited vitamin B_6 disorders that result in PLP deficiency have been genetically defined and characterized. This includes pyridox(am)ine phosphate oxidase (PNPO; affects PLP synthesis and recycling), disorders affecting PLP import into the brain (hypophosphatasia and glycosylphosphatidylinositol anchor synthesis defects), a disorder of an intracellular PLP-binding protein (PLPBP deficiency, previously called PROSC or PLPHP deficiency) and disorders where metabolites accumulate that inactivate PLP i.e., aldehyde dehydrogenase 7A1 (ALDH7A1) deficiency and hyperprolinaemia type II.[105] The profile of neurotransmitters for some affected individuals with these disorders can be similar to that of patients with DDC deficiency.[106–108] This includes elevated CSF 3-methoxytyrosine and urinary vanillactic acid, and decreased CSF homovanillic (HVA) and 5-hydroxyindoleacetic acid. This can however be transient and may normalize on treatment with PLP or pyridoxine (another form of vitamin B_6). More recently a novel vitamin B_6 disorder has been identified, pyridoxal kinase deficiency; however, unlike the other B_6 disorders patients do not show epilepsy but instead are affected by a polyneuropathy which is responsive to treatment with PLP.[109] One of the patients reported with PLPBP deficiency has a prominent movement disorder without clear epilepsy and had a clinical and biochemical diagnosis compatible with DDC deficiency.[107]

While the metabolism of vitamin B_6 has been investigated extensively in bacteria, fungi and plants,[110,111] these are all organisms capable of de novo synthesis of PLP and therefore differ in many aspects to humans with regard to B_6 metabolism. Animal models have been used to study the inherited vitamin B_6 disorders, however ALDH7A1-deficiency is the only disorder to date that has been looked at in a murine model.[112] *Aldh7a1* knock-out mice do not have a seizure phenotype when fed on standard chow, that is, low lysine/high pyridoxine, however when challenged on a high lysine/low pyridoxine diet they have vigorous seizures and die. Seizures respond to treatment with pyridoxine and the survival of the mice improves. Biochemical analysis of these mice while fed a standard chow i.e., no seizure activity, shows evidence of a widely deranged amino acid profile. Perhaps unsurprisingly few accompanying changes are evident in the neurotransmitter profile of both brain and plasma with only significant differences in the levels of the catecholamines norepinephrine (noradrenaline) and normetanephrine observed, being present at higher concentrations in plasma. Given that the seizure phenotype of *Aldh7a1-ko* mice can be induced by using a high lysine/low pyridoxine diet prior to analysis, and that under these conditions, the *ko* mice have low PLP levels relative to wildtype mice it is likely that effects on neurotransmitter and monoamine metabolism will also occur. Indeed, mice with significantly reduced levels of PLP due to low levels of pyridoxal kinase (*Pdxk*) transcript caused by a combined deficiency of the three transcriptional regulatory proteins albumin D-site-binding protein, hepatic leukemia factor and thyrotroph embryonic factor have reduced levels of serotonin, dopamine and histamine in their brains. They are affected by lethal audiogenic and spontaneous epileptic seizures.[113]

In summary, while murine models for the vitamin B_6-dependent disorders are limited currently it is likely that the *ALDH7A1* knockout mouse, when challenged through dietary manipulation, will be a useful model to study monoamine neurotransmitter homeostasis. Development of murine models for PNPO- and PLPHP-deficiencies, disorders which have only been characterized in a variety of other models including zebrafish[114] and fruit fly[115,116] to date, will also help in these efforts.

11 | VESICULAR MONOAMINE TRANSPORTER (VMAT) FORMATION AND/OR PACKAGING DEFECTS

11.1 | VMAT1

The vesicular monoamine transporter 1 (VMAT1) or solute carrier family 18, member 1 (SLC18A1, *SLC18A1* gene) is a H^+-coupled transporter that packages cytosolic monoamines (serotonin, dopamine, norepinephrine, and histamine) into vesicles acting as a secondary active antiporter. The two VMAT isoforms, VMAT1 and VMAT2 (*SLC18A2*; see below), share 63.5% sequence identity and have 12 transmembrane α-helices that arrange in alternating-conformations, that is, cytoplasm-facing, occluded, and lumen-facing, allowing the transport of the monoamines into the vesicles.[37,117,118] While VMAT2 is mostly expressed in neurons of the CNS, VMAT1 is mainly expressed in neuroendocrine cells in the sympathetic- and peripheral nervous system but also to some extent in the CNS. Nevertheless, the function of both VMAT isoforms, VMAT1 and VMAT2, is essential for the correct activity of the monoaminergic systems.[37,119,120] Thus, the functional importance of VMAT1 in the CNS has become increasingly evident.[121–125]

