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Spectrum of *DDC* variants causing aromatic L-amino acid decarboxylase (AADC) deficiency and pathogenicity interpretation using ACMG-AMP/ACGS recommendations

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ABSTRACT

Pathogenic variants in dopa decarboxylase (*DDC*), the gene encoding the aromatic L-amino acid decarboxylase (AADC) enzyme, lead to a severe deficiency of neurotransmitters, resulting in neurological, neuromuscular, and behavioral manifestations clinically characterized by developmental delays, oculogyric crises, dystonia, and severe neurologic dysfunction in infancy. Historically, therapy has been aimed at compensating for neurotransmitter abnormalities, but response to pharmacologic therapy varies, and in most cases, the therapy shows little or no benefit. A novel human *DDC* gene therapy was recently approved in the European Union that targets the underlying genetic cause of the disorder, providing a new treatment option for patients with AADC deficiency. However, the applicability of human *DDC* gene therapy depends on the ability of laboratories and clinicians to interpret the results of genetic testing accurately enough to diagnose the patient. An accurate interpretation of genetic variants depends in turn on expert-guided curation of locus-specific databases.

The purpose of this research was to identify previously uncharacterized *DDC* variants that are of pathologic significance in AADC deficiency as well as characterize and curate variants of unknown significance (VUSs) to further advance the diagnostic accuracy of genetic testing for this condition.

DDC variants were identified using existing databases and the literature. The pathogenicity of the variants was classified using modified American College of Medical Genetics and Genomics/Association for Molecular Pathology/Association for Clinical Genomic Science (ACMG-AMP/ACGS) criteria. To improve the current variant interpretation recommendations, *in silico* variant interpretation tools were combined with structural 3D modeling of protein variants and applied comparative analysis to predict the impact of the variant on protein function.

A total of 422 variants were identified (http://biopku.org/home/pnddb.asp). Variants were identified on nearly all introns and exons of the *DDC* gene, as well as the 3′ and 5′ untranslated regions. The largest percentage of the identified variants (48%) were classified as missense variants. The molecular effects of these missense variants were then predicted, and the pathogenicity of each was classified using a number of variant effect predictors. Using ACMG-AMP/ACGS criteria, 7% of variants were classified as pathogenic, 32% as likely pathogenic, 58% as VUSs of varying subclassifications, 1% as likely benign, and 1% as benign. For 101 out of 108 reported genotypes, at least one allele was classified as pathogenic or likely pathogenic.

In silico variant pathogenicity interpretation tools, combined with structural 3D modeling of variant proteins and applied comparative analysis, have improved the current *DDC* variant interpretation recommendations, particularly of VUSs.

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Table 1
ACMG-AMP/ACGS Criteria for Classifying Pathogenic Arguments as Applied to *DDC* Variants [20,21].

Pathogenicity Very Strong Criteria	
PVS1:*Null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where loss-of-function is a known mechanism of disease.*	This criterion can be used since AADC deficiency is caused by a loss-of-function mechanism. It should also be noted that PVS1 cannot be used together with PM4 or PP3. Decisions regarding PVS1 classifications were made according to a previously published decision tree [22]. We applied the regular PVS1 criteria to 34 variants. Within these, PVS1 was applied as a very strong argument for 28 nonsense or frameshift variants, which are predicted to undergo NMD in a biologically relevant transcript. Three variants were classified as PVS1_strong, as c.1233dup/p.Arg412SerfsTer10, c.1233del/p.Arg412GlyfsTer2, and c.1426C>T/p.Arg476Ter might not undergo NMD, instead they could lead to a truncated/altered region and present with a minimum loss of 10% of the protein, which is likely critical for protein function. Three variants were classified as PVS1_moderate, including the cryptic splice site c.1042-2A>G and 2 variants that affected the start codon.
Pathogenicity Strong criteria	
PS2 and PS4	Were not applied due to the lack of *de novo* aspects as AADC deficiency is inherited in an autosomal recessive manner, meaning both parents are obligate carriers for their single variants.
PS1:*PS1 is applied when a different substitution at the same nucleotide position leads to the same amino acid change.*	PS1 was applied only for the missense variant p.Gly140Arg, as c.418G>C and c.418G>A result in the same amino acid change.
PS3:*Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product* [39].	In cases of AADC deficiency, PS3 was used mainly as a moderate criterion for 21 variants since activity measurements in serum should only be considered in the homozygous state and *Escherichia coli* expression (with enhanced PLP binding and low K_{cat}/K_m) studies for the specific variants were performed <10 times. If only 1 of these 2 experiments confirmed the loss-of-function, PS3_supporting was applied (12 variants). Exceptions, where PS3 was used as a strong criterion, include the founder variant c.714+4A>T/IVS6+4A>T as well as c.995A<G/p.Tyr332Cys, in which a variety of repeated measurements approve the loss-of-function on molecular level.
Pathogenicity Moderate criteria	
PM1:*Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.*	We applied this hot spot (HS) criterion for accumulation of LP/P variants in a region with maximum 5AA cluster and/or in the case that an additional task is defined for protein function (e.g., PLP binding, dimerization domains). We defined 8 different HSs that contained 22 variants: 1. HS: Cys100-Trp105 2. HS: Gly146-Ser149 3. HS: c.714+4A>T /IVS6+4A>T as founder variant 4. HS: Ala275 5. HS: Cys281-Glu283 6. HS: Arg285-Leu288 7. HS: Phe309 8. HS: Tyr346-Arg347
PM2:*Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium* is *used as moderate argument if maximum.*	PM2 was the most used criteria for classifying the *DDC* variants, owing to the variants being absent or at an extremely low frequency from controls in the gnomAD database. We defined sharp borders for PM2 "low frequency" as a full moderate criterion with a maximum of 5 individuals, which is weakened to supporting criterion with a maximum of 10 probands detected in the control population. Based on these criteria, a total of 348 variants were classified as PM2, with 319 classified as moderate and 29 classified as supporting.
PM3: Used as moderate criteria *for recessive disorders, detected in trans (compound-heterozygous) with a pathogenic variant, or when 2 homozygous patients were described.*	Great care was taken to not push compound heterozygous variants mutually, as PM3 can only be used for one variant in an individual compound-heterozygous genotype, unless the second variant is found in an alternate case with another likely pathogenic/pathogenic variant. It should also be noted that PM3 can only be used in connection with PM2. Since many variants were found in a homozygous state, PM3 was used as supporting criteria. PM3 was strengthened to a strong criteria in variants found in at least two homozygous as well as in two compound heterozygous patients. Altogether, PM3 was applied as strong criteria for 5 variants, as moderate criteria for 52 variants, and as supporting criteria for 18 variants.
PM4:*Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants.*	PM4 was used as moderate criteria for 5 variants where an extension was predicted. PM4 was used as supporting criteria for p.Tyr20del, since a loss of 1 amino acid is <10% of the protein and might have a small impact.
PM5:*Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.*	PM5 was applied for 23 novel missense variants, as the missense changes p.Arg39Pro, p.Pro47His, p.Ile57Thr, p.Asp59Asn, p.Val60Ala, p.Gly102Ser, p.Ser147Arg, p. Ser149Thr, p.Arg160Trp, p.Asp189Tyr, p.Arg285Trp, p.Arg347Gln, p.Gly354Cys, p. Arg358His, p.Leu408Ile p.Arg412Trp p.Arg447His, p.Arg453Cys, and p.Arg462Pro have previously been identified as pathogenic.
PM6:*Assumed de novo, but without confirmation of paternity and maternity*	PM6 was not applied as AADC deficiency is inherited in an autosomal recessive manner, meaning both parents are confirmed carriers for their variants.
Pathogenicity Supporting criteria	
PP1 and PP2	These criteria were not applied as there was no case in which there was co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease (PP1). Additionally, decipher has no significant missense constraint ($P > .001$) which allows the use of PP2 criteria, defined as a missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease.
PP3:*Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc).*	PP3 criteria were applied as moderate if all 4 *in silico* tools used predicted an effect on protein function (deleterious, probably damaging, LP/P, CADD >20). PP3 was drawn as supporting criteria if a minimum of 2 tools (SIFT, PolyPhen2, CADD, PyMol) predicted a

	likely pathogenic or pathogenic effect. SIFT and Polyphen2 predictions must agree; if these did not agree, neither tool was noted. Based on these criteria, 109 variants were classified as PP3 moderate, and 67 variants were classified as PP3 supporting. There is an exception for the variants in the start codon. Only 3 *in silico* predictions were given for these variants (3/3), but since all predicted pathogenic effects we decided to apply PP3 moderate to these variants as well.
PP4:*Patient phenotype or family history is highly specific for a disease with a single genetic etiology*.	In the case of AADC deficiency, we strengthened PP4 criteria to moderate, since all measurable parameters confirmed these defects via elevated or reduced levels of the following in plasma, cerebrospinal fluid, or urine: 1) 5-OH Trp; 2) L-DOPA/3-OMD or VLA; 3) serotonin/5-HIAA; 4) dopamine/HVA/norepinephrine/MHPG/epinephrine/VMA. A minimum of 2 parameters must confirm AADC deficiency to apply PP4 as supporting criteria in general. PP4 moderate criteria were applied to 22 variants, and PP4 supporting criteria were applied to 15 variants.
Pathogenicity Benign criteria	
BA1:*Allele frequency is > 5% in gnomAD*	BA1 was used as strong criteria for 10 variants presenting with >2000 entries in gnomAD.
BS1: BS1 criteria were modified to *"more than 400 but fewer than 2000 probands observed in the healthy population."*	BS1 strong criteria were applied to 4 variants.
BS2:*Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age.*	BS2 criteria were applicable as a strong benign argument for 1 variant that was observed in s healthy adult individual in a homozygous state in gnomAD: c.436 −12T>C/IVS4 −12T>C (1,739× heterozygous, 8× homozygous).
BP4:*Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc).*	BP4 criteria were applied if all 4 *in silico* prediction tools displayed LB/B with a CADD score around 0. Based on this definition, 6 variants were classified as BP4 supporting.
BP7:*A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.*	We detected 64 variants that resulted in silent (synonymous) substitutions and classified these as BP7 supporting.

The following benign criteria were not applied since this is a retrospective study and all cases were confirmed AADC deficiency:
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease.
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.
BP3: In-frame deletions/insertions in a repetitive region without a known function.
BP5: Variant found in a case with an alternate molecular basis for disease.
BS3: Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.
BS4: Lack of segregation in affected members of a family.
Accession IDs: NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.
3-OMD, 3-*O*-methyldopa; 5-HIAA, 5-hydroxyindoleacetic acid; 5-OH, 5-hydroxy; AADC, aromatic L-amino acid decarboxylase; B, benign; CADD, combined annotation-dependent depletion; HS, hot spot; HVA, homovanillic acid; LB, likely benign; L-DOPA, L-3,4-dihydroxyphenylalanine; LP, likely pathogenic; MHPG, 3-methoxy-4-hydroxyphenylglycol; NMD, nonsense-mediated mRNA decay; P, pathogenic; PLP, pyridoxal 5′-phosphate; PolyPhen2, Polymorphism Phenotyping v2; SIFT, scale-invariant feature transform; VMA, vanillylmandelic acid.

1. Introduction

Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare, inherited, autosomal-recessive, neurotransmitter synthesis disorder caused by pathogenic variants of the dopa decarboxylase (*DDC)* gene that result in reduced AADC enzyme activity [1,2]. The condition was originally described in 1990 by Hyland and Clayton in monozygotic twins who presented at age 2 months with severe hypotonia, developmental delays, and irritability. Analyses revealed reduced cerebrospinal fluid (CSF) levels of homovanillic acid and 5-hydroxyindoleacetic acid, reduced plasma levels of noradrenaline, and reduced whole blood levels of serotonin, as well as highly elevated levels of 3-dihydroxyphenylalanine, 5-hydroxytryptophan, and 3-*O*-methyldopa. Deficiency in AADC was confirmed by enzyme activity levels in liver tissue that were 1% of normal [3].

The AADC enzyme is homodimeric, with each monomer consisting of a 309-residue large domain containing the pyridoxal 5′-phosphate (PLP) binding site, a C-terminal small domain of 86 residues, and an 85-residue N-terminal domain packed on top of the large domain. Biochemical and bioinformatics studies [4–9] have revealed a variety of effects of pathogenic *DDC* variants, including structural and functional defects of the enzyme such as decreased catalytic efficiency, decreased PLP binding affinity, and misfolding defects.

As the AADC enzyme plays a central role in the biosynthesis of the monoamine neurotransmitters dopamine, epinephrine, norepinephrine, and serotonin [1,2,10], a deficiency of this enzyme results in severely reduced synthesis of those neurotransmitters [10]. Low levels of dopamine may affect cognitive function, voluntary movement, and emotional state. Decreased levels of epinephrine and norepinephrine may affect mood, attention, sleep patterns, cognition, and stress hormone levels. Lower levels of serotonin may affect mood, sleep, memory/learning, and body temperature, as well as cardiovascular and endocrine function [1,10].

The clinical presentation of AADC deficiency varies extensively. However, most patients show symptoms within the first year of life and can experience a range of neurological, neuromuscular, and behavioral manifestations. Typical signs and symptoms include hypotonia, movement disorders (e.g., oculogyric crisis, dystonia, and hypokinesia), developmental delay, and autonomic symptoms such as ptosis, excessive sweating, and nasal congestion [2].

Attempts to quantify the incidence of AADC deficiency have been made, despite the rarity of this condition. Although global prevalence remains unknown, the birth rates are estimated to be 1:32,000 in Taiwan, 1:42,000 to 1:190,000 in the United States, 1:116,000 in the European Union, and 1:162,000 in Japan [11]. In addition, AADC deficiency can be difficult to distinguish from other clinical presentations, such as cerebral palsy, and therefore diagnosis may be delayed for years. According to consensus guidelines, a diagnosis should be based on a positive result from ≥2 of 3 diagnostic modalities, which include analysis of CSF neurotransmitter metabolites, direct assessment of plasma AADC activity, and DNA sequence analysis (including introns/splice points) [2]. Untargeted metabolomic profiling of multiple metabolites in plasma provides a powerful additional approach to diagnose AADC deficiency [12,13]. In addition, urine organic acids analysis may detect elevated levels of vanillactic acid (VLA), vanilpyruvic acid (VPA), and N-acetylvanilalanine [14].

