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Prevalence of *DDC* genotypes in patients with aromatic L-amino acid decarboxylase (AADC) deficiency and *in silico* prediction of structural protein changes

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ABSTRACT

Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare autosomal recessive genetic disorder affecting the biosynthesis of dopamine, a precursor of both norepinephrine and epinephrine, and serotonin. Diagnosis is based on the analysis of CSF or plasma metabolites, AADC activity in plasma and genetic testing for variants in the *DDC* gene. The exact prevalence of AADC deficiency, the number of patients, and the variant and genotype prevalence are not known. Here, we present the *DDC* variant ($n = 143$) and genotype ($n = 151$) prevalence of 348 patients with AADC deficiency, 121 of whom were previously not reported. In addition, we report 26 new *DDC* variants, classify them according to the ACMG/AMP/ACGS recommendations for pathogenicity and score them based on the predicted structural effect. The splice variant c.714+4A>T, with a founder effect in Taiwan and China, was the most common variant (allele frequency = 32.4%), and c.[714+4A>T];[714+4A>T] was the most common genotype (genotype frequency = 21.3%). Approximately 90% of genotypes had variants classified as pathogenic or likely pathogenic, while 7% had one VUS allele and 3% had two VUS alleles. Only one benign variant was reported. Homozygous and compound heterozygous genotypes were interpreted in terms of AADC protein and categorized as: i) devoid of full-length AADC, ii) bearing one type of AADC homodimeric variant or iii) producing an AADC protein population composed of two homodimeric and one heterodimeric variant. Based on structural features, a score was attributed for all homodimers, and a tentative prediction was advanced for the heterodimer. Almost all AADC protein variants were pathogenic or likely pathogenic.

1. Introduction

Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM 107930) is an inherited disorder of neurotransmitter biosynthesis with autosomal recessive inheritance [1,2]. Pathogenic variants in the *DDC* gene encoding the enzyme AADC lead to a severe combined deficiency of serotonin and catecholamines, clinically characterized by infantile hypotonia, ptosis, oculogyric crises, dystonia and hyperkinetic movements, excessive sweating, stridor and nasal congestion, hypersomnolence or insomnia, autonomic dysfunction and developmental delay [3–7]. Serotonin and dopamine are formed following the hydroxylation of tryptophan and tyrosine by tryptophan and tyrosine hydroxylases, respectively, and by a subsequent decarboxylation of the corresponding intermediates 5-hydroxytryptophan and L-dopa by a pyridoxal phosphate (PLP)–dependent AADC [8].

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 with the same elements of the neighboring subunit to stabilize the dimeric structure [9,10]. Biochemical and bioinformatics studies have revealed a variety of effects of pathogenic AADC variants, including structural and functional defects of the enzyme, such as decreased catalytic efficiency, decreased PLP binding affinity, and misfolding defects [11].

Because of the AADC deficiency, a specific pattern of neurotransmitter metabolites in CSF and plasma is highly diagnostic: low homovanillic acid (HVA) acid and 5-hydroxyindoleacetic acid (5HIAA) and elevated L-dopa, 5-hydroxytryptophan, and 3-*O*-methyldopa (3OMD) in CSF and 3OMD in plasma [4,12,13]. 3OMD can be easily quantified in dried blood spots (DBSs) and used in the newborn screening for AADC deficiency [14–16].

It would be important to estimate the total number of AADC-deficient patients, because most patients experience an unrelenting disease course with poor or no response to conventional medical therapy, which includes dopamine agonists, monoamine oxidase inhibitors, pyridoxine derivatives and anticholinergic agents, melatonin, and benzodiazepines [4]. Gene therapy, using targeted delivery of an AAV2-AADC to either the bilateral midbrain or bilateral putamen has been investigated in several early-stage clinical trials. The results indicate that gene delivery can be safely performed and produce disease-modifying improvements in autonomous symptoms and motor function [17–20].

A total of 581 *DDC* variants have been identified (http://biopku.org/home/pnddb.asp; as of March 8, 2023). Variants have been identified on nearly all introns and exons of the *DDC* gene, as well as in the 3′ and 5′ untranslated regions. The largest percentage of the identified variants (48%) were classified as missense variants and the pathogenicity was classified using the ACMG/AMP/ACGS criteria [21].

The aim of this study was to estimate the total number of patients with AADC deficiency (published and unpublished), to calculate the genotype and variant frequencies and to predict (*in silico*) the effects of interallelic complementation in compound heterozygous patients on the AADC protein population.

2. Materials and methods

We searched PubMed with the terms “aromatic L-amino acid decarboxylase”, “AADC”, “DDC”, and “dopa-decarboxylase” in combination with “deficiency”, “genotype”, “mutations”, “variants”, “diagnosis”, “gene therapy” and “prevalence” between January 1990 and January 2023. We gave preference to papers published within the past 5 years but did not exclude some important but less recent publications. No restriction was placed on the language of publications.

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), and the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php). All variants were tested with LUMC Mutalyzer 3 (https://mutalyzer.nl/) 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 or provided by the authors of this study.

Table 1
DDC genotypes reported in 348 patients with AADC deficiency, frequency, and pathogenicity interpretation.

