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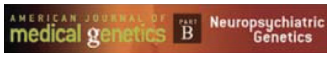

RESEARCH ARTICLE OPEN ACCESS

Molecular Profiling of Genes Associated With Methylphenidate Pathway Therapy and Discovery of New Variants in Amazonian Amerindian Populations

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ABSTRACT

In Attention Deficit Hyperactivity Disorder (ADHD), methylphenidate is one of the most widely used drugs, in which patient response significantly impacts prognosis. This study aimed to characterize the molecular profile of 10 genes associated with methylphenidate therapy. Whole-exome sequencing of 64 indigenous individuals from 12 different indigenous populations in the Amazon was used. A total of 143 genetic variants were identified, of which 123 were analyzed. Among these, three novel and population-specific variants were found, all with modifying impact, located in the ADGRL3 and ADRA2A genes. In addition, we identified a high-impact variant in the COMT gene, which exhibited statistical significance in the INDG population compared to other groups, along with five variants with moderate impact and 31 with modifying impact. Our findings highlight the unique genetic profile of indigenous populations in the Brazilian Amazon and provide valuable insights to optimize ADHD management and treatment strategies. This study contributes to improving therapeutic responses to methylphenidate and supports the development of precision medicine guidelines.

1 | Introduction

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric condition and one of the most prevalent mental disorders in children (Sürig et al. 2024). It is primarily characterized by a symptomatic triad consisting of attention deficit, hyperactivity, and impulsivity, with manifestations varying according to the three recognized subtypes: predominantly inattentive, predominantly hyperactive, and combined (López-López et al. 2019; Weibel et al. 2020).

The predominantly hyperactive ADHD subtype is marked by pronounced impulsivity and restlessness, both physically and mentally. In contrast, the inattentive subtype is associated with less prominent impulsivity but is characterized by drowsiness, lethargy, and slowed cognition and behavior. The combined subtype presents a mixture of symptoms from both inattentive and hyperactive types (Ilario et al. 2019; Weibel et al. 2020).

ADHD is typically diagnosed during school age, when demands for sustained attention and behavioral regulation increase

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(Sürig et al. 2024). Although it affects approximately 5% of children and adolescents, in recent years, there has been growing recognition of ADHD in adults, with an estimated prevalence of 2%–4% in this population (Weibel et al. 2020).

The etiology of ADHD is multifactorial, involving both genetic and environmental risk factors that contribute to susceptibility (Faraone and Larsson 2019). However, genetic factors are responsible for approximately 80% of ADHD cases according to Jornal da USP (2020). Genome-wide association studies (GWAS) have identified several genetic loci and potential candidate genes, highlighting the role of single nucleotide variants (SNVs) in the disorder's heritability. These findings have significant implications for treatment approaches and emerging clinical practices (Demontis et al. 2023; Zhong et al. 2020).

Currently, there is no curative treatment for ADHD. However, clinical guidelines recommend an individualized multimodal approach that integrates psychological and pharmacological interventions to manage symptoms effectively (Mechler et al. 2021). Methylphenidate is the most widely used first-line pharmacological treatment, and genetic factors may influence both its efficacy and associated side effects. This suggests that genetic evaluation may be beneficial in certain cases to personalize ADHD treatment (Shellenberg et al. 2020).

Several studies have demonstrated the role of key genes, including DRD4, in the response to and efficacy of methylphenidate treatment (Bonvicini et al. 2020; Dark et al. 2020). Other genes have been selected based on their involvement in the drug's mechanism of action, such as those related to dopamine and norepinephrine transporter inhibition, serotonin receptor type 1A agonism, and vesicular monoamine transporter 2 redistribution. These pharmacodynamic properties aim to reduce impulsivity and improve symptom control in ADHD (Shellenberg et al. 2020).

Despite significant advancements, research on precision medicine in Amazonian indigenous populations remains limited. Investigating the unique genetic characteristics of these groups and their association with ADHD can provide valuable insights into the broader genetic landscape, not only of Brazil but also of global populations. Expanding knowledge of the genetic diversity within these indigenous communities and their response to methylphenidate is crucial for developing more effective public health strategies for ADHD treatment.

