

ADHD risk alleles associated with opiate addiction: study of addicted parents and their children

Asher Ornoy¹, Victoria Finkel-Pekarsky¹, Einat Peles², Miriam Adelson², Shaul Schreiber^{2,3} and P. Richard Ebstein⁴

BACKGROUND: Polymorphisms in genes such as DAT1, 5HTTLPR, D4DR4, and MAO-A have been linked to attention deficit hyperactivity disorder (ADHD) and susceptibility for opiate addiction. We investigated in opiate-addicted parents and their children the rate of ADHD and genetic markers that could predict susceptibility to ADHD and/or opiate addiction.

METHODS: We studied 64 heroin-addicted, methadone-maintained parents, and their 94 children who had or had not been exposed prenatally to opiates. DNA extracted from mouthwash was assessed for genetic polymorphism for six polymorphic sites of four different genes. Study subjects also filled a variety of questionnaires assessing the rate of ADHD in the parents and children and the children's intelligence quotient.

RESULTS: Children of opiate-dependent mothers had a higher rate of ADHD compared to those of the opiate-dependent fathers. Opiate-dependent parents have a high risk of being carriers of most risk alleles examined except DRD4EX3 (allele 7). There was no difference whether the addicted parents had or did not have ADHD.

CONCLUSIONS: Serotonergic and dopaminergic risk alleles seem to be mainly related to opiate dependence with no effect on the occurrence of ADHD. People carrying those polymorphisms are susceptible to opioid addiction and not necessarily to ADHD.

The percent of women among opiate-dependent people is constantly rising, and hence the number of children born to opiate-addicted mothers. Drug dependency is influenced by both genetic and environmental factors (1–4). Many investigators have therefore looked for susceptibility genes for addiction, but studies were inconsistent. The main system in the brain associated with drug dependency and attention deficit hyperactivity disorder (ADHD) is the dopaminergic, and to a lesser extent, the serotonergic, gabaergic, glutamatergic, and cholinergic systems. Hence, many studies are carried out to try and delineate any genetic polymorphism related to drug dependency; an example is the fact that CYP2D6 deficiency has an important role in the protection against opiate dependency (5). Moreover, Li *et al.* (4) have found changes in genes related to dopamine

in heroin-addicted persons, differences in the polymorphism of DRD2 promoter, in dopamine receptor (D2-DRD2), dopamine transporter (DAT1), serotonin transporter SLC6A4, serotonin receptor 1B-HTR1B, serotonin receptor 3B-HTR3B, Catechol-O-methyl-transferase, COMPT, GABA-A receptor, γ 2-GABRG2, and mu opioid receptor (1,2,6) and over-representation of the D4 Dopamine Receptor exon III 7repeat allele (7). Most of these changes related to the dopaminergic system were also reported in ADHD, demonstrating possible similar genetic background for the interaction between opiate dependence and ADHD. In addition, important interactions between the dopaminergic and the opioid systems were reported (8).

Polymorphisms of genes related to ADHD have been reported by many investigators. Among them are the following: polymorphisms in the dopamine receptors D3, D4, and D5, the dopamine transporter DAT1, dopamine β hydroxylase (D β H) that converts dopamine to norepinephrine, monoamine oxidase related to the metabolism of dopamine (MAO-A), and the serotonin transporter 5HTTLPR (9–11). Of special importance seems to be the polymorphism for DRD4,7 repeat allele in relation to ADHD, as this allele is less active if compared to those that have the DRD4 repeat allele (9) and polymorphism for gene D4EX120 (allele2) in ADHD (12,13). Polymorphism for some of the above reported genes were also found to be associated to several psychiatric diseases (14).

In spite of the above-described findings, it should be emphasized that several investigators failed to demonstrate a close association of genes related to dopamine and serotonin metabolism with ADHD (15,16).

It is, however, well known that ADHD is a significant risk factor for drug abuse, and there is a high rate of ADHD among heroin-dependent persons (17–19), much higher than the 6–9% of ADHD in populations of normal children (20,21).

Studies on children and adolescents born to heroin-dependent mothers have shown repeatedly a very high rate of ADHD in the offspring. This was still significant even when compared to children born to low socio-economic status families or to heroin-dependent fathers that also have a higher rate of ADHD compared to control children (22,23). This high rate is partially reduced when the children born to heroin-dependent mothers

Served for partial fulfillment of the requirements for an MSc degree in Neurobiology of the Hebrew University.

¹Laboratory of Teratology, Department of Medical Neurobiology, Canada Israel Institute of Medical Research, Hebrew University Hadassah Medical School, Jerusalem, Israel;

²Adelson clinic for drug abuse treatment & Research, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ³Department of Psychiatry, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel;

⁴Department of Psychology, National University of Singapore, Singapore, Singapore. Correspondence: Asher Ornoy (ornoy@cc.huji.ac.il)

Received 26 November 2015; accepted 2 February 2016; advance online publication 11 May 2016. doi:10.1038/pr.2016.78

were adopted at a young age, but was still significantly higher compared to controls, emphasizing the possible relation to both the intrauterine exposure to heroin and the genetic component (22,23). The cognitive delay generally observed in children born to heroin-addicted parents probably stems from the poor environment the children were raised in, as their cognitive and learning abilities are similar to those of low socio-economic status or those born to heroin-dependent fathers (22,24). If the children born to heroin-dependent mothers were adopted at a young age and raised in drug free and good home environments, their cognitive abilities were similar to that of control, normal children (22,24). This emphasizes the importance of the home environment in the neurodevelopmental outcome of children born to drug-addicted parents.

The purpose of the present study was to investigate the relationship between genetic (alleles considered to be risk factors for ADHD and for opiate dependency) and environmental (intrauterine exposure to opiates) factors in children born to opiate-addicted mothers and/or fathers, with or without ADHD comparing the molecular findings between the children and their parents. The children born to opiate-dependent fathers, and therefore not exposed to opiates *in utero*, served together

with their fathers, as controls for the children exposed *in utero* to heroin with their mothers. An additional purpose was to examine if there are, out of the six polymorphisms studied, any genes that may serve as markers for heroin dependence.

RESULTS

The characteristics of the children born to opiate-dependent mothers and fathers as observed in the “general health questionnaire” are described in Table 1. Children born to opiate-dependent mothers were born earlier and had a lower birth weight. Opiate withdrawal symptoms were observed only in the children born to the addicted mothers. A higher rate of ADHD was reported among the children of opiate-dependent mothers compared to those of the opiate-dependent fathers. In the children born to families where both parents were addicted, the characteristics were similar to those of children born to opiate-addicted mothers, and were different from that in those born to opiate-addicted fathers. In these families, all parents smoked, and two thirds of their children were reported to have ADHD (results not shown).