Genotype (cDNA)	Genotype (Protein / Name)	ACMG/AMP/ACGS score	n	GF %	References
c.[714+4A>T];[714+4A>T]	IVS6+4A>T;IVS6+4A>T	**P/P**	74	21.3	[3,5,12,32–40] This study
c.[1040G>A];[1040G>A]	p.[Arg347Gln];[Arg347Gln]	**P/P**	18	5.2	[36,38,41–45] This study
c.[714+4A>T];[1234C>T]	IVS6+4A>T;p.Arg412Trp	**P/P**	13	3.7	[3,5,20,36,38,39] This study
c.[714+4A>T];[1297dup]	IVS6+4A>T;p.Ile433AsnfsTer60	**P/P**	13	3.7	[5,17,36,38,39] This study
c.[749C>T];[749C>T]	p.[Ser250Phe];[Ser250Phe]	**P/P**	9	2.6	[3,46,47] This study
c.[175G>A];[714+4A>T]	p.[Asp59Asn];IVS6+4A>T	**P/P**	7	2	[20,35,36,39] This study
c.[179T>C];[714+4A>T]	p.[Val60Ala];IVS6+4A>T	**P/P**	5	1.4	[5,38,39]
c.[242C>T];[242C>T]	p.[Pro81Leu];[Pro81Leu]	**P/P**	5	1.4	This study
c.[48C>A];[116G>C]	p.[Tyr16Ter];[Arg39Pro]	**P/P**	4	1.1	[48] This study
c.[106G>A];[714+4A>T]	p.[Gly36Arg];IVS6+4A>T	**P/P**	4	1.1	[36,38,39]
c.[175G>A];[175G>A]	p.[Asp59Asn];[Asp59Asn]	**P/P**	4	1.1	[49,50] This study
c.[175G>A];[1072C>T]	p.[Asp59Asn];[Arg358Cys]	**P/P**	4	1.1	This study
c.[208C>T];[208C>T]	p.[His70Tyr];[His70Tyr]	**P/P**	4	1.1	[3,49,51] This study
c.[304G>A];[304G>A]	p.[Gly102Ser];[Gly102Ser]	**P/P**	4	1.1	[3,39]
c.[714+4A>T];[1058T>C]	IVS6+4A>T;p.Leu353Pro	**P/LP**	4	1.1	[5,40] This study
c.[571-3C>G];[571-3C>G]	IVS5-3C>G;IVS5-3C>G	**LP/P**	4	1.1	[3,49]
c.[140C>A];[140C>A]	p.[Pro47His];[Pro47His]	**P/P**	3	1.1	This study
c.[230T>C];[823G>A]	p.[Phe77Ser];[Ala275Thr]	**LP/P**	3	0.9	[5,40] This study
c.[304G>A];[714+4A>T]	p.[Gly102Ser];IVS6+4A>T	**P/P**	3	0.9	[19,39,40]
c.[714+4A>T];[1312T>C]	IVS6+4A>T;p.Cys438Arg	**P/LP**	3	0.9	[41] This study
c.[823G>A];[823G>A]	p.[Ala275Thr];[Ala275Thr]	**P/P**	3	0.9	[3,52]
c.[1340G>A];[1340G>A]	p.[Arg447His];[Arg447His]	**P/P**	3	0.9	[3,50] This study
c.[206C>T];[206C>T]	p.[Thr69Met];[Thr69Met]	**P/P**	3	0.9	[53] This study
c.[1234C>T];[1234C>T]	p.[Arg412Trp];[Arg412Trp]	**P/P**	3	0.9	[3,41]
c.[73G>A];[1379T>G]	p.[Glu25Lys];[Val460Gly]	**LP/LP**	2	0.9	[28]
c.[105del];[710T>C]	p.[Tyr37ThrfsTer5];[Phe237Ser]	**P/LP**	2	0.6	[49]
c.[140C>A];[1072C>T]	p.[Pro47His];[Arg358Cys]	**P/P**	2	0.6	This study
c.[179T>C];[179T>C]	p.[Val60Ala];[Val60Ala]	**P/P**	2	0.6	[43] This study
c.[179T>C];[440G>T]	p.[Val60Ala];[Ser147Ile]	**P/P**	2	0.6	[29]
c.[202G>A];[254C>T]	p.[Val68Met];[Ser85Leu]	**LP/LP**	2	0.6	[14]
c.[231C>A];[231C>A]	p.[Phe77Leu];[Phe77Leu]	**P/P**	2	0.6	[33]
c.[260C>T];[446G>A]	p.[Pro87Leu];[Ser149Asn]	**P/LP**	2	0.6	[5] This study
c.[286G>A];[714+4A>T]	p.[Gly96Arg];IVS6+4A>T	**LP/P**	2	0.6	[38] This study
c.[322A>C];[812A>T]	p.[Ser108Arg];[Asp271Val]	**LP/LP**	2	0.6	This study
c.[322A>C];[876G>A]	p.[Ser108Arg];[Glu292=]	**LP/Hot VUS**	2	0.6	This study
c.[367G>A];[424G>A]	p.[Gly123Arg];[Gly142Arg]	**LP/LP**	2	0.6	[38] This study
c.[367G>A];[734C>T]	p.[Gly123Arg];[Thr245Ile]	**LP/LP**	2	0.6	[3,38]
c.[367G>A];[876G>A]	p.[Gly123Arg];[Glu292=]	**LP/Hot VUS**	2	0.6	This study
c.[647_653dup];[853C>T]	p.[Ala219HisfsTer36];[Arg285Trp]	**P/P**	2	0.6	This study
c.[714+4A>T];[811_812delinsTG]	IVS6+4A>T;p.Asp271Cys	**P/LP**	2	0.6	[3]
c.[714+4A>T];[853C>T]	IVS6+4A>T;p.Arg285Trp	**P/P**	2	0.6	[39] This study
c.[714+4A>T];[1339C>T]	IVS6+4A>T;p.Arg447Cys	**P/LP**	2	0.6	[3]
c.[1040G>A];[1073G>A]	p.[Arg347Gln];[Arg358His]	**P/P**	2	0.6	[54]
c.[1040G>A];?	p.[Arg347Gln];Ex2_3del	**P/P**	2	0.6	[3,5]
c.[1234C>T];[1297dup]	p.[Arg412Trp];[Ile433AsnfsTer60]	**P/P**	2	0.6	[55] This study
c.[989C>T];[989C>T]	p.[Pro330Leu];[Pro330Leu]	**P/P**	2	0.6	[31]
c.[1A>G];[181G>A]	p.[Met1Val];[Glu61Lys]	**LP/LP**	1	0.3	[38]
c.[2T>C];[277A>G]	p.[Met1Thr];[Met93Val]	**LP/LP**	1	0.3	[39]
c.[19C>T];[214C>T]	p.[Arg7Ter];[His72Tyr]	**P/P**	1	0.3	[38]
c.[19C>T];[299G>C]	p.[Arg7Ter];[Cys100Ser]	**P/LP**	1	0.3	[44]
c.[19C>T];[304G>A]	p.[Arg7Ter];[Gly102Ser]	**P/P**	1	0.3	[19]
c.[19C>T];[592G>A]	p.[Arg7Ter];[Ala198Thr]	**P/LP**	1	0.3	[42]
c.[19C>T];[841T>C]	p.[Arg7Ter];[Cys281Arg]	**P/LP**	1	0.3	This study
c.[19C>T];[1222C>A]	p.[Arg7Ter];[Leu408Ile]	**P/P**	1	0.3	[3]
c.[23G>A];[23G>A]	p.[Arg8Lys];[Arg8Lys]	**Hot VUS/Hot VUS**	1	0.3	This study
c.[44A>G];[44A>G]	p.[Asp15Gly];[Asp15Gly]	**LP/LP**	1	0.3	[30]
c.[58_60del];[714+4A>T]	p.[Tyr20del];[IVS6+4A>T]	**Hot VUS/P**	1	0.3	[5]
c.[73G>A];[315G>C]	p.[Glu25Lys];[Trp105Cys]	**LP/LP**	1	0.3	This study
c.[73G>A];[624del]	p.[Glu25Lys];[Ile209SerfsTer26]	**LP/P**	1	0.3	[38]
c.[73G>A];[1073G>A]	p.[Glu25Lys];[Arg358His]	**LP/P**	1	0.3	[38]
c.[97G>C];[1385G>C]	p.[Val33Leu];[Arg462Pro]	**LP/LP**	1	0.3	[27]
c.[106G>A];[106G>A]	p.[Gly36Arg];[Gly36Arg]	**P/P**	1	0.3	This study
c.[106G>A];[1340G>A]	p.[Gly36Arg];[Arg447His]	**P/P**	1	0.3	[41]
c.[113T>C];[714+4A>T]	p.[Leu38Pro];IVS6+4A>T	**LP/P**	1	0.3	[3]
c.[121C>A];[121C>A]	p.[Leu41Met];[Leu41Met]	**Tepid VUS/Tepid VUS**	1	0.3	[56]
c.[139C>G];[1339C>T]	p.[Pro47Ala];[Arg447Cys]	**LP/LP**	1	0.3	This study
c.[170T>C];[1234C>T]	p.[Ile57Thr];[Arg412Trp]	**LP/P**	1	0.3	[36]
c.[175G>A];[286G>A]	p.[Asp59Asn];[Gly96Arg]	**P/LP**	1	0.3	[42]
c.[175G>A];[288G>A]	p.[Asp59Asn];[Gly96=]	**P/Tepid VUS**	1	0.3	This study
c.[179T>C];[175G>A]	p.[Val60Ala];[Asp59Asn]	**P/P**	1	0.3	This study