Therefore, this study aims to investigate the genomic profile of key genes—SLC6A2, SLC6A3, SLC6A4, ADGRL3, COMT, CES1, ADRA2A, DRD4, DRD3, and DRD2—associated with methylphenidate therapy in a sample of Amazonian indigenous individuals.

2 | Materials and Methods

2.1 | Ethical Aspects

Ethical approval for this study was granted by the National Research Ethics Commission (CONEP) under protocol numbers 1062/2006 and 123/98. The volunteers and the respective leaders of the ethnic groups were informed about the research objectives with the assistance of a translator and provided written informed consent. The collection of biological material was conducted in accordance with the Declaration of Helsinki. Sample recruitment began in September 2017 and was completed in December 2018.

2.2 | Study and Reference Populations

This descriptive cross-sectional study included a total of 64 healthy individuals from the Amazon, forming the indigenous population group (INDG). These individuals belong to 12 distinct ethnic groups: 5 from the Asurini of Koatinemo, 7 from the Arara/Arara of Iriri, 6 from the Arawet', 16 from the Asurini of Trocar'a, 7 from the Awa-Guajá, 1 from the Munduruku, 2 from the Xikrins Odj'a, 5 from the Zo'e, 5 from the Wajãpi, 6 from the Xikrin of Cateté, 2 from the Karipuna, and 2 from the Juruna. Additional information on this population can be found in Rodrigues et al. (2020). These data samples were used in other articles produced by the Oncology Research Center of the Federal University of Pará, such as in the study by Rodrigues et al. (2022), Aguiar et al. (2023), de Lima et al. (2024), Carvalho et al. (2024) and Coelho et al. (2024). The exome results were compared with publicly available continental population data from the phase 3 release of the 1000 Genomes Project database (assembly GRCh38.p13; The 1000 Genomes Project Consortium, 2015), including 661 Africans (AFN), 347 Americans (AMR), 504 East Asians (EAS), 503 Europeans (EUR), and 489 South Asians (SAS). For variants not present in the 1000 Genomes platform, allele frequencies were obtained from the GnomAD v.3.1.2 database, which includes 20,744 Africans (AFN), 7647 Americans (AMR), 2604 East Asians (EAS), 34,029 non-Finnish Europeans (EUR), and 2419 South Asians (SAS).

2.3 | DNA Extraction and Exome Library Preparation

DNA extraction and exome library preparation for the INDG group followed the procedures described by Ribeiro-dos-Santos et al. (2020). For six additional individuals later included in the group, the extraction and library preparation processes were detailed in Rodrigues et al. (citation pending). Briefly, peripheral blood DNA was extracted using the phenol-chloroform method, and exome libraries were prepared using the Nextera Rapid Capture Exome (Illumina, San Diego, CA, USA) and SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, USA) kits, following the manufacturers' protocols. Sequencing reactions were performed on the NextSeq 500 platform using the NextSeq 500 High-Output v2 kit (300 cycles).

2.4 | Bioinformatic Analysis

Sequencing reads were first filtered to remove low-quality sequences using fastx_tools v.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/). Reads were then mapped and aligned to the reference genome (GRCh38) using the BWA v.0.7 tool (http://

bio-bwa.sourceforge.net/). Post-alignment, files were indexed and sorted using SAMtools v.1.2 (http://sourceforge.net/projects/samtools/). Duplicate sequences were removed (Picard Tools v.1.129; http://broadinstitute.github.io/picard/), mapping quality was recalibrated, and local realignment was performed (GATK v.3.2; https://gatk.broadinstitute.org/hc/en-us). Variant calling was conducted using GATK v.3.2, and annotation analysis was performed using Variant Viewer (ViVa).

Variants were annotated using three databases: SnpEff v.4.3T, Ensembl Variant Effect Predictor (Ensembl version 99), and ClinVar (v.201810). Pathogenicity predictions were obtained using multiple in silico tools, including SIFT (v.6.2.1), PolyPhen-2 (v.2.2), LRT (November 2009), Mutation Evaluator (v.3.0), Mutation Provider (v.2.0), FATHMM (v.2.3), PROVEAN (v.1.1.3), MetaSVM (v.1.0), M-CAP (v.1.4), and FATHMM-MKL (http://fathmm.biocompute.org.uk/about.html).

