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# The Dopamine Receptor D5 May Influence Age of Onset: An Exploratory Study on Indo-Caucasoid ADHD Subjects

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**Abstract**

The objective was to investigate contribution of the dopamine receptor 5 (DRD5) gene variants in the symptoms of attention-deficit/hyperactivity disorder (ADHD) probands since brain regions identified to be affected in these group of patients have higher expression of the DRD5 receptor. Out of 22 exonic variants, 19 were monomorphic in the Indo-Caucasoid individuals. rs6283 "C" and rs113828117 "A" exhibited significant higher occurrence in families with ADHD probands. Several haplotypes showed biased occurrence in the probands. Early and late onset groups exhibited significantly different genotypic frequencies. A new G>A substitution was observed in the control samples only. The late onset group exhibited higher scores for hyperactivity as compared to the early onset group. The authors infer that the age of onset of ADHD may at least partially be affected by DRD5 variants warranting further investigation on the role of DRD5 in the disease etiology.

**Keywords**

ADHD, DRD5, rs6283, rs113828117, rs1800762

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Attention-deficit/hyperactivity disorder (ADHD) is a symptomatic neurobehavioral disorder with features of inattention, hyperactivity/impulsivity, or both.[1,2] Symptoms often persist till adulthood in 30-50% cases although hyperactivity tends to diminish with age.[1-3] Though diagnosed often during the early childhood, a diagnosis can also be made at a later stage (>10 years),[2] which could be due to the presence of symptoms at a subthreshold level in the early age and a formal diagnosis of ADHD due to the prominence of symptoms under social, biological or psychological influences as they grow older. A review of published literature on long term follow up of individuals with ADHD revealed that about 40% develop personality disorder, substance abuse and/or criminality during adulthood,[4] making early recognition necessary for proper intervention.[5]

ADHD is highly heritable and since symptoms are alleviated with medications targeting mainly the dopamine/norepinephrine transporters, candidate gene search has been extensively carried out on the dopaminergic system.[6] Dopamine secreted mostly from the midbrain region, targets effectors cells of the forebrain especially the prefrontal cortex, basal ganglia, thalamic, hypothalamic regions, and part of the limbic system[7-10] and helps in the maintenance of homeostasis and higher order mental functions.[10]

The DRD type-I super family receptors (DRD1 and DRD5) which stimulate production of adenylate cyclase via G-protein coupling, thereby activating c-AMP dependent protein kinases,[11,12] are most abundantly found in major portion of the forebrain including pars compacta region of the substantia nigra, cerebral cortex, nucleus accumbens, hypothalamus, and striatum.[13] These regions govern cognitive functions like learning and memory, logical reasoning, social orientation, numerical analysis, and so on.[14,15] Recent reports also suggest a role of DRD5 in substance abuse, paranoid schizophrenia and ADHD.[16-18]

Expression of DRD5 is highly neuron specific within the limbic region of the brain,[13] hippocampus, the mamillary nuclei and the anterior pretectal nuclei.[19] DRD5 is encoded by an intronless gene located in the human chromosome (HSA) 4p16.1 having a length of over 1400 bp.[19-21] Two pseudogenes with 95% sequence homology have also been detected. An extensive bioinformatic exploration confirmed that the