Studies comparing wildtype mice with those lacking VMAT1 (*Vmat1-ko*) have helped to establish the

expression of VMAT1 in the brain, the role of VMAT1 in dopaminergic function and its relevance in neuropsychiatric disorders. Multani et al. characterized a *Vmat1-ko* mouse, which survived into adulthood.[126] qPCR and immunohistochemistry of these mice and their comparison to wildtype, with focus on hippocampal processes, revealed brain expression of VMAT1 in the wildtype and showed that deletion of VMAT1 leads to increased apoptosis in the dentate gyrus and reduced neurogenesis in the hippocampus, hereby supporting a key role of VMAT1 in the survival of hippocampal neurons. Behavioral characterizations of the *Vmat1-ko* also revealed that these mice manifest neurocognitive defects, even though VMAT2 expression was not altered, pointing to the contribution of VMAT1 to the neurocognitive deficits observed in neuropsychiatric disorders. In particular the spatial object recognition behavioral task, which has been linked to hippocampal function in mice,[127] was disrupted in the *Vmat1-ko* mouse.[126] The *Vmat1-ko* mice were further characterized[121] focusing on markers of dopaminergic function and neurotransmission, as well as dopamine-related locomotor behaviors. Analysis of tissue monoamines, measured both ex vivo and in vivo by microdialysis, showed that the *Vmat1-ko* had decreased dopamine levels in the frontal cortex compared with wildtype mice, in addition to increased expression of postsynaptic dopamine D2 receptors and decreased expression of TH in the frontal cortex. *Vmat1-ko* mice also showed an amplified locomotor response to amphetamine administration.[121] Collectively, these results indicated that dopaminergic signaling is severely altered, particularly in the frontal cortex, and to a lesser extent in the striatum of *Vmat1-ko* mice.

Thus, *Vmat1-ko* mice appear useful not only to study effects of abnormal monoaminergic signaling on hippocampal function,[126] but also for studies on the pathogenesis and/or treatment of psychiatric illnesses, including schizophrenia and bipolar disease,[121] since these disorders appear associated with dysfunctional dopaminergic signaling in the cerebral cortex.[128,129] However, despite the proof of functional involvement of VMAT1 in the CNS,[122] and the claimed associations with psychiatric disorders,[123,125] genome-wide association studies (GWAS) on psychiatric illnesses such as depression[130] and schizophrenia[131] among others, have not detected VMAT1 as a candidate gene. With this status in mind Sato et al. prepared a humanized knock-in *Vmat1-ki* mice via CRISPR/Cas9-mediated genome editing of VMAT1 to provide additional evidence for the association of specific human variants in this transporter with psychiatric disorders.[132] This humanized mouse included a single human-specific *SLC18A1* variant (p.N136I) that is absent in all known mammals, including primates. Characterization of the behavioral, neurophysiological, and molecular changes in the *Vmat1-ki* compared with wildtype mice, revealed variations in gene expression, enhanced monoamine neurotransmitter uptake into synaptic vesicles, increased monoaminergic signaling and neuronal activity in the amygdala, and a reduction of anxiety-like behaviors. The findings further support the functional importance of human-specific variants in VMAT1 and their contribution to the evolution of human socio-emotional traits.

11.2 | VMAT2

Brain dopamine–serotonin vesicular transport disease is an infantile onset neurodevelopmental disorder caused by biallelic loss of function variants in *SLC18A2*, which codes for the vesicular monoamine transporter 2 (VMAT2).[133] Monoamine transport requires correct packaging into synaptic vesicles by VMAT2. A phenotypic spectrum is seen for this disorder with some of the 58 affected individuals described to date responding to treatment with dopamine agonists while 16 (28%) have died prior to adolescence.[133–137] The affected children show global developmental delay, hypotonia, dystonia, oculogyric crisis, and autonomic dysfunction.[137] CSF neurotransmitter analysis is usually normal.[133,137] Urine levels of HVA and 5-HIAA may be high, and levels of adrenaline and dopamine may be low. There is a variable response to treatment with 35% responding to L-dopa and 37% responding to dopamine agonists.[137]