2.5 | Gene and Variant Selection

Gene selection for this study was based on a literature review using the PubMed database (pubmed.ncbi.nlm.nih.gov). Ten genes (SLC6A2, SLC6A3, SLC6A4, ADGRL3, COMT, CES1, ADRA2A, DRD4, DRD3, and DRD2) were selected due to their frequent association with the pharmacogenetics of methylphenidate. The selected genes are documented in a Table S1, detailing their functions, pathways involved, and supporting references. Single nucleotide variants (SNVs) were included based on the following criteria: (i) a minimum of 10 coverage reads (determined using fastx_tools v.0.13); (ii) classification as a modifier, moderate, or high-impact variant according to SnpEff; and (iii) inclusion of novel variants not previously documented. Variants that lacked frequency annotations in global population databases, such as gnomAD and 1000 Genomes, were removed from the main analysis. Variants were selected based on their potential impact. Variants considered to have high, moderate, and modified impact were selected, while those with low impact were listed in a supplementary table (Table S2). All duplicate variants were removed to ensure the accuracy and uniqueness of our dataset, preventing any bias in subsequent analyses.

2.6 | Statistical Analysis

Allele frequencies of genetic variants in the INDG group were determined using the allelic counting method and compared with reference populations (AFR, EUR, AMR, EAS, and SAS) from the 1000 Genomes Project and gnomAD databases. Interpopulation polymorphism variability was assessed using the Wright fixation index (FST), followed by Multidimensional Scaling Analysis. Allele frequency differences between populations were evaluated using Fisher's exact test, with Hochberg correction applied for multiple comparisons. A p-value <0.05 was considered statistically significant. Statistical analyses were conducted using Arlequin v.3.5.18 and RStudio v.4.2.3.

3 | Results

After selecting 143 variants across 11 genes, we analyzed 123 genetic variants in our study. Among them, 13 variants were identified in the SLC6A2 gene, 17 in SLC6A3, 9 in SLC6A4, 15 in ADGRL3, 14 in COMT, 38 in CES1, 2 in ADRA2A, 5 in DRD4, 4 in DRD3, and 6 in DRD2.

We focused on variants that exhibited significant correlations in at least 3 of the 5 continental populations analyzed (AFR, AMR, EAS, SAS, and EUR), resulting in 37 variants: 4 in SLC6A2, 4 in SLC6A3, 4 in SLC6A4, 6 in ADGRL3, 3 in COMT, 13 in CES1, 2 in DRD4, and 1 in DRD3.

Regarding impact prediction using the SnpEff software, 1 variant was classified as having a high impact, 5 as having a moderate impact, and 31 as having a modifier impact. Figure 1 illustrates the distribution of these variants in relation to their relative frequency in each gene, with particular emphasis on the COMT and CES1 genes. No variants with high, moderate, or modifier impact predictions were identified in the ADRA2A and DRD2 genes.

We identified a variant in the *COMT* gene (rs4818) with high impact, showing a significant difference in all correlations. In addition, five variants were classified as having moderate impact,

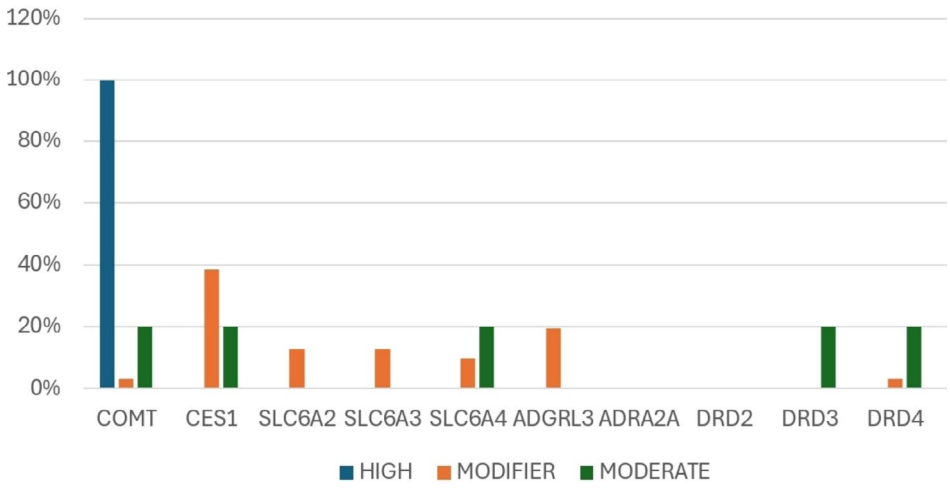

FIGURE 1 | Distribution of the relative frequency of variants with prediction of high, moderate, and modified impact on the *SLC6A2, SLC6A3, SLC6A4, ADGRL3, COMT, CES1, ADRA2A, DRD4, DRD3*, and *DRD2* genes.