Most patients experience an unrelenting disease course with poor or no response to conventional medical therapy, which includes dopamine agonists, monoamine oxidase inhibitors, and pyridoxine derivatives. Additional symptomatic treatments include anticholinergic agents, melatonin, and benzodiazepines [2]. The advent of human *DDC* gene

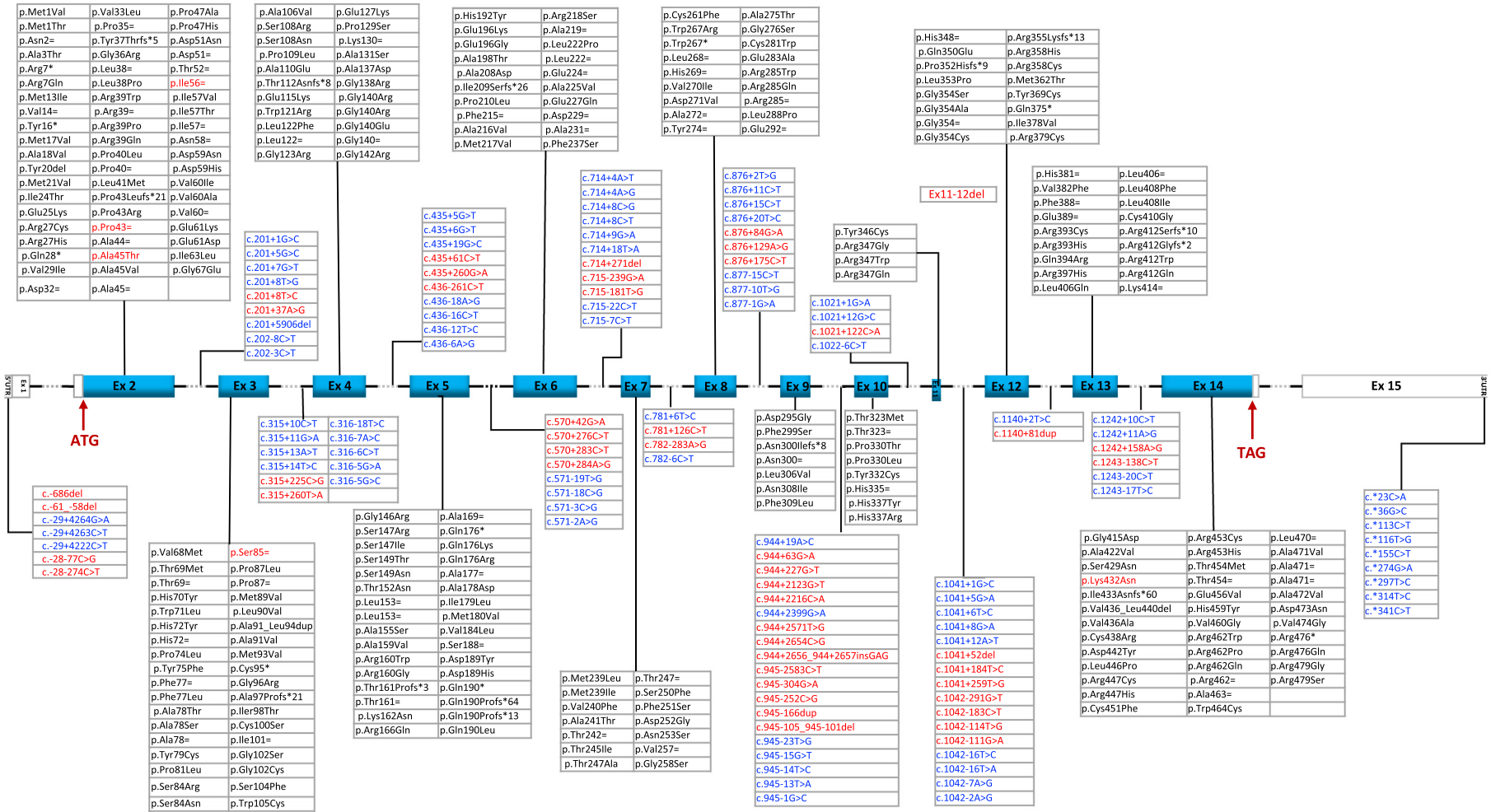

Fig. 1. Genomic Organization of the *DDC* Gene (NG_008742.1; NM_000790.4) and Variants Reported. Variants in black are associated with AADC deficiency, while variants in red are not associated with AADC deficiency. Variants in blue are located in intronic and untranslated regions (http://www.biopku.org/home/pnddb.asp). AADC, aromatic L-amino acid decarboxylase; *DDC*, dopa decarboxylase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

therapy represents a promising new treatment option for patients with AADC deficiency. Clinical studies of direct intraputaminal infusions of eladocagene exuparvovec, an adeno-associated virus type 2 vector containing the human *DDC* gene, have demonstrated acceptable safety and tolerability and encouraging improvement in motor milestones and cognitive symptoms, regardless of patient genotype [15–18]. Based on these results, eladocagene exuparvovec was authorized for use in the European Union, Iceland, Liechtenstein, and Norway by the European Commission in July 2022 [19]. A convection-enhanced delivery of AAV2-hAADC to the bilateral substantia nigra and ventral tegmental area is alternative option to restore AADC activity in these brain regions [20].

Given that genetic testing is a core diagnostic test for AADC deficiency, a comprehensive catalogue of the genetic variants associated with the disorder is critical for interpreting genetic testing results. Compiling these genetic variants requires expert-guided curation of locus-specific databases, since genetic interpretation of variant classification refers to and relies on impaired biomarkers as well as functional evidence. Additionally, many of the *DDC* variants in the ClinVar database are characterized as variants of unknown significance (VUSs). Improving the interpretation of these variants will provide a more complete understanding of which ones are associated with AADC deficiency and may aid in a faster, more accurate diagnosis for patients and their families. Thus, the aim of the present study was to identify and reclassify previously uncharacterized *DDC* variants to provide a deeper insight for pathogenicity classification and further expand the knowledge of these variants for more accurate diagnostic genetic testing in AADC deficiency.

2. Materials and methods

2.1. Variant and genotype collection

The *DDC* locus-specific database is based on information in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the Pediatric Neurotransmitter Disorders database (PNDdb; http://www.biopku.org/home/pnddb.asp), the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), and the Leiden Open Variation Database (LOVD; https://databases.lovd.nl/shared/genes/DDC). All variants were tested with LUMC Mutalyzer 3 (https://mutalyzer.nl/) by following Human Genome Variation Society guidelines (http://varnomen.hgvs.org/) (NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6). Genotypes of patients with confirmed AADC deficiency were identified from reports in the literature.

2.2. Variant and genotype classification and interpretation

The pathogenicity of variants was classified using the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP)/Association for Clinical Genomic Science (ACGS) recommendations [21,22]. Criteria such as PS2, PS4, and PM6 were not applicable as AADC deficiency is an autosomal recessive disorder. PP1 and PP2 as well as BP1, BP2, BP3, BP5, BS3, and BS4 were also not considered as they were not applicable to AADC deficiency. There were no discrepancies for PS1, PM4, PM5, BP7, and BS2 in AADC deficiency. PVS1 criteria can be used because a loss-of-function mechanism underlies AADC deficiency. PVS1 criteria was applied according to Tayoun et al. [23] and approved with the use of AutoPVS1 (http://autopvs1.genetics.bgi.com/). Further details regarding the application of ACMG-AMP/ACGS criteria to *DDC* variants can be found in Table 1. Genotypes of reported patients with confirmed AADC deficiency were also classified using ACMG-AMP/ACGS criteria.

2.3. In silico prediction

For *in silico* prediction, combined annotation-dependent depletion (CADD) scores (https://cadd.gs.washington.edu/) [24,25], ClinVar ACMG-AMP recommendations, Polymorphism Phenotyping v2 (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/) [26] and scale-invariant feature transform (SIFT) algorithms (https://sift.bii.a-star.edu.sg) [27,28] were used to predict the impact of the identified *DDC* variants on protein function. Scores were analyzed using correspondence analysis.

Possible molecular effects of specific variants were predicted using Maestro v12.2 and PyMol v2.0 software (Schrödinger) using the pig kidney AADC crystal structure as a template (pdb file 1Js6; RefSeq: NP_999019.2; Protein accession ID: AAB47157.1). The global location, microenvironment, and possible contacts of each residue subjected to substitution were analyzed.

Conservation analyses were performed using the Consurf Server (https://consurf.tau.ac.il/) and the PSI-BLAST as the homolog search algorithm between 35% and 95% of homology, the UniProt database, and CLUSTALW alignment software. Non-redundant homolog sequences were identified, and the amino acid conservation score was expressed on a scale of 1 (not conserved) to 9 (highly conserved).

2.4. Correspondence analysis of VEP severity scores

Severity levels of VEP scores were harmonized according to Suppl Table 1. Subsequently, a correspondence analysis (CA) was applied

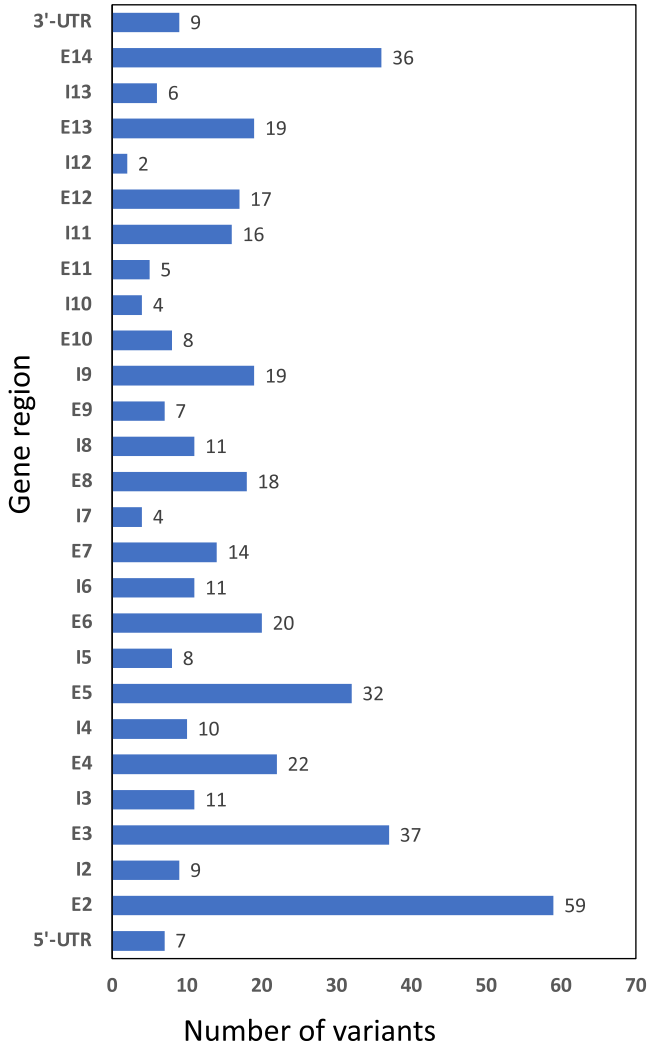

Fig. 2. Number of Variants Identified in Each Region of the *DDC* Gene. The number of identified variants in each exon and intron of the *DDC* gene, as well as the 3′ and 5′ UTRs, are shown. *DDC*, dopa decarboxylase; UTR, untranslated region.

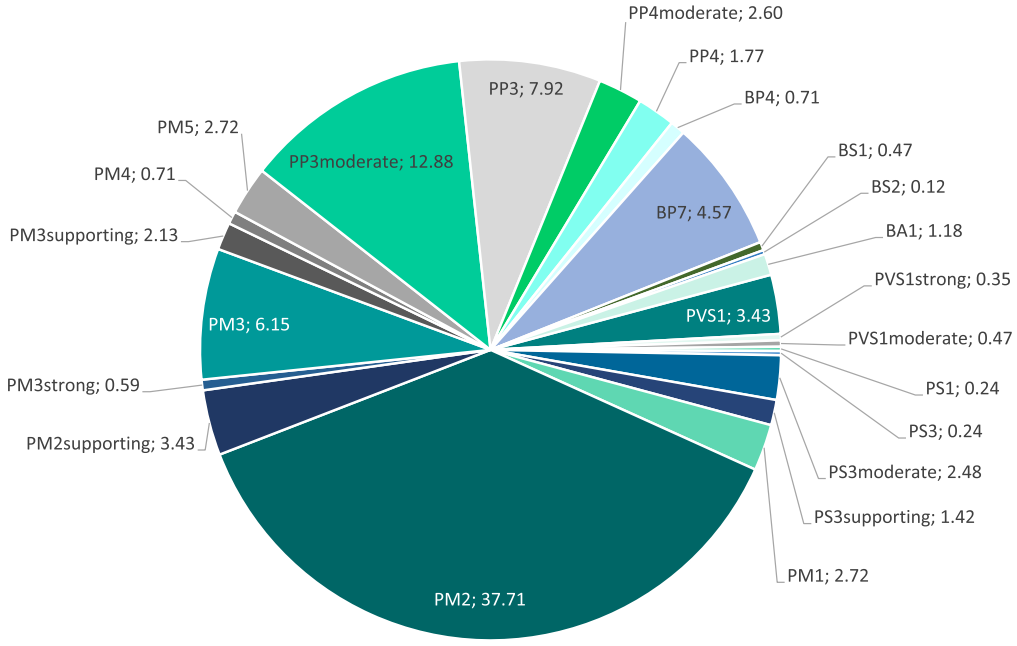

Fig. 3. *DDC* Variant Classification According to ACMG-AMP/ACGS Criteria. The percentage of identified *DDC* variants classified under each ACMG-AMP/ACGS category is shown. BA, stand-alone benign; BP, benign supporting; BS, strong benign; *DDC*, dopa decarboxylase; PM, pathogenicity moderate; PP, pathogenicity supporting; PS, pathogenicity strong; PVS, pathogenicity very strong.

over the contingency table (Suppl Table 2) of severity level (B, VUS, LP and P) and type of VEP (ACMG/ACGS, 3D-Analysis, Polyphen2, SIFT and CADD Score). The aim of CA is to visualize the relationship between categorical variables as a two-dimensional plot. This is achieved by mapping the high dimensional feature space of a contingency table into a lower dimensional space, while trying to preserve as much of the original variation as possible. Statistical analysis was computed with R language version 4.2.1, and CA was used from R package "FactoMineR" version 2.4 [29].