Table 2 summarizes the rate of ADHD among the children born to opiate-addicted mothers or fathers as observed from

Table 1. Characteristics of the children born to opiate addicted mothers and fathers

	Addicted fathers			Addicted mothers			P differences between addicted father and mother		
	Boys	Girls	P	Boys	Girls	P	P boys	P girls	P all
	26	20		16	24				
Age at examination	9.8 ± 3.3	10.5 ± 3.0	0.31	9.5 ± 3.3	9.0 ± 3.0	0.62	na	na	na
Gestational age at birth, weeks	39.1 ± 2.1	39.7 ± 0.9	0.44	38.2 ± 2.9 ^a	37.4 ± 3.2 ^a	0.29	0.316	0.003	0.004
Birth weight, grams	3,247 ± 531	3,249 ± 444	0.98	2,682 ± 638 ^a	2,698 ± 736 ^a	0.94	0.009	0.004	0.0001
Withdrawal symptoms	0	0	na	(11/16) 68.8% ^a	(10/24) 41.7% ^a	na	<0.0001	0.0003	<0.0001
Maternal cigarette smoking	(5/26) 19.2%	(3/20) 15%	na	(16/16) 100% ^a	(22/24) 92% ¹	na	<0.0001	>0.0001	0.0001
In utero exposure to opiates	0	0	na	(16/16) 100% ^a	(24/24) 100% ^a	na	>0.0001	<0.0001	0.0001
Attention deficit hyperactivity disorder symptoms according to parental report	(4/26) 15.4%	0%	na	(5/16) 31.3%	(3/24) 12.5 % ^a	na	0.971	0.036	0.142

na, not available.

^aP < 0.05 between addicted mothers and fathers.

Significant differences are in bold letters.

Table 2. Results of Conner's and short Achenbach questionnaire in children born to opiate-addicted parents

	Addicted father			Addicted mother			P difference between children of opiate addicted fathers and mothers		
	Girls	Boys	All	Girls	Boys	All	P girls	P boys	P all
	20	26	46	23	15	38			
Conner's average score	52.8 ± 11.2	56.9 ± 11.5	55.1 ± 11.5	59.2 ± 10.9	56.7 ± 13.8	58.2 ± 11.9	0.062	0.951	0.232
% children with attention deficit hyperactivity disorder	25	46.2	36.9	56.5 ^a	46.7	52.6	0.037	0.973	0.144
Minimal to maximal value (range)	42–82	41–82	41–82	44–90	30–86	30–86			
Achenbach externalizing	8.2 ± 7.4	14.2 ± 8.8	11.6 ± 8.7	12.1 ± 9.2 [*]	16.6 ± 9.8	13.8 ± 9.5	0.133	0.461	0.284
Achenbach internalizing	6.4 ± 7.7	11.3 ± 8.5	9.2 ± 8.4	8.9 ± 6.8	10.9 ± 8.7	9.6 ± 7.5	0.252	0.861	0.792
Externalizing (range)	1–31	1–30		1–38	2–34				
Internalizing (range)	0–28	0–33		1–22	1–35				

^aP < 0.05 between addicted mothers and fathers in girls.

Significant differences are in bold letters.

the Conner's and short Achenbach (SAC) questionnaires. The average scores on the Conner's were high in all groups; the score was higher in children born to opiate-addicted mothers compared to those with addicted fathers, but the difference did not reach statistical significance ($P = 0.062$). The percent of children with ADHD in those born to opiate-dependent mothers was similarly very high in boys and girls: 46.7% in girls and 56.5% among the boys. It was significantly higher in the girls born to heroin-addicted mothers compared to those born to heroin-dependent fathers, being 25% in girls of heroin-addicted fathers and 56.5% in girls born to heroin-addicted mothers ($P = 0.037$). The rate of ADHD in the girls born to two addicted parents was three times higher compared to the girls born to opiate-addicted fathers (83.3 vs. 25%, $P = 0.012$).

The results of the SAC (Table 2) demonstrate that the externalizing and internalizing scores of the children born to opiate-dependent mothers were higher than in those born to addicted fathers, but the differences were not statistically significant. The externalizing and internalizing scores on the SAC of children were significantly higher when both parents were addicted to opiates, being twice higher than that in children

of heroin-addicted fathers not prenatally exposed to heroin ($P < 0.05$, data not shown).

The Wechsler Intelligence Scale for Children Revised scores are shown in Table 3. Being on average below 90, they were significantly lower than expected in a population of normal school age children. There were no differences in the scores if the mother or father were addicted to opiates (Table 3), or if both parents were addicted (results not shown).

Table 4 shows the results of the parental Wender's questionnaire showing a very high rate of ADHD in the addicted parents. The score was higher in the addicted fathers compared to the addicted mothers, with 55% of the fathers having ADHD and 32% of the mothers. The rate of ADHD in the father, when both parents were addicted, was very high, i.e., ~80%. There was an **insignificant** correlation between the rate of ADHD in the opiate-dependent mothers and ADHD in their children, as only 25% of the mothers of children with ADHD had also ADHD, and 75% of the addicted mothers of children without ADHD had ADHD. No difference in the rate of ADHD was observed in the children of addicted fathers whether they had ADHD or not.

Table 5 shows the rate of the risk alleles in children with or without ADHD born to addicted fathers or mothers. MAO-A

Table 3. Wechsler Intelligence Scale for Children Revised scores in children of opiate dependent mothers and fathers

	Addicted father			Addicted mother			<i>P</i> comparison between children of addicted fathers and addicted mothers		
	Girls	Boys	All	Girls	Boys	All			
<i>N</i>	20	26	46	23	15	38	<i>P</i> girls	<i>P</i> boys	<i>P</i> all
Intelligence quotient score	88.9 ± 14.2	89.0 ± 12.9	88.9 ± 13.3	88.9 ± 11.1	88.7 ± 12.3	88.8 ± 11.4	0.994	0.942	0.963
Minimal to maximal (range)	61–107	69–115		71–120	70–105				

Table 4. Results of Wender Utah questionnaire for parental ADHD

	Opiate-dependent fathers	Opiate-dependent mothers	Both parents are opiate dependent
<i>N</i>	27	28	6
Wender's average score	21.9 ± 49.7	34.5 ± 16.7	46.4 ± 19.0
ADHD in group (%)	55%	32%	80%
Minimal to maximal score (range)	13–91	7–67	8–74

ADHD, attention deficit hyperactivity disorder.

