

Complex effects of dyslexia risk factors account for ADHD-traits: Evidence from two independent samples

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Abstract

Background: Developmental dyslexia (DD) and attention deficit/hyperactivity disorder (ADHD) are among the most common neurodevelopmental disorders, whose etiology involves multiple risk factors. DD and ADHD co-occur in the same individuals much more often than would be expected by chance. Several studies have found significant bivariate heritability, and specific genes associated with either DD or ADHD have been investigated for association in the other disorder. Moreover, there are likely to be gene-by-gene and gene-by-environment interaction effects (GxG and GxE, respectively) underlying the comorbidity between DD and ADHD. We investigated the pleiotropic effects of 19 SNPs spanning five DD genes (*DYX1C1*, *DCDC2*, *KIAA0319*, *ROBO1* and *GRIN2B*) and seven DD environmental factors (smoke, miscarriage, birth weight, breastfeeding, parental age, socioeconomic status and parental education) for main, either 1) genetic or 2) environmental, 3) GxG, and 4) GxE upon inattention and hyperactivity/impulsivity. We then attempted replication of these findings in an independent twin cohort.

Methods: Marker-trait association was analyzed by implementing the Quantitative Transmission Disequilibrium Test (QTDT). Environmental associations were tested by partial correlations. GxG were investigated by a general linear model equation and a family-based association test. GxE were analyzed through a general test for GxE in sib-pair-based association analysis of quantitative traits.

Results: *DCDC2*-rs793862 was associated with hyperactivity/impulsivity via GxG (*KIAA0319*) and GxE (miscarriage). Smoke was significantly correlated with hyperactivity/impulsivity. We replicated the *DCDC2*×*KIAA0319* interaction upon hyperactivity/impulsivity in the twin cohort.

Conclusions: DD genetic (*DCDC2*) and environmental factors (smoke and miscarriage) underlie ADHD-traits supporting a potential pleiotropic effect.

Keywords: developmental dyslexia; ADHD; association study; gene-by-environment interaction; gene-by-gene interaction; pleiotropy

Abbreviations:

ADHD = Attention Deficit/Hyperactivity Disorder

CPRS-R:L = Conners' Parent Rating Scales-Revised: Long version

DD = Developmental Dyslexia

DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

DSM-IV-HI = DSM-IV-hyperactivity/impulsivity

DSM-IV-I = DSM-IV-inattention

FDR = False Discovery Rate

GxE = gene-by-environment interaction

GxG = gene-by-gene interaction

miscarriage = risk of miscarriage

SES = socioeconomic status

smoke = maternal smoke during pregnancy

SNP = Single Nucleotide Polymorphism

QNTS = Québec Newborn Twin Study

QTDT = Quantitative Transmission Disequilibrium Test

WISC-R = Wechsler Intelligence Scale for Children, Revised

WISC-III = Wechsler Intelligence Scale for Children, Third edition

Introduction

Developmental dyslexia (DD) and attention deficit/hyperactivity disorder (ADHD) are among the most common neurodevelopmental disorders. DD affects about 5-12% of individuals and it is characterized by impaired reading acquisition, in spite of adequate neurological and sensorial conditions, educational opportunities, and normal intelligence. ADHD is characterized by continuous and age-inappropriate deficiency in sustained attention, and/or hyperactive and impulsive behaviors, and it affects approximately 2-10% of school-aged children (American Psychiatric Association, 2013).

Substantial heritability has been reported for both disorders, with estimates ranging from 0.18 to 0.72 for DD (Plomin & Kovas, 2005) and from 0.71 to 0.90 for ADHD (Greven et al., 2011,2012; Thapar et al., 2013). As it is typical for complex heritable disorders, a polygenic multifactorial model best describes the familial aggregation of both DD (Plomin & Kovas, 2005) and ADHD (Thapar et al., 2013).

It is well established, from observations in both clinical and community samples, that DD and ADHD co-occur in the same individuals much more often than would be expected by chance (Grigorenko, 2012). Indeed, across studies around 25-40% of children with either DD or ADHD also meet criteria for the other disorder (Pennington, 2006), and the comorbidity is more pronounced for inattention than for hyperactivity/impulsivity (Rosenberg et al., 2012; Plourde et al., 2015). The underlying causes of this co-occurrence are however only partially explained. The multiple-deficit model has been proposed as a framework to understand comorbidity (Pennington, 2006) and data are accumulating now in favor of shared etiological risk factors in ADHD-DD (Peterson & Pennington, 2012; Thapar et al., 2013; Kere, 2014; Li et al., 2014), and in the normal variation of related abilities (Plomin & Kovas, 2005; Plourde et al., 2015).