distributed among five genes: 1 in the *CES1* gene (rs754896838), 1 in the *COMT* gene (rs6267), 1 in the *DRD3* gene (rs6280), 1 in the *DRD4 gene* (rs572586776), and 1 in the *SLC6A4* gene (rs6355). In total, 31 variants were described with modifier impact, 4 located in the *SLC6A2* gene, 4 in *SLC6A3*, 3 in *SLC6A4*, 6 in *ADGRL3*, 1 in *COMT*, 12 in *CES1*, and 1 in *DRD4*.

These variants are listed in Table 1, which presents characteristics such as SNP ID, type of variant, chromosomal region, impact prediction by the SnpEff software, and frequency of alleles for the indigenous population, in addition to the five continental populations of the 1000 Genomes and GnomAD platform (AFR, AMR, EAS, EUR, and SAS). Variants with low impact are detailed in Table S2.

The variant in the *COMT* gene (rs4818), presented in Table 1, showed a high impact, being characterized by the change in nucleotide from C to G. Among the variants analyzed, 3 are new and possibly exclusive to the indigenous population: 2 are in the *ADGRL3* gene, in positions 61,813,904 and 62,063,484, and 1 is in the *ADRA2A* gene, in position 111,077,982 (Table 2).

A multidimensional scaling (MDS) was performed based on the fixation index (FST) of the variants, highlighting the genomic differences between the analyzed populations. From the selected genes, we observed that the African group is clearly separated from the others, reflecting its position as the population with an ancestral and oldest genetic profile in the world. Likewise, the indigenous population appears at the other end of the graph, indicating a genetic difference from the continental populations. The AMR and EUR groups are close, which validates the European contribution to mixed populations. Finally, the distance between the indigenous population and the EAS and SAS groups is observed, which further validates their genetic difference (Figure 2).

4 | Discussion

ADHD is a mental disorder, and its drug treatment includes psychostimulants, which are highly effective in relieving symptoms (Soutullo et al. 2023). Although it is more common in children and adolescents, with a prevalence ranging from 2% to 7%, ADHD often persists into adulthood. The recognition and diagnosis of ADHD in this group have increased, representing a risk factor for other mental health disorders and leading to negative consequences such as low educational performance, work difficulties, relational problems, and criminal behavior, highlighting ADHD as a global public health concern (Sayal et al. 2018).

The treatment consists of psychostimulants that act on dopamine and norepinephrine in the brain (Weibel et al. 2020). Methylphenidate is the recommended first-line treatment and the most widely used in many countries, with robust evidence supporting a favorable risk–benefit ratio (Elsayed et al. 2020; de Oliveira et al. 2021; Weibel et al. 2020).

Although the diagnosis of ADHD has advanced, studies on genetically heterogeneous populations or indigenous peoples with ADHD remain scarce, making genomics crucial for the implementation of precision medicine in methylphenidate therapy. According to Cohen-Paes et al. (2022), considering the distinct genetic contribution of native peoples compared to the ethnically homogeneous populations analyzed in studies, this group is often neglected in research. Additionally, the Brazilian population is characterized by a high degree of ethnic admixture (Santos, 2010 apud Rodrigues et al. 2019), primarily composed of ancestral groups of European settlers, African slaves, and Amerindians (Native Americans). Therefore, promoting pharmacogenetic studies specific to the native population also contributes to research on diagnostic and therapeutic responses in the Brazilian population as a whole. In this context, this study aims to evaluate the genomic profile of the indigenous population, pioneering the investigation of the exome of 10 genes relevant to methylphenidate therapy in underexplored populations.