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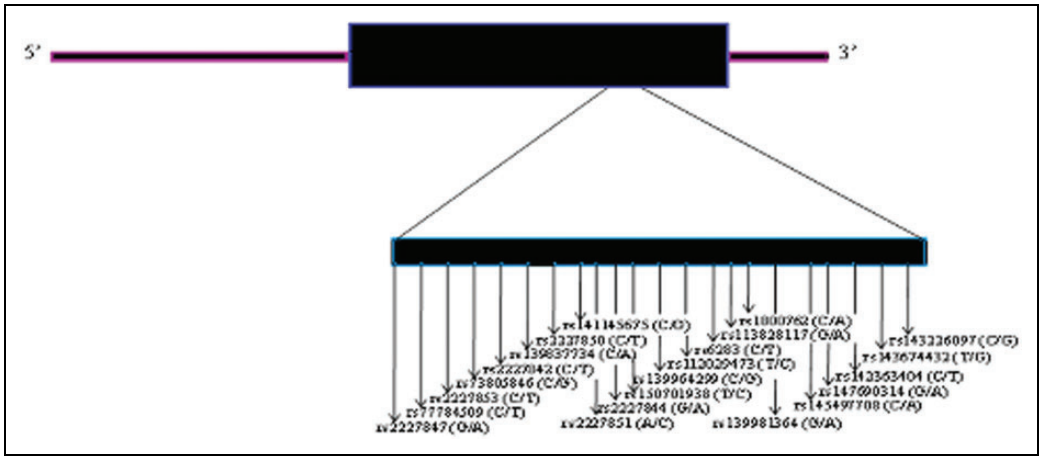


**Figure 1.** DRD5 single nucleotide polymorphisms analyzed in the present study.

segmental duplications/pseudogenes are localized in the HSA1 and HSA2 respectively.[22]

DRD5 has been recognized as 1 of the candidate genes for ADHD in the Caucasian population.[6] However, to date, no report is available on the contribution of DRD5 in the Indo-Caucasoid ADHD probands. The present investigation was aimed at finding out the role of DRD5 variants in the etiology of ADHD, primarily focusing on the age of disease onset and symptom severity.

## Materials and Methods

### Subjects

Nuclear families with ADHD probands were recruited after assessment through DSM-IV-TR[1] followed by psychological evaluations through (1) Conners' Parent Rating Scale–Revised,[23] for assessing the level of hyperactivity, inattention, and behavioral problems as well as disease severity by scoring the index, and (2) Intelligence Quotient assessment by the Wechsler Intelligence Scale for children above 5 years.[24] Children below 5 years were assessed for Developmental Quotient by the Developmental Screening Test for children.[25] Individuals suffering from any psychiatric problem, pervasive developmental disorders, any form of intellectual disability (IQ < 70) were excluded from the study. Subjects included were 134 parent-proband trios, 14 with 1 parent and 2 affected subjects only. Mean age of the probands was 7.7 $\pm$ 2.3 years, with a male to female ratio of 10:1, and all the probands were drug naïve at the time of recruitment. Unrelated control individuals (n = 100, IQ $\geq$ 90, mean age 8.9 $\pm$ 6.8 years, male to female ratio 10:3) evaluated similarly were recruited for population based analysis. All the cases and controls belonged to the Indo-Caucasoid ethnic category. Informed written consent for participation was obtained for all the recruited subjects. The Institutional Human Ethical Committee approved the study protocol.

### Genotyping

Peripheral blood collected from ADHD subjects, their parents, and control individuals was processed for genomic DNA isolation from leukocytes.[26] Based on the published protocol,[22] genomic DNA was treated with *NcoI* restriction enzyme in excess to cleave the pseudogenes but sparing the DRD5 exonic stretch, the trace amount of uncut DNA removed by T7 endonuclease and used for polymerase chain reaction (PCR) amplification. PCR products were subjected to Di-deoxy chain termination method of sequencing followed by analysis by 3130 DNA sequence analyzer. Twenty-two exonic single nucleotide polymorphisms (Figure 1), denoted by their respective rs numbers obtained from the dbSNP database (dbSNP: http://www.ncbi.nlm.nih.gov/SNP/), were investigated.

### Estimation of Endophenotypes and Age of Onset

Level of hyperactivity, inattention and severity of the disorder were assessed through Conners' Parent Rating Scale–Revised and for further analysis, *t* scores for each individual score were used. Age of onset of the disorder was determined based on the feedback received from the parents during recruitment; children reported to have syndromic features before 7 years were categorized as early onset group (mean age 5.09 $\pm$ 2.8 years), while children being syndromic by and after 7 years (mean age 10 $\pm$ 2.9 years) were categorized as the late onset group.