Investigating the extent to which observable phenotypic correlations are attributable to shared etiological backgrounds, and addressing the issue of pleiotropy, are amongst the major aims of contemporary genetic research (Pennington, 2006, 2015). Several studies have found significant bivariate heritability of ADHD- and DD-traits in normative samples, which is more pronounced for inattention (estimates from .39 to .60) than for hyperactivity/impulsivity (estimates from .05 to .35) (Willcutt et al., 2010b; Paloyelis et al., 2010; Greven et al., 2011, 2012; Plourde et al., 2015). Molecular genetic studies have mapped specific risk loci for DD and ADHD, and some of these are overlapping between the two disorders, e.g., 3p, 6p, 12p, 15q, suggesting that these latter regions could be the potential sites of the liability underlying ADHD-DD

comorbidity (for recent reviews see Kere, 2014; Li et al., 2014). Moreover, specific genes associated with either DD or ADHD have been investigated for association in the other disorder. DD genes *DYX1C1*, *DCDC2* and *KIAA0319* have been associated with inattention and hyperactivity/impulsivity in a Canadian sample of families with at least one member affected by either DSM-IV-inattention (DSM-IV-I), or DSM-IV-hyperactivity/impulsivity (DSM-IV-HI), or DSM-IV-combined (Wigg et al., 2004, 2008; Couto et al., 2009). As for the ADHD gene *DRD4*, inconsistent results have been reported in DD families. Although evidence for linkage has been reported in a sample of 100 families having at least two siblings affected with DD (Hsiung et al., 2004), no significant associations were found in two independent samples of families with DD (Marino et al., 2003; Hsiung et al., 2004). Notably, none of these studies controlled for concurrent measures of reading or ADHD-related traits, limiting the straightforwardness of their findings.

Besides the main genetic effect, there are likely to be gene-by-gene and gene-by-environment interaction effects (GxG and GxE, respectively) underlying ADHD-DD comorbidity (Pennington, 2006). Additive genetic effects explains only a small proportion of the heritability underlying complex traits (Plomin, 2013), clearly highlighting a major limitation of the polygenic model (Manolio et al., 2009; Zuk et al., 2009; Plomin, 2013). This is known as “the missing heritability problem” (Maher, 2008). Moreover, genes can contribute not only directly, but they are also likely to be modulated by, as well as operate by altering sensitivity to, measured environmental risk or protective factors. Until now, GxE have been documented for several disorders, including DD (Pennington et al., 2009; Friend et al., 2008; Mascheretti et al., 2013) and ADHD (Rosenberg et al., 2011; Grizenko, 2012), and they are likely to prove to be important in a broader range of multifactorial conditions (Rutter, 2006). However, as of yet, similar frameworks for exploring the pleiotropic effect of putative risk factors have never been used. Indeed, even if GxG and GxE have been investigated independently in DD (Kremen et al., 2005; Harold et al., 2006; McGrath et al., 2007; Friend et al., 2008; Ludwig et al., 2008; Powers et al., 2013, 2015; Mascheretti et al., 2013, 2015a; Jacobsen et al., 2015) and ADHD (Rosenberg et al., 2012; Jacobsen et al., 2015), to our knowledge, their pleiotropic effects across phenotypes have not been tested.

We therefore hypothesize that DD genes and environmental factors could have pleiotropic effects on ADHD-traits, including main, GxG and GxE effects. For the first time, in this study, we tested the pleiotropic effects of 19 SNPs of five well-replicated DD genes (*DYX1C1*, *DCDC2*, *KIAA0319*, *ROBO1* and *GRIN2B*), and seven DD environmental factors, i.e., maternal smoking during pregnancy (smoke), risk of

miscarriage (miscarriage), birth weight, breastfeeding, parental age, socioeconomic status (SES) and parental education, on concurrent measurements of ADHD-traits in families of DD. Since DD and ADHD often co-occur (Grigorenko, 2012), the proper conservative approach to target ADHD-traits more sharply is to include a composite score of all reading measures as covariate in all analyses. This ensures that potential pleiotropic effects of DD-candidate genes upon ADHD-traits are not limited to its phenotypic overlap with reading. We then attempted replication of nominal significant findings in one independent sample, i.e., the Québec Newborn Twin Study cohort (QNTS; Boivin et al., 2013).