3. Results

A total of 422 *DDC* variants were identified from information provided in ClinVar, LOVD, and the PNDdb (http://biopku.org/home/pnddb.asp) (Suppl Table 3). These variants were mapped onto their corresponding location of the human *DDC* gene, which spans >107 kb (Fig. 1). Variants were identified in nearly all introns and exons of the *DDC* gene, as well as the 5′ and 3′ untranslated regions. The highest number of variants were identified in exon 2 ($n = 59$), while the fewest variants ($n = 2$) were identified in exon 1 and intron 12 (Fig. 2).

All 422 *DDC* variants were first classified according to ACMG-AMP/ACGS criteria and listed according to observed pathogenicity (Suppl Table 4). These ACMG-AMP/ACGS criteria were used 846 times to classify the identified *DDC* variants. The largest number of variants were classified with moderate pathogenicity PM2 ($n = 348$) as a score for no allelic frequency in healthy population, followed by supporting pathogenicity PP3 ($n = 176$) for deleterious in silico prediction (Fig. 3). Additional information describing how the ACMG-AMP/ACGS criteria were applied in the context of AADC deficiency can be found in Table 1.

To date, 108 unique genotypes have been reported in patients with confirmed AADC deficiency. Of note, the splice variant c.714+4A>T is frequent in South Asian populations, particularly in Taiwan. A list of these genotypes and the ACMG-AMP/ACGS scoring used to describe the pathogenicity of each is listed in Table 2. Of note, at least one allele was classified as pathogenic or likely pathogenic in 101 of 108 reported genotypes. The remaining genotypes were a combination of two VUSs; no variants were classified as benign or likely benign.

Of the 422 identified variants, 48% ($n = 204$) were classified as missense variants. Fifteen percent ($n = 62$) were synonymous variants (5× within splice site [last or first AA of the exon] for positions Glu292, Thr323, His348, His381, and Lys414), and 13% ($n = 53$) were intronic splice site variants. The coding effects of 16% of the variants ($n = 68$) were unknown. Of these 68 variants, 69% ($n = 47$) were deep intronic variants. The coding effects of the remaining variants were classified as frameshift (3%, $n = 14$), extension (2%, $n = 9$), nonsense (2%, n = 9), and inframe (n = 3) (Fig. 4).

The predicted or analyzed molecular effects of 201 of the missense *DDC* variants using Maestro v12.2 and PyMol v2.0 software (Schrödinger), together with the results of the conservation analyses, are shown in Table 3 [1,6–9,30–39]. Variants p.Met1Thr and p.Met1Val were not included in the analysis as they cause the loss of the start codon, yielding no protein or a truncated protein due to the altered start and two nucleotide aberrations (c.418G>A and c.418G>C) are coding for the same mutant protein (p.Gly140Arg). The pathogenicity of each missense *DDC* variant was then identified using *in silico* variant effect predictors (VEPs). Table 4 lists each missense variant, ordered by ACMG-AMP/ACGS pathogenic classification, along with its pathogenicity classification according to its CADD scores, 3D crystal structure analysis, ClinVar recommendations, PolyPhen2, and SIFT. Within 204 missense variants, we classified 7% (14/204) as pathogenic, 32% (66/204) as likely pathogenic, 5% (11/204) as hot VUSs, 16% (32/204) as warm VUSs, 18% (36/204) as tepid VUSs, 12% (25/204) as cool VUSs, 5% (10/204) as cold VUSs, 2% (5/204) as ice cold VUSs, 1% (2/204) as likely benign, and 1% (3/204) as benign. Twenty variants classified as VUS in the ClinVar were reclassified as pathogenic or likely pathogenic using ACMG-AMP/ACGS scoring.

The results of the CA are shown in a biplot in Fig. 5. The first two dimensions account for about 98% of the total variation (dimension 1: 61.46%, dimension 2: 37.81%) indicating a successful representation of the contingency table. The biplot indicates three groups of scores: CADD and 3D-analysis, Polyphen2 and SIFT, and ACMG as a separate

Table 2
DDC genotypes ($n = 108$) reported in patients with AADC deficiency.

Genotype (cDNA)	Genotype (Protein)	ACMG-AMP/ACGS Score	
c.[1A>G];[181G>A]	p.[Met1Val];[Glu61Lys]	LP (6)	Tepid VUS (3)
c.[2T>C];[277A>G]	p.[Met1Thr];[Met93Val]	LP (7)	Warm VUS (4)
c.[19C>T];[1222C>A]	p.[Arg7Ter];[Leu408Ile]	P (10)	P (10)
c.[19C>T];[214C>T]	p.[Arg7Ter];[His72Tyr]	P (10)	LP (8)
c.[19C>T];[592G>A]	p.[Arg7Ter];[Ala198Thr]	P (10)	LP (6)
c.[48C>A];[116G>C]	p.[Tyr16Ter];[Arg39Pro]	P (10)	LP (7)
c.[58_60del];[714+4A>T]	p.[Tyr20del];IVS6+4A>T	Hot VUS (5)	P (13)
c.[73G>A];[1379T>G]	p.[Glu25Lys];[Val460Gly]	Hot VUS (5)	LP (6)
c.[73G>A];[315G>C]	p.[Glu25Lys];[Trp105Cys]	Hot VUS (5)	LP (8)
c.[73G>A];[1073G>A]	p.[Glu25Lys];[Arg358His]	Hot VUS (5)	LP (8)
c.[73G>A];[624del]	p.[Glu25Lys];[Ile209SerfsTer26]	Hot VUS (5)	P (10)
c.[97G>C];[1385G>C]	p.[Val33Leu];[Arg462Pro]	Hot VUS (5)	LP (6)
c.[105del];[710T>C]	p.[Tyr37ThrfsTer5];[Phe237Ser]	P (10)	LP (6)
c.[106G>A];[714+4A>T]	p.[Gly36Arg];IVS6+4A>T	LP (9)	P (13)
c.[106G>A];[1340G>A]	p.[Gly36Arg];[Arg447His]	LP (9)	LP (9)
c.[113T>C];[714+4A>T]	p.[Leu38Pro];IVS6+4A>T	LP (7)	P (13)
c.[121C>A];[121C>A]	p.[Leu41Met];[Leu41Met]	Cold VUS (1)	Cold VUS (1)
c.[128del];[1040G>A]	p.[Pro43LeufsTer21];[Arg347Gln]	P (10)	P (12)
c.[140C>A];[140C>A]	p.[Pro47His];[Pro47His]	LP (9)	LP (9)
c.[140C>A];[1072C>T]	p.[Pro47His];[Arg358Cys]	LP (9)	LP (6)
c.[170T>C];[1234C>T]	p.[Ile57Thr];[Arg412Trp]	LP (6)	P (12)
c.[175G>A];[714+4A>T]	p.[Asp59Asn];IVS6+4A>T	LP (7)	P (13)
c.[175G>A];[286G>A]	p.[Asp59Asn];[Gly96Arg]	LP (7)	LP (7)
c.[175G>A];[175G>A]	p.[Asp59Asn];[Asp59Asn]	LP (7)	LP (7)
c.[179T>C];[1234C>T]	p.[Val60Ala];[Arg412Trp]	P (10)	P (12)
c.[179T>C];[311C>T]	p.[Val60Ala];[Ser104Phe]	P (10)	P (11)
c.[179T>C];[179T>C]	p.[Val60Ala];[Val60Ala]	P (10)	P (10)
c.[179T>C];[714+4A>T]	**p.[Val60Ala];IVS6+4A>T**	P (10)	P (13)
c.[179T>C];[440G>T]	p.[Val60Ala];[Ser147Ile]	P (10)	P (10)
c.[201+5G>C];[1073G>A]	IVS2+5G>C;[Arg358His]	LP (8)	LP (8)
c.[201+5G>C];[201+5G>C]	IVS2+5G>C;IVS2+5G>C	LP (8)	LP (8)
c.[201+5G>C];[206C>T]	IVS2+5G>C;[Thr69Met]	LP (8)	P (10)
c.[202G>A];[254C>T]	p.[Val68Met];[Ser85Leu]	Tepid VUS (3)	Warm VUS (4)
c.[206C>T];[1337T>C]	p.[Thr69Met];[Leu446Pro]	P (10)	LP (6)
c.[206C>T];[439A>C]	p.[Thr69Met];[Ser147Arg]	P (10)	LP (8)
c.[206C>T];[206C>T]	p.[Thr69Met];[Thr69Met]	P (10)	P (10)
c.[206C>T];[823G>A]	p.[Thr69Met];[Ala275Thr]	P (10)	LP (8)
c.[208C>T];[208C>T]	p.[His70Tyr];[His70Tyr]	LP (8)	LP (8)
c.[214C>T];[214C>T]	p.[His72Tyr];[His72Tyr]	LP (8)	LP (8)
c.[231C>A];[231C>A]	**p.[Phe77Leu];[Phe77Leu]**	P (11)	P (11)
c.[231C>A];[446G>C]	p.[Phe77Leu];[Ser149Thr]	P (11)	LP (7)
c.[236A>G];[714+4A>T]	p.[Tyr79Cys];IVS6+4A>T	LP (6)	P (12)
c.[236A>G];[755A>G]	p.[Tyr79Cys];[Asp252Gly]	LP (6)	LP (6)
c.[242C>T];[242C>T]	p.[Pro81Leu];[Pro81Leu	LP (9)	LP (9)

c.[250A>C];[1063dup]	p.[Ser84Arg];[Arg355LysfsTer13]	Hot VUS (5)	P (10)
c.[260C>T];[446G>C]	p.[Pro87Leu];[Ser149Thr]	LP (6)	LP (7)
c.[260C>T];[286G>A]	p.[Pro87Leu];[Gly96Arg]	LP (6)	LP (7)
c.[260C>T];[799T>C]	p.[Pro87Leu];[Trp267Arg]	LP (6)	LP (8)
c.[272C>T];[823G>A]	p.[Ala91Val];[Ala275Thr]	LP (8)	LP (8)
c.[272C>T];[1228T>G]	p.[Ala91Val];[Cys410Gly]	LP (8)	LP (6)
c.[286G>A];[714+4A>T]	p.[Gly96Arg];IVS6+4A>T	LP (7)	P (13)
c.[286G>A];[665T>C]	p.[Gly96Arg];[Leu222Pro]	LP (7)	P (10)
c.[289del];[629C>T]	p.[Ala97ProfsTer21];[Pro210Leu]	P (10)	Tepid VUS (3)
c.[293T>C];[343G>A]	p.[Ile98Thr];[Glu115Lys]	Warm VUS (4)	Warm VUS (4)
c.[299G>C];[1021+1G>A]	p.[Cys100Ser];IVS10+1G>A	LP (8)	P (10)
c.[304G>A];[304G>A]	**p.[Gly102Ser];[Gly102Ser]**	P (12)	P (12)
c.[304G>A];[714+4A>T]	p.[Gly102Ser];IVS6+4A>T	P (12)	P (13)
c.[315G>C];[385C>T]	p.[Trp105Cys];[Pro129Ser]	LP (8)	LP (6)
c.[315G>C];[73G>A]	p.[Trp105Cys];[Glu25Lys]	LP (8)	Hot VUS (5)
c.[322A>C];[812A>T]	p.[Ser108Arg];[Asp271Val]	Warm VUS (4)	Warm VUS (4)
c.[323G>A];[1041+1G>C]	p.[Ser108Asn];IVS11+1G>C	LP (6)	P (12)
c.[330_334dup];[330_334dup]	p.[Thr112AsnfsTer8];[Thr112AsnfsTer8]	P (11)	P (11)
c.[361T>C];[1040G>A]	p.[Trp121Arg];[Arg347Gln]	Hot VUS (5)	P (12)
c.[364C>T];[714+4A>T]	p.[Leu122Phe];IVS6+4A>T	Hot VUS (5)	P (13)
c.[367G>A];[734C>T]	p.[Gly123Arg];[Thr245Ile]	LP (7)	LP (6)
c.[367G>A];[876G>A]	p.[Gly123Arg];[Glu292=]	LP (7)	Cool VUS (2)
c.[419G>A];[1375C>T]	p.[Gly140Glu];[His459Tyr]	Warm VUS (4)	Tepid VUS (3)
c.[424G>A];[1234C>T]	p.[Gly142Arg];[Arg412Trp]	LP (6)	P (12)
c.[436G>C];[1297dup]	p.[Gly146Arg];[Ile433AsnfsTer60]	LP (7)	LP (6)
c.[436G>C];[436G>C]	p.[Gly146Arg];[Gly146Arg]	LP (7)	LP (7)
c.[439A>C];[439A>C]	p.[Ser147Arg];[Ser147Arg]	LP (8)	LP (8)
c.[478C>G];[565G>T]	p.[Arg160Gly];[Asp189Tyr]	LP (6)	LP (6)
c.[478C>T];[1040G>A]	p.[Arg160Trp];[Arg347Gln]	LP (6)	P (12)
c.[568_569insCGAT];[1040G>A]	p.[Gln190ProfsTer64];[Arg347Gln]	P (10)	P (12)
c.[571-3C>G];[571-3C>G]	IVS5-3C>G;IVS5-3C>G	Tepid VUS (3)	Tepid VUS (3)
c.[665T>C];[665T>C]	p.[Leu222Pro];[Leu222Pro]	P (10)	P (10)
c.[714+4A>T];[848A>C]	IVS6+4A>T;[Glu283Ala]	P (13)	LP (8)
c.[714+4A>T];[1233dup]	IVS6+4A>T;[Arg412SerfsTer10]	P (13)	LP (8)
c.[714+4A>T];[714+4A>T]	**IVS6+4A>T;IVS6+4A>T**	P (13)	P (13)
c.[714+4A>T];[1312T>C]	IVS6+4A>T;[Cys438Arg]	P (13)	LP (6)
c.[714+4A>T];[752T>C]	IVS6+4A>T;[Phe251Ser]	P (13)	LP (6)
c.[714+4A>T];[801G>A]	IVS6+4A>T;[Trp267Ter]	P (13)	P (10)
c.[714+4A>T];[853C>T]	IVS6+4A>T;[Arg285Trp]	P (13)	P (11)
c.[714+4A>T];[1058T>C]	IVS6+4A>T;[Leu353Pro]	P (13)	LP (6)
c.[714+4A>T];[1106A>G]	IVS6+4A>T;[Tyr369Cys]	P (13)	LP (6)
c.[714+4A>T];[1234C>T]	**IVS6+4A>T;[Arg412Trp]**	P (13)	P (12)
c.[714+4A>T];[1297dup]	**IVS6+4A>T;[Ile433AsnfsTer60]**	P (13)	LP (6)
c.[714+4A>T];[1339C>T]	IVS6+4A>T;[Arg447Cys]	P (13)	LP (8)
c.[749C>T];[749C>T]	**p.[Ser250Phe];[Ser250Phe]**	P (10)	P (10)
c.[781+6T>C];[781+6T>C]	IVS7+6T>C;IVS7+6T>C	LP (7)	LP (7)