### Statistical Analysis

Genotypic frequencies were analyzed for the Hardy-Weinberg equilibrium and for sites maintaining the equilibrium ($P \geq .05$), further population- and family-based analyses were performed using the Unphased program.[27] Power of relevant data were calculated at 5% significance level.[28] Linkage disequilibrium between the single nucleotide polymorphisms was obtained by the program Haploview.[29] Contingency correlation test was performed to find out association between gene variants and groups with different age of disease onset. Comparative analysis on trait scores was performed by the Mann-Whitney test and correlation was calculated by the Pearson's correlation coefficient test.

## Results

Out of 22 single nucleotide polymorphisms, only 3, rs6283 (C/T), rs113828117 (G/A) and rs1800762 (C/A), were polymorphic in the studied population. Genotypic frequencies followed the Hardy-Weinberg equilibrium for all (Table 1).

**Table 1.** Population-Based Comparative Analysis of DRD5 Variants.

| Single nucleotide polymorphism ID | Allele/ genotype | Control (n = 100) | ADHD probands (n = 150) | $\chi^2(P)$[a] | Power α @ .05 | Parents of probands (n = 282) | $\chi^2(P)$[a] | Power α @ .05 | Hardy-Weinberg equilibrium |
|---|---|---|---|---|---|---|---|---|---|
| rs6283 | C | 0.47 | 0.67 | **8.16 (.004)** | 93.8 | 0.65 | **6.57 (.01)** | 88.1 | OK |
| | T | 0.53 | 0.33 | | | 0.35 | | | |
| | CC | 0.25 | 0.49 | **14.4 (.001)** | 93.4 | 0.46 | **17.40 (.0002)** | 97.0 | |
| | CT | 0.43 | 0.33 | | | 0.39 | | | |
| | TT | 0.32 | 0.16 | | | 0.15 | | | |
| rs113828117 | G | 0.98 | 0.83 | **13.1 (.0001)** | 99.3 | 0.83 | **13.1 (.0001)** | 99.3 | OK |
| | A | 0.02 | 0.17 | | | 0.17 | | | |
| | GG | 0.96 | 0.73 | **20.8 (.0001)** | 98.8 | 0.72 | **30.52 (2.3524e-007)** | 99.0 | |
| | GA | 0.04 | 0.20 | | | 0.21 | | | |
| | AA | 0.00 | 0.07 | | | 0.07 | | | |
| rs1800762 | C | 0.97 | 0.98 | 0.21 (.65) | — | 0.97 | 0.00 (1.00) | — | OK |
| | A | 0.03 | 0.02 | | | 0.03 | | | |
| | CC | 0.95 | 0.98 | 1.71 (.43) | — | 0.95 | 0.01 (.92) | — | |
| | CA | 0.04 | 0.02 | | | 0.05 | | | |
| | AA | 0.01 | 0.00 | | | 0.0 | | | |
| SNPD5 | G | 0.92 | 1.00 | **8.33 (.004)** | 94.2 | 1.00 | **8.33 (.004)** | 94.2 | OK |
| | A | 0.08 | 0.00 | | | 0.00 | | | |
| | GG | 0.88 | 1.00 | **12.8 (.002)** | 98.2 | 1.00 | **12.8 (.002)** | 98.2 | |
| | GA | 0.09 | 0.00 | | | 0.00 | | | |
| | AA | 0.03 | 0.00 | | | 0.00 | | | |

Significant differences are presented in bold. [a]In comparison to the control and stands after Benjamini and Hochberg's correction for multiple testing.

A new substitution (G/A), mentioned as SNPD5, was detected in the studied population.

