Cervantes-Henríquez, M. L., Acosta-López, J. E., Martínez-Banfi, M. L., Vélez, J. I., Mejía-Segura, E., Lozano-Gutiérrez, S. G., Sánchez-Rojas M., Zurbarán M. A, Zurek E. E., Arcos-Burgos M., Pineda, D. A. & Puentes-Rozo1 P. J. (2018). ADHD Endophenotypes in Caribbean Families. *Journal of attention disorders*, 1087054718763741.

INTRODUCTION

Attention deficit/hyperactivity disorder (ADHD, OMIM 143465) is a phenotypically complex(Acosta, Arcos-Burgos, & Muenke, 2004) and highly prevalent neurodevelopmental disorder that affects 10-17% of children and adolescents worldwide,(Acosta et al., 2011; Arcos-Burgos et al., 2010; Bukstein, 2012; Jain et al., 2011; Pelham & Fabiano, 2008; Visser, Bitsko, Danielson, & Perou, 2010) and frequently persisting into adulthood.(Sibley et al., 2012) Heritability estimates indicate that ADHD symptoms are highly heritable (h^2=0.85-0.90),(Rhee, Waldman, Hay, & Levy, 1999) and that offspring of ADHD affected individuals are six to eight times more likely to develop the condition than those of unaffected individuals.(Biederman & Faraone, 2005) Studies that have ascertained nuclear, extended, and multigenerational families from ADHD affected probands, and clustering several ADHD affected family members, demonstrated that families are an effective resource to define the genetic basis of ADHD.(Arcos-Burgos et al., 2002; Cannon, Gasperoni, van Erp, & Rosso, 2001; Castellanos & Tannock, 2002)

The term endophenotypes was first coined ~50 years ago to explain insects' evolution,(John & Lewis, 1966) and introduced to psychiatry by Gottesman & Shields in 1967.(Gottesman & Shields, 1967) In complex neuropsychiatric conditions such as ADHD, endophenotypes might be defined as

neuropsychological, behavioural, cognitive or neuroanatomical quantitative "measurable components" associated/correlated with the disorder(Castellanos & Tannock, 2002; Miller & Rockstroh, 2016; Walters & Owen, 2007) that occur at a higher frequency in individuals with the disease than in the general population, are heritable, state-independent (that is, manifest in individuals whether the illness is active), tend to co-segregate with the illness within families, and lie in the causal pathway between gene and disease.(Flint & Munafo, 2007; Lee Gregory, Burton, Shapiro, Rowland, & Coyle, 2015; Walters & Owen, 2007) Given that endophenotypes are in general continuous variables instead of categorical traits, do not depend of the inherent difficulties of a symptoms-based clinical diagnosis, and have the ability to differentiate between potential diagnoses that present with similar symptoms,(Brotman et al., 2008; Gottesman & Gould, 2003) it has been hypothesised that they are well suited to study the genetic and neurophysiological basis of psychiatric traits such as ADHD.(Castellanos & Tannock, 2002; Mastronardi et al., 2016; Pineda et al., 2011; Sibley et al.)

Several ADHD studies have identified potential cognitive endophenotypes in neuropsychological tasks such as continuous vigilance, inhibitory control, alteration of temporal perception, delay aversion, working memory alterations, interval timing deficits, fluid intelligence to sustained attention and visual-motor skills.(Acosta-López et al., 2010; Castellanos & Tannock, 2002; Henriquez-Henriquez et al., 2014; Pironti et al., 2014)·(Hwang-Gu & Gau, 2015)·(Mastronardi et al., 2016) Recently, we and others have identified ADHD endophenotypes in families ascertained from two well characterized genetic isolates, the *Paisa* community in Antioquia, Colombia,(Mastronardi et al., 2016)·(Pineda et al., 2011) and the Central Valley in Costa Rica.(Peskin et al., 2015)·(Arcos-Burgos &

Muenke, 2002) In these studies, several measures of cognitive intelligence, attention, visual and motor skills, verbal coefficient, sustained visual attention, and visuospatial problem resolution reported high heritability values and strong association to the ADHD status.(Pineda et al., 2011)·(Peskin et al., 2015) These initial findings support and confirm both: the heterogeneity and complexity of ADHD, as a syndrome and from the cognitive point of view,(Acosta et al., 2004)·(Pennington, 2006; E. J. Sonuga-Barke, Sergeant, Nigg, & Willcutt, 2008; Willcutt, Pennington, et al., 2010) and the important role that genetic factors play in the aetiology of this neuropsychiatric condition.(Mastronardi et al., 2016)·(Barkley., 1997; Doyle et al., 2005; Nigg, 2001; Willcutt, Betjemann, et al., 2010; Willcutt, Pennington, et al., 2010)

The racial composition in Latin-America is extremely complex and geographically heterogeneous as well as it is the cultural heritage.(Sibley et al.) It is well known that Colombia was colonized by Spaniards with variable geographical proportions of genetic admixture with the aboriginal Amerindian populations. This racial and genetic conundrum was later convoluted by the arrival of African populations as consequence of the slaves trading. The racial admixture in Colombia was more pronounced in communities inhabiting the Caribbean coast that had a strong influx of African populations arriving to Cartagena, one of the main trade centres of slaves.(Sibley et al.; Villalón, 2008) Further, earlier in the XX century, the arrival of Arabian populations to the Caribbean brought more diversity to these communities' gene pool. Thus, this differential pattern of admixture that happens in the Colombian Caribbean coast shaped the culture and genetic population structure in a particular and

differential way when compared to other regions of the country.(Barragán-Duarte, 2007; Sibley et al.; Villalón, 2008).

In this study, we explored the definition of ADHD cognitive endophenotypes in a family-based sample of 408 individuals ascertained from a community inhabiting the city of Barranquilla, Colombia. With a population of ~2.4 million where many populations that settled the Atlantic coast converge,(Villalón, 2008) Barranquilla is the biggest city in the Colombian Caribbean coast. Our overarching hypothesis was that there were racial and community specific endophenotypes able to represent a significant variance of the ADHD symptomatology and subtypes, and of the genetics underpinning ADHD susceptibility.

SUBJECTS AND METHODS

Subjects

Four-hundred and eight individuals belonging to 120 nuclear families from Barranquilla, Colombia and its metropolitan area, with at least a single ADHD affected individual, were recruited in this study. Barranquilla is a modern city of ~2.4 million people located in the Atlántico state, at the northern Caribbean coast. The Barranquilla population is the result of a racial admixture between Aboriginal Amerindian communities with Spaniards and Africans, and later with other communities (i.e., Syrian-Lebanese, Sephardi Jews, Germans, Italians and Britons). (Villalón, 2008) Most of the families belonged to medium socioeconomic stratum with an average monthly family income of ~US$1,000-3,000. All individuals in this study participated voluntarily and provided informed written consent either directly or from their parents (in the case of children; <18 years old). This study was approved by The Ethics Committee of Universidad Simón Bolívar at Barranquilla, Colombia (approval # 00032 of October 13, 2011).

Clinical assessment

ADHD diagnosis

The Diagnostic Interview for Children and Adolescents version IV (DICA-IV)(Palacio et al., 2004; Reich, 2000) was used as the Gold Standard to assess the ADHD diagnosis in children and adults. This interview gathers information about patients from a systematic examination of symptoms, making use of a binary classification system of symptoms (0 = absence; 1 = presence) to explore the commencement and end of them to allow an optimal clinical evaluation. Among others, the DICA-IV

cover childhood disorders, mood, anxiety, nutritional behaviour, psychotic disorders, and psychosocial stress -in conjunction, these areas allow the identification of ADHD and its inattentive, hyperactive and inattentive/hyperactive (combined) subtypes. In the case of children and adolescents, the DICA-IV structure interview was completed by children's parents who reported children's symptoms and consequences in the academic, legal and work-related areas, as well as alcohol and tobacco consumption and its consequences.(Palacio et al., 2004; Reich, 2000; Tacchini, Coppola, Musazzi, Altamura, & Invernizzi, 1994) This information was subsequently used to define the index case (proband). Presumptive ADHD diagnosis in children was assessed DICA-IV with a self-report evaluating, retrospectively, parents' behaviour during grades 1 to 11.(Acosta-Lopez et al., 2013) Persistent symptoms impacting family, social and work-related environments were also recorded.