c.[782G>T];[1060G>A]	p.[Cys261Phe];[Gly354Ser]	Warm VUS (4)	Hot VUS (5)
c.[799T>C];[799T>C]	p.[Trp267Arg];[Trp267Arg]	LP (8)	LP (8)
c.[823G>A];[823G>A]	p.[Ala275Thr];[Ala275Thr]	LP (8)	LP (8)
c.[843C>G];[1085T>C]	p.[Cys281Trp];[Met362Thr]	LP (6)	LP (6)
c.[925T>C];[925T>C]	p.[Phe309Leu];[Phe309Leu]	LP (8)	LP (8)
c.[1039C>G];[1039C>G]	p.[Arg347Gly];[Arg347Gly]	P (12)	P (11)
c.[1040G>A];[1040G>A]	**p.[Arg347Gln];[Arg347Gln]**	P (12)	P (12)
c.[1040G>A];[1073G>A]	p.[Arg347Gln];[Arg358His]	P (12)	LP (8)
c.[1040G>A];[1123C>T]	p.[Arg347Gln];[Gln375Ter]	P (12)	P (10)
c.[1040G>A];[Ex11_12del]	p.[Arg347Gln;Ex11_12del]	P (12)	P (10)
c.[1060G>T];[1060G>T]	p.[Gly354Cys];[Gly354Cys]	LP (6)	LP (6)
c.[1073G>A];[1073G>A]	p.[Arg358His];[Arg358His]	LP (8)	LP (8)
c.[1123C>T];[1123C>T]	p.[Gln375Te];[Gln375Ter]	P (10)	P (10)
c.[1144G>T];[1144G>T]	p.[Val382Phe];[Val382Phe]	LP (6)	LP (6)
c.[1222C>T];[1222C>T]	p.[Leu408Phe];[Leu408Phe]	LP (7)	LP (7)
c.[1234C>T];[1297dup]	p.[Arg412Trp];[Ile433AsnfsTer60]	P (12)	LP (6)
c.[1340G>A];[1340G>A]	p.[Arg447His];[Arg447His]	LP (9)	LP (9)
c.[1385G>C];[1385G>C	p.[Arg462Pro];[Arg462Pro]	LP (6)	LP (6)

Legend

ACMG/ ACGS classification	Score	Color Map
P (P)	>10	
Likely P (LP)	6 to 9	
Hot VUS	5	
Warm VUS	4	
Tepid VUS	3	
Cool VUS	2	
Cold VUS	1	
Ice cold VUS	0	
Likely benign (LB)	-1 to -5	
Benign (B)	<-6	

Genotypes reported in at least 4 patients are **bolded**. Numbers in parentheses reflect the genotype's score on a point system fitted to the ACMG-AMP/ACGS variant classification guidelines [41].

group. ACMG is highly associated with VUS severity level, whereas CADD and 3-D analysis are more associated with LB and LP, and Polyphen2 and SIFT with P and B.

4. Discussion

This is the first study specifically designed to classify previously uncharacterized *DDC* variants. The variants were interpreted based on the current data available, assuming the DNA aberration would now be detected in the next-generation sequencing analysis. In total, 422 *DDC* variants were identified. These variants were characterized using ACMG-AMP/ACGS criteria, as well as analyzed using a number of *in silico* VEPs.

The ACMG-AMP/ACGS criteria were partially modified and *DDC* variant customized since several criteria were applicable for autosomal-dominant inheritance, while AADC deficiency is inherited in an autosomal-recessive manner. Specifically, PS3 and PP4 were only applied for variants in homozygous state (with PM3_supporting/PM3_moderate if two homozygous patients occur, instead of PS4) and/or PS3 if functional studies in different expression cell lines were performed. PS3 can only be used as strong criterion for functional studies when a variety of experiments have confirmed loss-of-function of the variant on the protein level several times in a homozygous manner, including activity, protein expression, and functional tests in transient cell lines. If all levels were not tested, application of PS3 is not recommended and was used as a moderate criterion. The use of PS3_moderate is also suggested for cell expression studies for different specific variants to detect enhanced PLP binding or low k_{cat}/K_m, since the experiments were performed less than 10 times [40]. If fewer than 2 experiments confirmed the loss-of function, PS3_supporting was applied. PS3 cannot be used for transient knock-out of the whole gene in cellular or animal models.

For this study, we strengthened PP4 criteria to moderate since all defined measurable parameters confirmed these defects via elevated or

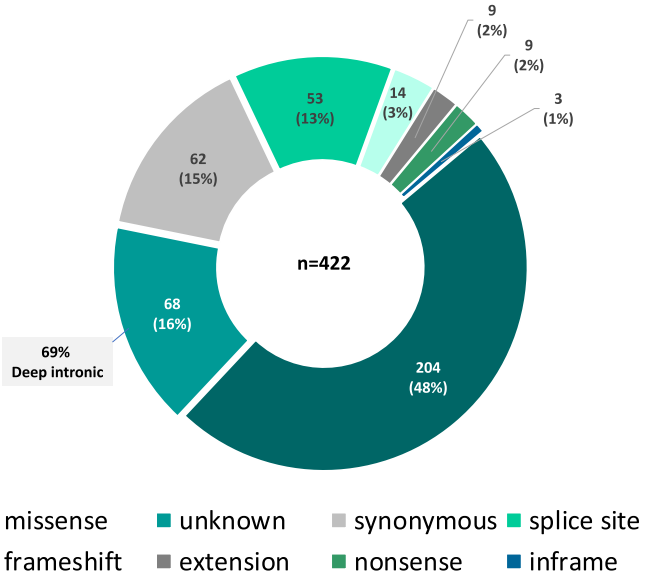

Fig. 4. Coding Effects of the *DDC* Gene Variants. Coding effects of the 420 identified *DDC* variants are shown. Of the 68 variants with unknown coding effects, 69% were deep intronic variants. *DDC*, dopa decarboxylase.

reduced levels of the following in plasma, cerebrospinal fluid, or urine: 1) 5-OH-Trp; 2) L-DOPA/ 3-OMD or VLA; 3) serotonin or 5-HIAA; 4) dopamine/ HVA/ norepinephrine/ MHPG/ epinephrine/ VMA (Fig. 6). We recommend that a minimum of two metabolites must confirm AADC deficiency to apply PP4 as regular supporting criteria.

It is important to note that the guidelines recommend not using PVS1 in combination with PM4 or PP3, and that PM3 should only be used if PM2 was applied (Table 1). We focused on establishing a useful criteria application for *DDC* variant interpretation and observed convincing results with fewer discrepancies. Noticeably, as an exception, is the missense variant c.629C>T;p.Pro210Leu, which we classified as tepid VUS as this variant should be investigated more deeply for the potential of a hypomorphic allele. As far as it is known, homozygosity for this variant does not cause disease, but we decided to not apply BS2 since this variant has been shown to cause loss of function (although found in the homozygous state in 25 individuals in gnomAD; Table 1). However, it should be highlighted that this allele in combination with a deleterious allele may cause disease.

Another inconsistency concerns discrepancies with Uniprot for PLP binding that have been noted for AA 148, 149, 246, 300 (303 N6 pyridoxal lysine), and were observed in the present study for AA 275, 283, 285, 309.

The signs and symptoms of AADC deficiency can be debilitating, and treatment options that do not target the underlying genetic cause of the

Table 3
Predicted or Analyzed Molecular Effects of 201 Missense *DDC* Variants on Structural and/or Functional Properties of the Enzyme.

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Ala3Thr	N-terminal	2	Only slight alterations of the monomer-monomer interface contacts are predictable.	LB
p.Arg7Gln	N-terminal	7	The substitution is expected to affect some interchain interactions between the N-terminal regions of the monomers.	LP
p.Met13Ile	N-terminal	6	The substitution is expected to have a slight structural impact on the interface between N-terminal regions of the monomers.	LB
p.Met17Val	N-terminal	6	The reduction of side chain footprint caused by the Met➔Val substitution could reduce the hydrophobic contacts of the original residue. However, local conformational changes are not expected.	LB
p.Ala18Val	N-terminal	6	The variant could affect the proper match between the N-terminal regions of the *DDC* monomers.	LP
p.Met21Val	N-terminal	#	In the pkDDC amino acid sequence, the 21st amino acid is a Leu residue [35]. For this reason, it is not possible to predict the effects of the p.Met21Val substitution.	VUS
p.Ile24Thr	N-terminal	7	This variant could affect both the local folding and the catalytic properties of the enzyme.	LP
p.Glu25Lys	N-terminal	5	The substitution generates a surface charge alteration. However, significant structural changes are not expected.	LB
p.Arg27His	N-terminal	4	The substitution is expected to abolish the salt bridge linkage Arg27-Glu61. Such interaction is an important junction point between α-helices 1–25 and 52–66. It is noteworthy that the latter segment precedes loop1 (66–84). Both folding and catalytic defects are predictable.	LP
p.Arg27Cys	N-terminal	4	Both folding and catalytic defects are predictable.	LP
p.Val29Ile	N-terminal	9	The steric hindrance generates by the substitution could not only alter the local conformation but also affect loop1. Structural and catalytic alterations are predictable.	LP
p.Val33Leu	N-terminal	8	Production of local steric hindrance leading to possible misfolding defect [1].	LP
p.Gly36Arg	N-terminal	9	Production of steric hindrance between N-terminal domain and loop3; possible catalytic and folding defects [1].	LP
p.Leu38Pro#	N-terminal	#	Strong reduction of the catalytic efficiency [7].	P
p.Arg39Gln	N-terminal	1	The variant could affect the monomer-monomer interaction by abolishing an interface electrostatic contact. Moreover, considering the strong reduction of the catalytic activity exhibited by the previously characterized p.Leu38Pro variant [7], it is possible to suggest that the Arg39 variant could also affect the catalytic activity.	LP
p.Arg39Trp	N-terminal	1	The variant could affect the monomer-monomer interaction by abolishing an interface electrostatic contact. Moreover, considering the strong reduction of the catalytic activity exhibited by the previously characterized p.Leu38Pro variant [7], it is possible to suggest that the Arg39 variant could also affect the catalytic activity.	LP
p.Arg39Pro	N-terminal	1	The variant could affect the monomer-monomer interface by abolishing an interchain salt bridge. Moreover, considering the strong reduction of the catalytic activity exhibited by the previously characterized p.Leu38Pro variant [7], it is possible to suggest that the Arg39 variant could also affect the catalytic activity.	LP
p.Pro40Leu	N-terminal	1	The variant could affect the local folding and the subunit interface. In addition, the reported effects of the p.Leu38Pro variant [7] suggest that alterations in this region could indirectly affect the active site.	LP

Table 3 (*continued*)

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Leu41Met	N-terminal	2	The variant could affect the local folding. In addition, the reported effects of the p.Leu38Pro variant [7] suggest that alterations in this region could indirectly affect the active site.	LP
p.Pro43Arg	N-terminal	7	The Pro→Arg substitution is predicted to affect both the local backbone conformation and the subunits interface.	LP
p.Ala45Thr	N-terminal	3	In the pkDDC amino acid sequence, the 45th amino acid is a threonine residue [35]. However, it is possible to suggest that p.Ala45Thr or p.Ala45Val substitution should not affect the local structural properties.	B
p.Ala45Val	N-terminal	3	In the pkDDC amino acid sequence the 45th amino acid is a threonine residue [35]. However, the p. Ala45Thr or p.Ala45Val substitution are expected to not affect the local structural properties.	B
p.Pro47Ala	N-terminal	9	Local alteration of the backbone conformation and, based on the previously characterized p. Pro47His variant [7], a reduction of the catalytic efficiency is expected.	P
p.Pro47His#	N-terminal	#	Reduction of the catalytic efficiency and low expression level in *Escherichia coli* system indicating a folding defect [7].	P
p.Asp51Asn	N-terminal	7	The substitution should have a minimal impact on the local microenvironment.	LB
p.Ile57Val	N-terminal	5	The substitution is not expected to affect the local structural properties.	B
p.Ile57Thr	N-terminal	5	Insertion of a polar residue in a hydrophobic environment; local folding alterations are predictable.	LP
p.Asp59His	N-terminal	8	The substitution generates significant steric hindrance and abolishes an interchain electrostatic contact. Local conformational changes and possible misfolding defect are predictable.	LP
p.Asp59Asn	N-terminal	8	The variant could abolish an interchain electrostatic contact. Local conformational changes and possible misfolding defect are predictable.	LP
p.Val60Ala#	N-terminal	#	Consistent decrease of the catalytic efficiency and of the PLP binding affinity [37].	P
p.Val60Ile	N-terminal	5	Val60 side chain belongs to a hydrophobic cleft at the interface of the subunits. The previously characterized p.Val60Ala pathogenic variants exhibited a reduction of both the catalytic activity and the coenzyme binding affinity [37]. It was suggested that the perturbation of the hydrophobic interaction network of Val60 could affect the conformation of loop1. On the contrary, p.Val60Ile substitution could maintain or reinforce the hydrophobic contacts network without generating significant steric hindrance.	LB
p.Glu61Lys	N-terminal	2	Loss of a surface salt bridge and alteration of the surface charge distribution. A conformational change of the N-terminal region is expected.	LP
p.Glu61Asp	N-terminal	2	Slight alterations of the N-terminal region conformation are possible.	LB
p.Ile63Leu	N-terminal	1	Little or no structural impact can be predicted.	B
p.Gly67Glu	N-terminal	9	The massive steric hindrance generated by the glutamate residue is expected to strongly affect the local folding.	LP
p.Val68Met	Loop1	3	Val68 is the first residue of loop1 (68–84); the bulker Met side chain generates steric hindrance, which could affect the conformation of the N-terminal part of loop1. Alterations in catalysis and PLP binding affinity are predictable.	LP
p.Thr69Met#	Loop1	#	The variant causes a 10-fold reduction of the catalytic efficiency and minimal structural alterations [7,34].	P
p.His70Tyr#	Loop1	#	Consistent reduction of the k_{cat} value leading to 30-fold reduction of the catalytic efficiency [37].	P
p.Trp71Leu	Loop1	8	The variant alters both the footprint and the contact of a residue belonging to loop1. Alterations in catalysis and PLP binding affinity are predictable.	LP
p.His72Tyr#	Loop1	#	Strong reduction of the PLP-binding affinity and of the catalytic efficiency [7].	P
p.Pro74Leu	Loop1	9	The substitution is predicted to generate consistent steric hindrance between loop1 and the C-terminal domain. Both structural and catalytic alterations are expected.	LP
p.Tyr75Phe	Loop1	6	Tyr75 is partly exposed on the protein surface, the more hydrophobic side chain of Phe could affect the local folding and in turn loop1 conformation. Both catalytic and structural alterations are predictable.	LP
p.Phe77Leu#	Loop1	#	Both k_{cat} and K_M values alterations leading to 100-fold reduction of the catalytic efficiency [37].	P
p.Ala78Ser	Loop1	9	The variant introduces a polar residue into an apolar region of the loop1. Based on the enzymatic phenotype of several loop1 variants previously analyzed [7,37], this variant could affect the substrate binding affinity, the rate of catalysis and the PLP binding affinity.	LP
p.Ala78Thr	Loop1	9	The substitution generates steric hindrance and introduces a polar residue into an apolar region of loop1. The variant could affect the substrate binding affinity, the rate of catalysis and the PLP binding affinity.	LP
p.Tyr79Cys#	Loop1	#	Remarkable decrease of the substrate binding affinity, catalytic efficiency, and PLP-binding affinity [7]	P
p.Pro81Leu#	Loop1	#	Reduction of the catalytic efficiency and PLP-binding affinity [7]	P
p.Ser84Asn	Loop1	5	Predicted alterations of loop1 conformation: possible catalytic impairment	LP
p.Ser84Arg	Loop1	5	Predicted alterations of loop1 conformation: possible catalytic impairment	LP
p.Ser85Leu	Large domain	9	Ser85 is located at the C-terminal end of loop1. The p.Ser85Leu substitution generates a massive steric hindrance. Such dramatic alteration could affect both the proper folding of the large domain and the active site architecture.	LP
p.Pro87Leu	Large domain	7	Predicted alterations of loop1 conformation: possible catalytic impairment [1].	LP
p.Met89Val	Large domain	5	Minimal conformational changes due to interface perturbation are expected.	LB
p.Leu90Val	Large domain	6	Minimal conformational changes due to interface perturbation are expected.	LB
p.Ala91Val#	Large domain	#	Remarkable reduction of the catalytic efficiency and coenzyme binding affinity [36].	P
p.Met93Val	Large domain	8	The variant could affect the proper match between the subunits in the dimer formation.	LP