Methods

The protocol was approved by the Scientific Review Board, and by the Bioethics Committee of the Scientific Institute, IRCCS Eugenio Medea.

Sample

This study is based on an ongoing project on the genetic basis of DD (Marino et al., 2003, 2004, 2005, 2007, 2011, 2012; Mascheretti et al., 2014, 2015b). To date, the sample consists of 493 unrelated Italian nuclear families of probands affected by DD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994) (mean age=11.45±3.43, male:female ratio=2:1), and 311 siblings (mean age=12.52±3.84; male:female ratio=1:1), of which 108 were affected by DD. Reading measures were available for all offspring from previous studies (Supplementary Information 1). Blood or mouthwash samples were obtained from all offspring and their biological parents. For the present study, families were contacted by phone and asked to participate in a new phase including an assessment of ADHD-traits.

Phenotypes' Definition

For each offspring, parents were asked to fill out the Conners' Parent Rating Scales-Revised: Long Version (CPRS-R:L; Conners 1990, 1998; for the Italian version see Nobile et al., 2007) which rates childhood behavioral problems in subjects aged 3-17 years old, including ADHD-traits (Conners et al., 1998). The scales are shown to have a good reliability and internal consistency (Nobile et al., 2007) and have been previously used for research purposes in the Italian population (Crippa et al., 2015). The CPRS-R:L consists of 80 items rated on a four-point Likert scale (from "0= never or rarely observed" to "3= very often"), and yields 14 sub-scales. For the current purpose, two subscales were considered, i.e., DSM-IV-inattention

(DSM-IV-I) and DSM-IV-hyperactivity/impulsivity (DSM-IV-HI). All scores were transformed into age- and gender-adjusted T-scores for analyses. Higher scores (T-score ≥ 65) indicate more problems.

Environmental data collection

Parents filled out an *ad hoc* questionnaire (Mascheretti et al., 2013, 2015c) investigating the following environmental variables: (1) smoke, (2) miscarriage, (3) birth weight, (4) breastfeeding, (5) parental age, (6) SES, and (7) parental education (for a detailed description see Supplementary Information 1). A sub-sample of 193 families (403 offspring) had complete environmental data. Descriptive statistics of the environmental variables and phenotypes of this sub-sample are outlined in Supplementary Table 1.

Genotyping

Genotyping data for 19 SNPs spanning *DYX1C1*, *DCDC2*, *KIAA0319*, *ROBO1*, and *GRIN2B* were available from previous studies and are described in detail elsewhere (Marino et al., 2005, 2012; Mascheretti et al., 2014, 2015b; Supplementary Information 1). Genotype error checking was completed in PEDSTATS (Wigginton & Abecasis, 2005) and inconsistent genotypes were not considered for further analyses. Allelic frequencies and Hardy-Weinberg equilibrium were calculated in parents (Table 1) by using PBAT (<http://www.biostat.harvard.edu/~clange/default.htm>; Lange et al., 2004). Genotype distributions did not significantly deviate from the Hardy Weinberg equilibrium (HWE).

Statistical analysis

Given that CPRS-R:L's sub-scales correlated with reading tasks (mean $r=.20$; Supplementary Table 2), a composite score of all reading measures was included as covariate in all analyses. To control for multiple testing, we adjusted the significance level of each type of analysis (i.e., genetic association, environment, GxE and GxG) by the false discovery rate (FDR) method (Storey, 2002). Indeed, FDR has a solid foothold and an increased power when many tests are performed, especially in the context of genomic data research, and represents an attractive alternative to control false positive error rates (Glickman et al., 2014).

Marker-trait association was investigated by QTDT - version 2.5.1 (Abecasis et al. 2000; Supplementary Information 1).

Correlation between environmental factors and ADHD-traits were tested by partial correlations controlling for the reading composite (Supplementary Table 2). All analyses have been implemented with SPSS version 20.0 (IBM Corp. Released 2011).

To explore the combined role of genetic and environmental factors on ADHD-traits, we used a general test for G×E interaction in sib pair-based association analysis of quantitative traits (van der Sluis et al., 2008; Mascheretti et al., 2013), which is an extension of the Fulker et al. (1999) maximum likelihood variance components analysis of quantitative traits that incorporates environmental main plus G×E effects, and where the association effect is orthogonally decomposed into between-family and within-family effects (Supplementary Information 1). Standardized residuals obtained from regressing the reading composite on ADHD-traits were used (Supplementary Table 2). All analyses were implemented using the R environment (www.r-project.org). Linear-mixed models were estimated using the ‘lme’ function.