Population-based analysis revealed significant over representation of rs6283 "C" and rs113828117 "A" alleles in families with ADHD probands as compared to the control population (Table 1); power of these association tests was significantly high (>80). Allelic and genotypic frequencies of rs1800762 showed lack of any significant difference (Table 1). Population-based analysis of SNPD5 revealed presence of the "A" allele in the control population only while the "G" allele was detected in families with ADHD probands (Table 1; $P = .004$). Stratified analysis also revealed statistically significant differences in allelic and genotypic frequencies for the male probands ($P < .001$ for rs6283, rs113828117 & SNPD5, $P = .05$ for rs1800762). Family-based analyses failed to show any statistically significant over transmission (Supplemental Table 1).

Case-control comparison also revealed statistically significant difference in the frequencies of haplotypes formed between rs6283 "C," rs113828117 "A" and rs1800762 "C" (Table 2). Haplotypic combinations involving variants of the SNPD5 also showed significant differences between cases and controls (Table 2); ADHD probands exhibited significantly higher frequencies of haplotypes formed by SNPD5 "G," rs6283 "C" and rs113828117 "A," which was markedly significant for the male probands (P = 3.93e-005). Conversely, higher occurrence of haplotypes comprising of SNPD5 "A," rs6283 "T," rs113828117 "G" and rs1800762 "C" alleles were mostly noticed in the control group ($P > .05$). Family-based analysis failed to show any bias in haplotype transmission (data not shown).

Though rs6283, rs113828117, and rs1800762 were within 27 bases, linkage disequilibrium between them was medium (D' > .6) in the studied subjects (Supplemental Table 2); moreover the confidence value $r^2$ was low, indicating lack of actual linkage disequilibrium. Linkage disequilibrium between SNPD5 and other 3 single nucleotide polymorphisms was also very low (Supplemental Table 2; $r^2 < .03$).

## *Association of Alleles with Age of Onset*

Statistically significant difference in genotypic frequencies was found between the early and late onset groups for all the 3 sites (Table 3; $P < .05$); for both rs6283 and rs113828117, frequency of the derived allele in homozygous condition was higher in the early onset group as compared to the late onset group. For rs1800762, frequency of the derived allele in heterozygous condition was higher in the early onset group (Table 3).

Comparative analysis on phenotypic traits revealed that both early and late onset groups had significant impairment in inattention, hyperactivity and ADHD index as compared to age-matched controls (Table 4, $P < .001$). Analysis within ADHD subjects showed that score for hyperactivity was relatively higher ($P = .02$) in probands with late onset of the disorder as compared to those with early onset. In absence of any strong correlation between the age of the children and the level of inattention ($r = –.04$), positive correlations were found for hyperactivity and ADHD index ($r = .16, .12$, respectively); however the data were statically insignificant ($P > .05$).