(*continued on next page*)

Table 3 (*continued*)

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Gly96Arg#	Large domain	#	Remarkable increase of the K_M value leading to a strong reduction of the catalytic efficiency [37].	P
p.Ile98Thr	Large domain	5	Insertion of a polar residue into a hydrophobic cluster at the interface between the subunits. Structural alterations and folding defect can be expected.	LP
p.Cys100Ser	Loop2	5	The mutationaffects the catalytic efficiency of DDC maily through the reduction of the substrate binding affinity [42].	P
p.Gly102Ser#	Loop2	#	Reduction of the catalytic efficiency mainly due to a decreased substrate binding affinity [7].	P
p.Gly102Cys	Loop2	7	As demonstrated by the effects of the similar p.Gly102Ser variant previously characterized [6], p. Gly102Cys is expected to strongly affect the catalytic efficiency by reducing the substrate binging affinity.	P
p.Ser104Phe	Loop2	6	Alterations of the active site conformation are predictable. Moreover, the p.Gly102Ser and p. Ala110Glu pathogenic variants previously characterized exhibited a strong reduction of the catalytic efficiency mainly due to a drop of the substrate binding affinity [7,37].	LP
p.Trp105Cys	Loop2	9	Expected local conformational changes affecting loop2: possible both catalytic and folding defects [1].	LP
p.Ala106Val	Loop2	2	The steric hindrance of the bulkier Val side chain could affect loop2 conformation and microenvironment. Structural and catalytic alterations are predicted.	LP
p.Ser108Arg	Loop2	9	The variant generates significant steric hindrance between loop2 and loop1. A reduction of the catalytic efficiency is expected.	LP
p.Ser108Asn	Loop2	9	The variant generates significant steric hindrance between loop2 and loop1. A reduction of the catalytic efficiency is expected.	LP
p.Pro109Leu	Loop2	9	The substitution generates massive steric hindrance at the subunit interface and the proline substitution could alter the backbone conformation of loop2. Probable catalytic impairment and local structural alterations.	LP
p.Ala110Glu#	Loop2	#	No detectable catalytic activity and consistent reduction of the coenzyme binding affinity [37].	P
p.Glu115Lys	Large domain	9	The substitution abolishes a salt bridge involving loop3. Such perturbation could not only affect the local tertiary structure but also the catalysis.	LP
p.Trp121Arg	Large domain	9	Change of the local conformation and perturbation of the interface between the monomers are expected.	LP
p.Leu122Phe	Large domain	5	Expected alteration of the local tertiary structure: possible misfolding defect [1].	LP
p.Gly123Arg#	Large domain	#	Decrease of the catalytic efficiency due to a reduction of the substrate binding affinity [7].	P
p.Glu127Lys	Large domain	#	In the pkDDC amino acid sequence, the 127th amino acid is a glutamine residue [35]. For this reason, it is not possible to predict the effects of the p.Glu127Lys substitution.	VUS
p.Pro129Ser	Large domain	9	Predicted secondary structure alterations leading to a misfolding defect [1].	LP
p.Ala131Ser	Large domain	4	Although the 2 residues exhibit a similar footprint, the change in polarity and the microenvironment of Ala131 suggest that the variant could cause a local structural rearrangement.	LP
p.Ala137Asp	Large domain	3	The variant causes a surface charge alteration in proximity of the C-terminal end of the “catalytic loop” [29,32]. Possible catalytic defect	LP
p.Gly138Arg	Large domain	6	Possible structural alteration of the “catalytic loop” [29,32] causing a catalytic defect	LP
p.Gly140Arg	Large domain	9	The substitution generates a massive steric hindrance which should force a significant local structural rearrangement. Possible misfolding defect	LP
p.Gly140Glu	Large domain	9	The substitution generates a massive steric hindrance which should force a significant local structural rearrangement. Possible misfolding defect	LP
p.Gly142Arg	Large domain	9	Expected alteration of the secondary and tertiary structure: possible misfolding defects [1]	LP
p.Gly146Arg	Large domain	5	Production of steric hindrance at the dimer interface: possible misfolding defect and/or dimerization impairment [1]	LP
p.Ser147Arg#	Large domain	#	Strong reduction of both the catalytic efficiency and PLP-binding affinity [7,34]	P
p.Ser147Ile	Large domain	7	As previously observed from the characterization of the p.Ser147Arg pathogenic variants [6,34], the variant of Ser147 could seriously affect both catalysis and PLP binding affinity.	P
p.Ser149Thr	Large domain	8	Possible decrease of the PLP-binding affinity [1]	LP
p.Ser149Asn	Large domain	8	Possible decrease of the PLP-binding affinity [1]	LP
p.Thr152Asn	Large domain	6	Possible effects on catalysis and PLP binding affinity could be envisaged.	LP
p.Ala155Ser	Large domain	7	The substitution doesn't generate significant steric hindrance but the introduction of a polar residue could affect the conformation of the $\alpha\beta$-barrel system of the large domain.	LP
p.Ala159Val	Large domain	9	The substitution generates consistent steric hindrance affecting the $\alpha\beta$-barrel system of the large domain. This could alter the local conformation and the folding process.	LP
p.Arg160Gly	Large domain	8	The drastic substitution Arg→Gly could perturbate the subunit interface and, on the basis of the molecular effects of the characterized p.R160W [8], catalytic and folding defects are predictable.	P
p.Arg160Trp#	Large domain	#	Both catalytic and misfolding defects [34]	P
p.Lys162Asn	Large domain	2	The substitution abolishes a possible surface electrostatic contact. This could cause local conformational changes.	LP
p.Arg166Gln	Large domain	1	The substitution should not significantly alter the local conformation.	LB

Table 3 (*continued*)

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Gln176Lys	Large domain	5	The substitution could affect an interchain H-bond. Possible alteration of the local conformation and the dimer stability can be suggested.	LP
p.Gln176Arg	Large domain	5	The similar footprint and chemical properties of the 2 residues do not suggest substantial structural alterations caused by the variant.	LB
p.Ala178Asp	Large domain	1	The substitution is predicted to generate steric hindrance and electrostatic repulsion at the monomer interface, which could affect the both the local conformation and the dimerization process.	LP
p.Ile179Leu	Large domain	2	The variant is expected to have minimal or no structural/catalytic impact.	B
p.Met180Val	Large domain	5	In the pkDDC amino acid sequence, the 180th amino acid is a Leu residue [35]. However, the residue microenvironment should accommodate both Met or Val. No structural alterations are predictable.	B
p.Val184Leu	Large domain	9	The substitution is expected to generate significant steric hindrance with possible folding alterations.	LP
p.Asp189His	Large domain	7	Possible conformational changes of loop 209–218, which has been recently identified as an important region for the proper folding of the enzyme [37].	LP
p.Asp189Tyr	Large domain	7	Possible conformational changes of loop 209–218, which has been recently identified as an important region for the proper folding of the enzyme [36].	LP
p.Gln190Leu	Large domain	9	The substitution introduces an apolar residue inside a polar cleft. This is predicted to affect at least one intrachain polar contact and could alter the local folding.	LP
p.His192Tyr	Large domain	9	His192 is a highly conserved PLP binding residue. The variant could affect the PLP binding affinity and orientation.	LP
p.Glu196Gly	Large domain	6	The substitution alters the electrostatic surface charge distribution. However slight or no conformational changes are expected.	LB
p.Glu196Lys	Large domain	5	Surface charge alterations in proximity of the active site are predictable.	LP
p.Ala198Thr	Large domain	8	Introduction of a polar residue into a hydrophobic microenvironment close to the PLP-binding pocket: local conformational changes and reduction of the PLP-binding affinity are predictable [1].	LP
p.Ala208Asp	Large domain	8	No directed structural or contact changes are predictable. However, the substitution introduces a bulkier negative charged residue on the protein surface in proximity of the active site.	LP
p.Pro210Leu#	Large domain	#	Very low protein solubility in *E coli* expression system suggesting a possible severe folding defect. Minimal impact on the catalytic properties [37].	LP
p.Ala216Val	Large domain	1	The substitution could cause a local slight rearrangement of a flexible region.	LB
p.Met217Val	Large domain	5	The conservative substitution with Val should not affect the local folding. Minimal or no structural impact are predictable.	B
p.Arg218Ser	Large domain	4	The substitution should not significantly affect the local folding. A slight structural impact is expected.	LB
p.Leu222Pro#	Large domain	#	Predicted secondary structure alterations possible leading to a misfolding defect [1]. The prediction is supported by the very low expression level found in *E coli* expression system [37].	P
p.Ala225Val	Large domain	7	The variant generates moderate steric hindrance between 2 important secondary structure elements. A folding alteration is possible.	LP
p.Glu227Gln	Large domain	4	The substitution is predicted to have not significant impact on the local structural properties of *DDC*.	B
p.Phe237Ser#	Large domain	6	Insertion of a polar residue into a hydrophobic region: possible misfolding defect [1]. The prediction is supported by the very low expression level found in *E coli* expression system [37].	P
p.Met239Ile	Large domain	3	The residue is immersed into a hydrophobic microenvironment, which is predicted to accommodate both Ile and Leu residue without significant structural impact.	B
p.Met239Leu	Large domain	5	The residue is immersed into a hydrophobic microenvironment, which could accommodate both Ile and Leu residue without significant structural impact.	B
p.Val240Phe	Large domain	7	The bulkier Phe side chain generates a massive steric hindrance between two secondary structural elements in proximity of the active site. Both folding and catalytic defect are expected.	LP
p.Ala241Thr	Large domain	7	The substitution generates minimal steric hindrance but the insertion of a polar residue into a hydrophobic region could cause possible folding defect.	LP
p.Thr245Ile	Large domain	9	Introduction of apolar residue into a polar cleft in proximity of the active site. Both local conformational changes and catalytic defects are probable.	P
p.Thr247Ala	Large domain	6	In the pkDDC amino acid sequence, the 247th amino acid is a serine residue [35]. For this reason, it is not possible to predict the effects of the p.Thr247Ala substitution.	VUS
p.Ser250Phe#	Large domain	#	Slight reduction of the catalytic efficiency and protein aggregation propensity [9].	LP
p.Phe251Ser#	Large domain	#	Modest reduction of the catalytic efficiency and PLP binding affinity [37].	LP
p.Asp252Gly	Large domain	9	Loss of a polar contact between two secondary structure elements. Possible misfolding defect [1].	LP
p.Asn253Ser	Large domain	1	The substitution is predicted to have a modest impact on the local structural properties of the enzyme.	LB
p.Gly258Ser	Large domain	5	The substitution could affect the proper folding of the $\alpha\beta$-barrel system of the large domain.	LP
p.Cys261Phe	Large domain	8	The large Phe footprint generates a massive hindrance inside the $\alpha\beta$-barrel system of the large domain. A folding defect is predictable.	LP
p.Trp267Arg#	Large domain	#	Low protein solubility in *E coli* expression system indicating a folding defect, 7-fold reduction of the catalytic efficiency and substantial reduction of the PLP-binding affinity [37].	P

(*continued on next page*)

Table 3 (*continued*)