(continued on next page)

Table 1 (*continued*)

Genotype (cDNA)	Genotype (Protein / Name)	ACMG/AMP/ACGS score	n	GF %	References
c.[179T>C];[311C>T]	p.[Val60Ala];[Ser104Phe]	**P/P**	1	0.3	[14]
c.[179T>C];[1234C>T]	p.[Val60Ala];[Arg412Trp]	**P/P**	1	0.3	[36]
c.[179T>C];[1297dup]	p.[Val60Ala];[Ile433AsnfsTer60]	**P/P**	1	0.3	This study
c.[201+5G>C];[201+5G>C]	IVS2+5G>C;IVS2+5G>C	**LP/LP**	1	0.3	[14]
c.[201+5G>C];[1073G>A]	IVS2+5G>C];[Arg358His]	**LP/P**	1	0.3	[42]
c.[206C>T];[439A>C]	p.[Thr69Met];[Ser147Arg]	**P/P**	1	0.3	[3]
c.[206C>T];[1337T>C]	p.[Thr69Met];[Leu446Pro]	**P/LP**	1	0.3	[38]
c.[214C>T];[214C>T]	p.[His72Tyr];[His72Tyr]	**P/P**	1	0.3	[3]
c.[223T>G];[223T>G]	p.[Tyr75Asp];[Tyr75Asp]	**LP/LP**	1	0.3	This study
c.[223T>G];[339G>T]	p.[Tyr75Asp];[Glu113Asp]	**LP/LP**	1	0.3	This study
c.[236A>G];[714+4A>T]	p.[Tyr79Cys];[IVS6+4A>T]	**P/P**	1	0.3	[3]
c.[236A>G];[755A>G]	p.[Tyr79Cys];[Asp252Gly]	**P/LP**	1	0.3	[34]
c.[250A>C];[250A>C]	p.[Ser84Arg];[Ser84Arg]	**LP/LP**	1	0.3	This study
c.[250A>C];[1063dup]	p.[Ser84Arg];[Arg355LysfsTer13]	**LP/P**	1	0.3	[36]
c.[256T>A];[989C>G]	p.[Tyr86Asn];[Pro330Arg]	**LP/LP**	1	0.3	This study
c.[260C>T];[260C>T]	p.[Pro87Leu];[Pro87Leu]	**P/P**	1	0.3	This study
c.[260C>T];[286G>A]	p.[Pro87Leu];[Gly96Arg]	**P/LP**	1	0.3	[26]
c.[260C>T];[446G>C]	p.[Pro87Leu];[Ser149Thr]	**P/LP**	1	0.3	[38]
c.[272C>T];[1228T>G]	p.[Ala91Val];[Cys410Gly]	**LP/LP**	1	0.3	[57]
c.[285C>A];[823G>A]	p.[Cys95Ter];[Ala275Thr]	**LP/P**	1	0.3	This study
c.[286G>A];[679G>C]	p.[Gly96Arg];[Glu227Gln]	**LP/Hot VUS**	1	0.3	This study
c.[289del];[629C>T]	p.[Ala97ProfsTer21];[Pro210Leu]	**P/Tepid VUS**	1	0.3	[33]
c.[293T>C];[343G>A]	p.[Ile98Thr];[Glu115Lys]	**Hot VUS/Hot VUS**	1	0.3	This study
c.[296G>A];[714+4A>T]	p.[Gly99Asp];IVS6+4A>T	**LP/P**	1	0.3	This study
c.[299G>C];[1021+1G>A]	p.[Cys100Ser];IVS10+1G>A	**LP/P**	1	0.3	[36]
c.[304G>A];[869G>A]	p.[Gly102Ser];[Gly290Glu]	**P/LP**	1	0.3	This study
c.[310T>C];[479G>A]	p.[Ser104Pro];[Arg160Gln]	**LP/LP**	1	0.3	This study
c.[315G>C];[385C>T]	p.[Trp105Cys];[Pro129Ser]	**LP/LP**	1	0.3	[58]
c.[323G>A];[1041+1G>C]	p.[Ser108Asn];IVS11+1G>C	**LP/P**	1	0.3	This study
c.[328G>A];[1140G>A]	p.[Ala110Thr];[Lys380=]	**LP/Warm VUS**	1	0.3	This study
c.[330_334dup];[1040G>A]	p.[Thr112AsnfsTer8];[Arg347Gln]	**P/P**	1	0.3	[38]
c.[339G>T];[339G>T]	p.[Glu113Asp];[Glu113Asp]	**LP/LP**	1	0.3	This study
c.[361T>C];[1040G>A]	p.[Trp121Arg];[Arg347Gln]	**LP/P**	1	0.3	[43]
c.[361T>C];[1085T>C]	p.[Trp121Arg];[Met362Thr]	**LP/LP**	1	0.3	This study
c.[364C>T];[714+4A>T]	p.[Leu122Phe];IVS6+4A>T	**LP/P**	1	0.3	[19]
c.[419G>A];[1375C>T]	p.[Gly140Glu];[His459Tyr]	**LP/LP**	1	0.3	[59]
c.[436G>C];[714+4A>T]	p.[Gly146Arg];IVS6+4A>T	**LP/P**	1	0.3	This study
c.[436-16C>T];[1040G>A]	IVS4-16C>T];[Arg347Gln]	B/P	1	0.3	This study
c.[446G>A];[714+4A>T]	p.[Ser149Asn];IVS6+4A>T	**LP/P**	1	0.3	This study
c.[446G>C];[714+4A>T]	p.[Ser149Thr];IVS6+4A>T	**LP/P**	1	0.3	This study
c.[476C>T];[476C>T]	p.[Ala159Val];[Ala159Val]	**LP/LP**	1	0.3	This study
c.[476C>T];[1325_1326insG]	p.[Ala159Val];[Asp442GlufsTer51]	**LP/LP**	1	0.3	This study
c.[478C>G];[565G>T]	p.[Arg160Gly];[Asp189Tyr]	**LP/LP**	1	0.3	[36]
c.[478C>T];[1040G>A]	p.[Arg160Trp];[Arg347Gln]	**LP/P**	1	0.3	[60]
c.[551T>C];[551T>C]	p.[Val184Ala];[Val184Ala]	**LP/LP**	1	0.3	This study
c.[557A>G];?	p.[Tyr186Cys];[Ex10_11del]	**LP/P**	1	0.3	This study
c.[557A>G];?	p.[Tyr186Cys];[Ex11_12del]	**LP/P**	1	0.3	This study
c.[564_568dup];[863T>C]	p.[Gln190ProfsTer13];[Leu288Pro]	**P/LP**	1	0.3	[43]
c.[564_568dup];[1040G>A]	p.[Gln190ProfsTer13];[Arg347Gln]	**P/P**	1	0.3	[43]
c.[629C>T];[853C>T]	p.[Pro210Leu];[Arg285Trp]	**Tepid VUS/P**	1	0.3	This study
c.[665T>C];[665T>C]	p.[Leu222Pro];[Leu222Pro]	**P/P**	1	0.3	[33]
c.[714+4A>T];[749C>T]	IVS6+4A>T;p.Ser250Phe	**P/P**	1	0.3	This study
c.[714+4A>T];[752T>C]	IVS6+4A>T;p.Phe251Ser	**P/LP**	1	0.3	[13]
c.[714+4A>T];[801G>A]	IVS6+4A>T;p.Trp267Ter	**P/P**	1	0.3	[37]
c.[714+4A>T];[848A>C]	IVS6+4A>T;p.Glu283Ala	**P/P**	1	0.3	[5]
c.[714+4A>T];[1061G>A]	IVS6+4A>T;p.Gly354Asp	**P/LP**	1	0.3	This study
c.[714+4A>T];[1093G>A]	IVS6+4A>T;p.Val365Ile	**P/LP**	1	0.3	This study
c.[714+4A>T];[1106A>G]	IVS6+4A>T;p.Tyr369Cys	**P/LP**	1	0.3	[34]
c.[714+4A>T];[1233dup]	IVS6+4A>T;p.Arg412SerfsTer10	**P/LP**	1	0.3	[19]
c.[734C>T];[734C>T]	p.[Thr245Ile];[Thr245Ile]	**LP/LP**	1	0.3	This study
c.[781+6T>C];[781+6T>C]	IVS7+6T>C;IVS7+6T>C	**LP/LP**	1	0.3	[41]
c.[782G>T];[1060G>A]	p.[Cys261Phe];[Gly354Ser]	**LP/LP**	1	0.3	This study
c.[782-6C>T];[1217T>A]	IVS7-6C>T;p.Leu406Gln	**Warm VUS/LP**	1	0.3	[19]
c.[799T>C];[799T>C]	p.[Trp267Arg];[Trp267Arg]	**LP/LP**	1	0.3	This study
c.[823G>A];[1040G>A]	p.[Ala275Thr];[Arg347Gln]	**P/P**	1	0.3	This study
c.[843C>G];[1085T>C]	p.[Cys281Trp];[Met362Thr]	**LP/LP**	1	0.3	[38]
c.[849G>C];[1066_1068del]	p.[Glu283Asp];[Arg356del]	**LP/LP**	1	0.3	This study
c.[853C>T];[1234C>T]	p.[Arg285Trp];[Arg412Trp]	**P/P**	1	0.3	This study
c.[853C>T];?	p.[Arg285Trp];Ex10_11del	**P/P**	1	0.3	This study
c.[1039C>G];[1039C>G]	p.[Arg347Gly];[Arg347Gly]	**P/P**	1	0.3	[61]
c.[1039C>T];[1039C>T]	p.[Arg347Trp];[Arg347Trp]	**P/P**	1	0.3	This study