**Table 2.** Population-Based Comparative Analysis on Haplotype Frequencies (Only Significant Values Are Presented).

| Single nucleotide polymorphism combination | Haplotype | Controls | Probands | $\chi^2(P)$ | Power |
|---|---|---|---|---|---|
| rs6283 - rs113828117 | C-G | 0.40 | 0.58 | 3.74 (.05) | 55.4 |
| | C-A | 0.00 | 0.12 | 9.64 (.002) | 93.6 |
| | T-G | 0.60 | 0.30 | 10.69 (.001) | 95.7 |
| rs6283 - rs1800762 | C-C | 0.43 | 0.76 | 14.68 (.0001) | 99.7 |
| | T-C | 0.57 | 0.24 | 14.68 (.0001) | 99.7 |
| rs113828117 - rs1800762 | G-C | 1.00 | 0.91 | 10.66 (.001) | 97.9 |
| | A-C | 0.00 | 0.09 | 10.66 (.001) | — |
| rs6283 - rs113828117 - rs1800762 | C-G-C | 0.40 | 0.58 | 3.82 (.05) | 56.3 |
| | C-A-C | 0.00 | 0.12 | 9.36 (.002) | 92.9 |
| | T-G-C | 0.60 | 0.30 | 10.73 (.001) | 95.8 |
| SNPD5 - rs6283 | G-C | 0.45 | 0.76 | 12.96 (.0003) | 98.3 |
| | G-T | 0.53 | 0.24 | 11.45 (.0007) | 96.8 |
| SNPD5 - rs113828117 | G-A | 0.00 | 0.08 | 10.06 (.002) | 94.5 |
| | A-G | 0.04 | 0.00 | 4.7 (.03) | — |
| SNPD5 - rs1800762 | G-C | 0.96 | 1.00 | 5.43 (.02) | 81.4 |
| | A-C | 0.04 | 0.00 | 5.43 (.02) | — |
| SNPD5 - rs6283 - rs113828117 | G-C-A | 0.00 | 0.12 | 9.07 (.002) | 88.7 |
| | G-T-G | 0.56 | 0.30 | 8.09 (.004) | 84.5 |
| SNPD5 - rs6283 - rs1800762 | G-C-C | 0.45 | 0.76 | 12.81 (.0003) | 98.2 |
| | G-T-C | 0.53 | 0.24 | 11.27 (.0007) | 96.6 |
| SNPD5 - rs113828117 - rs1800762 | G-A-C | 0.00 | 0.08 | 10.03 (.001) | — |
| | A-G-C | 0.04 | 0.00 | 4.79 (.03) | — |
| SNPD5 - rs6283 - rs113828117 - rs1800762 | G-C-A-C | 0.12 | 0.00 | 8.78 (.003) | — |
| | G-T-G-C | 0.30 | 0.57 | 8.06 (.004) | 84.3 |

**Table 3.** Analysis of Association Between Variants and Age of Onset of the Disorder as Well as Other Traits.

| | | Frequency of cases with different age of onset | | | Trait score (mean ± SE) | | |
|---|---|---|---|---|---|---|---|
| ID | Genotype | Early | Late | $\chi^2(P)$ | Inattention | Hyperactivity | ADHD index |
| rs6283 | CC | 0.53 | 0.39 | **8.46 (.015)** | 71.29 ± 1.88 | 73.90 ± 1.88 | 71.12 ± 1.23 |
| | CT | 0.29 | 0.49 | | 71.29 ± 1.77 | 72.52 ± 2.41 | 70.06 ± 1.67 |
| | TT | 0.18 | 0.12 | | 72.41 ± 3.06 | 72.0 ± 4.28 | 69.92 ± 2.70 |
| rs11328117 | GG | 0.75 | 0.67 | **6.32 (.042)** | 72.33 ± 1.32 | 72.62 ± 1.72 | 70.56 ± 1.23 |
| | GA | 0.17 | 0.30 | | 69.44 ± 3.5 | 73.94 ± 2.97 | 70.31 ± 1.57 |
| | AA | 0.08 | 0.03 | | 67.25 ± 2.86 | 72.13 ± 4.81 | 69.5 ± 2.16 |
| rs1800762 | CC | 0.99 | 0.91 | **6.74 (.009)** | 71.16 ± 1.20 | 72.94 ± 1.41 | 70.16 ± 0.92 |
| | CA | 0.01 | 0.09 | | 75.75 ± 5.74 | 72.5 ± 9.95 | 75.75 ± 7.35 |
| | AA | 0 | 0 | | — | — | — |

Significant differences are presented in bold.

**Table 4.** Difference in Phenotypic Traits of ADHD Probands as Compared to Age-Matched Controls (Mean ± SE).

| | | Group | | | | | |
|---|---|---|---|---|---|---|---|
| | | Early (3-6.11 years) | | | Late (≥7 years) | | |
| Subjects | n (E/L) | IA | HA | AI | IA | HA | AI |
| Control | 100 (76/24) | 51.06 ± 1.56 | 52.73 ± 3.13 | 52.4 ± 2.57 | 45.73 ± 0.71 | 51.07 ± 2.03 | 48.67 ± 1.33 |
| ADHD probands[a] | 150 (69/81) | 71.43 ± 1.94 | 70.63 ± 1.78** | 69.17 ± 1.28 | 72.57 ± 1.43 | 77.03 ± 2.62** | 72.7 ± 1.5 |

AI, ADHD index ; E, early; HA, hyperactivity; IA, inattention; L, late. [a]For ADHD probands age indicated are the age of onset of disease.
$P < .001$ for all case-control comparisons. Comparison between early and late onset groups for HA. **$P = .02$.