The DICA-IV interview has successfully been used in Colombia by the Grupo de Neurociencias de Antioquia in clinical and genetic studies of ADHD in the Paisa genetic isolate.(Palacio et al., 2004) ADHD diagnosis were performed by two experienced neuropsychologists (PP-R and JA-L), who were trained by a Child Psychiatrist (DPA) from the Grupo de Neurociencias de Antioquia until a κ concordance coefficient > 0.9 was reached for ADHD, ODD and CD diagnoses, and $\kappa > 0.75$ for other psychiatric diagnosis of the A criterion in the DSM-IV. The DICA-IV is highly reliable for each diagnostic category (Crobach's $\alpha > 0.75$) as it has questions, counter questions, validation questions and skip questions regarding every symptom of each criterion in every diagnostic category, in addition to a series of standardised examples in each category, specially designed to determine burden criteria. Following the C criteria of DSM-IV, ADHD symptoms in children and adolescents were evaluated by

their parents and teachers using the ADHD diagnosis was Colombian version of the Behavioural Assessment System for Children (BASC),(Pineda, Kamphaus, et al., 1999) and the ADHD checklist.(APA, 2000; DSM-IV, 2002)

Neurological evaluation

Anamnesis of personal and familiar pathological events was assessed using a neurological interview, which included prenatal, perinatal, childhood and language anomalies. First, mothers accompanied children during the neurological interview to facilitate the interaction with the examiner during the physical/neurological evaluation. Next, information about child's behaviour at home and at other scenarios (social events, parties, birthdays, etc.) is obtained from the mother. Subsequently, a new evaluation involving both parents is performed; information about parents' behaviour is obtained from the child's grandparents by telephonic interview to retrospectively assess parents' hyperactivity, inattention and impulsivity symptoms. Finally, a physical examination of senses, joints and cardiopulmonary, digestive, reproductive and nervous systems is further performed to every child, together with a neurological evaluation assessing cranial pairs, visual auditory syndromes, motor-sensitive skills, muscular tone, reflexes and soft neurological signs.(Puentes Rozo, 2018)

Neuropsychological tests

We used the mental control subtests of an adapted version of the Wechsler Memory Scale 3rd edition (WMS-III),(Wechsler, 2004) in addition to the Wechsler Intelligence Scale for Children 3rd edition (WISC-III)(Wechsler, 1991) for children between 6-16 years old, the short-version of the Wechsler Adult Intelligence Scale 3rd edition (WAIS-III),(Wechsler, 2003) the Trail Making Test parts A and B,(R. Reitan, 1958; R. M. Reitan, 1955; Ralph M Reitan & Wolfson, 1985, 1995, 2004) visuoverbal

memory test, the Rey-Osterrieth complex figure test (ROCFT) for copy and immediate recall,(Osterriech, 1944) the Token test, phonological and semantic fluency verbal tests,(Franke et al.) and the Wisconsin Card Sorting Test (WCST)(Heaton, Avitable, Grant, & Matthews, 1999) and Stroop's words and colours test.(Golden, 1999) The full neuropsychological protocol is presented in Supplementary Table 2. The vocabulary, comprehension, arithmetic, digits and analogies subtests of the WISC-III/WAIS-III were used to assess verbal intelligence quotient (VIQ) and rule out mental retardation, and the incomplete figures, block design, and symbol search and puzzle tests, were used to assess the performance intelligence quotient.(Khodiyar et al.) A prorated full scale intelligence quotient (FSIQ) was estimated.(Wechsler, 2004)·(Wechsler, 1955)

Neurological and neuropsychological evaluations were performed at the Unit of Cognitive Neurosciences of the Caribbean Group of Neurosciences, Simon Bolivar University, Barranquilla, Colombia, in two sections of ~1.5 hours long each. To avoid any potential distraction or interference during the clinical evaluation period, participants were evaluated in a room with constant illumination, a temperature of 18°C and isolated from external noise.

Procedure

Eleven Spanish-speaking public schools located at disparate areas of Barranquilla, Colombia and its metropolitan area were visited. These schools provide educational services to population of medium (three to five) socioeconomic strata. This study was advertised in the Grupo Neurociencias del Caribe's website. Out of the schools visited, seven agreed to participate in our study. Once their participation was approved by delegated authorities, an informative meeting with teachers from each school to explain the objectives and dynamic of the study took place. Subsequently, teachers provided a complete

list of children 6-11 years old (1st to 6th grades) attending their classes for the last six months, and whom they would think could have any issue that may affect their academic performance or their behaviour at school. Out of this list, we administered 845 checklist questionnaires(Pineda, Henao, et al., 1999) to children, children's parents and teachers from these seven ascertained schools. A georeferenced map showing the location of ascertained families is shown in Supplementary Figure 1. Parents and other family members of children with scores higher than the 85th percentile in the checklist (this value is an indicator of an ADHD positive diagnosis)(Pineda, Henao, et al., 1999) were further assessed and provided with all relevant information about the study. ADHD diagnosis in family members was assessed using the DICA-IV interview and the DSM-IV criteria.(Pineda, Henao, et al., 1999) After reviewing both the clinical evaluation and the psychiatric interview, each individual's diagnosis was discussed among a staff of well-experienced clinicians for confirmation. Our full neuropsychological evaluation protocol is presented in Supplementary Table 2.

Statistical analysis

Measures of location and dispersion were employed to summarize continuous variables. Those variables meeting the assumptions of normality and homogeneity of variance were compared using the *t* test for independent samples or the nonparametric Mann–Whitney *U* test otherwise. Normality and homogeneity of variance were tested with the Shapiro–Wilks and the Bartlett tests, respectively. Uncorrected Cohen's *d* was calculated to measure the effect size for all variables. To avoid the effect of potential confounding variables such as age and gender, *P*-values were corrected using analysis of

covariance (ANCOVA). Frequencies and proportions were estimated for categorical variables. Categorical variables were compared using a χ^2 test.

We used Advanced Recursive Partitioning Approach (ARPA) to construct a predictive tree-based model of ADHD status in our cohort. Gender, age and potential cognitive endophenotypes were used as predictors. ARPA offers fast solutions to reveal hidden complex substructures and provides non-biased statistical analyses of high dimensional seemingly unrelated data, and is widely used in predictive analyses as it accounts for non-linear hidden interactions better than alternative methods and is independent of the type of data and of the data distribution type.(Rao, 1998) ARPA was applied using the Classification and Regression Tree (CART),(L. Breiman, Friedman, Olshen, & Stone, 1984) Random Forest (L. Breiman, 2001; Satterfield, Cantwell, & Satterfield) and TreeNet(Friedman, 1999) modules implemented in the Salford Predictive Modeller® software suite (Salford Systems, San Diego, CA, USA). A short description of CART, RF and TreeNet is provided in the Supplementary Material. The final model was chosen based on a battery performance measures presented in Supplementary Table 1.

Heritability estimation

To estimate heritability of neurological and neuropsychological variables in our sample, the ASSOC module in the Statistical Analysis of Genetic Epidemiology (SAGE) software(Elston & Gray-McGuire, 2004) Briefly, ASSOC evaluates the association between a continuous trait and one or more covariates from pedigree data in the presence of familial correlations, and simultaneously estimates familial variance components (and hence familial correlations and heritability)(Elston & Gray-McGuire, 2004)

Parameters in the segregation model evaluated by ASSOC are estimated by maximum likelihood under the assumption that parameters follow multivariate normality.(Bochud, 2012; Elston & Gray-McGuire, 2004; R. C. Elston, J. M. Satagopan, & S. Sun, 2012; Robert C Elston, Jaya M Satagopan, & Shuying Sun, 2012)

RESULTS

Subjects

Four hundred and eight individuals (175 [43%] females, 233 [57%] males) from 120 nuclear families were included in this study (Table 1). Of those, 236 (57.84%) individuals were diagnosed as ADHD affected (161 [68.2%] males, 75 [31.8%] females; 105 [44.5%] were diagnosed as ADHD inattentive, 32 [13.6%] as ADHD hyperactive, and 99 [41.9%] as ADHD combined type). No children or adults were under medication. Among affected individuals, the estimated male-to-female ratio was 2.146 (95%CI = 1.65-2.85, $P<0.001$). As expected, the ADHD diagnosis distribution differed by gender ($\chi^2=$ 27.16, degrees of freedom [df] = 1, $P = 1.87x10^{-7}$). The average age at diagnosis in the whole sample was 26.64±15.5 (range: 6-60), and no statistically significant difference was found by gender (females: 25.34±16.77 years; males: 28.37±13.5 years; $W = 18707$, $P = 0.1537$). The average family size in the 120 nuclear families was 3.4±0.64 individuals (range 3-6), with 80 (66.7%) trios, 34 (28.3%) quartets, four (3.3%) families with five members and two (1.7%) families with six members.(Pineda et al., 2016; Puentes Rozo, 2018) Furthermore, 32 (26.7%) families had one affected individual, 63 (52.5%) had two, 21 (17.5%) families had three and 4 (3.3%) families had four affected individuals; 88 (73.3%) families had more than one member affected with ADHD. Analyses of the probands' relatives (n=288) indicate that 120 of them are diagnosed with ADHD (77 males, 43 females, 41.6%), with an age at diagnosis of 34.11±12.04, which differed between males and females (35.34±13.05 vs. 32.83±10.78, $P<0.0001$).

