Analysis of shared homozygosity regions in Saudi siblings with attention deficit hyperactivity disorder
Jameela M.A. Shinwari, Eman A.A. Al Yemi, Faten M. Alnaemi, Dejene Abebe, Basma S. Al-Abdulaziz, Bashayer R. Al Mubarake, Mohammad Ghaziuddin, and Nada A. Al Tassan

**Aim** Genetic and clinical complexities are common features of most psychiatric illnesses that pose a major obstacle in risk-gene identification. Attention deficit hyperactivity disorder (ADHD) is the most prevalent childhood-onset psychiatric illness, with high heritability. Over the past decade, numerous genetic studies utilizing various approaches, such as genome-wide association, candidate-gene association, and linkage analysis, have identified a multitude of candidate loci/genes. However, such studies have yielded diverse findings that are rarely reproduced, indicating that other genetic determinants have not been discovered yet. In this study, we carried out sib-pair analysis on seven multiplex families with ADHD from Saudi Arabia. We aimed to identify the candidate chromosomal regions and genes linked to the disease.

**Patients and methods** A total of 41 individuals from multiplex families were analyzed for shared regions of homozygosity. Genes within these regions were prioritized according to their potential relevance to ADHD.

**Results** We identified multiple genomic regions spanning different chromosomes to be shared among affected members of each family; these included chromosomes 3, 5, 6, 7, 8, 9, 10, 13, 17, and 18. We also found specific regions on chromosomes 8 and 17 to be shared among affected individuals from more than one family. Among the genes present in the regions reported here were involved in neurotransmission (GRM3, SIGMAR1, CHAT, and SLC18A3) and members of the HLA gene family (HLA-A, HLA-DPA1, and MICC).

**Conclusion** The candidate regions identified in this study highlight the genetic diversity of ADHD. Upon further investigation, these loci may reveal candidate genes that enclose variants associated with ADHD. Although most ADHD studies were conducted in other populations, our study provides insight from an understudied, ethnically interesting population. *Psychiatr Genet* 27:131–138

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*Behavioral Genetics Unit, Department of Genetics, *a*Department of Psychiatry, King Faisal Specialist Hospital & Research Center, *b*The National Center for Genomic Technology, King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia and *c*Department of Psychiatry, University of Michigan, Ann Arbor, Michigan, USA

Correspondence to Jameela M.A. Shinwari, MSc, Department of Genetics, King Faisal Specialist Hospital and Research Centre, PO Box 3354, Riyadh 11211, Saudi Arabia
Tel: +966 1 4647272 x37232; fax: +966 1 4424585; e-mail: sjameela@kfshrc.edu.sa

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**Introduction** Attention deficit hyperactivity disorder (ADHD) is a common childhood psychiatric condition associated with significant morbidity. The main symptoms of inattention, hyperactivity, and distractibility usually come to medical attention before the age of 7 years. Although it is regarded as a childhood-onset disorder, symptoms often persist in varying degrees. ADHD occurs in all countries and cultures and its prevalence rates vary according to the study population. In some developed countries, such as the USA, its prevalence rate among children aged 4–17 is estimated to be 9.5% (Pastor et al., 2015). Although no systematic epidemiological studies have been conducted in Saudi Arabia, clinic-based studies and anecdotal experience suggest comparable rates (between 4 and 12% of local children aged 6–12 years) (Al Hamed et al., 2008).

The exact cause of ADHD is not known. However, like most psychiatric disorders, both environmental and genetic factors have been implicated. Environmental factors include diet, toxins, maternal exposure to alcohol or cigarette smoke, as well as pregnancy-related and delivery-related complications (Banerjee et al., 2007). Evidence for genetic origins of the disorder comes from familial and twin studies which place the genetic susceptibility of ADHD ahead of other risk factors with a heritability estimate of about 76% (Morrison and Stewart, 1973; Cantwell, 1975;
Molecular genetic studies performed over the last two decades have clarified the complex and heterogeneous nature of ADHD by identifying several ADHD-linked loci and variants using candidate-gene association studies (CGAS) (Gizer et al., 2009), linkage-analysis and metaanalysis (Zhang et al., 2011), genome-wide association studies (GWAS) (Neale et al., 2010), exome sequencing (Lyon et al., 2011), and copy number variation (CNV) studies (Williams et al., 2010).