## Discussion

DRD5 mainly functions on neurons of the midbrain substantia nigra, limbic system including the thalamus, hypothalamus, amygdala, hippocampus, basal ganglia, and anterior cingulated cortex.[11-15,17] DRD5 was also localized in the medium spiny and large cholinergic neurons of the prefrontal cortex,[13] indicating its dual role in both excitatory postsynaptic potential and inhibitory postsynaptic potentials. In vitro studies located target cells in the neocortical and hippocampal tissues.[30] Anterior cingulate cortex is a region responsible for selective attention while basal ganglion is the source of dopamine through the nigro-striatal pathway. As children/adolescents with ADHD suffer from attention related problems, mainly selective and sustained attention involving anterior cingulate cortex and dorsolateral prefrontal cortex, presence of DRD5 in both these regions makes the receptor a good target for study. Moreover, since the limbic system is grossly involved in emotional arousal and emotion related memory formation, altered signaling through DRD5 may perturb this function, leading to comorbid mood disorder in subjects with ADHD. Deficiency in D5 receptors was hypothesized to contribute to learning problems in an animal model of ADHD,[31] indicating a potential role of DRD5 dysfunction in ADHD associated learning difficulties.

In the present study, ADHD probands showed higher level of inattention and hyperactivity in comparison to the age-matched healthy children. Comparative analysis between the early and late onset subgroups revealed that, while inattention was a common challenge for both, scores for hyperactivity was significantly higher in the late onset group (7-14 years), which could be attributed to recruitment of treatment naïve probands at an age (7-14 years) when disruptive symptoms were more prominent. In the Diagnostic and Statistical manual for Mental Disorders V,[2] the age of onset criteria has been revised to ''several inattentive or hyperactive-impulsive symptoms were present prior to 12 years,'' thereby indicating that symptoms leading to clinically significant impairment may appear after the age of 7 years and in this study's subjects the number of probands was higher in the late onset group. Furthermore, the ratings in the present study were based solely on the feedback received from parents, reflecting observations in 1 setting only. Against this backdrop, the current observation warrants further investigation in large cohort of ADHD subjects to understand the actual differences between children and adolescents, since more hyperactivity and impulsivity were reported earlier in younger children.[32]

Stratification based on the age of onset of the disorder revealed statistically significant differences in genotypic frequencies between the early and late onset groups for all the 3 sites. rs6283 ''CC'' was over represented in the probands with early onset of the disorder, while the late onset group showed higher frequency of the heterozygous ''CT'' genotype. rs1800762 and rs11328117 also exhibited higher frequencies of the heterozygous genotype in the late onset group leading to the possibility of contribution of the ancestral allele/s in the early expression of symptoms. While comparing the scores for each trait on the basis of genotype, mild variation was also observed. Comparative analysis showed over presence of ''C-A-C'' and ''C-G-C'' in the ADHD patients while frequency of ''T-G-C'' was more in the healthy controls. Thus the authors may hypothesize that allelic shift, even in heterozygous condition, may affect expression of phenotypic traits in the ADHD probands.