Table 5. A comparison of risk alleles between children with and without ADHD whether the mother or the father were addicted

	Addicted father		<i>P</i> in the group	Addicted mother		<i>P</i> in the group
	ADHD <i>n</i> (%)	No ADHD <i>n</i> (%)		ADHD <i>n</i> (%)	No ADHD <i>n</i> (%)	
Child is carrier of:						
5HTT (allele 2)	12 (70.6%)	15 (55.6%)	0.319	9 (50.0%)	11 (61.1%)	0.502
ST in 2 VNTR (allele 12)	15 (88.2%)	27 (96.4%)	0.285	17 (89.5%)	16 (94.1%)	0.615
MAO-A (allele 4)	12 (70.6%) ^a	9 (32.1%)	0.012	10 (52.6%)	6 (35.3%)	0.296
DAT1 (allele 10)	14 (100.0%)	22 (88.0%)	0.177	15 (75.0%)	15 (83.3%)	0.529
DRD4 EX3 (allele 7)	6 (35.3%)	4 (14.3%)	0.102	3 (15.0%)	10 (58.8%) ^b	0.005
DRD4 EX120 (allele 2)	11 (64.7%)	20 (71.4%)	0.637	13 (65.0%)	11 (61.1%)	0.801

Results are described as numbers and (percentage) of children carrying each one of the "risk" alleles.

^aSignificantly higher than without ADHD. ^bSignificantly higher than without ADHD.

ADHD, attention deficit hyperactivity disorder.

Significant differences are in bold letters.

(allele 4) was increased in the ADHD children born to addicted fathers compared to children without ADHD in the same group ($P = 0.012$) but not in the children born to addicted mothers, and DRD4EX3 (allele7) was increased in the children without ADHD born to opiate-addicted mothers in comparison to children of addicted fathers ($P = 0.005$). A simple Bonferroni correction for multiple testing for the six tested polymorphisms leaves only the noncorrected P values equal to or smaller than 0.0083 significant, hence the results for DRD4 EX3 allele 7 are significant ($P = 0.03$, after correction) whereas the results for MAO-A allele are only marginally significant ($P = 0.06$ after correction).

Table 6 shows the rate of risk alleles for ADHD in the boys and girls in relation to maternal or paternal opiate addiction. There was a significant difference only in DRD4 EX3 (allele7) that was higher in the girls born to addicted mothers; 37.5% of daughters of drug-addicted mothers carried DRD4 EX3 (allele7) compared to only 10% of girls that had heroin-addicted fathers ($P = 0.036$). No differences in risk alleles were observed between the boys and girls born to opiate-addicted fathers and children born to families where both parents were addicted (results not shown). Bonferroni correction for multiple testing leaves the results for DRD4 EX3 allele 7 insignificant ($P = 0.2$).

Table 7 shows the percent of risk alleles in the children with or without ADHD born to opiate-addicted fathers and

mothers that are carriers of the risk alleles. Generally, there was a high inheritance of the following risk alleles: ST in 2 VNTR (allele12), DAT1 (allele10), and DRD4 EX120 (allele2); an intermediate inheritance of 5HTTP (allele2) and MAO-A (allele 4) and a low inheritance of DRD4 EX3 (allele7). There were little differences between children with or without ADHD born to opiate-addicted mothers or fathers. However, there was a reduced rate of carrier state for DAT1 (allele10) in the children with ADHD and addicted mothers ($P = 0.043$) compared to ADHD children of addicted fathers, and an increased rate of DRD4 EX3 (allele7) in the children without ADHD of addicted mothers ($P = 0.002$) compared to children without ADHD of addicted fathers. Bonferroni correction for multiple testing leaves the results for DRD4 EX3 allele7 significant ($P = 0.01$). No differences were found between the children born to addicted fathers who are carriers of the risk alleles and those born to both addicted parents (results not shown).

Table 8 compares the carrier status of the risk allele in the children whose parents are or are not carriers. As expected, there is generally a high rate of carrier state in children whose mothers or fathers are carriers of the different risk alleles compared to their carrier state if the parent is not a carrier. If the opiate-addicted parents are not carriers of the risk alleles, there is generally a low risk for their child to be a carrier, except for

Table 6. Rate of "risk alleles" for ADHD in the boys and girls born to opiate-dependent mothers or fathers

% of risk alleles (type)	Addicted father			Addicted mother			Difference between children of fathers vs. mothers		
	Girls	Boys	All	Girls	Boys	All	P girls	P boys	P all
<i>n</i>	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)			
5HTTP (allele 2)	12 (60.0%)	15 (62.5%)	27 (61.4%)	14 (63.6%)	8 (50.0%)	22 (57.9%)	0.808	0.433	0.749
ST in 2 VNTR (allele 12)	20 (100%)	22 (88.0%)	42 (93.3%)	21 (87.5%)	14 (100%)	35 (92.1%)	0.101	0.177	0.829
MAO-A (allele 4)	6 (30.0%)	15 (60.0%)	21 (46.7%)	12 (52.2%)	5 (33.3%)	17 (44.8%)	0.142	0.103	0.86
DAT1 (allele 10)	16 (88.9%)	20 (95.2%)	36 (92.3%)	19 (79.2%)	12 (75.0%)	31 (77.5%)	0.403	0.074	0.067
DRD4 EX3 (allele 7)	2 (10.0%)	8 (32.0%)	10 (22.2%)	9* (37.5%)	4 (26.7%)	13 (33.3%)	0.036	0.722	0.255
DRD4 EX120 (allele 2)	14 (70.0%)	17 (68.0%)	31 (68.9%)	16 (66.7%)	9 (56.3%)	25 (62.5%)	0.813	0.466	0.533

Results are described as numbers and (percentage) of children carrying each one of the "risk" alleles.

*Significantly higher than girls of addicted fathers.

ADHD, attention deficit hyperactivity disorder.

Significant differences are in bold letters.

Table 7. A comparison of risk alleles in children with or without ADHD if their addicted father or mother is a carrier of the risk alleles

Parent is a carrier of:	Addicted father		Addicted mother		P children with ADHD	P children without ADHD
	Child with ADHD	Child without ADHD	Child with ADHD	Child without ADHD		
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)		
5HTTP (allele 2)	12 (70.6%)	15 (55.6%)	9 (50.0%)	11 (61.1%)	0.214	0.712
ST in 2 VNTR (allele 12)	15 (88.2%)	27 (96.4%)	17 (89.5%)	16 (94.1%)	0.906	0.715
MAO-A (allele 4)	12 (70.6%)	9 (32.1%)	10 (52.6%)	6 (35.3%)	0.269	0.828
DAT1 (allele 10)	14 (100.0%)	22 (88.0%)	15 (75.0%) ^a	15 (83.3%)	0.043	0.663
DRD4 EX3 (allele 7)	6 (35.3%)	4 (14.3%)	3 (15.0%)	10 (58.8%) ^b	0.152	0.002
DRD4 EX120 (allele 2)	11 (64.7%)	20 (71.4%)	13 (65.0%)	11 (61.1%)	0.985	0.466

^aSignificantly lower than child with ADHD of addicted father. ^bSignificantly higher than child without ADHD of addicted father.