To assess G×G, we applied a two-step approach (Mascheretti et al., 2015a): (1) a general linear model equation whereby the trait is predicted by the main effect of the number of rare alleles of two genes and by the effect of their interaction, and (2) a family-based association test that takes into account both between-family and within-family genetic orthogonal components (De Lobel et al., 2012; Supplementary Information 1). First, all possible pairwise G×G are tested, and then significant G×G pairwises are submitted to family-based analyses to control for stratification bias and to strengthen the reliability of significant findings. Standardized residuals obtained from regressing the reading composite on ADHD-traits (Supplementary Table 2) were used as dependent variables. All analyses were implemented using the R environment (www.r-project.org). Linear-mixed models were estimated using the ‘lme’ function.

Results

238 unrelated nuclear families with 468 offspring all of Italian ancestry participated in this new study. One-hundred and fifty siblings (65.2%) met the diagnostic criteria of DD. Consistent with previous data (Pennington 2006), ADHD-traits were reported in 30.5% of subjects with DD according to the CPRS-R:L-DSM-IV-Total (T-score ≥ 65), and inattention was more prevalent than hyperactivity/impulsivity (35.8% and 20.7%, respectively). Table 2 shows the descriptive statistics of phenotypic measures in the total sample. DSM-IV-I showed significant associations with the common alleles ‘G’ of both rs3743205 and rs57809907 (*DYX1C1*; $\chi^2=5.34$; nominal p-value=0.02; 98 informative families; genetic effect=6.21, and $\chi^2=6.57$; nominal p-value=0.01; 129 informative families; genetic effect=6.12, respectively) and with the rare allele ‘A’ of rs5796555 (*GRIN2B*; $\chi^2=4.05$; nominal p-value=0.04; 246 informative families; genetic effect=3.11), which did not survive FDR correction (Supplementary Table 3). Similarly, DSM-IV-HI showed a significant

association with the rare allele 'C' of rs6803202 (*ROBO1*; $\chi^2=4.96$; nominal p-value=0.03; 250 informative families; genetic effect=2.77), which did not survive FDR correction (Supplementary Table 3).

DSM-IV-I showed significant associations with smoke and miscarriage (nominal p-values=0.02 and 0.01, respectively), while DSM-IV-HI was significantly associated with smoke (nominal p-value<0.01). After FDR correction, only the correlation between smoke and DSM-IV-HI survived ($r=0.19$, q-value=0.01; Supplementary Table 4).

Several nominal significant GxE were found upon DSM-IV-I and DSM-IV-HI (Supplementary Table 5). After FDR correction, only rs793862 (*DCDC2*) with miscarriage upon DSM-IV-HI survived. In particular, allele 'G' interacts with the risk of miscarriage ($\beta=-1.70$, SE=0.44, q-value=0.05; Supplementary Table 5) to worsen hyperactivity/impulsivity of 1.70 SD. In order to account for the presence of G-E correlations, we investigated the relationship between the between-family component of rs793862 as a 'proxy' variable of the parents' genotype, and miscarriage by the Pearson χ^2 test. No association was found ($\chi^2=1.56$, df=5, p-value=0.91) suggesting that G-E correlations might be considered negligible for this pair of predictors.

Several nominal significant GxG were found upon both DSM-IV-I and DSM-IV-HI, although none survived FDR correction (Supplementary Table 6).

All nominal p-values and FDR-adjusted q-values for main, GxE and GxG analyses are reported in Supplementary Information 2.

Replication of nominal significant findings in the QNTS cohort (Boivin et al., 2013)

QNTS is an ongoing prospective longitudinal follow-up of a birth cohort of twins (n=662) born between 1995 and 1998 in the greater Montreal area, Québec, Canada, whose goal is to document developmental aspects of cognitive, behavioral and social-emotional traits. Inclusion criteria were the fluent use of French or English by the mother and no major medical complications at birth. Blood or mouthwash samples were obtained from 322 twins and their biological parent. Parental authorized consents were obtained for all the included twin pairs. The QNTS had been previously genotyped for a host of DD genetic (unpublished data) and environmental factors (Boivin et al., 2013). For the purpose of this study, we included families of dizygotic twins for which inattention, hyperactivity/impulsivity and reading measures were available, between ages 6 and 8 years, and with either genetic or environmental factors overlapping those measured in the Italian sample.