The *Vmat2*-homozygous complete knock-out mouse (*Vmat2-ko*) is poorly viable postnatally. The newborn *Vmat2-ko* pups appear small and hypoactive and more prone to hypothermia.[138] Their growth was severely stunted, and the majority died within 1–3 days of birth with maximal survival to 12 days. Brain neurotransmitters show absent norepinephrine, dopamine, and serotonin whereas the metabolite levels remain approximately the same (DOPAC and HVA) or elevated (5-HIAA), suggesting an increase in the rate of degradation.[138] Heterozygous mice survive to adulthood with normal weight gain, fertility, and locomotor activities similar to wild-type littermates. In heterozygotes, amphetamine produces enhanced locomotion but diminished behavioral reward,10%–15% die suddenly between 2 and 4 months with prolonged Q-T but these do not relate directly to clinical VMAT2 deficiency.[139]

A transgenic mouse line expressing 5% of VMAT2, the *Vmat2*-LO mouse, survives into adulthood.[140] These mice have striatal dopamine levels reduced by 85% with reduced DOPAC and HVA, and also exhibit an age-dependent decline in dopamine at 24 months. Several compensatory mechanisms were observed including an

increase in TH activity, dopamine turnover, and an age-dependent decline in dopamine transporter (DAT) expression and activity. The chronic dopamine dysregulation contributed to neuronal degeneration in older animals with progressive loss of TH-positive neurons within the substantia nigra pars compacta. Behaviorally, *Vmat2*-LO mice exhibit many of the Parkinsonian motor phenotypes. Beginning at 2 months of age, they show reduced locomotor activity, which is L-DOPA responsive but significant motor behavioral deficits do not present until 24 months age, associated with neuronal loss.[140]

Overall, mice lacking VMAT1 (*Vmat1-ko*) have aided to establish the functional importance of VMAT1 in the CNS, supporting a key role of VMAT1 in the survival of hippocampal neurons, and its relevance in neuropsychiatric disorders. The VMAT2 models, on the other hand, do not seem to recapitulate the clinical disease, with the full knockout (*Vmat2-ko*) being so deleterious that pups are not viable to weaning with absent norepinephrine, dopamine and serotonin. Although the alternative 5% *Vmat2*-LO model has relevant biochemical and behavioral features, the age of onset of significant motor deficits and dopaminergic neuronal degeneration is at 24 months. This is incongruous to the childhood onset of disease observed clinically but potentially does provide a means to study consequences of VMAT2 deficiency and identify therapeutic targets.

12 | DOPAMINE TRANSPORTER DEFICIENCY SYNDROME

Dopamine transporter deficiency syndrome (DTDS) is caused by biallelic loss-of-function variants in the *SLC6A3* gene which encodes the dopamine transporter (DAT) that causes defective presynaptic uptake of dopamine resulting in accumulation of dopamine in the synaptic cleft.[141] This underlies the CSF neurotransmitter profile that is characteristic of DTDS related infantile parkinsonism dystonia showing high levels of HVA, normal levels of 5-HIAA and a raised HVA:5-HIAA ratio >5. In vitro modeling of disease-causing missense variants reveal defects in expression, glycosylation, and impaired dopamine uptake. The classical DTDS phenotype typically manifests during early infancy with irritability, feeding difficulties, hypotonia, and delayed motor development. A hyperkinetic movement disorder develops with features of chorea, dystonia, ballismus, and orolingual dyskinesia.[142] Over time, the condition evolves to parkinsonism-dystonia with the development of dystonic posturing, bradykinesia, distal tremor, rigidity, and hypomimia. The movement disorder progresses to akinesia in late childhood/early adolescence. Some children also experience episodic status dystonicus and eye movement disorders with oculogyric crisis, ocular flutter, eyelid myoclonus, and saccade initiation failure.[143] Atypical DTDS phenotypes have also been reported showing later onset disease with phenotype–genotype correlation related to residual DAT activity.[144,145]

The DAT knock-out mouse (*Dat-ko*) model recapitulates many features of the human disease as it shows an early locomotor disorder of hyperactivity and 33% reduced survival by 10 weeks of age.[146] These mice exhibit weight loss and progressive motor disorder including bradykinesia, gait abnormalities, hind limb clasping, scoliosis, and tremor recapitulating DTDS related infantile parkinsonism-dystonia.[147] The model also has raised brain HVA levels with normal 5-HIAA as observed in patients.[6] The *Dat-ko* mouse has been extensively characterized as a model for addiction and following the identification of the disease-causing gene in DTDS utilized to develop novel therapies such as viral gene therapy, which are progressing toward clinical application, for DTDS.[6]