Table 1 (*continued*)

Genotype (cDNA)	Genotype (Protein / Name)	ACMG/AMP/ACGS score	n	GF %	References
c.[1040G>A];[1123C>T]	p.[Arg347Gln];[Gln375Ter]	**P/P**	1	0.3	This study
c.[1040G>A];[1242G>A]	p.[Arg347Gln];[Lys414=]	**P/Warm VUS**	1	0.3	This study
c.[1040G>A];[1040G>A]	p.[Arg347Gln];[Arg347Gln]	**P/P**	1	0.3	This study
c.[1060G>T];[1060G>T]	p.[Gly354Cys];[Gly354Cys]	**LP/LP**	1	0.3	[49]
c.[1073G>A];[1073G>A]	p.[Arg358His];[Arg358His]	**P/P**	1	0.3	[38]
c.[1123C>T];[1123C>T]	p.[Gln375Ter];[Gln375Ter]	**P/P**	1	0.3	[62]
c.[1144G>T];[1144G>T]	p.[Val382Phe];[Val382Phe]	**LP/LP**	1	0.3	[63]
c.[1243-10A>G];[1243-10A>G]	IVS13-10A>G;IVS13-10A>G	**Warm VUS/Warm VUS**	1	0.3	This study
c.[1297dup];[714+4A>T]	p.[Ile433AsnfsTer60];IVS6+4A>T	**P/P**	1	0.3	[5]
c.[1385G>C];[1385G>C]	p.[Arg462Pro];[Arg462Pro]	**LP/LP**	1	0.3	[3]

NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.
P: pathogenic; LP: likely pathogenic, B: benign; VUS: variant of unknown significance.

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 as reported previously [21].

Conservation analyses were performed with the Consurf Server (https://consurf.tau.ac.il/) using the human AADC (*h*AADC) amino acid sequence as input data (id: p20711). A conservation score from 1 (variable residue) to 9 (conserved residue) was attributed to each residue. Protein visualization, measurement and rotamer analysis for *in silico* prediction were performed using PyMOL 2.0 (The PyMOL Molecular Graphics 50 System, Version 2.0 Schrödinger, LLC, New York, NY, 2021). The template used was the available porcine AADC (*p*AADC) crystal structure (pdb 1JS6). The type of substitution, residue localization, microenvironment, and interactions were analyzed.