Using linkage analysis, many genomic regions were linked to ADHD, but only 24 were significantly related to the disorder. Several regions among them, including 16p13 (Smalley et al., 2002; Ogdie et al., 2003), 17p11 (Arcos-Burgos et al., 2004; Ogdie et al., 2004), 5p13 (Ogdie et al., 2006), and 16q23.1-qter (Zhou et al., 2008), presented consistent findings when linkage was replicated. Candidate genes within these regions included dopamine transporter SLC6A3, which was reported in over 70 studies (Li et al., 2014).

CGAS linked many genes to ADHD, of which 50% were later found to be significant. Although most of the identified genes were associated with varying degrees with specific pathways thought to be involved in ADHD pathology, a substantial discrepancy still exists for the same gene locus. Examples of identified genes include DRD1 in the dopaminergic neurotransmission system (Ribases et al., 2012); HTR2A, DDC, and MAOB (Ribases et al., 2009) in the serotonergic neurotransmission system; and SLC6A2 and ADRA1B in the noradrenergic system (Hawi et al., 2013). To counter inconsistencies between studies, a complete meta-analysis was conducted using data from multiple CGAS yielding a number of genes, including DAT1, DRD4, DRD5, SHMT, HTR1B, and SNAP25, with significant gene–disorder associations (Gizer et al., 2009).

False-positive findings are considered one of the main limitations of linkage and candidate-gene studies resulting in selection bias for genes that are thought to be involved in ADHD-related pathways. Alternatively, GWAS are unbiased regarding candidate-gene selection (Hall et al., 2013). Although some GWAS failed to detect significant ADHD associations (Mick et al., 2010; Neale et al., 2010), others were more promising. One example is genome-wide association scans of pooled DNA samples. Such studies identified novel risk genes and further supported the existence of a common effect for CDH13 and ASTN2 cell adhesion molecules and CTNNA2 and KALRN synaptic plasticity regulators in ADHD (Lesch et al., 2008) and significantly linked intronic markers of CDH13 and GFOD1 to ADHD (Lasky-Su et al., 2008).

Other genetic variations, including rare inherited structural variants, are thought to play a role in ADHD risk. Some studies reported as many as 222 rare inherited CNVs (Elia et al., 2010), whereas others reported only 17 (de novo or inherited from a single affected parent) to be in association with ADHD (Lesch et al., 2011).

Aggregation of ADHD characteristics in families, both within and across generations, has been observed. This trend was supported by twin studies reporting high concordance rates as well as familial studies showing first-degree relatives of an individual with ADHD to be at a greater risk for the disorder compared with those of controls (Franke et al., 2012). Therefore, utility of consanguineous populations for the elucidation of the genetic basis of complex neurodevelopmental disorders has proven useful in uncovering causal or risk genes. In this study, we intended to take advantage of two important features of the Saudi population: (a) the highly inbred nature of this population (57.7% of marriages are consanguineous, 28.4% of which are among first cousins) (El-Hazmi et al., 1995), (b) in addition to the familial structures and social conditions, rendering it close to an ideal population for studying ADHD. Moreover, no systematic genetic study has been conducted so far on this population, with the exception of a few reports (El-Tarras et al., 2012; Al-Owain et al., 2013; Alhraiwil et al., 2015). Hence, we set out to identify the chromosomal regions and genes contributing to ADHD in Saudi families by performing homozygosity analysis and candidate-gene prioritization.

Patients and methods

Patients

This study was performed in accordance with the regulations of the King Faisal Specialist Hospital and Research Centre (KFSSH&RC) Ethics Committee and in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html) (RAC # 2120001). All participants signed a written informed consent form. KFSSH&RC is a 900-bed hospital located in Riyadh, Saudi Arabia, that receives patients for diagnosis and treatment from all over the Middle East.