ADHD, attention deficit hyperactivity disorder.

Significant differences are in bold letters.

ST in VNTR (allele12) and DRD4 (allele2), when a high percent of the children born to noncarrier parents are carriers. Of exception is MAO-A (allele4) where the risk is only 33.3% if the father is a carrier, compared to 40% if the father is not a carrier. Bonferroni's correction for multiple testing leaves the results in the mothers for MAO-A Allele4, DRD4 EX3 allele 7, and DAT1 allele 10 significant ($P < 0.05$) and 5HTTP allele 2 and DRD4 EX3 allele 7 for the fathers ($P < 0.05$).

If the parents are opiate dependent, they have a high risk of being carriers of most risk alleles examined except D4 EX3 (allele7) that was low (Table 9). There was, however, no difference whether the addicted parents had or did not have ADHD, pointing to the possible associations of these risk alleles mainly to opiate dependence.

Table 10 shows the scores on the different tests of the children that are, or are not carriers of the different risk alleles. As observed for 5 HTTPLR (allele2), the score on the Conner's and the Achenbach internalizing of the children born to two addicted parents was significantly higher compared to those where the father is addicted. Similar results were found regarding the risk allele ST in 2 VNTR (allele12) and with all other risk alleles (Table 10). Regarding risk alleles MAO-A (allele4), and DRD4EX3 (allele7), the score on the externalizing Achenbach was also higher in the children born to two opiate-addicted parents. None of these alleles has any effect on the cognitive abilities.

Interestingly, the Conner's questionnaire score was higher in the noncarriers of MAO-A (allele4) of the children born to addicted mothers. A comparison between the risk alleles in children born to addicted mothers and fathers showed no effects of the risk alleles on the occurrence of ADHD.

DISCUSSION

In our study, we do not have a comparison group of a control normal population of children and adults. Although in previous studies we compared the results of the different questionnaires on children of heroin-dependent fathers and mothers to control children, we did not study the distribution of the risk alleles in the normal Israeli Jewish population. Hence, our comparison can be performed either to the occurrence of the risk alleles in a normal mixed European population (The Allele Frequency Database, www.alfred.med.yale.edu), (Supplementary Table S1 online) or to a normal but relatively homogeneous population of Yemenite Jews in Israel. Regarding MAO-A (allele 4) and 5 HTTPLR (allele2) we also have data on a mixed Israeli population (25). When comparing each one of these figures to the data in our study, it is obvious that the rate of the risk alleles in our study of opiate-addicted parents and their children is higher for HTTPLR (allele2), DAT (allele10), ST in2VNTR (allele12), and D4 EX3 (allele7) than in any of these control populations, implying that these alleles might indeed be associated with opiate dependence and possibly with ADHD.

Our findings suggest that prenatal exposure to heroin might have adverse effects, causing higher rates of ADHD among children, especially girls, compared to children born to opiate-dependent fathers, implying that intrauterine exposure increases the rate and severity of ADHD. This is in line with our previous studies on early school age children as well as in adolescents (22,23). Moreover, the lack of correlation between maternal ADHD and ADHD in the offspring further indicates that the effect is not mainly hereditary and the rate is increased by prenatal exposure to heroin. When both parents were

Table 8. Correlations between risk allele carriers in the opiate addicted mothers or fathers and the carrier status of their children

Risk alleles	Mother addicted		<i>P</i> between mother carrier or not	Father addicted		<i>P</i> between father carrier or not?
	Mother is not a carrier	Mother is a carrier		Father is not a carrier	Father is a carrier	
	<i>n</i> (%)	<i>n</i> (%)		<i>n</i> (%)	<i>n</i> (%)	
HTTP5 allele 2	5 (55.6%)	21 (77.8%)	0.197	1 (14.3%)	20 (80.0%)	0.001
ST in VNTR allele 12	1 (100%)	34 (94.4%)	0.809	5 (71.4%)	26 (96.3%)	0.039
MAO-A allele 4	3 (13.6%)	10 (71.4%)	0.001	6 (40.0%)	6 (33.3%)	0.692
DAT1 allele 10	1 (20.0%)	27 (84.4%)	0.002	2 (50.0%)	23 (82.0%)	0.146
DRD4EX3 allele 7	2 (9.1%)	8 (53.3%)	0.003	5 (18.5%)	5 (71.4%)	0.006
DRD4 EX120 allele 2	7 (58.3%)	23 (92.0%)	0.014	9 (69.2%)	20 (95.2%)	0.038

Significant differences are in bold letters.

Table 9. Rate of risk allele carrier in the addicted mother or father in relation to their ADHD diagnosis

Risk alleles	Addicted mother with/without ADHD		<i>P</i>	Addicted father with without ADHD		<i>P</i>
	No ADHD	ADHD		No ADHD	ADHD	
	<i>n</i> (%)	<i>n</i> (%)		<i>n</i> (%)	<i>n</i> (%)	
5HTTP (allele 2)	14 (73.7%)	8 (72.7%)	0.955	11 (91.7%)	12 (75.0%)	0.255
ST in 2 VNTR (allele 12)	20 (100%)	11 (100%)	1	11 (84.6%)	14 (82.3%)	0.869
MAO-A (allele 4)	6 (31.6%)	7 (63.6)	0.088	6 (50.0%)	11 (64.7%)	0.428
DAT1 (allele 10)	17 (85.0%)	10 (90.9%)	0.639	11 (84.6)	15 (100%)	0.115
DRD4 EX3 (allele 7)	8 (40.0%)	5 (45.5%)	0.768	3 (23.1%)	4 (23.5%)	0.977
DRD4 EX120 (allele 2)	15 (75.0%)	6 (54.5%)	0.176	6 (45.2%)	12 (70.6%)	0.244

Table 10. The presence of risk alleles in children of opiate addicted mothers, fathers, and both parents in relation to the behavioral questionnaires and Wechsler test