3. Results

We tabulated full genotypes from 348 patients with AADC deficiency, 121 (35%) of whom were previously not reported in the literature (Table 1). Clinical information was not provided for all patients and was thus not a part of this study. All patients had a confirmed diagnosis of AADC deficiency.

The four most common genotypes (out of 151) were c.[714+4A>T];[714+4A>T] (GF = 21.3%), p.[Arg347Gln];[Arg347Gln] (GF = 5.2%), c.714 + 4A > T/p.Arg412Trp and c.714+4A>T/p.Ile433AsnfsTer60 (GF = 3.8% each). The Asian c.714+4A>T splice variant was the most common *DDC* variant with an allele frequency (AF) of 32.3% (Table 2) and was part of 30 different genotypes. Forty-eight percent (167) of patients were homozygotes for 41 different genotypes, and 181 were compound heterozygotes. In 15 patients (not included in this study) *DDC* variants were detected in only one allele.

Twenty-six new *DDC* variants (19 missense, 2 frameshift, 2 synonymous, 1 in-frame, 1 intronic, and 1 large deletion) were reported in this study (Table 3). New variants were scored for pathogenicity according to ACMG/AMP/ACGS guidelines; 22 were classified as pathogenic (P) or likely pathogenic (LP), and 4 were classified as variant of unknown significance (VUS).

In addition, crystal structure inspection was carried out to evaluate the modification determined by the genetic alteration of the AADC homodimeric protein. Of the 26 new variants, 6 did not have a 3D score since no complete AADC protein could be synthesized: 3 variants were synonymous or could affect splicing sites, 1 was a large deletion, and 2 were frameshifts that led to incomplete AADC polypeptide chains. The remaining 20 missense mutations and one in frame variant were mainly classified as P (9) and LP (10), and only one was classified as likely benign (LB) (c.23G>A/p.Arg8Lys).

The 151 genotypes were compared with the pathogenicity of their variants, and 90% (136) were a combination of two P or LP variants (Fig. 1). Only one genotype include a benign (B) variant, and in the remaining genotypes, there was one VUS variant (7%) or two VUS alleles (3%).

At the protein level, out of 41 homozygous genotypes (27%), 14% (6) were loss-of-function genotypes since no AADC protein was synthesized (splicing, deletions, insertions, and frameshifts) and were pathogenic. The remaining 35 variants (except 1 LB) predominantly led to a functionally compromised AADC protein and were classified as P (20) and LP (14) using the 3D score, in agreement with ACMG/AMP/ACGS scoring for the respective genetic variants.

Compound heterozygous genotypes (110, 73% of the total) were partitioned into: i) those possessing a splice, deletion, or frameshift mutation on both alleles (4 out of 110) that could be considered a loss-of-function since no AADC protein could be produced and ii) those possessing a splice, deletion, or frameshift mutation on one allele and a missense mutation on the other allele. These could be considered as functionally hemizygotes that, like homozygotes, produce only one type of AADC homodimer. Of these 59 combinations (55% out of 110), ranking based on 3D structural features identified 30 P combinations and 28 LP combinations and 1 LB combination in terms of AADC protein structural outcomes. As shown in Table 4, the 45 compound heterozygous genotypes, for which prediction is more complex, were comparable to the 3D scores predicted for the genetic variants that give rise to the respective homodimeric AADC proteins [21]. For the heterodimers, we introduced a scoring value that accounts for the effects caused by two different amino acid substitutions in the AADC dimeric assembly and focuses on possible direct effects at the active site. This criterion is based on the localization of each residue in the dimeric 3D structure and cannot account for long-range effects, which are not predictable by crystallographic data. Residues

Table 2
Allele frequency (AF) of *DDC* variants reported in 348 patients with AADC deficiency.