Our analyses identified one previously described variant categorized as high impact, five with moderate impact, and 31 with modifier impact. Additionally, we identified three novel variants that have never been described and appear to be exclusive to this population. Regarding variants with modifier impact, we found a significant number that corroborates the findings of other studies on indigenous populations, such as the study by Coelho et al. (2024), which reported a total of 36 modifier-impact variants and three population-specific variants, as well as the study by de Lima et al. (2024), which identified 32 modifier-impact variants, four of which were novel in the indigenous population. The ADRA2A and DRD2 genes were investigated, but the variants identified had low-impact predictive values, as described in the Table S2.

Our results demonstrated that the rs4818 variant in the COMT gene, in addition to presenting a high clinical impact, is statistically significant in the indigenous population when compared to the AFR, AMR, EUR, and SAS populations. Catechol-O-methyltransferase (COMT) metabolizes catechol estrogens and the catecholamines epinephrine, norepinephrine, and dopamine by catalyzing the transfer of a methyl group from S-adenosyl methionine to the catechol moieties (Hall et al. 2019). Research by Fageera et al. (2021) indicates that the interaction between the COMT and DRD3 genes can significantly influence ADHD expression and response to treatment. Understanding this genetic relationship is essential to personalizing therapies and optimizing outcomes for each individual.

Additionally, we identified five variants with moderate impact (rs6267, rs754896838, rs6280, rs572586776, and rs6355). The rs6267 variant, with moderate impact, is also located in the COMT gene and was statistically significant in the indigenous population when compared to all continental populations. Similarly, the rs754896838 variant, categorized as having a moderate impact, is located in the CES1 gene (carboxylesterase 1). This gene is involved in methylphenidate metabolism in the liver, and studies by Mechler et al. (2021) and Shellenberg et al. (2020) suggest that the presence of mutations and variants in this gene may be associated with impaired methylphenidate metabolism, increasing susceptibility to adverse reactions and influencing drug metabolism rates, plasma levels, and treatment efficacy.

The rs6280 and rs572586776 variants, both with moderate impact, are located in the DRD3 and DRD4 genes, respectively,

TABLE 1 | Results of allele frequencies of genetic variants with high, modified and moderate impact on the Indigenous population (INDG) and continental populations (African (AFR), American (AMR), East Asian (EAS), European (EUR) and South Asian (SAS)) described in the 1000 genomes and GnomAD database.

Gene	Chrom	Position	Reference	Variant	SNP id	Var type	Impact	INDG[a]	AFR[a]	AMR[a]	EAS[a]	EUR[a]	SAS[a]
COMT	chr22	19,962,740	G	T	rs6267	SNV	Moderate	0.116	0.001	0.033	0.035	0.007	0.001
COMT	chr22	19,964,374	G	C	rs4646315	SNV	Modifier	0.421	0.124	0.222	0.123	0.187	0.249
COMT	chr22	19,963,684	C	G	rs4818	SNV	High	0.023	0.830	0.705	0.659	0.597	0.685
DRD4	chr11	640,349	G	A	rs1870723	SNV	Modifier	0.2	0.526	0.684	0.811	0.755	0.844
DRD4	chr11	637,361	[b]	C	rs572586776	SNV	Moderate	0.260	0.971	0.883	0.868	0.935	0.911
CES1	chr16	55,833,075	T	C	rs12149370	SNV	Modifier	0.166	0.878	0.929	0.890	0.918	0.892
CES1	chr16	55,816,932	G	A	rs754896838	SNV	Moderate	0.083	0	0	0	0	0
CES1	chr16	55,833,101	T	C	rs12149373	SNV	Modifier	0.25	0.862	0.857	0.779	0.836	0.789
CES1	chr16	55,828,693	G	A	rs3848300	SNV	Modifier	0.666	0.209	0.196	0.412	0.136	0.249
CES1	chr16	55,820,561	T	C	rs4784587	SNV	Modifier	0.25	0.03954	0.081	0.005	0.106	0.045
CES1	chr16	55,816,796	T	G	rs2302719	SNV	Modifier	0.112	0.478	0.693	0.381	0.775	0.522
CES1	chr16	55,832,982	T	TA	rs56278207	INDEL	Modifier	0.317	0.126	0.526	0.306	0.669	0.485
CES1	chr16	55,821,238	TG	T	rs3217164	INDEL	Modifier	0.096	0.048	0.464	0.185	0.523	0.320
CES1	chr16	55,810,697	G	T	rs2244613	SNV	Modifier	0.437	0.249	0.272	0.601	0.161	0.389
CES1	chr16	55,833,130	A	C	rs3815583	SNV	Modifier	0.083	0.030	0.218	0.45	0.18	0.263
CES1	chr16	55,810,705	G	A	rs2244614	SNV	Modifier	0.382	0.234	0.532	0.187	0.613	0.349
CES1	chr16	55,820,334	G	T	rs2307242	SNV	Modifier	0.166	0.055	0.108	0.005	0.180	0.065
CES1	chr16	55,820,347	G	A	rs2307237	SNV	Modifier	0.166	0.061	0.108	0.006	0.173	0,068
ADGRL3	chr4	61,912,876	AG	A	rs1182547245	INDEL	Modifier	0.1	0	0	0.00005016	0	0
ADGRL3	chr4	61,579,528	T	C	rs10018746	SNV	Modifier	0.125	0.439	0.514	0.624	0.290	0.425
ADGRL3	chr4	61,996,246	A	T	rs56038622	SNV	Modifier	0.081	0.209	0.225	0.339	0.228	0.217
ADGRL3	chr4	61,996,223	G	C	rs17226398	SNV	Modifier	0.013	0.775	0.775	0.661	0.772	0.783
ADGRL3	chr4	61,677,239	C	A	rs4860425	SNV	Modifier	0.403	0.129	0.245	0.118	0.173	0.141
ADGRL3	chr4	61,517,597	G	C	rs335312	SNV	Modifier	0.394	0.15	0.378	0.062	0.288	0.146
SLC6A3	chr16	55,699,677	T	C	rs1800887	SNV	Modifier	0.039	0.432	0.857	0.957	0.784	0.649
SLC6A3	chr5	1,416,027	C	A	rs40358	SNV	Modifier	0.625	0.316	0.215	0.391	0.157	0.145
SLC6A3	chr5	1,394,700	A	G	rs1042098	SNV	Modifier	0.023	0.500	0.754	0.892	0.686	0.774