Other disease causing heterozygous *SLC6A3* variants have been identified in association with neuropsychiatric disease such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and bipolar disorder (BD).[148] These heterozygous missense variants result in more discrete transporter functional changes such as aberrant dopamine efflux and knock-in models have been generated including p.A559V. This variant was identified in ADHD, ASD, and BD.[149,150] In vitro cell modeling showed that the variant *Dat*-p.A559V mediated leak of cytoplasmic dopamine.[150] Heterozygous *Dat-ki* (p.A559V) mice were equivalent to wildtype mice while homozygous mice do exhibit increased darting speed upon imminent handling, they do not recapitulate the associated neuropsychiatric disorders reported.[150,151] An ASD-associated de novo mutation *Dat*-p.T356M has also been modeled.[152] Heterozygous mice did not show any differences to wildtype. Homozygous *Dat-ki* (p.T356M) mice displayed impaired striatal dopamine neurotransmission and are hyperactive and exhibit repetitive behaviors with repetitive rearing behavior, reduced marble burying and loss of social dominance.[152] These findings support the role of dopamine neurotransmission in neuropsychiatric disorders of ADHD and ASD and further our understanding of the role of dopamine and DAT within the spectrum of parkinsonian and neuropsychiatric disorders.

In summary, the combination of *Dat-ko* and *Dat-ki* models have been key in understanding the role of dopamine homeostasis in neurological and neuropsychiatric disorders. The *Dat-ki* model has contributed significantly to the understanding of DTDS, development of novel

targeted gene therapy and the role of DAT in addiction. The knock-in models have been invaluable in confirming the role of *Dat* missense variants associated with ASD and provide models to further study pathological consequences and therapeutic targets in DAT related disorders.

13 | CONCLUSION

Murine models remain key for investigating inherited diseases and have contributed enormously to the advancement of the understanding of pathophysiology and disease mechanisms. In this review we have focused on existing murine models of monoamine neurotransmitter disorders. Nevertheless, in a few instances, we have mentioned animal models other than mice—such as flies, worms, or fish—for a specific defect where we find that such animal mutants mimic phenotypes found in human subjects and may thus be useful as a platform for treatment studies. Besides animal models, pluripotent stem cells (iPSCs) derived from patients have recently been shown to be appropriate disease models that may recapitulate deficiency phenotypes. Such studies include investigations of neurotoxicity of L-Phe on human iPSCs,[153] disorders for BH_4 cofactors deficiency,[154,155] TH deficiency and response to L-Dopa treatment,[156] pharmacochaperone treatment and gene therapy in DTDS,[6] and iPSCs derived from DNAJC12 patients[157,158] (for an overview see also[157]). Expanding the spectrum of variant-specific mice in the future, in combination with diverse animal and humanized cell models such as these, will help to ensure our understanding of these diseases continues to advance and will pave the way for development of better therapies for monoamine neurotransmitter disorders.

AUTHOR CONTRIBUTIONS

All authors have equally contributed to this review article.

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CONFLICT OF INTEREST STATEMENT

Joanne Ng is an inventor on patent application titled "Gene therapy for DTDS (GB2101958.3)," has consultancy agreements with Albion Venture Capital, sponsored research agreements with Synpromics/Askbio Europe, Rocket Pharma, Helex Bio and Bloomsbury Genetic Therapies and holds equity in Bloomsbury Genetic Therapies. Manju A. Kurian is an inventor on patent application titled "Gene therapy for DTDS (GB2101958.3)," was sponsored by Agilis to attend the AADC Deficiency International Advisory Board (2018), received honoraria for consultancy and speaking at PTC-sponsored symposia and is a co-founder of Bloomsbury Genetic Therapies. Aurora Martinez is co-founder and CSO of Pluvia Biotech and is inventor on patent: "Hyperphenylalaninemia and treatments thereof" (WO2017/029202A1). Beat Thöny and Philippa Mills have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available reference number.

INFORMED CONSENT AND ANIMAL RIGHTS

This article does not contain new studies with human or animal subjects performed by any of the authors.

COMPLIANCE WITH ETHICS GUIDELINES

This review does not contain new data and/or studies with human or animal subjects.

ORCID

B. Thöny https://orcid.org/0000-0002-5001-086X
A. Martinez https://orcid.org/0000-0003-1643-6506

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