cDNA-Aberration	Variant	n	%
c.714+4A>T	IVS6+4A>T	225	32.3
c.1040G>A	p.Arg347Gln	48	6.9
c.1234C>T	p.Arg412Trp	24	3.4
c.175G>A	p.Asp59Asn	22	3.2
c.749C>T	p.Ser250Phe	19	2.7
c.1297dup	p.Ile433AsnfsTer60	17	2.4
c.179T>C	p.Val60Ala	15	2.2
c.304G>A	p.Gly102Ser	13	1.9
c.823G>A	p.Ala275Thr	11	1.6
c.242C>T	Pro81Leu	10	1.4
c.139C>G	p.Pro47Ala	9	1.3
c.206C>T	p.Thr69Met	8	1.1
c.208C>T	p.His70Tyr	8	1.1
c.571-3C>G	IVS5-3C>G	8	1.1
c.106G>A	p.Gly36Arg	7	1.0
c.853C>T	p.Arg285Trp	7	1.0
c.1340G>A	p.Arg447His	7	1.0
c.19C>T	p.Arg7Ter	6	0.9
c.260C>T	p.Pro87Leu	6	0.9
c.367G>A	p.Gly123Arg	6	0.9
c.1072C>T	p.Arg358Cys	6	0.9
c.1073G>A	p.Arg358His	6	0.9
c.73G>A	p.Glu25Lys	5	0.7
c.286G>A	p.Gly96Arg	5	0.7
c.48C>A	p.Tyr16Ter	4	0.6
c.116G>C	p.Arg39Pro	4	0.6
c.231C>A	p.Phe77Leu	4	0.6
c.322A>C	p.Ser108Arg	4	0.6
c.734C>T	p.Thr245Ile	4	0.6
c.876G>A	p.Glu292=	4	0.6
c.1058T>C	p.Leu353Pro	4	0.6
c.201+5G>C	IVS2+5G>C	3	0.4
c.214C>T	p.His72Tyr	3	0.4
c.223T>G	p.Tyr75Asp	3	0.4
c.230T>C	p.Phe77Ser	3	0.4
c.250A>C	p.Ser84Arg	3	0.4
c.339G>T	p.Glu113Asp	3	0.4
c.446G>A	p.Ser149Asn	3	0.4
c.476C>T	p.Ala159Val	3	0.4
c.1123C>T	p.Gln375Ter	3	0.4
c.1312T>C	p.Cys438Arg	3	0.4
c.1339C>T	p.Arg447Cys	3	0.4
c.1385G>C	p.Arg462Pro	3	0.4
c.23G>A	p.Arg8Lys	2	0.3
c.44A>G	p.Asp15Gly	2	0.3
c.105del	p.Tyr37ThrfsTer5	2	0.3
c.121C>A	p.Leu41Met	2	0.3
c.202G>A	p.Val68Met	2	0.3
c.236A>G	p.Tyr79Cys	2	0.3
c.254C>T	p.Ser85Leu	2	0.3
c.299G>C	p.Cys100Ser	2	0.3
c.315G>C	p.Trp105Cys	2	0.3
c.361T>C	p.Trp121Arg	2	0.3
c.424G>A	p.Gly142Arg	2	0.3
c.440G>T	p.Ser147Ile	2	0.3
c.446G>C	p.Ser149Thr	2	0.3
c.551T>C	p.Val184Ala	2	0.3
c.557A>G	p.Tyr186Cys	2	0.3
c.564_568dup	p.Gln190ProfsTer13	2	0.3
c.629C>T	p.Pro210Leu	2	0.3
c.647_653dup	p.Ala219HisfsTer36	2	0.3
c.665T>C	p.Leu222Pro	2	0.3
c.710T>C	p.Phe237Ser	2	0.3
c.781+6T>C	IVS7+6T>C	2	0.3
c.799T>C	p.Trp267Arg	2	0.3
c.811_812delinsTG	p.Asp271Cys	2	0.3
c.812A>T	p.Asp271Val	2	0.3
c.989C>T	p.Pro330Leu	2	0.3
c.1040G>A	p.Arg347Gln	2	0.3
c.1060G>T	p.Gly354Cys	2	0.3
c.1085T>C	p.Met362Thr	2	0.3
c.1144G>T	p.Val382Phe	2	0.3
c.1243-10A>G	IVS13-10A>G	2	0.3
c.1379T>G	p.Val460Gly	2	0.3
?	Ex2_3del	2	0.3
?	Ex10_11del	2	0.3
c.1A>G	p.Met1Val	1	0.1
c.2T>C	p.Met1Thr	1	0.1
c.58_60del	p.Tyr20del	1	0.1
c.97G>C	p.Val33Leu	1	0.1
c.113T>C	p.Leu38Pro	1	0.1
c.170T>C	p.Ile57Thr	1	0.1
c.181G>A	p.Glu61Lys	1	0.1
c.256T>A	p.Tyr86Asn	1	0.1
c.272C>T	p.Ala91Val	1	0.1
c.277A>G	p.Met93Val	1	0.1
c.285C>A	p.Cys95Ter	1	0.1
c.288G>A	p.Gly96=	1	0.1
c.289del	p.Ala97ProfsTer21	1	0.1
c.293T>C	p.Ile98Thr	1	0.1
c.296G>A	p.Gly99Asp	1	0.1
c.310T>C	p.Ser104Pro	1	0.1
c.311C>T	p.Ser104Phe	1	0.1
c.323G>A	p.Ser108Asn	1	0.1
c.328G>A	p.Ala110Thr	1	0.1
c.330_334dup	p.Thr112AsnfsTer8	1	0.1
c.343G>A	p.Glu115Lys	1	0.1
c.364C>T	p.Leu122Phe	1	0.1
c.385C>T	p.Pro129Ser	1	0.1
c.419G>A	p.Gly140Glu	1	0.1
c.436G>C	p.Gly146Arg	1	0.1
c.436-16C>T	IVS4-16C>T	1	0.1
c.439A>C	p.Ser147Arg	1	0.1
c.478C>T	p.Arg160Trp	1	0.1
c.478C>G	p.Arg160Gly	1	0.1
c.479G>A	p.Arg160Gln	1	0.1
c.565G>T	p.Asp189Tyr	1	0.1
c.592G>A	p.Ala198Thr	1	0.1
c.624del	p.Ile209SerfsTer26	1	0.1
c.679G>C	p.Glu227Gln	1	0.1
c.752T>C	p.Phe251Ser	1	0.1
c.755A>G	p.Asp252Gly	1	0.1
c.782-6C>T	IVS7-6C>T	1	0.1
c.782G>T	p.Cys261Phe	1	0.1
c.801G>A	p.Trp267Ter	1	0.1
c.841T>C	p.Cys281Arg	1	0.1
c.843C>G	p.Cys281Trp	1	0.1
c.848A>C	p.Glu283Ala	1	0.1
c.849G>C	p.Glu283Asp	1	0.1
c.863T>C	p.Leu288Pro	1	0.1
c.869G>A	p.Gly290Glu	1	0.1
c.989C>G	p.Pro330Arg	1	0.1
c.1021+1G>A	IVS10+1G>A	1	0.1
c.1039C>G	p.Arg347Gly	1	0.1
c.1039C>T	p.Arg347Trp	1	0.1
c.1039C>G	p.Arg347Gly	1	0.1
c.1039C>T	p.Arg347Trp	1	0.1
c.1041+1G>C	IVS11+1G>C	1	0.1
c.1060G>A	p.Gly354Ser	1	0.1
c.1061G>A	p.Gly354Asp	1	0.1
c.1063dup	p.Arg355LysfsTer13	1	0.1
c.1066_1068del	p.Arg356del	1	0.1
c.1093G>A	p.Val365Ile	1	0.1
c.1106A>G	p.Tyr369Cys	1	0.1
c.1140G>A	p.Lys380=	1	0.1
c.1217T>A	p.Leu406Gln	1	0.1
c.1222C>A	p.Leu408Ile	1	0.1
c.1228T>G	p.Cys410Gly	1	0.1
c.1233dup	p.Arg412SerfsTer10	1	0.1
c.1242G>A	p.Lys414=	1	0.1
c.1325_1326insG	p.Asp442GlufsTer51	1	0.1
c.1337T>C	p.Leu446Pro	1	0.1
c.1375C>T	p.His459Tyr	1	0.1
?	Ex11_12del	1	0.1

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

Table 3
New *DDC* variants reported in this study.