	Both parents addicted		Addicted father		Addicted mother		P-addicted mother vs. addicted father (control)	P both parents addicted vs. addicted father
	Carrier of risk allele	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 6	n = 3	n = 27	n = 17	n = 21	n = 16		
HTTPLR 5 (allele 2)								
Conner's score	69.83 ± 12.97	58.67 ± 6.35	57.07 ± 12.17	52.29 ± 10.71	57.35 ± 14.24	59.13 ± 9.7	0.81	0.031
Wechsler	93.80 ± 10.71	85.67 ± 7.02	88.00 ± 12.86	91.79 ± 13.52	88.67 ± 10.08	90.2 ± 14.82	0.793	0.619
Achenbach-externalizing	17.67 ± 9.33	17 ± 13	10.96 ± 8.5	13.12 ± 9.49	15.55 ± 11.38	11.69 ± 7.08	0.146	0.088
Achenbach-internalizing	19.17 ± 10.53	19.67 ± 2.52	10.33 ± 8.84	6 ± 4.97	11.50 ± 8.69	7.88 ± 5.63	0.567	0.045
ST in 2 VNTR (risk allele 12)	Carrier	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 9	n = 0	n = 42	n = 3	n = 33	n = 3		
Conner's	66.11 ± 12.09	None	54.33 ± 11.28	66.67 ± 12.01	58.39 ± 12.76	57.67 ± 5.86	0.082	0.008
Wechsler	90.75 ± 9.87	None	88.94 ± 13.69	89.00 ± 5.66	88.53 ± 9.98	91.67 ± 24.6	0.643	0.921
Achenbach-externalizing	17.44 ± 9.84	None	11.12 ± 8.77	19.67 ± 4.16	13.52 ± 9.95	16.00 ± 08.72	0.221	0.055
Achenbach-internalizing	19.33 ± 8.43	None	9.07 ± 8.81	11.00 ± 2.65	9.93 ± 7.48	09.67 ± 10.78	0.289	0.003
MAO-A (allele 4)	Carrier	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 4	n = 5	n = 21	n = 24	n = 16	n = 20		
Conner's	64.5 ± 14.18	67.40 ± 11.72	60.05 ± 12.09	50.88 ± 9.48	60.50 ± 15.48	56.10 ± 8.78	0.024	0.008
Wechsler	91.25 ± 13.91	90.25 ± 5.74	90.44 ± 10.37	87.75 ± 15.46	88.19 ± 10.11	89.06 ± 13.02	0.983	0.872
Achenbach-externalizing	17.75 ± 14.19	17.20 ± 6.5	14.52 ± 10.12	9.21 ± 6.67	15.88 ± 10.95	11.00 ± 7.17	0.159	0.015
Achenbach-internalizing	23.25 ± 7.46	16.20 ± 8.53	10.57 ± 7.35	8.00 ± 9.44	12.56 ± 8.97	7.35 ± 5.72	0.484	0.039
DAT1 (allele 10)	Carrier	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 8	n = 1	n = 36	n = 3	n = 30	n = 8		
Conner's score	65.75 ± 12.9	69	55.97 ± 12.38	44.67 ± 3.06	57.70 ± 11.83	60.13 ± 13.09	0.329	0.044
Wechsler score (IQ)	90.75 ± 9.87		88.03 ± 13.08	97 ± 4.24	90.15 ± 10.75	83.86 ± 13.02	0.738	0.784
Achenbach-externalizing (score)	18.38 ± 10.08	10	11.50 ± 8.39	6 ± 6.08	14.40 ± 9.47	11.38 ± 9.91	0.132	0.072
Achenbach-internalizing (score)	20.13 ± 8.64	13	08.44 ± 8	7 ± 5.57	09.30 ± 6.27	10.75 ± 11.58	0.267	0.002
DRD4 EX3 (allele7)	Carrier	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 1	n = 8	n = 10	n = 35	n = 13	n = 25		
Conner's score	69	65.75 ± 12.88	62.10 ± 11.86	53.17 ± 10.91	54.08 ± 13.54	60.58 ± 10.89	0.066	Not valid
Wechsler score		90.75 ± 9.89	80.33 ± 13.82	90.67 ± 12.77	93.54 ± 12.2	85.75 ± 9.89	0.059	Not valid
Achenbach-externalizing	10	18.38 ± 10.08	14.70 ± 8.45	10.83 ± 8.8	14.75 ± 10.68	13.16 ± 9.27	0.893	Not valid
Achenbach-internalizing	13	20.13 ± 8.64	12.50 ± 6.95	8.26 ± 8.79	07.33 ± 5	10.84 ± 8.44	0.055	Not valid
DRD4 EX120 (allele 2)	Carrier	Noncarrier	Carrier	Noncarrier	Carrier	Noncarrier		
	n = 8	n = 1	n = 31	n = 14	n = 24	n = 14		
Conner's	66.50 ± 12.87	63	54.45 ± 11.96	56.71 ± 11.08	59.37 ± 12.55	56.21 ± 11.04	0.096	0.017
Wechsler	90.86 ± 10.65	90	87.00 ± 13.85	92.83 ± 11.79	86.48 ± 12.22	92.92 ± 8.64	0.675	0.832
Achenbach-externalizing	18.75 ± 9.65	7	11.55 ± 8.99	12 ± 8.59	12.88 ± 9.26	15.29 ± 10.10	0.467	0.047
Achenbach-internalizing	19.00 ± 8.94	22	09.74 ± 9.1	8 ± 7.29	09.33 ± 8.38	10.14 ± 6.02	0.953	0.016

Significant differences are in bold letters.

addicted, the rate of ADHD in the offspring rose to 80% with increased severity, probably as a result of the genetic contribution from both parents. An additional possibility is that the very high rate of ADHD is related to postnatal environmental impact. A child growing up in the household where both parents are addicted to opiates is in a disadvantageous position compared to a child whose mother has no heroin addiction.

The similar reduced intelligence quotient observed in the offspring whether the father or the mother were addicted implies that the decrease in the cognitive abilities are not necessarily related to heroin exposure but rather to poor postnatal environment. This is indeed in line with our previous studies (22,24) where we found that reduced cognitive abilities were equally observed in opiate-exposed children as well as children born to addicted fathers or children suffering from environmental deprivation. Moreover, when children born to heroin-dependent mothers were adopted at a young age, their cognitive abilities were similar to that of control normal children (22,24). Thus, it seems that prenatal exposure to heroin does not impair the child's cognitive abilities.

The rate of ADHD among opiate-dependent mothers and fathers is much higher than that observed in the general population. This emphasizes again the interaction between ADHD and drug dependence, as observed in many studies (17–19). The rate is higher in addicted males compared to addicted females, in line with the general higher prevalence of ADHD in males. The sex ratio is lost in children exposed *in utero* to heroin, as the rate among boys and girls was similar, about 50%, demonstrating that the increase in girls specifically resulted from the exposure to heroin.

The high rate of risk alleles in the heroin-addicted parents was not related to the presence of ADHD, as judged from the Wender's questionnaire, because there was no significant difference in the rate of these alleles if ADHD was present or absent in the parents. It points to the possibility that carriers of these alleles are at risk for drug dependence, regardless whether they have ADHD or not. Several investigators have shown the increased risk for drug dependency among carriers of such risk allele. For example, carriers of MAO-A allele4 (long arm) that affect serotonin metabolism, are at risk for drug dependence at a young age and for aggressive behavior (25). A similar finding of increased aggressiveness among the carriers of this allele was reported by others (1,26). Similarly, DRD4 repeat (allele7) was also associated with an increased risk for drug dependence. We should, however, remember that many studies on opiate dependence have found polymorphism in the DRD3 gene (27) that was not studied by us.

