

DR D2 polymorphism in Egyptian with Attention-Deficit Hyperactivity Disorder Children sample

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Abstract

Background: ADHD is a highly heritable disorder. Several genes were found to be involved; one of the commonest genes is dopamine D2 gene which was found to be associated with ADHD.

Objectives: The purpose of this study was the correlation of the children with ADHD DRD2 gene polymorphism. We hypothesized a positive correlation of DRD2 polymorphisms in ADHD patients.

Methodology: Our research sample included 100 children aged between 4 and 12 years (8.1 ± 1.8). The ADHD group consisted of 50 cases; diagnosed by Diagnostic and Statistical Manual of Mental Disorders, fourth Edition TR (DSM- IV- TR), and the 50 control of comparable age and sex. PCR- RFLP analysis was done for the detection of the DRD2 polymorphism

Results: A1 allele was more encountered in cases compared to controls, while the A2 allele was significantly more prevalent among controls. A2 allele was statistically insignificant more encountered among mildly inattentive cases, and mild and moderate hyperactivity.

Conclusion: ADHD was significantly associated with dopamine D2 TaqI A Alleles polymorphism in the current study.

Keywords: Attention- Deficit Hyperactivity Disorder (ADHD), D2 Receptor Gene, Polymorphism, Genotype, Phenotype.

دراسة العلاقة بين مرض نقص الانتباه وفرط الحركة مع الجين الدال على الدوبامين D2

الخلفية: اضطراب فرط الحركة ونقص الانتباه هو اضطراب وراثي. تم العثور على العديد من الجينات المشاركة؛ واحد من الجينات الأكثر شيوعاً هو جين دوپامين D2 الذي وجد أنه يرتبط باضطراب فرط الحركة ونقص الانتباه.

الأهداف: كان الغرض من هذه الدراسة هو دراسة تعدد الأشكال الجينية (الدوبامين) لدى الأطفال الذين يعانون من اضطراب فرط الحركة ونقص الانتباه مع افتراضيه وجود علاقة إيجابية بينهما.

المنهجية: شملت عينة البحث لدينا 100 طفل تتراوح أعمارهم بين 4 و 12 سنة. تتألف مجموعة الحالات من 50 حالة؛ تم تشخيصها من قبل الدليل التشخيصي والإحصائي للاضطرابات العقلية، الطبعة الرابعة، بينما الكنترول 50 طفل غير مصاب بفرط الحركة من العمر والجنس للمقارنة. وقد تم الكشف عن تعدد الأشكال الجينية للدوبامين باستخدام مجموعة تنقية GeneJET™ Genomic DNA.

النتائج: وجد الأليل 1 مهما أكثر في الحالات مقارنة بالضوابط، بينما كان الأليل 2 أكثر انتشاراً بين عناصر التحكم. كان الأليل 2 غير ذي دلالة إحصائية أكثر في حالات اللامبالاة البسيطة، وفرط النشاط المعتدل والخفيف.

الخلاصة: ارتبط اضطراب فرط الحركة ونقص الانتباه بشكل كبير مع تعدد الأشكال الدوبامين في الدراسة الحالية.

الكلمات الدالة: اضطراب نقص الانتباه وفرط الحركة، جينات، تعدد الأشكال، التركيب الوراثي، النمط الظاهري.

Introduction:

ADHD is one of the most common neurodevelopmental disorders in young people. It is a developmental disorder characterized by persistent pattern of inattention and/ or hyperactivity- impulsivity. In the majority of cases, symptoms begin in childhood and continue to affect a person's functioning well into adulthood (APA, 2013; Quinn and Madho, 2014; Clara et.al., 2015).

ADHD is diagnosed approximately three times more in boys than in girls. About 30- 50% of people diagnosed in childhood continue to have symptoms into adulthood and between 2- 5% of adults have the condition (National Collaborating Centre for Mental Health, 2014).

Studies suggest that untreated behavioral problems pose significant sociocultural, academic, employment, relationship, and life coping skill deficits (Waite et.al., 2013).

Despite being the most commonly studied and diagnosed psychiatric disorder in children and adolescents, the cause in the majority of cases is unknown; however, it is believed to involve interactions between genetic and environmental factors (Taylor and Eric, 2014). There are several hypotheses to explain occurrence of ADHD. One of them is the dopamine hypothesis which install that dysfunctions in dopamine systems are responsible for some of the symptoms (Volkow et.al., 2010).