cDNA Aberration	Variant	Exon/Intron	Variant effect	ACMG/AMP/ACGS scoring	3D scoring	Polyphen	SIFT	CADD
c.23G>A	p.Arg8Lys	E2	Missense	Hot VUS (5)	LB	B	T	8.4
c.44A>G	p.Asp15Gly	E2	Missense	LP (7)	LP	PrD	D	29.8
c.223T>G	p.Tyr75Asp	E3	Missense	LP (6)	LP	B	D	22.5
c.230T>C	p.Phe77Ser	E3	Missense	LP (8)	P	PrD	D	28.4
c.256T>A	p.Tyr86Asn	E3	Missense	LP (6)	LP	PrD	D	27.0
c.288G>A	p.Gly96=	E3	Synonymous	Tepid VUS (3)	–	–	–	15.8
c.296G>A	p.Gly99Asp	E3	Missense	LP (6)	LP	PoD	D	25.9
c.310T>C	p.Ser104Pro	E3	Missense	LP (9)	P	PoD	D	27.7
c.328G>A	p.Ala110Thr	E4	Missense	LP (7)	P	PrD	D	24.8
c.339G>T	p.Glu113Asp	E4	Missense	LP (7)	LP	PoD	D	21.8
c.479G>A	p.Arg160Gln	E5	Missense	LP (9)	P	PrD	D	26.2
c.551T>C	p.Val184Ala	E5	Missense	LP (7)	LP	PrD	D	28.1
c.557A>G	p.Tyr186Cys	E5	Missense	LP (7)	LP	PrD	D	28.7
c.647_653dup	p.Ala219Hisfs*36	E6	Frame shift	P (10)	–	–	–	–
c.811_812delinsTG	p.Asp271Cys	E8	Missense	LP (7)	P	PrD	D	–
c.841T>C	p.Cys281Arg	E8	Missense	P (10)	P	PrD	D	26.7
c.849G>C	p.Glu283Asp	E8	Missense	LP (9)	LP	PrD	D	23.1
c.869G>A	p.Gly290Glu	E8	Missense	LP (7)	LP	PrD	D	25.3
c.989C>G	p.Pro330Arg	E10	Missense	LP (6)	P	PrD	T	23.9
c.1061G>A	p.Gly354Asp	E12	Missense	LP (9)	P	PrD	D	27.5
c.1066_1068del	p.Arg356del	E12	In frame	LP (7)	P	–	–	–
c.1093G>A	p.Val365Ile	E12	Missense	LP (7)	LP	PrD	D	25.8
c.1140G>A	p.Lys380=	E12	Synonymous	Warm VUS (4)	–	–	–	25.9
c.1243-10A>G	IVS13-10A>G	I13	Intronic	Warm VUS (4)	–	–	–	–
c.1325_1326insG	p.Asp442GlufsTer51	E14	Frame shift	LP (6)	–	–	–	–
?	Ex11_12del	E11/E12	Large deletion	P (10)	–	–	–	–

NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.
P: pathogenic; LP: likely pathogenic, B: benign; LB: likely benign; VUS: variant of unknown significance; PrD: probably damaging; PoD: possibly damaging; T: tolerated; D: damaging.

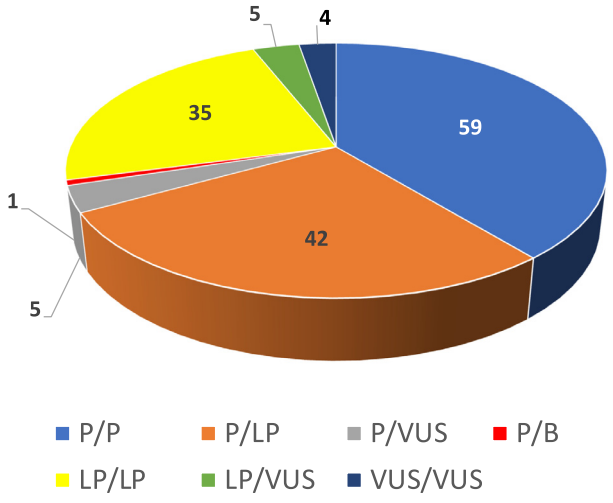

Fig. 1. *DDC* genotypes according to the ACMG/AMP/ACGS classification. P: pathogenic; LP: likely pathogenic, B: benign; VUS: variant of unknown significance.

belonging to important structural elements of the active site [10] or demonstrated to affect it [22] and amino acids belonging to or flanking loops 1, 2, and 3 could exert strong mutual effects, since some of them could contribute *in trans*. Analyzing the possible patterns, a combination that preserved the functionality of at least one active site in the heterodimeric protein resulted in a better score. In particular, the heterodimers resulted in 29 LP, 10 LB and 6 P proteins.

Using the information from this study, 56 *DDC* variants from the PNDdb database were reclassified. Within these, 17 variants could be strengthened from LP to P, 18 variants from VUS to P or LP, and 21 variants remained in the same class (11 VUS, 9 LP and 1 P) (Supplemental Table S1).

Table 4
Prediction of the structural and functional effect of *DDC* genotypes on protein level.

Compound heterozygotes with two missense variants		3D score		Active site score			3D score Heterodimer[c]
		Homodimer 1	Homodimer 2	Homodimer 1[a]	Homodimer 2[a]	Heterodimer[b]	
p.Asp59Asn	p.Arg358Cys	LP	LP	0	2	**1**	**LP**
p.Phe77Ser	p.Ala275Thr	P	P	2	2	**2**	**P**
p.Glu25Lys	p.Val460Gly	LB	LP	0	0	**0**	**LB**
p.Pro47His	p.Arg358Cys	P	LP	0	2	**1**	**LP**
p.Val60Ala	p.Ser147Ile	P	P	0	2	**1**	**LP**
p.Val68Met	p.Ser85Leu	LP	LP	2	2	**2**	**P**
p.Pro87Leu	p.Ser149Asn	LP	LP	2	2	**2**	**P**
p.Ser108Arg	p.Asp271Val	LP	P	2	2	**1**	**LP**
p.Gly123Arg	p.Gly142Arg	P	LP	0	0	**0**	**LB**
p.Gly123Arg	p.Thr245Ile	P	P	0	2	**1**	**LP**
p.Arg347Gln	p.Arg358His	P	P	2	2	**1**	**LP**
p.Glu25Lys	p.Arg358His	LB	P	0	2	**1**	**LP**
p.Val33Leu	p.Arg462Pro	LP	P	0	0	**0**	**LB**
p.Gly36Arg	p.Arg447His	LP	P	0	2	**1**	**LP**
p.Pro47Ala	p.Arg447Cys	P	P	0	2	**1**	**LP**
p.Ile57Thr	p.Arg412Trp	LP	P	0	0	**0**	**LB**
p.Asp59Asn	p.Gly96Arg	LP	P	0	2	**1**	**LP**
p.Val60Ala	p.Asp59Asn	P	LP	0	0	**0**	**LB**
p.Val60Ala	p.Ser104Phe	P	LP	0	2	**1**	**LP**
p.Val60Ala	p.Arg412Trp	P	P	0	0	**0**	**LB**
p.Thr69Met	p.Ser147Arg	P	P	2	2	**2**	**P**
p.Thr69Met	p.Leu446Pro	P	LP	2	0	**1**	**LP**
p.Tyr75Asp	p.Glu113Asp	LP	LP	2	0	**1**	**LP**
p.Tyr79Cys	p.Asp252Gly	P	LP	2	0	**1**	**LP**
p.Tyr86Asn	p.Pro330Arg	LP	P	2	2	**1**	**LP**
p.Pro87Leu	p.Gly96Arg	LP	P	2	2	**1**	**LP**
p.Pro87Leu	p.Ser149Thr	LP	LP	2	2	**1**	**LP**
p.Ala91Val	p.Cys410Gly	P	P	2	2	**2**	**P**
p.Gly96Arg	p.Glu227Gln	P	B	2	0	**1**	**LP**
p.Ile98Thr	p.Glu115Lys	LP	LP	2	2	**2**	**P**
p.Gly102Ser	p.Gly290Glu	P	LP	2	0	**1**	**LP**
p.Ser104Pro	p.Arg160Gln	P	P	2	0	**1**	**LP**
p.Trp105Cys	p.Pro129Ser	LP	LP	2	0	**1**	**LP**
p.Trp121Arg	p.Arg347Gln	LP	P	0	2	**1**	**LP**
p.Trp121Arg	p.Met362Thr	LP	P	0	2	**1**	**LP**
p.Gly140Glu	p.His459Tyr	LP	LP	0	0	**0**	**LB**
p.Arg160Gly	p.Asp189Tyr	P	LP	0	0	**0**	**LB**
p.Arg160Trp	p.Arg347Gln	P	P	0	2	**1**	**LP**
p.Pro210Leu	p.Arg285Trp	P	P	0	0	**0**	**LB**
p.Cys261Phe	p.Gly354Ser	LP	LP	0	2	**1**	**LP**
p.Ala275Thr	p.Arg347Gln	P	P	2	2	**1**	**LP**
p.Cys281Trp	p.Met362Thr	P	P	0	2	**1**	**LP**
p.Glu283Asp	p.Arg356del	LB	LP	0	2	**1**	**LP**
p.Arg285Trp	p.Arg412Trp	P	P	0	0	**0**	**LB**
p.Glu25Lys	p.Trp105Cys	LB	LP	0	2	**1**	**LP**