When the parents are carriers of the risk alleles, there is a similarly high rate of carrier state among their offspring, which, again, is not related to the presence or absence of ADHD. A high rate of similarity between the carrier state of the parents and their children was also observed by others, (28) but inheritance of risk alleles for ADHD is a complex process as there seems to be a preferential transmission of paternal over maternal alleles (28,29).

There were little differences in the rate of risk alleles in the children born to heroin-addicted parents whether they had ADHD or not, except for MAO-A (allele4) and DRD4 EX3 (allele7) that were different between those with or without

ADHD. This raises the issue of the relevance of the different risk alleles in the etiology of ADHD, an issue that is still in debate. Our sample, however, is too small to enable us to draw firm conclusions, and the fact that there is a very high carrier state in the parents of these children makes it even more difficult to conclude, as the heritability of the risk alleles for ADHD seems to be complex. Perhaps in the future, more common mechanisms will be revealed between ADHD and heroin addiction to help identify by simple genetic testing the groups that might be at risk for drug dependence.

ADHD is more common in males than in females. In the children born to opiate-dependent mothers, the rate of ADHD was similarly high among males and females. This implies that intrauterine exposure to heroin, most probably increased the risk for ADHD as a result of the specific effects of heroin. Similar findings were observed by us previously in school age children born to heroin-dependent mothers compared to those born to heroin-dependent fathers (22).

Conclusions

Parents addicted to opiates have a high rate of ADHD. The rate is even higher in their children, being similar in males and females. Most addicted parents carry dopamine and serotonin gene polymorphism similar to that observed in children with ADHD, even if they do not have ADHD. Hence, people carrying these genes seem to be prone to opioid addiction. It seems important that children with ADHD who are carriers of these genes should receive appropriate treatment by stimulants at childhood, as early treatment might reduce the risk for opioid addiction at adolescence or adulthood. More studies are needed to substantiate these conclusions.

METHODS

The study included 158 study subjects, 64 heroin-addicted parents, and their 94 children that were raised in their homes. Families with at least one parent that during pregnancy was heroin-dependent were studied. Methadone maintenance treatment (MMT) is the best treatment for the chronic relapsing brain disorder called opioid addiction. Heroin-dependent pregnant women in Israel, as elsewhere, are referred to MMT clinics, where treatment with adequate methadone doses is being adjusted individually. During the study, all adult participants were treated in MMT clinics in Israel. The ages of the 94 children ranged from 5 to 16.5 y. The children were divided into three groups: children of addicted mothers exposed *in utero* to heroin, children of opiate-addicted fathers that were not exposed to heroin (our controls), and children with both parents being addicted to heroin. Data regarding the addiction status of each one of the parents was received by them and was verified by the MMT clinics. The parents were Sephardic and Ashkenazic Jews, all being born in Israel. The parents filled out a "general health questionnaire" related to pregnancy, delivery and various medical problems and the Wender Utah Rating Scale, a self-assessment of ADHD-related problems. Parents also completed the short form (SAC) of the Child Behavior Checklist (Achenbach questionnaire) for assessing behavioral problems and the Conner's Rating Scales for assessing ADHD in their children. The intelligence quotient score was assessed in all children using the Wechsler Intelligence Scale for Children Revised. Children and their parents had, in addition, the genetic studies on cells from mouth wash.

General Health Questionnaire

This questionnaire includes questions on the course of pregnancy and delivery, birth weight, gestational age at delivery, use of drugs of

abuse, ethanol and smoking in pregnancy, use of psychotropic and other drugs and whether their child has or does not have congenital anomalies, chronic diseases or ADHD, as described by us in previous studies (22,23).

Wechsler Intelligence Scales for Children Revised

The Wechsler Intelligence Scales for Children Revised is a widely used means of assessment for the cognitive development of children. The scale comprises 10 subtests, 5 of which measure verbal abilities and 5 that measure abilities reflected in performance. The assessment has well-established norms and evidence of reliability and validity both in the United States (30) and in Israeli research (22). In the present study, we administered all subtests from the verbal and performance section of the assessment from which we calculated the general intelligence quotient score presented here.

Short Child Behavior Checklist (Short Form Assessment for Children (SAC))

The Child Behavior Checklist (Achenbach) yields normed estimates of degree of behavior problems of the child or adolescent. The SAC, a 48-item questionnaire is administered to parents and assesses behavior problems of their children between the ages 4–16, mainly applicable to inattention and/or hyperactivity (ADHD) (31,32). Overall scores are designated as reflecting externalizing behaviors (acting out, delinquency, violence) and internalizing behaviors (withdrawal, somatic complaints, anxiety, and depression). A total score is based on the scores of all scales; the higher scores indicate that more problems are encountered by the person responding. There is evidence for the applicability of local norms that were used in the present study for the Israeli context (32). Both internalizing and externalizing scores are elevated in children with ADHD.

Conners Rating Scales for Assessing Attention Deficit and Hyperactivity Revised

This 80-item short-form scale was filled out by the parents of the children and is based on their perception of the behavior of the child (33,34). This widely used questionnaire has been used in various versions and has demonstrated high interitem reliability and evidence of validity (23). We used the following subscales: scales measuring attention span, hyperactivity, impulsivity, anxiety, and general ADHD measure. High scores indicate more ADHD problems for the child. Children with scores above 56 are considered to have ADHD.

Wender Utah Rating Scale

This instrument contains 61 questions for adults that relate to attention problems they had as children (35,36). The questionnaire serves as a measure of ADHD among adults. The Wender Utah Rating Scale has shown reasonable interitem reliability and validity in previous studies (23). High scores indicate more ADHD problems for the parent. A score of 46 or above is considered to be an indication of ADHD.

Genetic Studies

Mouth wash samples using Aquafresh as the mouth wash fluid, were collected from both heroin-addicted parents and their children. DNA from mouth wash collection was extracted by using the Master Pure Kit (Epicenter, Madison, WI). DNA extraction was carried out from the cells by adding 300 µl of tissue and cell lysis solution and proteinase K, then incubation with 1 µl of RNase, and 150 µl of protein precipitation reagent. The complex is centrifuged and 0.5 ml of isopropanol is added to the supernatant in order to precipitate the DNA. The DNA pellet is washed with 0.5 ml 70% ethanol and then immersed in 100 µl of TRIS EDTA buffer and stored for further studies. The amplification procedure included a 5-min prestart at 95 °C and 30 cycling conditions, as follows: 90 °C for 30 s; 55 °C for 30 s, and 72 °C for 90 s. A final extension was performed at 72 °C for 5 min (37).