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Val270Ile	Large domain	7	The substitution should be well tolerated by the local conformation without significant structural alteration.	LB
p.Asp271Val	Large domain	9	Substitution of a PLP binding residue critical for the catalytic mechanism. Dramatic impact on the catalytic activity and PLP binding affinity are expected.	P
p.Ala275Thr#	Large domain	#	Remarkable reduction of PLP-binding affinity [6].	P
p.Gly276Ser	Large domain	9	Introduction of the polar Ser side chain into an aromatic and hydrophobic cluster of residues: a folding defect is predictable.	LP
p.Cys281Trp	Large domain	8	Expected secondary and tertiary structure alterations. This is in line with the very low protein solubility in *E. coli* expression system [34]. Folding defect was suggested.	P
p.Glu283Ala#	Large domain	#	Low protein solubility in *E. coli* expression system indicating a folding defect, aggregation propensity of the apo form of the enzyme and substantial reduction of the PLP binding affinity [37].	P
p.Arg285Trp#	Large domain	#	High exposition of hydrophobic surface to the solvent indicating a folding defect, reduction of catalytic efficiency and PLP-binding affinity [7].	P
p.Arg285Gln	Large domain	7	Possible loss of multiple surface polar contacts resulting in the exposition of hydrophobic residues to the solvent. Moreover, the previous characterized p.Arg285Trp variant exhibited a reduction in PLP binding affinity and catalytic efficiency [7].	LP
p.Leu288Pro	Large domain	5	The introduction of a Pro residue could disrupt the helix 284–294. The variant is predicted to alter the local secondary structure.	LP
p.Asp295Gly	Large domain	7	The substitution alters the surface charge distribution and abolishes ≥1 intrachain polar contact. Local conformational changes are predictable.	LP
p.Phe299Ser	Large domain	7	The variant introduces a polar Ser residue into an aromatic and hydrophobic cluster of residues: a folding defect is predictable.	LP
p.Leu306Val	Large domain	6	The conservative substitution Leu➔Val is predicted to have a slight structural impact.	LB
p.Asn308Ile	Large domain	6	Loss of polar contacts in proximity of the active site: both the structural and catalytic alterations are predictable.	LP
p.Phe309Leu#	Large domain	#	Reduction of the catalytic efficiency mainly due to a decreased substrate binding affinity [6].	P
p.Thr323Met	Loop3	2	The substitution generates a consistent steric hindrance which could affect the residue microenvironment involving some loop2 residues. Structural and catalytic alterations are expected.	LP
p.Pro330Leu	Loop3	6	The variant causes a dramatic reduction of the catalytic efficiency [31].	P
p.Pro330Thr	Loop3	6	Pro330 is not visible in the electron density map of the pkDDC crystal structure [35]. However, based on the previously characterized p.Pro330Leu variant [31], the substitution is expected to strongly reduce the catalytic efficiency.	P
p.Tyr332Cys	Loop3	6	Previous studies demonstrated that Tyr332 is a critical residue for the DDC catalysis [30]. On this basis, the substitution is expected to strongly reduce/abolish the catalytic activity.	P
p.His337Tyr	Loop3	3	The residue is not visible in the electron density map of the pkDDC crystal structure [35]. Considering that the residue belongs to a flexible loop found to be critical for catalysis [29], the substitution could dramatically affect the catalytic activity of the enzyme.	LP
p.His337Arg	Loop3	3	The residue is not visible in the electron density map of the pkDDC crystal structure [35]. Considering that the residue belongs to a flexible loop found to be critical for catalysis [29], the substitution could dramatically affect the catalytic activity of the enzyme.	LP
p.Tyr346Cys	Loop3	5	The mutation is expected to abolish several hydrophobic contacts and, based on the previously analyzed variant Arg347Gln, Arg347Gly, Leu353Pro belonging to the loop3 [7,8,33,37,38], a strong catalytic impairment is predictable.	LP
p.Arg347Gln#	Loop3	#	Strong reduction of the catalytic efficiency [7,8]	P
p.Arg347Gly#	Loop3	#	Strong reduction of the catalytic efficiency [38]	P
p.Arg347Trp	Loop3	8	Based on the known molecular effects of previously analyzed substitution p.Arg34Gln and p. Arg347Gly [7,8,38], the more drastic substitution of Arg347 with Trp is expected to strongly compromise the DDC catalysis.	P
p.Gln350Glu	Loop 3	9	The substitution could affect ≥1 H-bond linkage that contribute to loop3 conformation. A catalytic defect is expected.	LP
p.Leu353Pro#	Loop3	#	Almost complete loss of the catalytic activity [37]	P
p.Gly354Ala	Loop3	7	The variant is expected to affect the catalytic activity and/or the PLP binding affinity.	LP
p.Gly354Cys	Loop3	7	The variant introduces the bulkier Cys side chain very close to the PLP housing. This probably affects both catalysis and PLP binding affinity.	LP
p.Gly354Ser	Loop3	7	The variant is expected to affect the catalytic activity and/or the PLP binding affinity.	LP
p.Arg358His#	Large domain	#	Strong reduction of both catalytic efficiency and coenzyme binding affinity [8].	P
p.Arg358Cys	Large domain	8	Based on the previously characterized p.Arg358His [8], the drastic substitution Arg➔Cys is predicted to affect both catalysis and coenzyme binding affinity.	LP
p.Met362Thr#	C-terminal	#	Reduced solubility in *E coli* expression system, slight alteration of the kinetic parameters and coenzyme binding affinity [34].	LP
p.Tyr369Cys	C-terminal	7	Loss of a polar contact and reduction of the local hydrophobicity. Possible misfolding defect	LP
p.Ile378Val	C-terminal	8	The conservative substitution Ile➔Val is predicted to have a slight structural impact.	LB
p.Arg379Cys	C-terminal	9	The previously characterized variant p.Glu283Ala exhibited folding defect, aggregation tendency of the apo-form and reduced coenzyme binding affinity [37]. These effects were attributed to the abolition of the Arg379-Glu283 salt bridge contact. Similar effects are predictable for the p. Arg379Cys variant.	P
p.Arg379His	C-terminal	9	The previously characterized variant p.Glu283Ala exhibited folding defect, aggregation tendency of the apo-form and reduced coenzyme binding affinity [37]. These effects were attributed to the abolition of the Arg379-Glu283 salt bridge contact. Similar effects are predictable for the p. Arg379His variant.	P

Table 3 (*continued*)

Protein variant	Domain	Conservation score 9 = highly conserved 1 = not conserved	Predicted or analyzed molecular effects	Predicted classification of pathogenicity†
p.Val382Phe	C-terminal	6	The substitution generates a massive steric hindrance between the large domain and the C-terminal domain. Local conformational changes and folding alterations are predictable.	LP
p.Arg393Cys	C-terminal	1	In the pig kidney amino acid sequence, the 393rd amino acid is a leucine residue [35]. For this reason, it is not possible to predict the effects of the Arg393➔Cys substitution.	VUS
p.Arg393His	C-terminal	1	In the pig kidney amino acid sequence, the 393rd amino acid is a leucine residue [35]. For this reason, it is not possible to predict the effects of the Arg393➔Cys substitution.	VUS
p.Gln394Arg	C-terminal	3	The substitution should not affect the local conformation and thus is expected to be well tolerated.	B
p.Arg397His	C-terminal	7	Loss of a polar contact with Asp395: local conformational changes and folding alterations are predictable.	LP
p.Leu406Gln	C-terminal	8	The substitution introduces a polar residue into a hydrophobic core. Conformational changes and/or folding alterations of the C-terminal domain are possible.	LP
p.Leu408Phe	C-terminal	9	Production of steric hindrance between the C-terminal domain and loop1 [1]. Based on the reported molecular effects of the p.Leu408Ile variant [7], a remarkable catalytic defect is expected.	P
p.Leu408Ile#	C-terminal	#	Strong reduction of the catalytic efficiency [7]	P
p.Cys410Gly#	C-terminal	#	Low protein solubility in *E coli* expression system indicating a folding defect; dramatic reduction of the coenzyme binding affinity [31].	P
p.Arg412Gln	C-terminal	9	Unlike the previously characterized p.Arg412Trp variant [7], the conservative substitution with Gln might not affect the local folding. Minimal or no structural impact are predictable.	LB
p.Arg412Trp#	C-terminal	#	15fold reduction of the catalytic efficiency, reduced solubility in *E coli* expression system [7].	LP
p.Gly415Asp	C-terminal	5	The massive steric hindrance of the Asp side chain could affect the relative position of some secondary structure elements of the C-terminal domain. Structural alterations could be envisaged.	LP
p.Ala422Val	C-terminal	1	The conservative substitution Ala➔Val is predicted to have slight or no impact on the local conformation.	LB
p.Ser429Asn	C-terminal	3	The substitution is predicted to have not significant impact on the local structural properties of AADC.	B
p.Lys432Asn	C-terminal	2	The substitution should have a minimal impact on the local conformation.	LB
p.Val436Ala	C-terminal	6	The substitution could abolish the hydrophobic contact with Tyr79 of loop1. A possible local alteration involving the active site could be envisaged.	LP
p.Cys438Arg	C-terminal	6	Predicted secondary and tertiary structure alterations possibly leading to a misfolding defect [1].	LP
p.Asp442Tyr	C-terminal	6	In the pkDDC amino acid sequence the 442nd amino acid is a glycine residue. For this reason, it is not possible to predict the effects of the p.Asp442Tyr substitution.	VUS
p.Leu446Pro	C-terminal	6	The introduction of the Pro446 residue could affect the folding of the β-strand system of the C-terminal domain. Both folding and catalytic defects are predictable.	LP
p.Arg447Cys	C-terminal	9	Based on the previously characterized p.Arg447His variant [7], a reduction of the substrate binding affinity and PLP binding affinity are expected.	P
p.Arg447His#	C-terminal	#	Reduction of both the substrate-binding affinity and the PLP-binding affinity [7].	P
p.Cys451Phe	C-terminal	7	Expected alteration of loop1 conformation. Catalytic defects are predictable.	LP
p.Arg453Cys#	C-terminal	#	Consistent drop of the catalytic efficiency and of the PLP binding affinity [37].	P
p.Arg453His	C-terminal	4	Expected alteration of loop1 conformation and based on the reported molecular effects of the p. Arg453Cys variant [37], catalytic defects are predictable.	LP
p.Thr454Met	C-terminal	4	In the pkDDC amino acid sequence, the 454th amino acid is a lysine residue. For this reason, it is not possible to predict the effects of the p.Thr454Met substitution.	VUS
p.Glu456Val	C-terminal	5	The substitution is not predicted to generate significant structural alterations.	LB
p.His459Tyr	C-terminal	9	The His459 is located at the interface between the C-terminal domain and the Loop1. The larger footprint of the Tyr side chain is expected to affect both the local conformation and the catalysis	LP
p.Val460Gly	C-terminal	7	Reduction of the local hydrophobicity possibly leading to a misfolding defect [1].	LP
p.Arg462Trp	C-terminal	1	In the pkDDC amino acid sequence [35], the 462nd amino acid is a leucine residue. For this reason, it is not possible to predict the effects of the p.Arg462Trp substitution.	VUS
p.Arg462Pro#	C-terminal	#	Reduction of the catalytic efficiency and of the PLP-binding affinity [7].	P
p.Arg462Gln	C-terminal	1	In the pig kidney DDC amino acid sequence [35] the 462nd amino acid is a leucine residue [35]. For this reason, it is not possible to predict the effects of the p.Arg462Gln substitution.	VUS
p.Trp464Cys	C-terminal	8	The substitution is predicted to affect several hydrophobic contacts. Conformational changes of the C-terminal domain are predictable.	LP
p.Ala471Val	C-terminal	5	Due to the steric hindrance generated by the substitution, local conformational alterations are expected to occur.	LP
p.Ala472Val	C-terminal	1	The substitution should not affect the local conformation and thus is expected to be well tolerated.	B
p.Asp473Asn	C-terminal	1	In the pkDDC crystal structure, the 473rd amino acid is a glutamate [35]. However, this surface region should accommodate aspartate or asparagine without significant structural alteration.	B
p.Val474Gly	C-terminal	1	In the pkDDC amino acid sequence, the 474th amino acid is a leucine residue [35]. For this reason, it is not possible to predict the effects of the p.Val474Gly substitution.	VUS
p.Arg476Gln	C-terminal	1	In the pkDDC amino acid sequence, the 476th amino acid is an alanine residue [35]. For this reason, it is not possible to predict the effects of the p.Arg476Gln substitution.	VUS
p.Arg479Ser	C-terminal	1	In the pkDDC crystal structure, the 479th amino acid is not visible in the electron density map [32]. For this reason, it is not possible to predict the effects of the p.Arg479Ser substitution.	VUS
p.Arg479Gly	C-terminal	1	In the pkDDC crystal structure, the 479th amino acid is not visible in the electron density map [32]. For this reason, it is not possible to predict the effects of the p.Arg479Gly substitution.	VUS

Protein accession ID: AAB47157.1.

B, benign; *DDC*, dopa decarboxylase; LB, likely benign; LP, likely pathogenic; P, pathogenic; pkDDC, pig kidney *DDC*; PLP, pyridoxal 5′-phosphate; VUS, variant of unknown significance.

†**Pathogenic:** 1) Significant predictable structural impact, 2) high conservation score 3) involvement of residues whose pathogenic variants are already identified; **Likely pathogenic**: 1) Significant predictable structural impact, 2) low/medium/high/ conservation score; **Likely benign**: 1) modest predictable structural impact, 2) Low/medium conservation core; **Benign** 1) None predictable structural impact 2) low/medium conservation score; **VUS:** involvement of some residues that in human *DDC* are different from the corresponding ones in pkDDC.

#Classification of pathogenicity based on the structural and/or functional effects observed by means of in vitro studies.

Table 4
Pathogenicity Scoring of 203 Missense *DDC* Variants According to Different *In Silico* VEP Tools.