Neuropsychological differences between affected and unaffected individuals

We found statistically significant differences between ADHD affected and unaffected individuals after controlling for age and gender in neurological and neuropsychological tasks measuring mental control, visuospatial ability (i.e., ROCFT), visuoverbal memory, verbal fluency tasks (VFTs) by phonological and semantic guidance, planning and abstraction (i.e., WCST), and intelligence (i.e., IQ in the WISC-III and WAIS-III)(see Table 2 and Figure 1a). ADHD affected individuals had a lower score than unaffected individuals in the Total 9/9 test (4.44±2.54 vs. 5.93±2.17, P=0.028), and in the numbers from 20 to 1 test (2.13±0.99 vs. 2.55±0.7, P=0.034) of the mental control subtest. Likewise, ADHD affected individuals had a lower score than unaffected individuals in the ROCF copy (20.65±8.49 vs. 26.31±6.5, P = 0.002) and ROCF evocation (immediate recall; 9.15±6.05 vs. 13.38±6.16, $P = 2.4x10^{-5}$) subtests, but not in the ROCF type (2.64±1.53 vs. 1.62±0.94, P = 0.018) subtest or in the number of attempts needed to accomplish the visuoverbal memory test (3.18±1.77 vs. 2.7±0.91, P = 0.027). Conversely, unaffected individuals obtained an average score higher than affected individuals in the phonological VFTs (12.34±9.84 vs. 16.81±13.65, P = 0.024), the 36/36 Token test (30.46±3.66 vs. 32.04±4.21, P = 0.001), and correct answers (73.46±23.69 vs. 80.9±20.42, P=0.014) of the WCST (i.e., planning and abstraction cognitive domain; see Supplementary Table 2). As expected, ADHD affected individuals performed poorer than ADHD unaffected individuals in the number of errors (53.6±23.21 vs. 46.58±20.34, P=0.031) and the number of correct answers at the conceptual level (58.26±29.81 vs. 66.56±27.4, P=0.044) of the WCST. Analogously, ADHD affected individuals had lower performance

than unaffected individuals in the FSIQ of the WISC-III (children) and WAIS-III (adults) with low-to-high effect sizes (Table 2 and Figure 1a).

Heritability estimates

Strong statistical evidence supporting genetics effects and hereditary transmission (measured by the heritability parameter, h^2) was found in several neuropsychological variables (tasks) used to clinically characterise our sample (Table 2 and Figure 1b). These variables include the score in the numbers 1 to 20 (h^2=0.351, P = 0.006) and alphabet errors (h^2=0.546, P <0.00001) measuring mental control; the differed evocation at 20 test (h^2=0.546, P <0.00001) assessing visuoverbal memory; *the total number of errors* (h^2=0.765, P <0.00001) and perseverance (h^2=0.546, P <0.00001) assessing phonological fluency; *the total number of errors* (h^2=0.264, P=0.01) and perseverance (h^2=0.669, P <0.00001) subtests assessing semantic verbal fluency; the Token 36/36 test (h^2=0.355, P=0.002); and all but the *figure completion test* (h^2=0.176, P=0.094) in the WAIS subtests, indicating that most of the measures assessing FSIQ had significant heritability. Within the WAIS-III subtests, the highest heritability value was estimated in the vocabulary test (h^2=0.452, P=1.7x10^{-4}) and the minimum in the reverse digits test (h^2=0.171, P=0.048). No significant genetic effects and hereditary transmission were found in neuropsychological variables assessing ROCF, continuous auditory execution or the verbal semantics (Table 2 and Figure 1b). Our results suggest both a simultaneous differential pattern in ADHD diagnosis and genetic effects and hereditary transmission (significant heritability) in the numbers from 20 to 1 mental control subtest, the 36/36 Token test and in most of the WAIS-III subtests (see Table 2 and Figure 1c).

Predictive model for ADHD diagnosis

Based on the performance measures presented in Supplementary Table 1, a five-level tree with seven terminal nodes was derived by CART to differentiate ADHD affected individuals from unaffected in our cohort of 120 nuclear families. Splitting nodes involved age at diagnosis, sex and traits 4 (numbers from 20 to 1; Table 2), 44 (digits; Table 2), 45 (vocabulary; Table 2) and 47 (arithmetic; Table 2) (Figure 2a). This predictive model was validated via RF and TreeNet, producing comparable results (data not shown). Interestingly, these last four variables defining splitting nodes were also found to be associated with ADHD and exhibited a significant heritability (that is, constitute endophenotypes; see Table 2 and Figure 1c).

Out of the 408 individuals clinically assessed, 58% of them were diagnosed as ADHD affected and 42% as unaffected (node 1, Figure 2a). In the first split, children < 14 years old have 87% chance of being diagnosed with ADHD regardless of gender (terminal node 3, 37% of all sample), whilst those ≥14 years have a 59% change of being diagnosed as ADHD unaffected (node 2, 63% of total sample). Within this node, individuals with more than 12 points in trait 44 (digits; Table 2) have a 70% chance of being classified as ADHD unaffected (node 4; 38% of total sample), compared to 58% of being ADHD affected (node 5, 25%). On the other hand, males with < 12 points in trait 44 (digits; Table 2) and <14 years old have a 74% (terminal node 11, 12% of total sample) of being diagnosed as ADHD affected. Likewise, females with <12 points in trait 44 (digits; Table 2), < 38 points in trait 45 (vocabulary; Table 2) and ≥12 points in trait 47 (arithmetic; Table 2) have a 91% chance of being diagnosed as ADHD affected (terminal node 43; 3% of total sample) (Figure 2a; bottom). Finally, females with <38 points in trait 45 (vocabulary; Table 2), ≥12 points in trait 47 (arithmetic; Table 2)

and <1.5 points in trait 4 (numbers from 20 to 1; Table 2) are classified as ADHD affected (terminal node 85, 1% of all sample) (Figure 2a; bottom).

Figure 2b depicts the variable importance and Receiver Operating Characteristic (ROC) curves for the CART strategy for the learn and test data sets. Although similar results were obtained with all strategies, CART performed better than RF and TreeNet (Supplementary Figure 2). The performance measures for the testing and learning data sets using the CART strategy are shown in Figure 2c (see also Supplementary Table 1). For the learning data set, the estimated AUC was 81.5 (95%CI=77.6-85.3), with values of 81.4 (95%CI=77.5-85.0) for the classification rate, a sensitivity of 82.5 (95%CI=76.7-88.0), specificity of 80.5 (95%CI=75.3-85.5) and precision of 86.1 (95%CI=81.6-90.7), with overlapping 95%CI in most of these measures for the learning data set based on 10-fold cross-validation (Figure 2c). Further analysis indicated that this predictive model outperforms that including sex and gender only (Supplementary Figure 3). Altogether, these measures indicate substantial predictive power of these cognitive endophenotypes to differentiate ADHD affected from ADHD unaffected individuals.

DISCUSSION

The purpose of this study was to define cognitive endophenotypes in a set of nuclear families ascertained from ADHD probands recruited from Barranquilla, Colombia. We characterized, by clinical neuropsychology methods, visuoconstructional skills, visuoverbal memory, language, executive function and intelligence domains. We found strong evidence that tasks of mental control, language, and intelligence meet the criteria for endophenotypes. Despite the well-known limitations of CART (that is, being a nonparametric technique, lacking the ability of forcing variables into the model, and high variance across samples),(L. Breiman et al., 1984; Gordon, 2013; Hayes, Usami, Jacobucci, & McArdle, 2015; Ojha, 2018) these clinical variables accurately predict the ADHD status in this community (Figure 2 and Supplementary Figure 3). While we replicated endophenotypes described for other Colombian -and in general Latino- communities, there were new neuropsychological endophenotypes that perform as new major players in outlining ADHD and its neurobiological basis. This new discovered endophenotypes might be specific, but the role they might play in other ADHD cohorts and studies in other communities will define their importance.