The prevalence of ADHD varies from 0.2- 28% in Arab countries. The prevalence of ADHD in children is between 1.70 and 17.8% according to DSM- IV TR studies and of individuals diagnosed with ADHD in childhood the disorder continues into adolescence by 78- 85%, and for 70- 50% of these children, it continues into adulthood (Lara et.al., 2009; Karakas et.al., 2015).

ADHD is a highly heritable disorder. Studies of twins, families, and adoptive children or siblings have estimated a heritability ranging from 60% to 90% making it one of the highest among psychiatric disorders (Geschwind and Flint, 2015).

Dopamine plays important roles in executive functions, motor control, motivation, arousal, reinforcement, and reward, as well as lower- level functions including lactation, sexual gratification, and nausea (Schultz, 2007).

Aim of the Study:

Study the relation between dopamine D2 receptor and ADHD in Egyptian samples.

Design of Study:

Case control study which was conducted at regular working hours for ADHD patients in a period from June 2015 to December 2017.

Subjects:

- ⊠ Patients: Fifty diagnosed ADHD children according to DSM IV TR diagnostic criteria were recruited from the Child Psychiatry Clinic of the Faculty of Postgraduate Childhood Studies, Ain Shams University (50 cases ADHD)
- ⊠ Controls: Fifty of cross matches age and sex were enrolled as well.

1. Inclusion Criteria:

- a. All patients were diagnosed as ADHD according to DSM- IV TR diagnostic criteria.
- b. Both Sexes.
- c. Age Group (4- 12) years

2. Exclusion criteria: We excluded any ADHD child associated with IQ below than 80, diagnosis of pervasive developmental disorders, and presence of any other medical or neurological diseases.**Methods:**

All patients will subjected to the following:

1. Full medical history taking.
2. Clinical Examination.
3. Psychological Assessment:
 - a. DSM- IV TR criteria for ADHD: to settle the diagnosis of the disorder.
 - b. Psychosocial function assessment using Pediatric Symptom Checklist (PSCL): it designed to facilitate the recognition of cognitive, emotional, and behavioral problems so that appropriate interventions can be initiated as early as possible (Little et.al., 1994).
 - c. Conner's Rating Scales- Revised (CRS- R) (parent form): It was completed by parents to assist in evaluating children with ADHD (Conners, 1997).
 - d. ADHD assessment using the ADHD Rating Scale- IV (Collett et.al, 2003).
 - e. IQ test using Stanford- Binet Intelligence Scale V5, The Arabic version (Abu El- Neil, 2011)
4. Genetic study: All of the cases and controls were subjected to dopamine receptor D2 (DRD2) TAq1A1 genotyping by polymerase chain reaction followed by restriction fragment length polymorphism for genotyping (PCR- RFLP) analysis as previously described (Grandy et.al. 1993).
5. Data Analysis: The collected data was organized, tabulated and analyzed using Statistical Package for Social Science (SPSS version 22) (SPSS, 2013). The obtained results were considered statistically insignificant at p- values> 0.05, significant at p- values< 0.05 and highly significant at p- values< 0.01.
6. Ethical Consideration: Written informed consent was obtained from parents after explanation of the aim of the study and its benefits.

Results:

The sample of children with ADHD included 43 males (86%), and 7 females (14%); their mean age (8.1± 1.8) years. There were 50 controls, 38 of them were boys (66%), and 12 were females (34%) with a mean age of 8.7± 1.9 years. Consanguinity and family medical history were statistically insignificantly different between cases and controls (p= 0.231). According to cognitive functions, cases showed more significant prevalence in inattention (96.0%) and poor academic performance (98.0%) compared to controls (P< 0.001). Frequency distribution of the different

score of DSM- IV criteria as encountered among cases with ADHD are presented as 20% are inattentive type, 80% were combined ADHD.

ADHD rating scale showed that 80% of cases were associated with significant inattention, (64%) of cases with significant hyperactivity; with significant total ADHD index (74%).

Analysis of dopamine D2 TAq1A polymorphism revealed that, the percentage of A1 allele was (42%) versus (27%) among controls. While A2 allele was more prevalent in controls (73%), (p= 0.037, statistically insignificant). The inattention component of the Conner's score showed that A1 allele was more encountered among moderately and severely inattentive patients (43.8%, 42.3% respectively), while A2 allele was more encountered among mildly inattentive cases (p- value= 0.901 statistically insignificant). The A1 allele was statistically insignificant in hyperactivity subtype of Conner rating scale which appeared in (50%; 33.3% respectively) of cases with mild and severe symptoms, while A2 allele was more prevalent in cases with mild and moderate symptoms (66.7%; 66.7), (p= 0.145 statistically insignificant).