Variant	ACMG-AMP/ACGS scoring*	3D analysis	CADD score	Polyphen2	SIFT	ClinVar
p.Gly102Ser	Pathogenic (12)	P	26.3	Probably damaging	Deleterious	P
p.Arg347Gln	Pathogenic (12)	P	27.1	Probably damaging	Tolerated	P
p.Arg412Trp	Pathogenic (12)	LP	33.0	Probably damaging	Deleterious	P
p.Phe77Leu	Pathogenic (11)	P	23.2	Probably damaging	Tolerated	VUS
p.Ser104Phe	Pathogenic (11)	LP	31.0	Possibly damaging	Deleterious	N/A
p.Arg285Trp	Pathogenic (11)	P	31.0	Probably damaging	Deleterious	VUS
p.Arg347Gly	Pathogenic (11)	P	23.9	Probably damaging	Deleterious	N/A
p.Val60Ala	Pathogenic (10)	P	23.7	Possibly damaging	Deleterious	N/A
p.Thr69Met	Pathogenic (10)	LP	27.2	Probably damaging	Deleterious	VUS
p.Ser147Ile	Pathogenic (10)	P	24.8	Possibly damaging	Deleterious	LP
p.Leu222Pro	Pathogenic (10)	P	28.1	Probably damaging	Deleterious	N/A
p.Ser250Phe	Pathogenic (10)	LP	28.8	Probably damaging	Deleterious	P
p.Tyr332Cys	Pathogenic (10)	P	29.2	Probably damaging	Deleterious	N/A
p.Leu408Ile	Pathogenic (10)	P	26.0	Probably damaging	Deleterious	N/A
p.Gly36Arg	Likely Pathogenic (9)	LP	27.0	Probably damaging	Deleterious	N/A
p.Pro47His	Likely Pathogenic (9)	P	25.3	Probably damaging	Deleterious	N/A
p.Pro81Leu	Likely Pathogenic (9)	P	29.3	Probably damaging	Deleterious	LP
p.Arg447His	Likely Pathogenic (9)	P	29.0	Probably damaging	Deleterious	N/A
p.His70Tyr	Likely Pathogenic (8)	P	27.4	Probably damaging	Deleterious	N/A
p.His72Tyr	Likely Pathogenic (8)	P	25.2	Probably damaging	Deleterious	N/A
p.Ala91Val	Likely Pathogenic (8)	P	24.3	Probably damaging	Deleterious	VUS
p.Cys100Ser	Likely Pathogenic (8)	P	24.3	Possibly damaging	Deleterious	N/A
p.Gly102Cys	Likely Pathogenic (8)	P	27.0	Probably damaging	Deleterious	N/A
p.Trp105Cys	Likely Pathogenic (8)	LP	34.0	Probably damaging	Deleterious	N/A
p.Ser147Arg	Likely Pathogenic (8)	P	26.1	Probably damaging	Deleterious	P
p.Trp267Arg	Likely Pathogenic (8)	P	26.6	Probably damaging	Deleterious	N/A
p.Ala275Thr	Likely Pathogenic (8)	P	25.1	Probably damaging	Deleterious	P
p.Glu283Ala	Likely Pathogenic (8)	P	27.1	Probably damaging	Deleterious	N/A
p.Phe309Leu	Likely Pathogenic (8)	P	31.0	Possibly damaging	Tolerated	P
p.Arg347Trp	Likely Pathogenic (8)	P	25.9	Probably damaging	Deleterious	VUS
p.Arg358His	Likely Pathogenic (8)	P	31.0	Probably damaging	Deleterious	P
p.Arg447Cys	Likely Pathogenic (8)	P	32.0	Probably damaging	Deleterious	N/A
p.Leu38Pro	Likely Pathogenic (7)	P	29.2	Probably damaging	Deleterious	N/A
p.Arg39Pro	Likely Pathogenic (7)	LP	26.9	Probably damaging	Deleterious	VUS
p.Asp59Asn	Likely Pathogenic (7)	LP	23.9	Probably damaging	Deleterious	LP
p.Gly96Arg	Likely Pathogenic (7)	P	25.8	Probably damaging	Tolerated	P
p.Gly123Arg	Likely Pathogenic (7)	P	24.7	Probably damaging	Deleterious	VUS
p.Ser149Asn	Likely Pathogenic (7)	LP	25.1	Probably damaging	Tolerated	N/A
p.Ser149Thr	Likely Pathogenic (7)	LP	24.8	Possibly damaging	Tolerated	P
p.Gly146Arg	Likely Pathogenic (7)	LP	32.0	Probably damaging	Tolerated	N/A
p.Leu288Pro	Likely Pathogenic (7)	LP	0.739	Probably damaging	Deleterious	N/A
p.Leu408Phe	Likely Pathogenic (7)	LP	28.3	Probably damaging	Deleterious	N/A
p.Met1Thr	Likely Pathogenic (7)	LP	23.7	Probably damaging	Deleterious	N/A
p.Met1Val	Likely Pathogenic (6)	LP	22.1	Probably damaging	Deleterious	N/A
p.Arg39Trp	Likely Pathogenic (6)	LP	28.4	Probably damaging	Deleterious	VUS

p.Pro47Ala	Likely Pathogenic (6)	P	24.3	Probably damaging	Deleterious	N/A
p.Ile57Thr	Likely Pathogenic (6)	LP	22.7	Possibly damaging	Deleterious	N/A
p.Asp59His	Likely Pathogenic (6)	LP	23.9	Probably damaging	Deleterious	VUS
p.Tyr79Cys	Likely Pathogenic (6)	P	31.0	Probably damaging	Deleterious	N/A
p.Pro87Leu	Likely Pathogenic (6)	LP	27.5	Probably damaging	Deleterious	P
p.Pro129Ser	Likely Pathogenic (6)	LP	24.8	Probably damaging	Deleterious	N/A
p.Gly140Arg	Likely Pathogenic (6)	LP	24.6	Probably damaging	Deleterious	VUS
p.Arg160Trp	Likely Pathogenic (6)	P	27.7	Probably damaging	Deleterious	VUS
p.Arg358Cys	Likely Pathogenic (6)	LP	33.0	Probably damaging	Deleterious	N/A
p.Tyr346Cys	Likely Pathogenic (6)	LP	26.2	Probably damaging	Deleterious	VUS
p.Leu353Pro	Likely Pathogenic (6)	P	31.0	Probably damaging	Deleterious	N/A
p.Gly354Cys	Likely Pathogenic (6)	LP	29.2	Probably damaging	Deleterious	N/A
p.Met362Thr	Likely Pathogenic (6)	LP	25.6	Probably damaging	Deleterious	VUS
p.Tyr369Cys	Likely Pathogenic (6)	LP	25.9	Probably damaging	Deleterious	VUS
p.Ser108Asn	Likely Pathogenic (6)	LP	22.5	Probably damaging	Deleterious	N/A
p.Ala110Glu	Likely Pathogenic (6)	P	25.3	Probably damaging	Deleterious	N/A
p.Gly142Arg	Likely Pathogenic (6)	LP	25.2	Probably damaging	Deleterious	VUS
p.Arg160Gly	Likely Pathogenic (6)	P	25.7	Probably damaging	Deleterious	N/A
p.Asp189Tyr	Likely Pathogenic (6)	LP	25.3	Probably damaging	Deleterious	N/A
p.Ala198Thr	Likely Pathogenic (6)	LP	25.2	Probably damaging	Deleterious	N/A
p.Phe237Ser	Likely Pathogenic (6)	LP	29.0	Probably damaging	Deleterious	VUS
p.Thr245Ile	Likely Pathogenic (6)	P	27.9	Probably damaging	Deleterious	N/A
p.Phe251Ser	Likely Pathogenic (6)	LP	27.8	Probably damaging	Deleterious	N/A
p.Asp252Gly	Likely Pathogenic (6)	LP	28.9	Probably damaging	Deleterious	N/A
p.Cys281Trp	Likely Pathogenic (6)	P	23.2	Probably damaging	Deleterious	N/A
p.Arg285Gln	Likely Pathogenic (6)	LP	25.3	Probably damaging	Deleterious	VUS
p.Cys410Gly	Likely Pathogenic (6)	P	31.0	Probably damaging	Deleterious	P
p.Cys438Arg	Likely Pathogenic (6)	LP	27.6	Probably damaging	Deleterious	N/A
p.Val382Phe	Likely Pathogenic (6)	LP	25.5	Probably damaging	Deleterious	N/A
p.Leu446Pro	Likely Pathogenic (6)	LP	31.0	Probably damaging	Deleterious	N/A
p.Arg453Cys	Likely Pathogenic (6)	P	31.0	Probably damaging	Deleterious	LP
p.Val460Gly	Likely Pathogenic (6)	LP	25.3	Possibly damaging	Deleterious	N/A
p.Arg462Pro	Likely Pathogenic (6)	P	0.322	Benign	Tolerated	N/A
p.Glu25Lys	Hot VUS (5)	LB	21.6	Benign	Tolerated	VUS
p.Val33Leu	Hot VUS (5)	LP	22.8	Benign	Deleterious	N/A
p.Arg39Gln	Hot VUS (5)	LP	24.2	Probably damaging	Tolerated	VUS
p.Ser84Arg	Hot VUS (5)	LP	26.3	Possibly damaging	Tolerated	N/A
p.Leu122Phe	Hot VUS (5)	LP	23.9	Probably damaging	Tolerated	N/A
p.Pro330Leu	Hot VUS (5)	P	26.3	Probably damaging	Deleterious	N/A
p.Gly354Ser	Hot VUS (5)	LP	23.9	Probably damaging	Tolerated	N/A
p.Gly354Ala	Hot VUS (5)	LP	26.6	Probably damaging	Tolerated	VUS
p.Arg412Gln	Hot VUS (5)	LB	32.0	Probably damaging	Deleterious	VUS
p.Arg453His	Hot VUS (5)	LP	23.9	Possibly damaging	Tolerated	VUS
p.Ile24Thr	Warm VUS (4)	LP	25.6	Probably damaging	Deleterious	LP
p.Val29Ile	Warm VUS (4)	LP	24.8	Possibly damaging	Deleterious	VUS
p.Ile57Val	Warm VUS (4)	B	2.474	Benign	Tolerated	VUS
p.Gly67Glu	Warm VUS (4)	LP	26.5	Probably damaging	Deleterious	VUS

p.Trp71Leu	Warm VUS (4)	LP	29.9	Probably damaging	Deleterious	VUS
p.Pro74Leu	Warm VUS (4)	LP	31.0	Probably damaging	Deleterious	VUS
p.Ala78Thr	Warm VUS (4)	LP	27.2	Probably damaging	Deleterious	VUS
p.Ala78Ser	Warm VUS (4)	LP	26.0	Probably damaging	Deleterious	VUS
p.Ser85Leu	Warm VUS (4)	P	29.7	Probably damaging	Deleterious	N/A
p.Met93Val	Warm VUS (4)	LP	23.1	Possibly damaging	Deleterious	N/A
p.Ile98Thr	Warm VUS (4)	LP	26.7	Probably damaging	Deleterious	N/A
p.Ser108Arg	Warm VUS (4)	LP	25.1	Probably damaging	Deleterious	VUS
p.Pro109Leu	Warm VUS (4)	LP	27.3	Probably damaging	Deleterious	VUS
p.Glu115Lys	Warm VUS (4)	LP	25.5	Probably damaging	Deleterious	N/A
p.Trp121Arg	Warm VUS (4)	LP	28.4	Probably damaging	Deleterious	VUS
p.Gly138Arg	Warm VUS (4)	LP	23.3	Possibly damaging	Deleterious	VUS
p.Gly140Glu	Warm VUS (4)	LP	24.2	Probably damaging	Deleterious	VUS
p.Ala159Val	Warm VUS (4)	LP	26.2	Probably damaging	Deleterious	VUS
p.Asp189His	Warm VUS (4)	LP	24.9	Probably damaging	Deleterious	VUS
p.Gln190Leu	Warm VUS (4)	LP	32.0	Possibly damaging	Deleterious	VUS
p.His192Tyr	Warm VUS (4)	LP	25.5	Probably damaging	Deleterious	VUS
p.Val240Phe	Warm VUS (4)	LP	24.4	Probably damaging	Deleterious	VUS
p.Ala241Thr	Warm VUS (4)	LP	22.8	Possibly damaging	Deleterious	VUS
p.Cys261Phe	Warm VUS (4)	LP	33.0	Probably damaging	Deleterious	N/A
p.Asp271Val	Warm VUS (4)	P	25.9	Probably damaging	Deleterious	N/A
p.Gly276Ser	Warm VUS (4)	LP	25.1	Probably damaging	Deleterious	VUS
p.Asp295Gly	Warm VUS (4)	LP	32.0	Probably damaging	Deleterious	VUS
p.Phe299Ser	Warm VUS (4)	LP	32.0	Probably damaging	Deleterious	VUS
p.Pro330Thr	Warm VUS (4)	P	25.1	Probably damaging	Deleterious	VUS
p.Gln350Glu	Warm VUS (4)	LP	25.4	Probably damaging	Deleterious	VUS
p.Arg379Cys	Warm VUS (4)	P	32.0	Probably damaging	Deleterious	VUS
p.Leu406Gln	Warm VUS (4)	LP	32.0	Probably damaging	Deleterious	VUS
p.Cys451Phe	Warm VUS (4)	LP	27.0	Probably damaging	Deleterious	VUS
p.Met13Ile	Tepid VUS (3)	LB	23.4	Possibly damaging	Tolerated	VUS
p.Ala18Val	Tepid VUS (3)	LP	1.717	Probably damaging	Deleterious	VUS
p.Arg27His	Tepid VUS (3)	LP	26.6	Probably damaging	Tolerated	VUS
p.Pro40Leu	Tepid VUS (3)	LP	23.0	Possibly damaging	Tolerated	VUS
p.Asp51Asn	Tepid VUS (3)	LB	23.4	Benign	Deleterious	VUS
p.Glu61Lys	Tepid VUS (3)	LP	25.4	Probably damaging	Tolerated	N/A
p.Val68Met	Tepid VUS (3)	LP	24.0	Benign	Deleterious	N/A
p.Ser84Asn	Tepid VUS (3)	LP	16.0	Benign	Tolerated	VUS
p.Ala106Val	Tepid VUS (3)	LP	19.1	Benign	Tolerated	VUS
p.Ala131Ser	Tepid VUS (3)	LP	2.953	Benign	Tolerated	VUS
p.Ala137Asp	Tepid VUS (3)	LP	2.736	Benign	Tolerated	VUS
p.Ala155Ser	Tepid VUS (3)	LP	21.7	Benign	Deleterious	VUS
p.Lys162Asn	Tepid VUS (3)	LP	21.3	Probably damaging	Tolerated	VUS
p.Ala178Asp	Tepid VUS (3)	LP	18.1	Benign	Tolerated	VUS
p.Glu196Gly	Tepid VUS (3)	LB	28.7	Probably damaging	Deleterious	VUS
p.Ala208Asp	Tepid VUS (3)	LP	16.6	Benign	Tolerated	VUS
p.Pro210Leu	Tepid VUS (3)	LP	24.5	Benign	Tolerated	LB
p.Ala216Val	Tepid VUS (3)	LB	21.0	Benign	Tolerated	VUS