The findings in this study can be framed from two perspectives. Firstly, there is strong evidence supporting significant phenotypic differences between ADHD affected and unaffected individuals in the cognitive domains of mental control (total score and numbers from 20 to 1 of the mental control test), visuomotor skills (copy type, and scores of copy and evocation in ROCF), visuoverbal memory (number of trials in the visuoverbal memory test), phonological and semantic verbal fluency (total score of the verbal fluency test), as well as in language comprehension (Token test total score),

abstraction and problem solving (number of correct responses, total errors and conceptual level responses in the WCTS), comprehension and verbal reasoning (analogies, vocabulary, digits span and arithmetic, and comprehension subtests of the WISC-III/WAIS-III) and execution and perceptive reasoning (figure completion, block design, symbol search and objects assembly subtests of the WISC-III/WAIS-III)(Table 2 and Figure 1a). These results are not only consistent with other studies evaluating potential ADHD cognitive endophenotypes,(Peskin et al., 2015; Pineda et al., 2011) but also show how heterogeneous the ADHD phenotype is. In the mid-to-long term, these phenotypic differences might allow researchers to dissect the spectrum of cognitive and behavioural phenotypes in ADHD,(Cervantes-Henríquez, Acosta-López, Aguirre-Acevedo, Pineda-Álvarez, & Puentes Rozo, 2008; Puentes, 2009; Puentes-Rozo, Barcelo-Martinez, & Pineda, 2008) and contribute to the better understanding of the aetiology, subtypes and severity of this neuropsychiatric condition.(E. J. Sonuga-Barke, Dalen, Daley, & Remington, 2002)

Secondly, we also identified statistically significant heritability indexes in the domains of mental control (numbers from 20 to 1 and abecedary errors), visuoverbal memory (differed evocation at 20), phonological and semantic verbal fluency (total errors and perseverance), language comprehension (Token test), verbal comprehension (vocabulary, analogies, arithmetic and digits span subtests of the WISC-III/WAIS-III) and execution (incomplete figures and objects assembly subtests of the WISC-III/WAIS-III) (Table 2 and Figure 1b). Although these findings are closely related to previous findings,(Doyle et al., 2005; Peskin et al., 2015; Pineda et al., 2011; Pineda et al., 2007; Rommelse, 2008; Rommelse et al., 2008) and provide supporting evidence regarding the genetic component of

ADHD and how the offspring from parents affected with ADHD inherit this condition(Ramos-Quiroga, Ribases-Haro, Bosch-Munso, Cormand-Rifa, & Casas, 2007), only the domains of attention, tasks of verbal comprehension and some tasks of the execution scale in this Caribbean community are similar to the findings in the Paisa genetic isolate,(Pineda et al., 2011; Pineda et al., 2007) and those of a recent study in the Central Valley of Costa Rica,(Peskin et al., 2015) which reported high heritability values for attention and verbal IQ. In this study we found that verbal comprehension tasks, but verbal IQ, are highly heritable. It is intriguing how closely related the findings in the Paisa and this Costa Rican communities are compared to those in our Caribbean families, and that, unlike endophenotypes that were highly heritable in our sample, only two symptoms of the DSM-IV were so (Supplementary Table 3). This latter result supports the hypothesis that symptoms are not sufficient to determine genetic effects and hereditary transmission in ADHD, and that other approaches are needed.(Acosta et al., 2011)

The fact that several tasks of the Wechsler Intelligence Scale for Children (WISC; Table 2) but not the IQ were found to be potential endophenotypes suggests the existence of different IQ profiles among ADHD subtypes,(Pennington, 2006; Sonuga-Barke, Bitsakou, & Thompson, 2010; E. J. Sonuga-Barke et al., 2002; E. J. Sonuga-Barke et al., 2008) and a potential association mainly with attention as a processing system, with temporal processing(Castellanos & Tannock, 2002)and with working memory, thus interfiring with the normal learning processes and limiting the ability of individuals to easily adapt to the environment. In this sense, intelligence may not be critically compromised but diminished due to the aforementioned difficulties, and lead to learning disorders

(Castellanos & Tannock, 2002; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Temporal-processing and working memory deficits are associated with ADHD,(Castellanos & Tannock, 2002) which may partially explain why some attention and working memory tasks and not IQ resulted to be potential endophenotypes in our sample. Tulsky, Saklofske & Zhu(Tulsky, Saklofske, & Zhu, 2003) suggest that the IQ can be determined not only by cognitive aspects, but also by other motivational factors. Thus, difficulties in attention and working memory are related to the cognitive and academic impairment cluster observed in individuals with severe inattention than in individuals with hyperactivity/impulsivity,(Castellanos & Tannock, 2002) which is consistent with the inattention profile of our sample.

Although genetic factors are implicated in the aetiology of ADHD and its comorbidities,(Acosta et al., 2011; Arcos-Burgos et al., 2002; Arcos-Burgos et al., 2010; Jain et al., 2011; Palacio et al., 2004) environmental, epigenetic, cultural and educational factors may offer an explanation about the heterogeneity of the disorder.(Acosta et al., 2004) The fact that the number of endophenotypes compromising several cognitive domains in our sample is considerably less than the phenotypes evaluated (Table 2), puts us one step closer to genetic factors explaining ADHD variability.(Doyle et al., 2005) Studying distinctive ADHD profiles, such that the inattentive type in our sample, may potentially lead to the identification of genetic factors and physiopathological processes underlying ADHD. However, it is important to acknowledge that, given the multifactorial nature of ADHD, our approach of comparing cognitive impairments in individuals with ADHD to impairments in unaffected relatives is limited. As a complementary approach, we conducted a factor analysis and studied the

heritability of the derived factors (Supplementary Table 4 and Supplementary Figure 4). Interestingly, only factors constructed from phonetic fluency tasks, semantic verbal fluency, WCST and WISC-III/WAIS-III were found to be heritable.

Finding that some tasks of the WISC test were found to be heritable (Table 2 and Figure 1b) is consistent with the postulate that individuals having a family history of ADHD increases susceptibility to develop the condition as well as presenting major social and vocational difficulties than individuals with no family history.(Arcos-Burgos et al., 2002; Bochud, 2012; Faraone et al., 1993; Lopera et al., 1999; Lopez-Campo, Gomez-Betancur, Aguirre-Acevedo, Puerta, & Pineda, 2005; Peskin et al., 2015; Pineda et al., 2011; Willcutt et al., 2005) We found significant heritability values in sustained visual attention, speed of information processing and resolution of visuospatial subtests of the Wechsler scale that might be used in genetic research of ADHD (i.e., fine-mapping and genome-wide linkage and associaton studies) as efficient phenotypic indicators. Following this approach, new neurobiological and genetic markers for ADHD can be defined and subsequently increase the power to detect genetic loci conferring susceptibility to the disorder.(Mastronardi et al., 2016)

The importance of our findings can be summarised as follows. First, the study was performed in a sample of 120 nuclear families from the metropolitan area of Barranquilla, Colombia, with at least one individual affected with ADHD. These families have been clinically characterised using extensive neuropsychological batteries during the last five years,(Pineda et al., 2016; Puentes Rozo, 2018) and constitute, to the best of our knowledge, the largest collection of nuclear families with ADHD in South America today. Because of their structure and admixture composition (~63% African descendants is

with a vast Amerindian contribution),(Barragán Duarte) these families constitute a powerful resource for genetic studies of ADHD. Second, this is one of few studies examining the heritability of cognitive measures as probable endophenotypes(Kuntsi et al., 2010) and might be useful to support future molecular studies aiming to uncover the final causes of ADHD. Future studies will include conducting linkage and association genetic analysis between common, rare and functional exomic variants to these cognitive endophenotypes, and possibly deep sequencing of genes harbouring these variants in this set of families. This will be crucial for accurate diagnostic, treatment, improve long-term outcomes and for outlining public health policies.(Arango-Dávila, Rojas, & Moreno, 2008; Posada, 2013)

Acknowledgements

We express our highest appreciation to all families enrolled in this study. This study was financed by COLCIENCIAS, project *"Fenotipos Complejos y Endofenotipos del Trastorno por Déficit de Atención e Hiperactividad y su Asociación con Genes Mayores y de Susceptibilidad"*, grant 1253-5453-1644, contract RC 384-2011 conferred to Grupo de Neurociencias del Caribe, Universidad Simón Bolívar, Barranquilla. MLC-H, JAL and EM-S are doctoral students at Universidad del Norte, Barranquilla, Colombia, Universidad Maimónides in Buenos Aires, Argentina and Universidad De Flores in Buenos Aires, Argentina. Some of this work is to be presented in partial fulfilment of the requirements for the PhD degree. The sponsor of the study has no role in the study design, data collection, data analysis, data interpretation, or writing of the paper. MLC-H, JAL, JIV and PPR have full access to all the data in the study and are responsible for submitting this work for publication.