(Continues)

TABLE 1 | (Continued)

Gene	Chrom	Position	Reference	Variant	SNP id	Var type	Impact	INDG[a]	AFR[a]	AMR[a]	EAS[a]	EUR[a]	SAS[a]
SLC6A3	chr5	1,409,012	T	C	rs429699	SNV	Modifier	0.773	0.002	0.095	0.232	0.001	0.013
SLC6A4	chr17	30,222,880	G	T	rs6354	SNV	Modifier	0.918	0.328	0.14	0.119	0.213	0.158
SLC6A4	chr17	30,222,791	C	T	rs28914827	SNV	Modifier	0.083	—	0.004	—	0.017	0.014
SLC6A4	chr17	30,211,514	C	T	rs140701	SNV	Modifier	0.190	0.296	0.530	0.818	0.41	0.51
SLC6A4	chr17	30,221,792	C	G	rs6355	SNV	Moderate	0.083	—	0.004	—	0.018	0.014
DRD3	chr3	114,171,968	C	T	rs6280	SNV	Moderate	0.071	0.182	0.573	0.693	0.664	0.580
SLC6A2	chr16	55,698,034	G	A	rs998424	SNV	Modifier	0.435	0.074	0.360	0.254	0.36	0.187
SLC6A2	chr16	55,702,000	C	T	rs2242447	SNV	Modifier	0.75	0.149	0.712	0.559	0.688	0.455
SLC6A2	chr16	55,692,063	A	G	rs5564	SNV	Modifier	0.166	0.018	0.046	0.178	0.062	0.078
SLC6A2	chr16	55,694,169	T	G	rs28613651	SNV	Modifier	1	0.045	0	1000	0.001	1000

Note: (—) No annotation.
Abbreviations: AFR, African population; AMR, American population; EAS, East Asian population; EUR, European population; INDG, Indigenous Amazonian population; SAS, South Asian population.
[a]Minor allele frequencies.
[b]CGCGGGGGGCATCT.

and exhibited statistically significant differences compared to all continental populations. Both genes encode proteins that regulate synaptic dopamine concentration and signaling, a key neurotransmitter in attention modulation and motor behavior (Naumova et al. 2019; Shellenberg et al. 2020). According to a genetic study by Fageera et al. (2018), there is a strong association between rs6280—particularly in T allele carriers—and reduced dopamine sensitivity, which is consequently linked to an increased risk of ADHD and a lower response to methylphenidate. Additionally, a randomized study by Naumova et al. (2019) confirmed that, among children with ADHD, variations in the DRD4 gene modulate behavioral outcomes in response to treatment, with children homozygous for the long allele showing better treatment outcomes compared to SS and SL genotype carriers.