This led to a final sample composed of 193 dizygotic twin pairs with complete data on inattention and hyperactivity/impulsivity, reading, rs793862, rs9461045, birth weight, smoke, parental age and SES (Supplementary Table 7). The Social Behavior Questionnaire (SBQ; Tremblay et al., 1991) was used to rate inattention and hyperactivity/impulsivity (SBQ-I and SBQ-HI, respectively). Teachers rated the level of ADHD-traits within the past six months in Kindergarten and Grade 1 on a three-point Likert scale (from “0= never or not true” to “2= often or very true”). A mean score between teacher ratings collected at both grades was used for further analysis corrected for reading in Grade 2 (see Plourde et al., 2015 and Supplementary Information 1 for a description of the phenotypic measures). A thorough description of ADHD dimensions, reading measures and their correlation is available in Supplementary Information 1 and Supplementary Table 8, respectively.

The finding rs793862 x rs9461045 upon hyperactivity/impulsivity that was found significant in the Italian sample was tested for replication in the twin cohort (Supplementary Table 6).

Rs793862 x rs9461045 were in HWE in both samples, although rs793862 neared significance for deviation in the Italian sample and conditioned the between and within components distributions in offspring (Supplementary Table 9). To control for stratification bias and insure the reliability of findings, we tested GxG between rs793862 and rs9461045 by the family-based association test. Similarly to what we observed in the Italian sample, we found a significant interaction ($\beta=0.82$, $SE=0.32$, nominal $p\text{-value}=0.01$) in the QNTS cohort, which survived FDR correction ($q\text{-value}=0.03$; Supplementary Information 3). This finding means that each additional transmission of the minor allele in the pairwise produces an additional worsening upon DSM-IV-I and SBQ-HI of 0.45 and 0.82 SD, respectively, compared to the main effect.

Discussion

In a genetically informed study of 238 Italian families of DD, we explored the hypothesis that five genes (*DYX1C1*, *DCDC2*, *KIAA0319*, *ROBO1* and *GRIN2B*) and seven environmental factors (smoke, miscarriage, birth weight, breastfeeding, parental age, SES, and parental education) known to influence DD, could also be associated with ADHD-traits across the whole distribution of liability, *via* main, GxG and GxE effects. After controlling for reading traits, we found significant main and interactive associations upon hyperactivity/impulsivity involving *DCDC2*, *KIAA0319*, smoke and miscarriage, suggesting that these factors exert pleiotropic effects and that complex effects are at play and might be responsible for ADHD-DD

comorbidity. Most importantly, we replicated the GxG effect between *DCDC2* and *KIAA0319* upon hyperactivity/impulsivity in the QNTS cohort. Noteworthy, although we relied upon a conservative statistical corrections for multiple testing to infer significance, further validating by replication in an independent cohort adds strength to our GxG findings, as replication provided the strongest evidence that the results are not due to type I error (e.g., Eicher et al., 2014).

In particular, we found that hyperactivity/impulsivity is affected by smoke, which represents one of the most consistent and well-replicated environmental risk factor for ADHD, although the nature of the association is still under debate (Thapar et al., 2009a,b). Moreover, hyperactivity/impulsivity is modulated by *DCDC2* in interaction with miscarriage. By providing hints about the time window within which miscarriage-related putative hazards potentially exert their action, i.e., the prenatal period, these results may shed light into some time-sensitive, neurobiological mechanisms underpinning hyperactivity/impulsivity. The prenatal period is indeed a time frame of great anatomical and functional changes in terms of brain development. To the extent that a role in neuronal migration (Meng et al., 2005; Burbridge et al, 2008; Wang et al., 2011) and in ciliary function (Massinen et al., 2011) has been suggested for *DCDC2*, we might hypothesize that miscarriage sets off a cascade of risk events, possibly via epigenetic mechanisms, which negatively modulate *DCDC2* expression, eventually influencing fetal brain cytoarchitecture and development. Previous studies reported detrimental effects of maternal behaviors correlated to prenatal hazards such as miscarriage, acting as hidden predictors upon ADHD (Thapar et al., 2013). From this perspective, our results are consistent with the diathesis-stress model (Rende & Plomin, 1992), whereby a hostile environment may lead to greater genetic liability, which would remain otherwise undetected in more supportive environments.