The A1 allele is more prevalent in ADHD index with insignificant difference in cases which appeared in (45.9%) of cases with severe symptoms (p- value= 0.017), while A2 allele was more encountered in cases with mild and moderate symptoms (75.0%; 68.2%) (p= 0.389).

Table (1) Frequency distribution of the studied polymorphism as regards Conner rating scale (inattentive subtype).

Alleles	Conner's rating scale (inattention subtype)						Chi- Square Test	
	Mild		Moderate		Severe		x ²	P-Value
	No.	%	No.	%	No.	%		
A1 (N= 42)	2	33.3%	7	43.8%	33	42.3%	0.208	0.901
A2 (N= 58)	4	66.7%	9	56.3%	45	57.7%		

Chi- square test (χ²) was used for statistical comparison

* Statistically insignificant at P> 0.05, p< 0.01= statistically highly significant

Table (2) Frequency distribution of the studied polymorphism as regards Conner's rating scale (ADHD index subtype)

Alleles	Conner's rating scale (ADHD index)						Chi- Square Test	
	Mild		Moderate		Severe		x ²	P-Value
	No.	%	No.	%	No.	%		
A1 (N= 42)	1	25.0%	7	31.8%	34	45.9%	1.884	0.389
A2 (N= 58)	3	75.0%	15	68.2%	40	54.1%		

Chi- square test (χ²) was used for statistical comparison

* Statistically insignificant at P> 0.05, p< 0.01= statistically highly significant

Discussion:

ADHD is the most common neurobehavioral disorder of childhood that is characterized by persistent hyperactivity, impulsivity and inattention (Cunningham and Jensen, 2012; Gendy et.al., 2107).

The prevalence of ADHD ranges between 0.9% and 20% which raises concerns about the consistency of estimates and the validity of diagnoses (Wolraich et.al., 2012).

Dopamine (DA) is a key neurotransmitter modulating essential functions of the central nervous system (CNS), like voluntary movement, reward, several cognitive functions and goal- oriented behaviors (Volkow et.al., 2009)

In the current study, it is suggested that there are correlation between DRD2 receptor gene polymorphisms and Egyptian ADHD children.

In the present study; the sample of children with ADHD included 43 males (86%), and 7 females (14%); their mean age (8.1± 1.8) years. There were 50 controls, 38 of them were boys (66%), and 12 were females (34%) with a mean age of 8.7± 1.9 years. This is similar to the study of Keun et.al (2007) in which the mean age was 8.4 years.

Our study showed that, consanguinity, and family medical history reported statistically insignificant differences between cases and controls (χ²=2.929; p= 0.231). Nafi and Shaheen, (2011) reported in their study of all school children aged (6- 12) years (both males and females) in Al Qaser district in South Jordan, and found the prevalence of consanguinity in ADHD student was 34.8%.

Cognitive functions showed significantly higher prevalence of inattention (96.0%)& academic performance was poor (98.0%) in studied cases compared to controls (P< 0.001). This is in agreement with a study done by Mugnaini et.al. (2006) on primary school students in Italy who found that academic performance problems were more prevalent among girls with ADHD.

A1 allele was more encountered among moderately and severely inattentive patients according to Conner rating scale (43.8%, 42.3% respectively), while A2 allele was more encountered among mildly inattentive cases (χ²= 0.208, p- value= 0.901 statistically insignificant).

The study showed that there was statistically insignificant difference between ADHD patients and controls as regards A1allele, which was associated with mild and severe hyperactivity subtype of Conner rating scale ADHD symptoms (50%; 33.3% respectively), while A2 allele was more prevalent in cases with mild and moderate symptoms (66.7%; 66.7) (p- value= 0.145 statistically insignificant). This is in agreement with a study done by Denys et.al. (2005) who examined a sample of 150 patients and 150 controls who suffering from impulsivity, and found in their study that A1 allele of the DRD2 did not find any similarity between cases and controls.

Conclusion:

ADHD was significantly associated with dopamine D2 TAq1 A Alleles polymorphism in the current study.