Compliance with Ethical Standards

The authors assert that all procedures contributing to this work have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The views and opinions expressed in this article are those of the authors and should not be construed to represent the views of any of the sponsoring organizations, agencies, or governments.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

TABLES

Table 1. Demographic characteristics of 408 individuals included in this study.

	Affected *n*=236	Unaffected *n*=172	Statistic index	*P*	Effect Size
Gender	Frequency (%)	Frequency (%)	χ^2		
Male	161 (68.22)	72 (41.86)	27.156	<0.00001	-
Female	75 (31.78)	100 (58.14)			
	Mean (SD)	Mean (SD)	Mann-Whitney's *U*		
Age	21.14 (12.15)	34.19 (15.4)	29746	<0.0001	0.941

420 Table 2. Performance on neurological and neuropsychological tasks of 408 individuals from the
421 Colombian Caribbean.

#	Task	Affected (*n*=236)	Unaffected (*n*=172)	*d*	*P*	Heritability	
						h^2(SE)	*P*
	Mental Control	Mean (SD)	Mean (SD)				
1	Total score 9/9	4.44 (2.54)	5.93 (2.17)	0.624	0.028	0.182 (0.117)	0.059
2	Time – Numbers from 20 to 1	16.68 (14.49)	11.29 (6.28)	0.459	0.147	[a]	[a]
3	Errors – Numbers from 20 to 1	0.25 (1.56)	0.13 (0.47)	0.103	0.817	[a]	[a]
4	Score – Numbers from 20 to 1	2.13 (0.99)	2.55 (0.7)	0.483	0.034	0.351 (0.138)	0.006
5	Time – Abecedary	15.14 (15.78)	10.73 (9.18)	0.329	0.420	[a]	[a]
6	Errors – Abecedary	0.78 (2.06)	0.48 (1.36)	0.171	0.443	0.546 (0.089)	$1.0x10^{-7}$
7	Score – Abecedary	1.43 (1.37)	2.12 (1.23)	0.525	0.192	[a]	[a]
8	Time – Counting	29.49 (24.42)	22 (12.18)	0.371	0.054	[a]	[a]
9	Errors – Counting	1.44 (2.84)	1.52 (2.99)	0.026	0.941	[a]	[a]
10	Score – Counting	0.84 (1.14)	1.22 (1.27)	0.319	0.237	[a]	[a]
	A Continuous Auditory Performance Test						
11	Correct answers	13.53 (3.01)	15.15 (1.53)	0.649	0.062	[a]	[a]
12	Omissions	2.32 (2.8)	0.85 (1.53)	0.626	0.083	[a]	[a]
13	Comissions	1.94 (2.03)	1.22 (1.59)	0.385	0.246	[a]	[a]
	Rey-Osterrieth Complex Figure						
14	Copy (type)	2.64 (1.53)	1.62 (0.94)	0.770	0.018	0.172 (0.119)	0.075
15	Copy (time)	192.02 (85.41)	165.08 (88.97)	0.310	0.855	[a]	[a]
16	Copy (score)	20.65 (8.49)	26.31 (6.5)	0.734	0.002	0.142 (0.137)	0.150
17	Evocation (time)	115.81 (53.56)	129.66 (56.92)	0.252	0.058	[a]	[a]
18	Evoction (score)	9.15 (6.05)	13.38 (6.16)	0.694	$2.4x10^{-5}$	0.185 (0.115)	0.055
	Visuoverbal Memory						
19	Initial volume	6.44 (1.64)	6.66 (1.41)	0.142	0.123	0.141 (0.113)	0.105
20	Maximum volume 10/10	9.98 (0.16)	9.91 (0.81)	0.131	0.469	[a]	[a]
21	Number of trials	3.21 (1.69)	2.81 (1.09)	0.275	0.027	0.065 (0.109)	0.274
22	Organizational index	0.64 (0.35)	0.79 (0.77)	0.267	0.115	[a]	[a]
23	Differed evocation at 20	8.67 (1.82)	9.44 (1.32)	0.478	0.063	0.773 (0.064)	$1.0x10^{-7}$
	Phonetic Fluency Tasks						

24	Total score	16.07 (14.02)	24.15 (17.99)	0.511	0.024	[a]	[a]
25	Total errors	2.57 (3.06)	2.76 (2.99)	0.065	0.466	0.765 (0.050)	$1.0x10^{-7}$
26	Missing categories	11.6 (13.03)	13.53 (16.09)	0.134	0.768	[a]	[a]
27	Perseverance	0.92 (1.53)	1.18 (1.39)	0.177	0.418	0.653 (0.079)	$1.0x10^{-7}$
	Semantic Verbal Fluency Test						
28	Total score	18.28 (15.27)	24.84 (18.4)	0.394	0.032	[a]	[a]
29	Total errors	1.44 (1.66)	1.49 (1.58)	0.033	0.362	0.264 (0.115)	0.011
30	Missing categories	22.8 (36.52)	18.55 (28.52)	0.128	0.305	[a]	[a]
31	Perseverance	0.8 (1.36)	0.81 (1.26)	0.013	0.640	0.669 (0.057)	$1.0x10^{-7}$
32	Token Test 36/36	31.36 (3.8)	33.51 (2.68)	0.637	0.001	0.355 (0.124)	0.002
	Wisconsin Card Sorting Test						
33	Correct responses	73.46 (23.69)	80.9 (20.42)	0.333	0.014	[a]	[a]
34	Total errors	53.6 (23.21)	46.58 (20.34)	0.318	0.031	[a]	[a]
35	Nonperseverative errors	29.6 (20.17)	24.05 (15.64)	0.302	0.073	[a]	[a]
36	Perseverative errors	25.15 (22.58)	23.24 (13.33)	0.100	0.234	[a]	[a]
37	Categories	5.26 (18.6)	4.89 (2.92)	0.026	0.174	[a]	[a]
38	Perseverative errors (%)	21.41 (33.89)	19.43 (15.05)	0.072	0.885	[a]	[a]
39	Conceptual level responses	58.26 (29.81)	66.56 (27.4)	0.288	0.044	[a]	[a]
40	Conceptual level responses (%)	46.8 (23.67)	53.01 (20.91)	0.276	0.083	[a]	[a]
41	Failures to keep the principle	1.43 (1.52)	1.39 (1.28)	0.028	0.832	0.138 (0.107)	0.099
	WISC-III and WAIS-III subtests						
42	Digit Span Total – Forward	6.84 (1.73)	7.8 (1.92)	0.526	$3.7x10^{-4}$	0.492 (0.107)	$1.0x10^{-5}$
43	Digit Span Total – Backward	4.53 (1.88)	5.24 (1.87)	0.375	0.001	0.171 (0.102)	0.048
44	Total	11.32 (3.06)	13.12 (3.33)	0.564	$1.6x10^{-5}$	0.416 (0.109)	$6.8x10^{-5}$
45	Vocabulary	28.28 (10.63)	35.51 (10.99)	0.670	0.005	0.452 (0.126)	$1.7x10^{-4}$
46	Comprehension	17.75 (6.27)	21.01 (5.88)	0.533	0.019	0.210 (0.107)	0.025
47	Arithmetic	12.94 (4.52)	12.87 (3.87)	0.016	0.007	0.365 (0.116)	0.001
48	Similarities	16.16 (6.98)	20.55 (5.89)	0.671	0.002	0.366 (0.130)	0.003
49	Figure completion	18.81 (4.86)	20.58 (3.45)	0.410	0.036	0.235 (0.133)	0.039
50	Block design	30.89 (14.28)	37.99 (12.86)	0.518	$1.8x10^{-4}$	[a]	[a]
51	Symbol search	21.45 (8.73)	25.96 (8.81)	0.514	0.015	0.176 (0.133)	0.094
52	Objects assembly	25.56 (8.8)	29.92 (9.13)	0.488	0.012	0.323 (0.132)	0.007
	Intelligence Quotient (IQ)						
53	Verbal	98.51 (16.81)	97.44 (12.33)	0.071	0.078	[a]	[a]

54	Performance	100.98 (16.69)	101.42 (11.45)	0.030	0.128	[a]	[a]
55	Full scale	99.15 (16.85)	98.86 (12.14)	0.019	0.040	[a]	[a]

[a] Parameter could not be maximized in SAGE. [b] Corrected for gender and age. [c] Corrected for ADHD status, gender and age. *d:* Cohen's effect size; h^2*:* heritability estimated value; SE: Standard error. Potential endophenotypes are highlighted in blue. *P*-values < 0.05 are shown in bold. Task numbers highlighted in red are included in the predictive model for ADHD status (see Figure 2).