Finally, concerning GxG finding, the impairment due to *KIAA0319* and *DCDC2* is mostly driven by synergistic rather than main or additive effects (effect sizes range between 0.45 and 0.82 for GxG, and 0.01-0.06 for main effects). These data fit with the reported independence of interaction and main effects at both the statistical (Moffitt et al., 2005) and the biological levels, and further support the investigation of GxG as a crucial approach to catch hidden heritability (Plomin, 2013).

Conclusion

In summary, our data sustain pleiotropic effects upon ADHD for DD susceptibility factors. The estimated statistical power of our study is around 95% both in the Italian sample and in the QNTS cohort (PBAT Power

Calculator, <http://www.biostat.harvard.edu/Bfbat/pbat.htm>; G*POWER version 3.1.5, <http://www.ats.ucla.edu/stat/gpower/>). Nevertheless, some limitations need to be addressed. First, univariate association analyses should be considered exploratory, and in the future, may be confirmed by newly developed methods for multivariate association analyses of multiple related traits. Second, the significance of the current association findings should be interpreted cautiously until they can be replicated in ADHD families.

Key points:

- DD and ADHD are heritable, complex, neurodevelopmental disorders, which are frequently comorbid
- Data are now in favor of shared etiological risk factors, which can be either genetic or environmental
- *DCDC2* is associated with hyperactivity/impulsivity through both G×G (with *KIAA0319*) and G×E (miscarriage) effects.
- Maternal smoke during pregnancy is significantly correlated with hyperactivity/impulsivity.
- DD factors show pleiotropic effects upon ADHD-traits.

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Table 1. Allele frequencies and Hardy-Weinberg equilibrium's p-values in parents.

	Marker	Allele	Allele frequency	HWE
DYX1C1	rs3743205	G	0.93	0.85
		A	0.07	
	rs57809907	G	0.90	0.97
		T	0.10	
	rs189983504	C	0.89	0.35
		G	0.11	
DCDC2	READ1	deletion	0.07	0.82
	rs793862	A	0.28	0.07
		G	0.72	
KIAA0319	rs4504469	C	0.63	0.18
		T	0.37	
	rs203813	G	0.68	0.97
		T	0.33	
	rs9461045	C	0.80	0.99
		T	0.20	
	rs2143340 ^s	A	0.84	0.99
		G	0.17	
ROBO1	rs6803202	T	0.51	0.72

		C	0.49	
	rs9853895	C	0.60	0.55
		T	0.34	
	rs333491	A	0.51	0.27
		G	0.49	
	rs7644521	T	0.86	0.94
		C	0.14	
GRIN2B	rs5796555	-	0.72	0.25
		A	0.27	
	rs1012586	G	0.69	0.30
		C	0.31	
	rs2268119	A	0.79	0.56
		T	0.21	
	rs2216128	T	0.77	0.87
		C	0.23	
	rs11609779	C	0.80	0.99
		T	0.20	
	rs2192973	C	0.78	0.77
		T	0.22	

⁵ Marker rs2143340A/G is located on intron 2 of the *TTRAP* gene.

Table 2. Descriptive statistics of the selected CPRS-R:L' scale in total sample.

	Total Sample (n=468)					Probands (n=238)		Siblings (n=230)		Affected* (n=318)		Not-affected (n=150)	
	<i>min</i>	<i>max</i>	<i>Mean (SD)</i>	<i>Skew</i>	<i>Kurtosis</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
	Age [†] =141.21(±39.81)					Age [†] =131.61(±31.40)		Age [†] =151.43(±45.02)		Age [†] =134.54(±33.80)		Age [†] =154.32(±46.29)	
	Sex [‡] =63.7%					Sex [‡] =70.6%		Sex [‡] =56.5%		Sex [‡] =68.6%		Sex [‡] =54.4%	
DSM-IV-I	38	98	57.71 (13.93)	0.70	-0.26	61.75	13.79	52.98	12.56	60.92	13.89	50.34	10.91
DSM-IV-HI	37	96	52.85 (12.23)	1.08	0.78	54.63	12.32	50.78	11.82	54.26	12.50	49.60	11.07

DSM-IV-I=CPRS-R:L's DSM-IV-inattention sub-scale; DSM-IV-HI=CPRS-R:L's DSM-IV-hyperactivity/impulsivity sub-scale.

All scores were transformed into age- and gender-adjusted T-scores according to Italian population norm

*The affection status was assigned according to the criteria outlined in the text.

[†]Age was expressed in months.

[‡]Percentage of the male was reported.