p.Arg218Ser	Tepid VUS (3)	LB	23.3	Probably damaging	Tolerated	VUS
p.Ala225Val	Tepid VUS (3)	LP	22.8	Benign	Tolerated	VUS
p.His459Tyr	Tepid VUS (3)	LP	24.8	Probably damaging	Tolerated	N/A
p.Gly258Ser	Tepid VUS (3)	LP	27.8	Probably damaging	Tolerated	VUS
p.Leu306Val	Tepid VUS (3)	LB	19.5	Possibly damaging	Deleterious	VUS
p.Asn308Ile	Tepid VUS (3)	LP	31.0	Probably damaging	Deleterious	VUS
p.His337Tyr	Tepid VUS (3)	LP	22.8	Benign	Tolerated	VUS
p.His337Arg	Tepid VUS (3)	LP	24.0	Benign	Tolerated	LB
p.Ile378Val	Tepid VUS (3)	LB	26.8	Probably damaging	Tolerated	VUS
p.Arg379His	Tepid VUS (3)	P	29.3	Probably damaging	Deleterious	VUS
p.Arg393Cys	Tepid VUS (3)	VUS	24.0	Possibly damaging	Tolerated	VUS
p.Arg397His	Tepid VUS (3)	LP	27.2	Probably damaging	Deleterious	VUS
p.Gly415Asp	Tepid VUS (3)	LP	25.8	Benign	Tolerated	VUS
p.Val436Ala	Tepid VUS (3)	LP	28.0	Probably damaging	Tolerated	VUS
p.Asp442Tyr	Tepid VUS (3)	VUS	31	Probably damaging	Deleterious	VUS
p.Glu456Val	Tepid VUS (3)	LB	22.3	Benign	Deleterious	VUS
p.Arg462Trp	Tepid VUS (3)	VUS	13.97	Benign	Tolerated	VUS
p.Trp464Cys	Tepid VUS (3)	LP	24.3	Possibly damaging	Deleterious	VUS
p.Val474Gly	Tepid VUS (3)	VUS	24.5	Probably damaging	Deleterious	VUS
p.Met21Val	Cool VUS (2)	VUS	0.108	Benign	Tolerated	VUS
p.Arg27Cys	Cool VUS (2)	LP	28.1	Probably damaging	Tolerated	VUS
p.Pro43Arg	Cool VUS (2)	LP	25.6	Probably damaging	Deleterious	VUS
p.Ala45Thr	Cool VUS (2)	B	0.001	Benign	Tolerated	N/A
p.Ala45Val	Cool VUS (2)	B	13.88	Benign	Deleterious	VUS
p.Ile63Leu	Cool VUS (2)	B	18.9	Benign	Tolerated	VUS
p.Tyr75Phe	Cool VUS (2)	LP	19.01	Benign	Tolerated	VUS
p.Met89Val	Cool VUS (2)	LB	14.98	Benign	Tolerated	VUS
p.Leu90Val	Cool VUS (2)	LB	16.07	Benign	Tolerated	VUS
p.Glu127Lys	Cool VUS (2)	VUS	7.603	Benign	Tolerated	VUS
p.Thr152Asn	Cool VUS (2)	LP	25.9	Probably damaging	Deleterious	VUS
p.Arg166Gln	Cool VUS (2)	LB	5.484	Benign	Tolerated	VUS
p.Gln176Lys	Cool VUS (2)	LP	16.79	Benign	Deleterious	VUS
p.Ile179Leu	Cool VUS (2)	B	15.47	Benign	Tolerated	VUS
p.Met180Val	Cool VUS (2)	B	15.94	Benign	Tolerated	VUS
p.Glu196Lys	Cool VUS (2)	LP	26.6	Probably damaging	Deleterious	VUS
p.Glu227Gln	Cool VUS (2)	B	17.96	Benign	Tolerated	N/A
p.Thr247Ala	Cool VUS (2)	VUS	9.838	Benign	Tolerated	VUS
p.Asn253Ser	Cool VUS (2)	LB	10.2	Benign	Tolerated	VUS
p.Thr323Met	Cool VUS (2)	LP	14.99	Benign	Tolerated	VUS
p.Arg393His	Cool VUS (2)	VUS	6.344	Benign	Tolerated	VUS
p.Gln394Arg	Cool VUS (2)	B	20.2	Benign	Tolerated	VUS
p.Ala422Val	Cool VUS (2)	LB	14.08	Benign	Tolerated	VUS
p.Ser429Asn	Cool VUS (2)	B	5.389	Benign	Tolerated	VUS
p.Arg479Ser	Cool VUS (2)	VUS	14.96	Benign	Tolerated	VUS
p.Ala3Thr	Cold VUS (1)	LB	0.002	Benign	Tolerated	LB/VUS
p.Arg7Gln	Cold VUS (1)	LP	24.5	Probably damaging	Tolerated	VUS
p.Leu41Met	Cold VUS (1)	LP	23.2	Probably damaging	Tolerated	VUS

p.Val60Ile	Cold VUS (1)	LB	0.183	Benign	Tolerated	VUS
p.Gln176Arg	Cold VUS (1)	LB	14.5	Benign	Deleterious	VUS
p.Val184Leu	Cold VUS (1)	LP	24.6	Benign	Deleterious	B/LB
p.Met239Leu	Cold VUS (1)	B	20.3	Benign	Tolerated	LB
p.Ala471Val	Cold VUS (1)	LP	23.1	Possibly damaging	Tolerated	VUS
p.Ala472Val	Cold VUS (1)	B	2.235	Benign	Tolerated	N/A
p.Arg476Gln	Cold VUS (1)	VUS	0.001	Benign	Tolerated	VUS
p.Val270Ile	Ice Cold VUS (0)	LB	7.903	Benign	Tolerated	VUS
p.Lys432Asn	Ice Cold VUS (0)	LB	19.43	Possibly damaging	Tolerated	VUS
p.Thr454Met	Ice Cold VUS (0)	VUS	7.424	Benign	Tolerated	VUS
p.Asp473Asn	Ice Cold VUS (0)	B	2.474	Benign	Tolerated	VUS
p.Arg479Gly	Ice Cold VUS (0)	VUS	10.52	Benign	Tolerated	LB
p.Glu61Asp	Likely Benign (-4)	LP	16.85	Benign	Tolerated	B/LB
p.Met239Ile	Likely Benign (-4)	B	17.99	Benign	Tolerated	B
p.Met17Val	Benign (-10)	LB	1.244	Benign	Tolerated	B
p.Met217Val	Benign (-10)	B	17.24	Benign	Tolerated	B
p.Arg462Gln	Benign (-10)	VUS	0.204	Benign	Tolerated	B/LB

*for description of ACMG/ACGS criteria see Material and Methods section.

Accession IDs: NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.

Legend

ACMG-AMP/ACGS classification	Score	3D analysis	ClinVar	PolyPhen2	SIFT	CADD	Color Map
Pathogenic (P)	>10	P	P	Probably damaging	Deleterious		
Likely pathogenic (LP)	6 to 9	LP	LP	Possibly damaging		LP (>20)	
Hot VUS	5						
Warm VUS	4						
Tepid VUS	3						
Cool VUS	2						
Cold VUS	1						
Ice cold VUS	0	VUS	VUS				
Likely benign (LB)	-1 to -5	LB	LB			LB (<20)	
Benign (B)	<-6	B	B	B	Tolerated		
N/A			Not listed				

*for description of ACMG-AMP/ACGS criteria see Material and Methods section.
Accession IDs: NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.
3D analysis: Maestro v12.2 software (Schrödinger) and PyMol v2.0 software (Schrödinger); ACGS: Association for Clinical Genomic Science; ACMG: American College of Medical Genetics; CADD: Combined Annotation Dependent Depletion (https://cadd.gs.washington.edu/); ClinVar: Public archive of the relationships among human variations and phenotypes (https://www.ncbi.nlm.nih.gov/clinvar/); NTD: neurotransmitter disorder; Polyphen2: Polymorphism phenotyping v2 (http://genetics.bwh.harvard.edu/pph2/); SIFT: Sorting intolerant from tolerant (https://sift.bii.a-star.edu.sg/); VUS: Variant of unknown significance; B: B; LB: L; P: P; LP: LP. VEP: variant effect prediction.

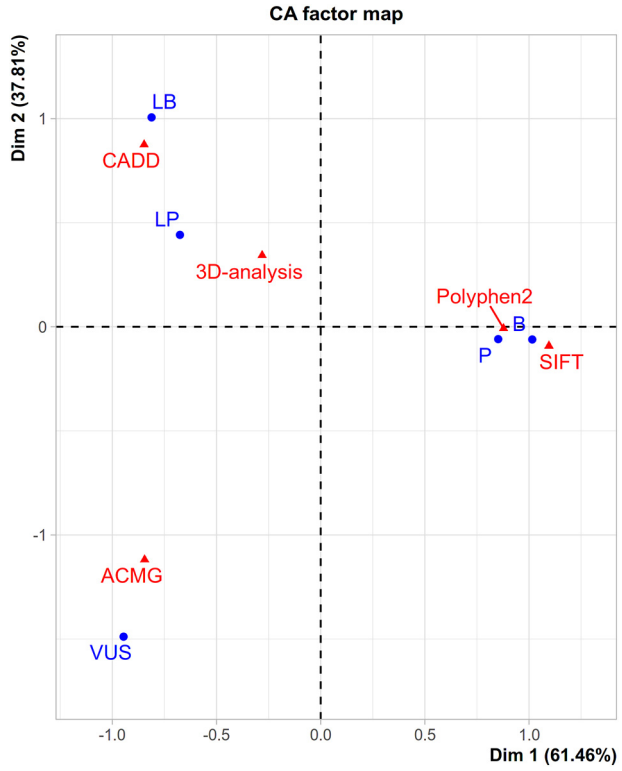

Fig. 5. Biplot of the correspondence analysis (CA). The first two dimensions can account for about 98% of the total variation. Three groups can be identified: ACMG, CADD with 3D-analysis, and Polyphen2 with SIFT. B, benign; LB, likely benign; LP, likely pathogenic; P, pathogenic; VUS, variant of unknown significance.

disorder have limited efficacy [2]. However, a gene therapy for the treatment of AADC deficiency is now approved, with a recent study demonstrating that greater clinical benefits are achieved the earlier it is administered [18,20]. Thus, early diagnosis, which may be dependent in part on interpretation of *DDC* genetic testing results, is critical for improved patient outcomes.

It should be, however, noted that *DDC* variant classification according to the ACMG-AMP/ACGS does not necessarily describe the severity of the metabolic phenotype, as this depends on the residual enzyme activity, which is in turn defined by the corresponding gene variant. Unfortunately, no residual enzyme activity was reported for most missense variants, and a genotype-phenotype correlation is difficult to elucidate due to a relatively low number of patients with the same genotype. Additionally, most of the identified patients are compound heterozygotes for two different variants, and it has been shown in other inherited metabolic disorders with a large cohort of patients that the milder of the two variants is always dominant over the more severe one and thus determines the metabolic phenotype (interallelic complementation and epigenetic factors excluded) [41].

A ClinGen *DDC* variant curation expert panel (VCEP) was recently established (https://clinicalgenome.org/affiliation/50118/) and will provide high quality and systematic curation of *DDC* variants.

5. Conclusions

AADC deficiency is difficult to diagnose and likely more prevalent than is generally acknowledged. An accurate diagnosis is critical to ensure patients receive appropriate treatment as early as possible. As AADC deficiency is a recessive genetic disorder, an accurate diagnosis is also important so that families can be informed and educated about recurrence risks. Human *DDC* gene therapy represents a promising new avenue for the treatment of AADC deficiency and is agnostic to the genotype of the affected patient. Timely diagnosis and initiation of appropriate therapy depend on a robust understanding of the association between *DDC* variants, their molecular effects, and the various AADC deficiency phenotypes.

A total of 422 *DDC* variants are now reported in the PNDdb (http://www.biopku.org/home/pnddb.asp). The use of *in silico* variant interpretation tools, combined with structural 3D modeling of variant proteins and applied comparative analysis, improved the current *DDC* variant interpretation recommendations, particularly of VUSs.

Disclosures

Nastassja Himmelreich, Riccardo Montioli, Sven Garbade, and Carla Carducci have nothing to disclose. Carla B. Voltattorni is a member of the Scientific Advisory Council of the Oxalosis & Hyperoxaluria Foundation. Nenad Blau has served as a consultant for Merck Serono SA, BioMarin Pharmaceuticals Ltd., Censa Pharmaceuticals, Synlogic Inc., Nestle, Ipsen Pharmaceuticals, Homology Medicine Inc., Sangamo Therapeutics, SOM Biotech, and PTC Therapeutics/Agilis Biotherapeutics. Jeffrey Kopesky is a full-time employee of PTC Therapeutics, Inc. Sarah Elsea is a recipient of research grant funding from PTC Therapeutics and is an employee of Baylor College of Medicine which derives revenue from genetic testing at Baylor Genetics.

Data availability

Data will be made available on request.

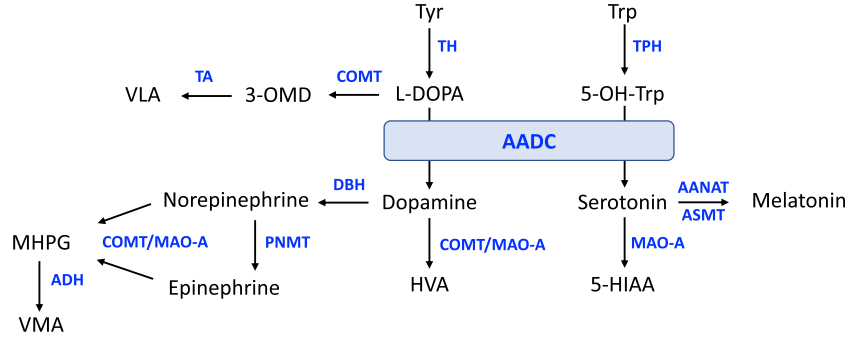

Fig. 6. Biosynthesis and catabolism of catecholamines and serotonin. 3-OMD:3-*O*-methyldopa, 5-HIAA: 5-hydroxyindoleacetic acid, 5-OH-Trp: 5-hydroxytryptophan, AADC: aromatic L-amino acid decarboxylase, AANAT: serotonin-N-acetyltransferase, ADH: alcohol dehydrogenase, ASMT: N-acetylserotonin *O*-methyltransferase, COMT: catechol *O*-methyltransferase, DBH: dopamine beta-hydroxylase, HVA: homovanillic acid, L-DOPA: 3,4-dihydroxyphenylalanine, MAO-A: monoamine oxidase A, MHPG: 3-methoxy-4-hydroxyphenylglycol, PNMT: phenylethanolamine N-methyltransferase, TA: transaminase, TH: tyrosine hydroxylase, TPH: tryptophan hydroxylase, VLA: vanillactic acid, VMA: vanillylmandelic acid.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2022.11.003.

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