FIGURE LEGENDS

Figure 1. Neurological and neuropsychological where (a) ADHD affected individuals differed from unaffected individuals; (b) genetic effects and hereditary transmission are present; and (c) fulfill the requirements to be considered as potential endophenotypes. Displayed numbers correspond to task # in Table 2.

Figure 2. Classification tree for predicting ADHD status in individuals from the Colombian Caribbean. Numbers within white squares represent the node number, the first line corresponds to the most frequent class (0: unaffected; 1: ADHD affected), the second line to the probability of each class within the node, and the third line to the percentage of the total sample size (n=408) within each node. Nodes where ADHD affected individuals are more likely to be classified are shown in blue. (b) Variable importance (left) and ROC curve (Ando, Ono, & Wright) for the CART strategy. Displayed numbers correspond to task # in Table 2. (c) Performance measures for the learning (blue) and test (pink) data sets. AUC: Area under the curve; CART: Classification and regression tree; CI: confidence interval; CR: Classification rate; ROC: Receiver operating characteristic.

REFERENCES

Acosta, M. T., Arcos-Burgos, M., & Muenke, M. (2004). Attention deficit/hyperactivity disorder (ADHD): complex phenotype, simple genotype? *Genet Med, 6*(1), 1-15. doi:10.1097/01.GIM.0000110413.07490.0B

Acosta, M. T., Velez, J. I., Bustamante, M. L., Balog, J. Z., Arco-Burgos, M., & Muenke, M. (2011). A two-locus genetic interaction between LPHN3 and 11q predicts ADHD severity and long-term outcome. *Translational Psychiatry*.

Acosta-Lopez, J., Cervantes-Henriquez, M., L., Jiménez-Figueroa, G., M., N., M., S., & P., P. (2013). Uso de una escala comportamental Wender Utah para evaluar en retrospectiva trastorno de atención-hiperactividad en adultos de la ciudad de Barranquilla. *La Revista Universidad y Salud,, 15*, 45-61.

Acosta-López, J., Cervantes-Henríquez, M., Sánchez-Rojas, M., Núñez-Barragán, M., Puentes Rozo, P., Aguirre-Acevedo, D., & Pineda, D. (2010). Alteraciones del Control Inhibitorio Conductual en Niños de 6 A 11 Años Con TDAH Familiar de Barranquilla. *psicogente, 13*(24), 274-291.

Ando, J., Ono, Y., & Wright, M. J. (2001). Genetic structure of spatial and verbal working memory. *Behav Genet, 31*(6), 615-624.

APA. (2000). Diagnostic and statistical manual of mental disorders (DSM). *Washington, DC: American psychiatric association, 4th ed.,*.

Arango-Dávila, C., A., Rojas, J., & Moreno, M. (2008). Análisis de los aspectos asociados a la enfermedad mental en Colombia y la formación en psiquiatría. *Revista colombiana de psiquiatría, 37*(4), 538-563.

Arcos-Burgos, M., Castellanos, F. X., Lopera, F., Pineda, D., Palacio, J. D., Garcia, M., . . . Muenke, M. (2002). Attention-deficit/hyperactivity disorder (ADHD): feasibility of linkage analysis in a genetic isolate using extended and multigenerational pedigrees. *Clin Genet, 61*(5), 335-343. doi:cge610503 [pii]

Arcos-Burgos, M., Jain, M., Acosta, M. T., Shively, S., Stanescu, H., Wallis, D., . . . Muenke, M. (2010). A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Molecular psychiatry, 15*(11), 1053-1066. doi:mp20106 [pii]10.1038/mp.2010.6

Arcos-Burgos, M., & Muenke, M. (2002). Genetics of population isolates. *Clinical genetics, 61*(4), 233-247.

Barkley. (1997). Attention-deficit/hyperactivity disorder, self-regulation, and time: toward a more comprehensive theory. *J Dev Behav Pediatr, 18*(4), 271-279.

Barragán Duarte, J. L. Mapa genético de los colombianos [Colombian genetic map]. Retrieved from http://historico.unperiodico.unal.edu.co/ediciones/105/15.html

Barragán-Duarte, J. L. (2007). Mapa genético de los colombianos. *UN Periódico*, 105.

Biederman, J., & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *Lancet, 366*(9481), 237-248. doi:10.1016/S0140-6736(05)66915-2

Bochud, M. (2012). Estimating heritability from nuclear family and pedigree data. *Statistical Human Genetics: Methods and Protocols*, 171-186.

Breiman, L. (2001). Random Forests. In R. E. Schapire (Ed.), *Machine Learning* (Vol. 45, pp. 5-32). Statistics Department, University of California, Berkeley, CA 94720: Kluwer Academic Publishers. Manufactured in The Netherlands.

Breiman, L., Friedman, J. H., Olshen, R. A., & Stone, C. H. (1984). *Classification and Regression Trees*. Belmont, CA: Wadsworth International Group, Inc.

Brotman, M. A., Guyer, A. E., Lawson, E. S., Horsey, S. E., Rich, B. A., Dickstein, D. P., . . . Leibenluft, E. (2008). Facial emotion labeling deficits in children and adolescents at risk for bipolar disorder. *Am J Psychiatry, 165*(3), 385-389. doi:10.1176/appi.ajp.2007.06122050

Bukstein, O. G. (2012). Attention deficit hyperactivity disorder and substance use disorders. *Current topics in behavioral neurosciences, 9*, 145-172. doi:10.1007/7854_2011_148

Cannon, T. D., Gasperoni, T. L., van Erp, T. G., & Rosso, I. M. (2001). Quantitative neural indicators of liability to schizophrenia: implications for molecular genetic studies. *Am J Med Genet, 105*(1), 16-19.

Castellanos, F. X., & Tannock, R. (2002). Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. *Nat Rev Neurosci, 3*(8), 617-628. doi:10.1038/nrn896

Cervantes-Henríquez, M., Acosta-López, J., Aguirre-Acevedo, D., Pineda-Álvarez, D., & Puentes Rozo, P. (2008). Fenotipo comportamental evaluado con una escala multidimensional de la conducta en niños y adolescentes de 30 familias con trastorno de atención-hiperactividad. *Acta Neurol Colomb, 24*, 53-62.

Doyle, A. E., Faraone, S. V., Seidman, L. J., Willcutt, E. G., Nigg, J. T., Waldman, I. D., . . . Biederman, J. (2005). Are endophenotypes based on measures of executive functions useful for molecular genetic studies of ADHD? *J Child Psychol Psychiatry, 46*(7), 774-803. doi:10.1111/j.1469-7610.2005.01476.x

DSM-IV. (2002). *Manual Diagnóstico y Estadístico de los Trastornos Mentales: Texto Revisado*: Masson.

Elston, R. C., & Gray-McGuire, C. (2004). A review of the 'Statistical Analysis for Genetic Epidemiology' (S.A.G.E.) software package. *Hum Genomics, 1*(6), 456-459.

Elston, R. C., Satagopan, J. M., & Sun, S. (2012). Genetic terminology. *Methods Mol Biol, 850*, 1-9. doi:10.1007/978-1-61779-555-8_1

Elston, R. C., Satagopan, J. M., & Sun, S. (2012). *Statistical human genetics*: Humana Press;.