NC_000007.14; NG_008742.1; NM_000790.4; NP_000781.2, GRCh38-v1.6.
[a] 0 means that the identical amino acid substitutions in both monomers of the homodimer are far from the active site region; 2 means that both active sites of the homodimer are affected by the identical amino acid substitutions on the two different monomers.
[b] 0 means that the amino acid substitutions in both monomers of the heterodimer are far from the active site region; 1 means that one active site of the heterodimer is affected by the combination of amino acid substitutions on the two different monomers while the other active site is not affected; 2 means that both active sites of the heterodimer are affected by the combination of amino acid substitutions on the two different monomers.
[c] The numerical active site score of heterodimer 0, 1, and 2 has been decoded as LB, LP and P, respectively.
Colors: white = the 3D score of the heterodimer is identical to those of both homodimers, cyan = the 3D score of the heterodimer is identical to that of the milder homodimer; red = the 3D score of the heterodimer is identical to that of the most severe homodimer; green = the 3D score of the heterodimer is an "average", being higher than that of the milder and lower than that the most severe homodimer.

4. Discussion

The global prevalence remains unknown, but 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 [13]. Based on the newborn screening pilot studies in Europe, the prevalence of AADC deficiency is probably much lower and on the order of 1:500,000 live births [15,16,23]. The condition is thought to be more prevalent in certain Asian populations, particularly Taiwan, China, and Japan, due to the founder variant c.714+4A>T [13,24]. A recent gnomAD-based study estimated worldwide incidence of AADC deficiency to be about 1:1,300,000 [25]. In view of emerging new therapies for AADC deficiency (the current therapeutic options are only partially effective), it is important to promote new diagnostic options in addition to standard testing for neurotransmitter metabolites in CSF [26], *e.g.*, untargeted metabolomics of plasma [27] or 3OMD in DBS [15], and to estimate the current number of diagnosed patients.

Our study documents that approximately 35% of AADC-deficient patients were not previously reported and many more are likely to not have a final diagnosis and are thus without appropriate treatment. Gene therapy, using targeted delivery of the AAV2-AADC gene vector to either the bilateral midbrain or bilateral putamen has been investigated in several early-stage clinical trials. The results indicate that gene delivery can be safely performed and produces disease-modifying improvements in autonomous symptoms and motor function [17–20]. Studies are ongoing to determine the optimal target site and dose. In an initial study of seven patients with severe motor impairment, midbrain gene delivery resulted in complete resolution of oculogyric crises in 86% of patients, attainment of independent sitting within 12 months in 57%, and improved mood and sleep [19].

A phase 1/2 trial is ongoing (NCT02852213). Following bilateral putaminal delivery of eladocagene exuparvovec, improvements in symptoms and motor function were reported in 26 patients who were enrolled in three consecutive trials (compassionate use, phase 1/2, phase 2b) [20]. A phase 2 trial is ongoing (NCT04903288).

Including the patients in this study, approximately 350 AADC-deficient patients with a full genotype have been reported. Although clinical information was not available for all patients, most suffered from a severe phenotype, and only a few were reported as having a mild or less severe form [28–31].

Of the 151 genotypes (Table 1), 15 (10%) are found in approximately 50% of all patients, and this is mostly due to a frequent Asian c.714+4A>T splice variant (AF = 32.3%) and the most frequent homozygous c.[714+4A>T];[714 + 4A > T] genotype (GF = 21.3%). Only one genotype included a benign c.436-16C>T variant in a combination with a pathogenic variant (c.1040G>A).

To predict the structural and functional effect of *DDC* genotypes at the protein level, a combination of criteria was used, including the structural change determined by the amino acid substitutions/deletions/frameshifts in terms of the altered network of bonds essential to preserve function, the evolutionary conservation of the altered amino acid, the chemical impact of the modification in terms of polarity and steric hindrance, and the presence of already identified pathogenic variants for each residue. If genotypes were interpreted in terms of AADC proteins, one should consider that the functional AADC enzyme is an obligate dimer with swapped structural parts among the two subunits at the interfacial active sites [32]. Predictions are more complex for compound heterozygous genotypes with missense mutations in both alleles, as both can theoretically synthesize AADC polypeptide chains [11] that result in three different AADC enzymes: two homodimers and a heterodimer. However, the better outcome in heterodimeric AADC (which could be related to interallelic complementation regarding activity [11]) is not sufficient itself to dictate enzymatic fitness, since this results also from the residual activity of the related two homodimeric variants. Interestingly, the comparison of the scores of the heterodimer with those of the two homodimers showed neutral, increased, or decreased pathogenicity, which may be related to both positive and negative complementation effects, although this has only been validated by functional assays in four species.

We hope this study will add to the current understanding of the genetic background of AADC deficiency and will initiate further studies of interallelic complementation in compound heterozygous genotypes.

Data availability

Data will be made available on request.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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