Reference:

1. American Psychiatric Association (2013): **Diagnostic and statistical manual of mental disorders**, 5th edn. Arlington, VA.
2. Clara I, Sanchez G, Alvarez RR, Insuga VS et.al., (2015): Attention deficit hyperactivity disorder: genetic association study in a cohort of Spanish children. **Behav Brain Funct**, vol 12.
3. Collett BR, Jeneva L. Ohan, And Kathleen M. Myers. (2003): Scales Assessing Attention- Deficit/ Hyperactivity Disorder. **Journal of the American Academy of Child and Adolescent Psychiatry**, 1015- 1037.
4. Conners CK (1997): **Conners' Rating Scales- Revised Technical Manual**. North Tonawanda, NY: Multi- Health Systems Inc.
5. Cunningham NR, Jensen: Attention deficit hyperactivity disorder in Kliegman. **Nelson textbook of pediatrics**. 19th ed. Philadelphia, PA: Saunders Elsevier: 108- 112.

6. Denys D, Nieuwerburgh FV, Deforce D, et.al; (2005): Association between the dopamine D2receptor Taq I A2 allele and low activity COMT allele with obsessive compulsive disorder in males. **European Neuropsychopharmacology**; 1- 5.
 7. EL- Gendy SD, Bitar EA, Awady MA, Bayomy HE, Agwa EM (2017): Attention- Deficit/ Hyperactivity Disorder: Prevalence and risk factors in Egyptian primary School Children. **The Egyptian Journal of community Medicine**; 35: 1.
 8. and Geschwind Flint J (2015): Genetics and genomics of psychiatric disease. **Science**: 349; 1489- 94.
 9. Grandy DK, Zhang Y, Civelli O (1993): PCR detection of the TaqA RFLP at the DRD2 locus. **Hum Mol Genet**; 2: 2197.
 10. Karakas S, Erdog E, Dog E. et.al., (2015): Relationship between diagnosis of ADHD in offspring and current and retrospective self-reports of parental ADHD. **J Child Fam Stud**: 24:3595- 3609.
 11. Keun- Ah Cheon, Boong- Nyun Kim and Soo- Churl Cho (2007): Association of 4- Repeat Allele of the Dopamine D4 Receptor Gene Exon III Polymorphism and Response to Methylphenidate Treatment in Korean ADHD Children. **Neuropsychopharmacology**; 32, 1377- 1383.
 12. Lara C, Fayyad J, de Graaf R, Kessler RC., et.al (2009): Childhood predictors of adult attention deficit/ hyperactivity disorder: Results from the World Health Organization World Mental Health Survey Initiative. **Biological Psychiatry**: 46- 54.
 13. Little M, Murphy JM, and Jellinek MS (1994): Screening 4 and 5 years old children for psychosocial dysfunction: A preliminary study with Pediatric Symptom Checklist. **Journal of Developmental and Behavioral Pediatrics**; 15: 191- 197.
 14. Mugnaini D, Masi G, Brovedani P, Chelazzi C, Matas M, Romagnoli C, et.al., (2006): Teacher reports of ADHD symptoms in Italian children at the end of first grade. **Europ Psychiatry**; 21: 419- 426.
 15. Nafi OA and Shaheen AM (2011): Prevalence of Attention Deficit Hyperactive Disorder (ADHD) in School Children in Al- Qaser District Jordan. **J. Med J**; 45 (1):3 7- 43.
 16. National Collaborating Centre for Mental Health (London) (2014): **National Clinical Practice Guideline Number 72**, Leicester: British Psychological Society.
 17. Quinn PQ& Madhoo M. (2014): **Review of Attention- Deficit/ Hyperactivity Disorder in women and girls: uncovering this hidden diagnosis**. Prim care companion CNS disord: 16 (3).
 18. Schultz W. (2007): Multiple dopamine functions at different time courses. **Annual Review of Neuroscience**; 30: 259- 88.
 19. SPSS (2013): **Statistical Package for the Social Science**; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows
 20. Taylor and Eric (2014): Attention deficit and hyperkinetic disorders in childhood and adolescence. **New Oxford Textbook of Psychiatry**.
 21. Volkow N, Wang G, Newcorn J, et.al., (2010): **Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway Molecular Psychiatry**: 16 (11)
 22. Waite R, Vlam RC, Irrera- Newcomb M, Babcock T (2013): The diagnosis less traveled: NPs' role in recognizing adult ADHD. **J Am Assoc Nurse Pract**: 25: 302- 8.
 23. Wolraich ML, McKeown RE, Visser SN, Bard D, Cuffe S, Neas B et.al (2012): The Prevalence of ADHD: Its Diagnosis and Treatment in Four School Districts Across Two States. **Journal of Attention Disorders**
٢٤. محمود السيد ابوالنيل (٢٠١١): مقياس ستانفورد بينيه للذكاء- الصورة الخامسة، المؤسسة العربية لأعداد وتقنين ونشر الاختبارات النفسية، القاهرة، مصر.