Faraone, S. V., Biederman, J., Lehman, B. K., Keenan, K., Norman, D., Seidman, L. J., . . . Chen, W. J. (1993). Evidence for the independent familial transmission of attention deficit hyperactivity disorder and learning disabilities: results from a family genetic study. *Am J Psychiatry, 150*(6), 891-895. doi:10.1176/ajp.150.6.891

Flint, J., & Munafo, M. R. (2007). The endophenotype concept in psychiatric genetics. *Psychol Med, 37*(2), 163-180. doi:10.1017/S0033291706008750

Franke, B., Vasquez, A. A., Johansson, S., Hoogman, M., Romanos, J., Boreatti-Hummer, A., . . . Reif, A. (2010). Multicenter analysis of the SLC6A3/DAT1 VNTR haplotype in persistent ADHD

suggests differential involvement of the gene in childhood and persistent ADHD. *Neuropsychopharmacology, 35*(3), 656-664. doi:10.1038/npp.2009.170

Friedman, J. H. (1999). *Greedy Function Approximation: a Gradient Boosting Machine.* Retrieved from Stanford:

Golden, C. J. (1999). *Stroop Test de Colores y Palabras: Manual de aplicación* (M. TEA Ediciones, España Ed.).

Gordon, L. (2013). *Using Classification and Regression Trees (CART) in SAS® Enterprise Miner For Applications in Public Health*. Paper presented at the SAS Global Forum 2013: Data Mining and Text Analytics.

Gottesman, II, & Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry, 160*(4), 636-645. doi:10.1176/appi.ajp.160.4.636

Gottesman, II, & Shields, J. (1967). A polygenic theory of schizophrenia. *Proc Natl Acad Sci U S A, 58*(1), 199-205.

Hayes, T., Usami, S., Jacobucci, R., & McArdle, J. J. (2015). Using Classification and Regression Trees (CART) and Random Forests to Analyze Attrition: Results From Two Simulations. *Psychol Aging, 30*(4), 911-929. doi:https://dx.doi.org/10.1037%2Fpag0000046

Heaton, R. K., Avitable, N., Grant, I., & Matthews, C. G. (1999). Further crossvalidation of regression-based neuropsychological norms with an update for the Boston Naming Test. *J Clin Exp Neuropsychol, 21*(4), 572-582. doi:10.1076/jcen.21.4.572.882

Henriquez-Henriquez, M. P., Billeke, P., Henriquez, H., Zamorano, F. J., Rothhammer, F., & Aboitiz, F. (2014). Intra-Individual Response Variability Assessed by Ex-Gaussian Analysis may be a New Endophenotype for Attention-Deficit/Hyperactivity Disorder. *Front Psychiatry, 5*, 197. doi:10.3389/fpsyt.2014.00197

Hwang-Gu, S. L., & Gau, S. S. (2015). Interval timing deficits assessed by time reproduction dual tasks as cognitive endophenotypes for attention-deficit/hyperactivity disorder. *PLoS One, 10*(5), e0127157. doi:10.1371/journal.pone.0127157

Jain, M., Velez, J. I., Acosta, M. T., Palacio, L. G., Balog, J., Roessler, E., . . . Muenke, M. (2011). A cooperative interaction between LPHN3 and 11q doubles the risk for ADHD. *Molecular psychiatry*. doi:10.1038/mp.2011.59

John, B., & Lewis, K. R. (1966). Chromosome variability and geographic distribution in insects. *Science, 152*(3723), 711-721. doi:10.1126/science.152.3723.711

Khodiyar, V. K., Maltais, L. J., Ruef, B. J., Sneddon, K. M., Smith, J. R., Shimoyama, M., . . . Lovering, R. C. (2007). A revised nomenclature for the human and rodent alpha-tubulin gene family. *Genomics, 90*(2), 285-289. doi:10.1016/j.ygeno.2007.04.008

Kuntsi, J., Wood, A. C., Rijsdijk, F., Johnson, K. A., Andreou, P., Albrecht, B., . . . Asherson, P. (2010). Separation of cognitive impairments in attention-deficit/hyperactivity disorder into 2 familial factors. *Arch Gen Psychiatry, 67*(11), 1159-1167. doi:10.1001/archgenpsychiatry.2010.139

Lee Gregory, M., Burton, V. J., Shapiro, B. K., Rowland, L. P., & Coyle, J. T. (2015). Developmental Disabilities and Metabolic Disorders. In *Neurobiology of Brain Disorders* (pp. 18-41).

Lopera, F., Palacio, L. G., Jimenez, I., Villegas, P., Puerta, I. C., Pineda, D., . . . Arcos-Burgos, M. (1999). [Discrimination between genetic factors in attention deficit]. *Rev Neurol, 28*(7), 660-664.

Lopez-Campo, G. X., Gomez-Betancur, L. A., Aguirre-Acevedo, D. C., Puerta, I. C., & Pineda, D. A. (2005). [Attention and executive function tests components in attention deficit/hyperactivity children]. *Rev Neurol, 40*(6), 331-339.

Mastronardi, C. A., Pillai, E., Pineda, D. A., Martinez, A. F., Lopera, F., Velez, J. I., . . . Arcos-Burgos, M. (2016). Linkage and association analysis of ADHD endophenotypes in extended and multigenerational pedigrees from a genetic isolate. *Molecular psychiatry, 21*(10), 1434-1440. doi:10.1038/mp.2015.172

Miller, G. A., & Rockstroh, B. S. (2016). The Neurobiology of Schizophrenia. In (pp. 17-38).

Nigg, J. T. (2001). Is ADHD a disinhibitory disorder? *Psychol Bull, 127*(5), 571-598.

Ojha, A. K. (2018). Use a Classification and Regression Tree (CART) for Quick Data Insights. Retrieved from https://www.isixsigma.com/methodology/lean-methodology/use-a-classification-and-regression-tree-cart-for-quick-data-insights/

Osterriech, P. A. (1944). Le test de copie d'une figure complexe. *Archives de Psychologie, 30*, 206-356.

Palacio, J. D., Castellanos, F. X., Pineda, D. A., Lopera, F., Arcos-Burgos, M., Quiroz, Y. T., . . . Muenke, M. (2004). Attention-deficit/hyperactivity disorder and comorbidities in 18 Paisa Colombian multigenerational families. *J Am Acad Child Adolesc Psychiatry, 43*(12), 1506-1515. doi:S0890-8567(09)61384-8 [pii]10.1097/01.chi.0000142279.79805.dc

Pelham, W. E., Jr., & Fabiano, G. A. (2008). Evidence-based psychosocial treatments for attention-deficit/hyperactivity disorder. *Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53, 37*(1), 184-214. doi:10.1080/15374410701818681

Pennington, B. F. (2006). From single to multiple deficit models of developmental disorders. *Cognition, 101*(2), 385-413. doi:10.1016/j.cognition.2006.04.008

Peskin, V. A., Ordonez, A., Mackin, R. S., Delucchi, K., Monge, S., McGough, J. J., . . . Mathews, C. A. (2015). Neuropsychological and dimensional behavioral trait profiles in Costa Rican ADHD sib pairs: Potential intermediate phenotypes for genetic studies. *Am J Med Genet B Neuropsychiatr Genet, 168B*(4), 247-257. doi:10.1002/ajmg.b.32305

Pineda, D. A., Acosta-López, J., Cervantes-Henríquez, M. L., Jiménez-Figueroa, G., Sánchez-Rojas, M., Pineda-Alhucema, W., . . . Puentes Rozo, P. J. (2016). Conglomerados de clases latentes en 408 miembros de 120 familias nucleares de Barranquilla con un caso índice afectado de trastorno de atencíon hiperactividad (TDAH). *Acta Neurol Colomb, 32*(4), 275-284.

Pineda, D. A., Henao, G. C., Puerta, I. C., Mejia, S. E., Gomez, L. F., Miranda, M. L., . . . Murrelle, L. (1999). [The use of brief questionnaire in the diagnosis of attention deficit. Study group of the Manizales University Foundation]. *Rev Neurol, 28*(4), 365-372.

Pineda, D. A., Kamphaus, R. W., Mora, O., Restrepo, M. A., Puerta, I. C., Palacio, L. G., . . . Holguin, J. A. (1999). [A system of multidimensional behavior assessment. A scale for parents of children from 6 to 11 years of age. Colombian version]. *Rev Neurol, 28*(7), 672-681.