The rs6355 variant, categorized as having a moderate impact, is present in the SLC6A4 gene and exhibited statistically significant differences compared to the AMR, EUR, and SAS populations. The SLC6A4 gene encodes the serotonin transporter, which is crucial for serotonin reuptake, thereby regulating its availability in brain synapses (Wang et al. 2021). Furthermore, studies have investigated other variants in this gene, such as Wang et al. (2021), demonstrating that the gene–gene interaction between ADRA2A rs553668G and SLC6A4 rs6354G is associated with an increased risk of ADHD. Therefore, investigating genetic variants such as rs6355 in the SLC6A4 gene underscores the relevance of personalized medicine in the management of neuropsychiatric disorders, allowing therapeutic strategies to be tailored to patients' individual needs.

Moreover, an important and notable finding in our analyses was the identification of three novel variants in the indigenous population, which are therefore possibly exclusive to this group, with modifying clinical impact. Among these, two are intronic variants in the ADGRL3 gene, at positions 61,813,904 and 62,063,484, while one is located in the ADRA2A gene, at position 111,077,982, in the UTR_5'PRIME region. The ADGRL3 gene is responsible for establishing precise glutamatergic signaling during neuronal development by encoding a protein that modulates interactions between adjacent neurons, thus affecting axon guidance, synaptogenesis, and synaptic plasticity (Meza-Aguilar et al. 2014 and Kappel et al. 2019). The ADRA2A gene plays a critical role in the regulation of noradrenergic neurotransmission, which is strongly linked to ADHD (Chen et al. 2023). Furthermore, this gene encodes the α2A-adrenergic receptor, which, when stimulated, mediates the increase in intrinsic excitability induced by methylphenidate (Yuan et al. 2021).

The results obtained in this study through MDS analysis clearly demonstrate that our study population is genetically distinct from other continental populations in relation to the genes investigated. The Brazilian admixed population, primarily from the Amazon, has the largest contribution of Amerindian ancestry in the country, averaging approximately 30% (Cardoso DC, 2020 apud Santos NP, 2010). This admixture directly impacts the fluctuation in the frequency of genetic variants that may contribute to a predisposition to various disorders, such as ADHD.

According to Cohen-Paes A (2022), understanding the molecular profile of a population is one of the foundations for inferences

TABLE 2 | Description of new variants found in the indigenous population of the Brazilian Amazon for genes related to methylphenidate therapy.

Gene	Chromosome	Position	Var Type	Region detailed	Reference	Variant	Impact	Change protein	Altera-tion	INDG
ADGRL3	chr4	61,813,904	SNV	INTRON	A	T	MODIFIER	c.1480+15A>T	—	0.0714
ADRA2A	chr10	111,077,982	SNV	UTR_5_ PRIME	C	T	MODIFIER	c.-15C>T	—	0.0161
ADGRL3	chr4	62,063,484	SNV	INTRON	C	G	MODIFIER	c.3815-6625C>G	—	0.1

Note: (—) No annotation.
Abbreviation: INDG, Indigenous Amazonian population.