The studies on dopamine receptors were performed according to methods described by Treister *et al.* (38) and those on serotonin transporter according to methods described by Bachner-Melman *et al.* (39). In brief, they are as follows:

DRD4 (38): The DRD4 exon III polymorphism was characterized using the following primers: DRD4 P1 5'-GGC GTT GCC GCT CTG AAT GC-3' and DRD4 P2 5'-GAG GGA CTG AGC TGG ACA ACC-3'. The reaction mixture (20 µl) contained 200 µmol/l dNTPs, 0.5

µmol/l primers, Mg/Cl and dimethyl sulfoxide (DMSO) 5% (10 µl), and 20 ng DNA. The amplification procedure included a 5-min prestart at 94 °C (for the hot start) and 35 cycling conditions, as follows: 94 °C for 30 s; 55 °C for 30 s; and 72 °C for 90 s. A final extension at 72 °C for 5 min was employed. The reaction mixture underwent electrophoresis in a 3% agarose gel. Six variants of a 48-bp repeat sequence have been reported: 2 (380 bp); 3 (428 bp); 4 (476 bp); 5 (524 bp); 6 (572 bp); and 7 (720 bp).

DRD4 EX 120 (38): Hundred nanograms of genomic DNA were amplified in a total volume of 25 ml with the following primers at 0.2 mmol/l: D4upstrFor2 (58-GTTGTCTGTCTTTCTCATT GTTTCCATTG-38) and D4upstrRev3 (58-GAAGGAGCAGGCAC CGTGAGC-38) with 0.2 mmol/l dNTPs, 5% DMSO, 0.5 U Taq polymerase in standard polymerase chain reaction (PCR) buffer containing 1.5 mmol/l MgCl. PCR cycling conditions are 5 min at 95 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 58 °C, and 60 s at 72 °C with a final extension for 10 min at 72 °C. Ten microliters of the PCR product were electrophoresed through a 1.5% agarose gel and stained with ethidium bromide. The PCR reaction yields distinct bands at 429 bp ("S", short allele) and 549 bp ("L", long allele). A faint diffuse band migrating more slowly than the 549 bp band is also seen in some heterozygotes and is presumed to be composed of heteroduplex molecules.

MAO-A (39): The MAO-A promoter region polymorphism was characterized using the following primers: MAO-A P1 5'-ACA GCC TGA CCG TGG AGA AG-3' and MAO-A P2 5'-GAA CGG ACG CTC CAT TCG GA-3'. The reaction mixture (20 µl) contained 200 µmol/l dNTPs, 0.25 µmol/l primers, 0.5 U TAQ Gold (Perkin-Elmer Life Sciences, Boston, MA), and 20 ng DNA. The amplification procedure included a 12-min prestart at 95 °C (for the hot start) and 30 cycling conditions, as follows: 95 °C for 35 s; 64 °C for 35 s; and 72 °C for 50 s. A final extension at 72 °C for 5 min was employed. The reaction mixture underwent electrophoresis in a 4% agarose gel. Five variants of a 30-bp repeat sequence have been reported: 2 (291 bp); 3 (321 bp); 3.5 (336 bp); 4 (351 bp); and 5 (381 bp).

Serotonin transporter (39): 5-HTTLPR. PCR amplification was carried out using a ReddyMix kit (Abgene). The primers used were: forward 59-GGCGTTGCCGCTCTGAATGC-39 and reverse 59-GAGGGACT-GAGCTGGACAACC-39. The reaction mixture contained the following components: 0.5 lM primers, 20 ng of DNA, and 5% DMSO in a total volume of 10 ml. ReddyMix buffer consisted of 75 mmol/l Tris-HCl (pH 8.8 at 25.8 °C), 20 mmol/l (NH₄)₂SO₄, and 0.01% (v/v) Tween 20. After an initial denaturation step of 94 °C for 5 min, amplification was carried out for 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s) in a PerkinElmer (Wellesley, CA) Cetus 9600 thermal cycler. A 5-min final extension at 72 °C was used. The reaction mixture was electrophoresed on a 3% agarose gel (Ameresco, Solon, OH) with ethidium bromide to screen for genotypes.

ST in2 VNTR (39): PCR amplification was carried out for the intron 2 VNTR with the following primers: forward 59-T CAGTATCACAGGCTGC- GAG-39 and reverse 59-TGTTCCCTAGT CTTACGCCAGTG-39. The reaction mixture contained the following components: 0.5 lM primers, 20 ng of DNA, and 5% DMSO in a total volume of 10 ml. ReddyMix buffer consisted of 75 mmol/l Tris-HCl (pH 8.8 at 25 °C), 20 mmol/l (NH₄)₂SO₄, and 0.01% (v/v) Tween 20. After an initial denaturation step of 94 °C for 5 min, amplification was carried out for 35 cycles (94 °C for 30 s, 61 °C for 30 s, and 72 °C for 30 s) in a PerkinElmer Cetus 9600 thermal cycler. A 10-min final extension at 72 °C was used.

We examined six different polymorphic sites at the above stated four candidate genes (Supplementary Table S2 online).

Statistical Analysis

Comparison between groups for continuous variables (such as the Conner's) was done using two-tail *t*-test or Wilcoxon signed test (for the small groups); when the groups were compared for categorical variables (e.g., gender, presence of genes), χ^2 test was used. Significance was set at $P < 0.05$. A Bonferroni correction for multiple testing was performed on the results of the genetic studies.

The study has been approved by the Institutional Review board of both Hadassah Hebrew University Medical Center and of the Tel Aviv Sourasky Medical Center. All parents gave their written informed consent to participate in this study.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

STATEMENT OF FINANCIAL SUPPORT

This study was supported by Grant number 0394247 from the Israeli Institute for prevention of drug abuse and alcoholism.