Pineda, D. A., Lopera, F., Puerta, I. C., Trujillo-Orrego, N., Aguirre-Acevedo, D. C., Hincapie-Henao, L., . . . Arcos-Burgos, M. (2011). Potential cognitive endophenotypes in multigenerational families: segregating ADHD from a genetic isolate. *Atten Defic Hyperact Disord, 3*(3), 291-299. doi:10.1007/s12402-011-0061-3

Pineda, D. A., Palacio, L. G., Puerta, I. C., Merchan, V., Arango, C. P., Galvis, A. Y., . . . Arcos-Burgos, M. (2007). Environmental influences that affect attention deficit/hyperactivity disorder: study of a genetic isolate. *Eur Child Adolesc Psychiatry, 16*(5), 337-346. doi:10.1007/s00787-007-0605-4

Pironti, V. A., Lai, M. C., Muller, U., Dodds, C. M., Suckling, J., Bullmore, E. T., & Sahakian, B. J. (2014). Neuroanatomical abnormalities and cognitive impairments are shared by adults with attention-deficit/hyperactivity disorder and their unaffected first-degree relatives. *Biol Psychiatry, 76*(8), 639-647. doi:10.1016/j.biopsych.2013.09.025

Posada, J., A. (2013). La salud mental en Colombia. *Biomédica, 33*(4), 497-498.

Puentes, P. (2009). Neuropsicología de las funciones ejecutivas. *Barranquilla, Colombia: Ediciones Universidad Simón Bolívar.[Links]*.

Puentes Rozo, P., Acosta-Lopez JE, Cervantes-Henriquez M, Martinez-Banfi,M, Lozano-Gutierrez, S, Jimenez -Figueroa, G, Pineda- Alhucema, W, Mejia-Segura, E, Zurbaran, MA, Zurek EE, Sanchez-Rojas M, Arcos-Burgos, M, Velez JI, Pineda, DA. (2018). Attention Deficit /Hyperactivity Disorder and Comorbidities in 120 Nuclear Families from a Caribbean Community *(to be Submitted)*.

Puentes-Rozo, P. J., Barcelo-Martinez, E., & Pineda, D. A. (2008). Behavioural and neuropsychological characteristics of children of both sexes, between 6 and 11 years of age, with attention deficit hyperactivity disorder]. *Rev Neurol, 47*(4), 175-184.

Ramos-Quiroga, J. A., Ribases-Haro, M., Bosch-Munso, R., Cormand-Rifa, B., & Casas, M. (2007). [Genetic advances in attention deficit hyperactivity disorder]. *Rev Neurol, 44 Suppl 3*, S51-52.

Rao, D. C. (1998). CAT scans, PET scans, and genomic scans. *Genetic epidemiology, 15*(1), 1-18. doi:10.1002/(SICI)1098-2272(1998)15:1<1::AID-GEPI1>3.0.CO;2-B

Reich, W. (2000). Diagnostic interview for children and adolescents (DICA). *J Am Acad Child Adolesc Psychiatry, 39*(1), 59-66. doi:S0890-8567(09)66101-3 [pii]10.1097/00004583-200001000-00017

Reitan, R. (1958). The validity of the trail making test as an indicator of organic brain damage.[8: 271-6]. 1958. *Perceptual and Motor skills*.

Reitan, R. M. (1955). The relation of the trail making test to organic brain damage. *J Consult Psychol, 19*(5), 393-394.

Reitan, R. M., & Wolfson, D. (1985). *The Halstead-Reitan neuropsychological test battery: Theory and clinical interpretation* (Vol. 4): Reitan Neuropsychology.

Reitan, R. M., & Wolfson, D. (1995). Category Test and Trail Making Test as measures of frontal lobe functions. *The Clinical Neuropsychologist, 9*(1), 50-56.

Reitan, R. M., & Wolfson, D. (2004). The Trail Making Test as an initial screening procedure for neuropsychological impairment in older children. *Archives of Clinical Neuropsychology, 19*(2), 281-288.

Rhee, S. H., Waldman, I. D., Hay, D. A., & Levy, F. (1999). Sex differences in genetic and environmental influences on DSM-III-R attention-deficit/hyperactivity disorder. *J Abnorm Psychol, 108*(1), 24-41.

Rommelse, N. N. (2008). Endophenotypes in the genetic research of ADHD over the last decade: have they lived up to their expectations? *Expert Rev Neurother, 8*(10), 1425-1429. doi:10.1586/14737175.8.10.1425

Rommelse, N. N., Arias-Vasquez, A., Altink, M. E., Buschgens, C. J., Fliers, E., Asherson, P., . . . Franke, B. (2008). Neuropsychological endophenotype approach to genome-wide linkage analysis identifies susceptibility loci for ADHD on 2q21.1 and 13q12.11. *Am J Hum Genet, 83*(1), 99-105. doi:10.1016/j.ajhg.2008.06.006

Satterfield, J. H., Cantwell, D. P., & Satterfield, B. T. (1974). Pathophysiology of the hyperactive child syndrome. *Arch Gen Psychiatry, 31*(6), 839-844.

Sibley, M. H., Pelham, W. E., Jr., Molina, B. S., Gnagy, E. M., Waschbusch, D. A., Garefino, A. C., . . . Karch, K. M. (2012). Diagnosing ADHD in adolescence. *Journal of consulting and clinical psychology, 80*(1), 139-150. doi:10.1037/a0026577

Sonuga-Barke, Bitsakou, P., & Thompson, M. (2010). Beyond the dual pathway model: evidence for the dissociation of timing, inhibitory, and delay-related impairments in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry, 49*(4), 345-355.

Sonuga-Barke, E. J., Dalen, L., Daley, D., & Remington, B. (2002). Are planning, working memory, and inhibition associated with individual differences in preschool ADHD symptoms? *Dev Neuropsychol, 21*(3), 255-272. doi:10.1207/S15326942DN2103_3

Sonuga-Barke, E. J., Sergeant, J. A., Nigg, J., & Willcutt, E. (2008). Executive dysfunction and delay aversion in attention deficit hyperactivity disorder: nosologic and diagnostic implications. *Child Adolesc Psychiatr Clin N Am, 17*(2), 367-384, ix. doi:10.1016/j.chc.2007.11.008

Tacchini, G., Coppola, M. T., Musazzi, A., Altamura, A. C., & Invernizzi, G. (1994). [Multinational validation of the Composite International Diagnostic Interview (CIDI)]. *Minerva Psichiatr, 35*(2), 63-80.

Tulsky, D. S., Saklofske, D. H., & Zhu, J. (2003). Chapter 2 – Revising a Standard: An Evaluation of the Origin and Development of the WAIS-III. In *Practical Resources for the Mental Health Professional* (pp. 43-92): Academic Press.

Villalón, J. (2008). Colonias extranjeras en Barranquilla. *Barranquilla, Colombia*.

Visser, S., Bitsko, R., Danielson, M., & Perou, R. (2010). Increasing Prevalence of Parent-Reported Attention-Deficit/Hyperactivity Disorder Among Children --- United States, 2003 and 2007. *Mortality and Morbidity Weekly Report, 59*, 1439-1443.

Walters, J. T., & Owen, M. J. (2007). Endophenotypes in psychiatric genetics. *Molecular psychiatry, 12*(10), 886-890. doi:10.1038/sj.mp.4002068

Wechsler, D. (1955). *Wechsler Adult Intelligence Scale. New York: The Psychological Corporation*
Wechsler, D. (1991). *Test de Inteligencia para niños WISC-III*: PAIDOS, Buenos Aires.
Wechsler, D. (2003). *Escala Wechsler de Inteligencia para adultos-III. 3° edición*: Manual Moderno, México.
Wechsler, D. (2004). *Escala de memoria de Wechsler-III*: Ediciones TEA, Madrid.
Willcutt, E. G., Betjemann, R. S., McGrath, L. M., Chhabildas, N. A., Olson, R. K., DeFries, J. C., & Pennington, B. F. (2010). Etiology and neuropsychology of comorbidity between RD and ADHD: the case for multiple-deficit models. *Cortex, 46*(10), 1345-1361. doi:10.1016/j.cortex.2010.06.009
Willcutt, E. G., Doyle, A. E., Nigg, J. T., Faraone, S. V., & Pennington, B. F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. *Biol Psychiatry, 57*(11), 1336-1346. doi:10.1016/j.biopsych.2005.02.006
Willcutt, E. G., Pennington, B. F., Duncan, L., Smith, S. D., Keenan, J. M., Wadsworth, S., . . . Olson, R. K. (2010). Understanding the complex etiologies of developmental disorders: behavioral and molecular genetic approaches. *J Dev Behav Pediatr, 31*(7), 533-544. doi:10.1097/DBP.0b013e3181ef42a1