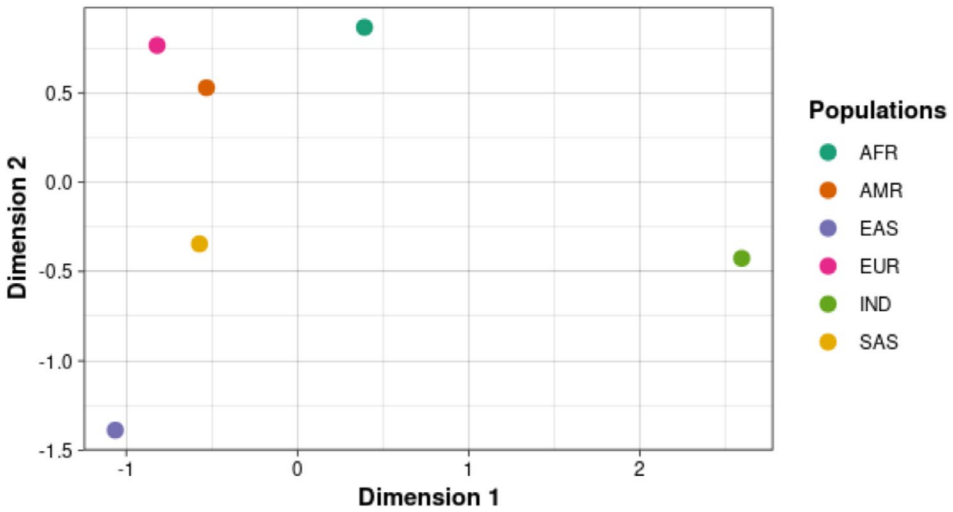

FIGURE 2 | Differences in the allele frequencies of the variants studied in the continental and in the Native American population, plotted in MDS.

about human evolutionary history, as it aids in the validation of molecular biomarkers of susceptibility to complex and rare diseases, as well as in the improvement of specific precision medicine protocols applied to these populations and to populations with high Amerindian ancestry, such as the Brazilian population. These findings highlight the importance of research focused on the genomic characteristics of Indigenous communities to improve our understanding of the genes that may influence the response to methylphenidate in the treatment of ADHD—a condition still poorly understood in these populations.

The data obtained in our research can contribute to the development of public policies targeted at these groups, aiming to establish precision medicine guidelines adapted to their unique genetic diversity, which differs significantly from that of other populations worldwide. One example of this could be the incorporation of local clinical protocols in regions with a high prevalence of individuals of Amerindian ancestry, such as the North of Brazil, where pharmacogenomic testing could be performed as part of the diagnostic process or therapeutic adjustment for ADHD, minimizing the adverse effects resulting from the inappropriate use of methylphenidate, as well as reducing spending on medications that may be misused in the public health system. Among the main foreseeable barriers are insufficient laboratory infrastructure, the high cost and difficulty in accessing genetic testing, ethnic and social inequality, and the invisibility of indigenous peoples in public health policies.

5 | Conclusions

This study analyzed genes associated with methylphenidate therapy for ADHD in an Amazonian indigenous population. Our findings highlight the distinct genetic profile of this population, identifying one high-impact variant and five moderate-impact variants, as well as three novel mutations with modified impact in the *ADGRL3* and *ADRA2A* genes, which had not been previously described. Investigating the genetic characteristics of an understudied population is essential for guiding clinical management and therapeutic approaches, with the goal of improving treatment response to Ritalin and contributing to the development of precision medicine guidelines. Therefore, our data underscore the urgent need for further research into the impact of these variants and their associations with methylphenidate use in indigenous individuals with ADHD, ultimately advancing personalized medicine tailored to the specific genetic background of this population.

Author Contributions

Aline Pasquini Santos: writing – original draft, visualization, and investigation. **Tatiane Carinta de Souza:** writing – original draft, visualization, and investigation. **Juliana Carla Gomes Rodrigues:** formal analysis. **Natasha Monte:** formal analysis. **Kaio Evandro Cardoso Aguiar:** review and editing, visualization. **Ana Caroline Alves da Costa:** writing – original draft. **Rita de Cássia Calderaro Coelho:** writing – original draft. **Marianne Rodrigues Fernandes:** writing – review

and editing. **Giovanna Gilioli da Costa Nunes:** writing – original draft. **André Maurício Ribeiro dos Santos:** bioinformatics analysis. **Sandro José de Souza:** sample collection. **Ândrea Ribeiro-dos-Santos:** exome analysis. **Sidney Emanuel Batista dos Santos:** supervision. **Rommel Mario Rodriguez Burbano:** supervision. **Ney Pereira Carneiro dos Santos:** writing – review and editing, supervision, project administration, methodology, funding acquisition, and conceptualization.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Information about genes, gene function and relation with *methylphenidate*. **Table S2:** Description of the 12 variants with low impact prediction present in the genes *ADGRL3, CES1, ADRA2A, DRD2, DRD3, SLC6A2* and *SLC6A3* on the Indigenous population (INDG) and continental populations (African (AFR), American (AMR), East Asian (EAS), European (EUR) AND South Asian (SAS)) described in the 1000 genomes and GnomAD database.