Disclosure: There is no conflict of interest of any of the authors in this study

REFERENCES

- Chien CC, Lin CH, Chang YY, Lung FW. Association of VNTR polymorphisms in the MAOA promoter and DRD4 exon 3 with heroin dependence in male Chinese addicts. *World J Biol Psychiatry* 2010;11(2 Pt 2):409–16.
- Kreek MJ, Zhou Y, Butelman ER, Levran O. Opiate and cocaine addiction: from bench to clinic and back to the bench. *Curr Opin Pharmacol* 2009;9:74–80.
- Li T, Zhu ZH, Liu X, et al. Association analysis of polymorphisms in the DRD4 gene and heroin abuse in Chinese subjects. *Am J Med Genet* 2000;96:616–21.
- Li T, Liu X, Zhao J, et al. Allelic association analysis of the dopamine D2, D3, 5-HT2A, and GABA(A)gamma2 receptors and serotonin transporter genes with heroin abuse in Chinese subjects. *Am J Med Genet* 2002;114:329–35.
- Tyndale RF, Droll KP, Sellers EM. Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. *Pharmacogenetics* 1997;7:375–9.
- Thapar A, Langley K, Asherson P, Gill M. Gene-environment interplay in attention-deficit hyperactivity disorder and the importance of a developmental perspective. *Br J Psychiatry* 2007;190:1–3.
- Kotler M, Cohen H, Segman R, et al. Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. *Mol Psychiatry* 1997;2:251–4.
- Picetti R, Schlusman SD, Zhou Y, et al. Addictions and stress: clues for cocaine pharmacotherapies. *Curr Pharm Des* 2013;19:7065–80.
- Faraone SV, Perlis RH, Doyle AE, et al. Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005;57:1313–23.
- Faraone SV, Khan SA. Candidate gene studies of attention-deficit/hyperactivity disorder. *J Clin Psychiatry* 2006;67 Suppl 8:13–20.
- Gizer IR, Ficks C, Waldman ID. Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet* 2009;126:51–90.
- McCracken JT, Smalley SL, McGough JJ, et al. Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). *Mol Psychiatry* 2000;5:531–6.
- Mill J, Fisher N, Curran S, Richards S, Taylor E, Asherson P. Polymorphisms in the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Neuroreport* 2003;14:1463–6.
- Xing QH, Wu SN, Lin ZG, et al. Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene in schizophrenia. *Schizophr Res* 2003;65:9–14.
- Kotler M, Manor I, Sever Y, et al. Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. *Am J Med Genet* 2000;96:278–81.
- Todd RD, Jong YJ, Lobos EA, Reich W, Heath AC, Neuman RJ. No association of the dopamine transporter gene 3' VNTR polymorphism with ADHD subtypes in a population sample of twins. *Am J Med Genet* 2001;105:745–8.
- Ameringer KJ, Leventhal AM. Associations between attention deficit hyperactivity disorder symptom domains and DSM-IV lifetime substance dependence. *Am J Addict* 2013;22:23–32.
- Carpentier PJ, van Gogh MT, Knapen LJ, Buitelaar JK, De Jong CA. Influence of attention deficit hyperactivity disorder and conduct disorder on opioid dependence severity and psychiatric comorbidity in chronic methadone-maintained patients. *Eur Addict Res* 2011;17:10–20.
- Peles E, Schreiber S, Sutzman A, Adelson M. Attention deficit hyperactivity disorder and obsessive-compulsive disorder among former heroin addicts currently in methadone maintenance treatment. *Psychopathology* 2012;45:327–33.
- Center for Disease Control and Prevention (CDC). Increasing prevalence of parent-reported attention-deficit/hyperactivity disorder among children—United States 2003 and 2007. *Morbidity Mortality Weekly Report (MMWR)*. 2010; 59:1439–1443.
- Wilens TE. The nature of the relationship between attention-deficit/hyperactivity disorder and substance use. *J Clin Psychiatry* 2007;68 Suppl 11:4–8.
- Ornoy A, Segal J, Bar-Hamburger R, Greenbaum C. Developmental outcome of school-age children born to mothers with heroin dependency: importance of environmental factors. *Dev Med Child Neurol* 2001;43:668–75.
- Ornoy A, Daka L, Goldzweig G, et al. Neurodevelopmental and psychological assessment of adolescents born to drug-addicted parents: effects of SES and adoption. *Child Abuse Negl* 2010;34:354–68.
- Ornoy A, Michailivskaya V, Lukashov I, Bar-Hamburger R, Harel S. The developmental outcome of children born to heroin-dependent mothers, raised at home or adopted. *Child Abuse Negl* 1996;20:385–96.
- Vanyukov MM, Maher BS, Devlin B, et al. The MAOA promoter polymorphism, disruptive behavior disorders, and early onset substance use disorder: gene-environment interaction. *Psychiatr Genet* 2007;17:323–32.
- Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase A gene promoter. *Hum Genet* 1998;103:273–9.
- Kuo SC, Yeh YW, Chen CY, et al. DRD3 variation associates with early-onset heroin dependence, but not specific personality traits. *Prog Neuropsychopharmacol Biol Psychiatry* 2014;51:1–8.
- Hawi Z, Segurado R, Conroy J, et al. Preferential transmission of paternal alleles at risk genes in attention-deficit/hyperactivity disorder. *Am J Hum Genet* 2005;77:958–65.
- Manor I, Tyano S, Mel E, et al. Family-based and association studies of monoamine oxidase A and attention deficit hyperactivity disorder (ADHD): preferential transmission of the long promoter-region repeat and its association with impaired performance on a continuous performance test (TOVA). *Mol Psychiatry* 2002;7:626–32.
- Sattler JM. *Wechsler Intelligence Scale for Children-Revised (WISC-R): Description: in Assessment of Children*. San Diego, CA: Psychological Co; 1992.
- Glisson C, Hemmelgarn, AL, Post JA. The short-form assessment for children: An assessment and outcome measure for child welfare and juvenile justice. *Res on Social Work Practice*. 2002; 12, 82–106.
- Zilber N, Auerbach J, Lerner Y. Israeli norms for the Achenbach Child Behavior Checklist: comparison of clinically-referred and non-referred children. *Isr J Psychiatry Relat Sci* 1994;31:5–12.
- Conners CK. *CRS-R, Conners' Rating Scales-Revised: Instruments for Use With Children and Adolescents*. Toronto, North Tonawanda, NY: Multi-Health Systems, Inc; 1997; 94–134.
- Goyette CH, Conners CK, Ulrich RF. Normative data on revised Conners parent and teacher rating scales. *J Abnorm Child Psychol* 1978;6:221–36.
- Ward MF, Wender PH, Reimherr FW. The Wender Utah Rating Scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. *Am J Psychiatry* 1993;150:885–90.
- Stein MA, Sandoval R, Szumowski E, et al. Psychometric characteristics of the Wender Utah Rating Scale (WURS): reliability and factor structure for men and women. *Psychopharmacol Bull* 1995;31:425–33.
- Treister R, Pud D, Ebstein RP, Eisenberg E. Dopamine transporter genotype dependent effects of apomorphine on cold pain tolerance in healthy volunteers. *PLoS One* 2013;8:e63808.
- Treister R, Pud D, Ebstein RP, et al. Associations between polymorphisms in dopamine neurotransmitter pathway genes and pain response in healthy humans. *Pain* 2009;147:187–93.
- Bachner-Melman R, Dina C, Zohar AH, et al. AVPR1a and SLC6A4 gene polymorphisms are associated with creative dance performance. *PLoS Genet* 2005;1:e42.