Earlier investigators principally analyzed 2 polymorphic microsatellites in the DRD5, located outside the duplication site.[33,34] A significant number of association studies were also performed[6,22,34-41] and meta-analysis of published data supported positive association of DRD5 with ADHD.[42,43] However, to date, association of DRD5 with ADHD has never been explored in the Indo-Caucasoid population. The authors' attempt was to understand the role of DRD5 exonic single nucleotide polymorphisms, rs2227847 (G/A), rs77784509 (C/T), rs2227853 (C/T), rs73805846 (C/G), rs2227842 (C/T), rs139837734 (C/A), rs2227850 (C/T), rs141145675 (C/G), rs2227851 (A/C), rs2227844 (G/A), rs150701938 (T/C), rs139964299 (C/G), rs112029473 (T/C), rs6283 (C/T), rs113828117 (G/A), rs1800762 (C/A), rs139981364 (G/A), rs145497708 (C/A), rs147690314 (G/A), rs142363404 (C/T), rs143674432 (T/G) and rs143226097 (C/G), located within 9784305 to 9785248 bases on the chromosome 4p16.1, in the etiology of ADHD. Other 6 single nucleotide polymorphisms were investigated in 30 families with ADHD probands and 171 unrelated controls and no significant contribution was observed.[22] In the present study, 150 Indo-Caucasoid ADHD probands along with their parents and 100 healthy unrelated controls were recruited. This investigation for the first time exhibited statistically significant association of 3 single nucleotide polymorphisms including rs113828117. Haplotype analysis also supported a clustering of rs6283 ''C'' and rs113828117 ''A'' alleles in the probands. A new polymorphic site (SNPD5) was detected, the ''A'' allele of which may essentially serve as a protective factor for the disorder since it was found only in the control group but not in families with ADHD probands. ADHD is believed to have a strong genetic basis with familial predisposition[44] and the observed clustering of risk alleles in families with ADHD probands provides further support to the notion.

Though the single nucleotide polymorphisms are located closely, the present study revealed very weak linkage disequilibrium between the 3 sites. Whether this is due to the presence of recombination hotspots between the sites or is the resultant of small sample size is a matter of conjecture which needs further validation with large cohort of samples.

In silico analyses in the present investigation through the online PESX program revealed that the rs6283 ''T'' allele creates a splice site in the putative exon, while rs1800762 is a missense polymorphism with a regulatory potential of 0.33. No functional contribution could be predicted for rs113828117 and SNPD5.

An earlier analysis on the American Caucasian population revealed significant association between single nucleotide polymorphisms surrounding the DRD5 gene and the age of disease onset;[45] in absence of any direct documentation, the

authors concluded that the observed variants may be in linkage disequilibrium with a functional DRD5 gene variant. This study, for the first time, documented association of DRD5 exonic single nucleotide polymorphisms with ADHD symptoms and thus probably have identified the "functional variant" mentioned by the previous investigators.[45]

## Conclusion

ADHD is an early onset neurobehavioral disorder affected by multiple factors; gene variants, environmental factors, maternal complication during pregnancy and many more finally give rise to a number of symptoms observed in a proband with ADHD.[6]

The cardinal features of ADHD, inattention, hyperactivity, and impulsivity, are regulated by brain regions showing expression of DRD5, making it one of the candidates for the disorder.[46] DRD5 is a structural gene with more than 10 times higher affinity for dopamine as compared to DRD1 and alteration in receptor expression may attenuate adenylate cyclase activity thereby affecting neurotransmission. Deleterious substitution, even in heterozygous form, can hamper function of the protein. In silico analysis in the present study revealed that both rs6283 and rs1800762 have the potential of altering protein expression. ADHD children harboring the rs6283 "C" and rs113828117 "A" alleles may have altered DRD5 signaling culminating in varied disease penetrance, scholastic under achievement and comorbid disorders. Based on the data obtained in the present study, further investigation on the role of DRD5 is warranted in the etiology of ADHD.

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### Author Contributions

SM and KS performed genotyping and statistical analysis and drafted the manuscript and contributed equally to this work. SS helped in subject recruitment. KM conceptualized the work, supervised its execution, and edited the manuscript. All the authors approved the final manuscript.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Ethical Approval

Approval from the Institutional Human Ethical Committee and parental consent were obtained for all patients.

### Supplemental Material

The online supplemental tables are available at http://jcn.sagepub.com/supplemental.

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