Seven multiplex Saudi families each with at least a minimum of two members diagnosed with ADHD and often at least one unaffected sibling were identified from consecutive referral to the child psychiatric clinic at KFSSH&RC, giving a total of 19 affected and 22 unaffected studied members. At the time of the study, the clinic was staffed by two US-trained and board-certified child and adolescent psychiatrists. The inclusion criteria stipulated that participants must be children and/or adolescents between the ages of 4 and 17 years of either sex born to Arabic-speaking parents of Saudi origin and living with at least one biological parent. Exclusion criteria included the existence of profound intellectual disability (estimated IQ < 50), major learning or communication deficits, severe self-injurious or other aggressive behaviors, sensory deficits, and severe physical disabilities.
Children who were non-Arabic speaking and/or came from expatriate families living in Saudi Arabia were also excluded. Details about family history, including the presence of consanguinity, were obtained from one or both parents and collected in a standardized manner. Hospital and school records were also examined. The categorization of intellectual disability was based on clinical assessment and/or an IQ test. A comprehensive psychiatric and physical examination was performed by the child psychiatrist with a psychiatry fellow, and, based on all the information available, a final diagnosis was given according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed., text revision (DSM-IV-TR; American Psychiatric Association, 2013). Diagnostic disagreements were resolved by follow-up evaluations and by discussion with other members of the team. Of the 19 affected participants, 16 were male and three were female. Nine of the affected participants were categorized as intellectually disabled.

Genomic DNA extraction
Blood samples were collected from 19 diagnosed cases and available family members (i.e., parents, affected siblings, and at least one unaffected sibling). For each, 3–5 ml of whole blood was collected in EDTA tubes. DNA was extracted from peripheral blood lymphocytes using the Gentra Systems Puregene DNA Isolation Kit in accordance with the manufacturer’s recommendations (Gentra Systems Inc., Minneapolis, Minnesota, USA).

Homozygosity analysis
DNA samples from cases and all available family members were evaluated using the Axiom Genome-Wide CEU 1 Human Mapping Array (Affymetrix, Santa Clara, California, USA). Briefly, the Axiom 2.0 reagent kit was used to amplify the target DNA followed by fragmentation. The pellets were then resuspended and hybridized according to the manufacturer’s recommendations (Affymetrix). Genotyping was performed using GeneTitan Multi-Channel Instrument (Affymetrix). GeneChip operating software and Genotyping Console (GTC) software (Affymetrix) were used for primary data analysis with an overall single-nucleotide polymorphism call rate of 95–99%. Data generated from the arrays were then analyzed using Affymetrix Genotyping Console (version 3.01) and/or a homozygosity mapping tool (Adic et al., 2005; Seelow et al., 2009). Each family was analyzed independently using Genotyping Console (GTC/Affymetrix) software. Genotyping was performed according to the manufacturer’s protocols, and the resulting genotypes were evaluated using homozygosity mapper software (http://www.homozygositymapper.org/) (Seelow et al., 2009). Affected (cases) and unaffected (controls) members in a given family were analyzed by the software, which returns regions of homozygosity shared between the cases but absent from their corresponding controls; that is, any homozygous stretches found to be present in both cases and controls were excluded, leaving only those unique to affected cases or shared between affected individuals within a family. In addition, regions of homozygosity shared among cases across families were manually detected and identified. In total, 22 unaffected family members were used as controls for their respective families.

ToppGene (Chen et al., 2009) was used to prioritize genes according to their relevance to the development of ADHD. Basically, the genes within the regions of interest, the ‘test genes’, were ranked based on their functional annotations similarity with a list of known disease-related genes, the ‘training set’. The training set contained genes reported to be associated with ADHD: namely, TXNPA7, DLGAP2, APBA2, TP1, NDNL2, PRODH, DGCR6, NRXN1, DOC2A, MAPT, SHANK3, GABRA5, GABRB3, GABRG3, GRIA3, CHRN7, SLC6A3, DBH, COMT, MAOA, DRD4, DRD5, DRD2, HTR1B, and SLC6A4. The top three candidate genes were reported in the present study.

Results
Description of families
In total, 41 individuals from seven multiplex families were examined in this study (Fig. 1). Family A comprised two affected sons of a consanguineous marriage who were aged 17 and 16 years. The pedigree spanned five generations, and the parents were unaffected. Family B comprised four siblings from unrelated parents, including an affected female (12 years old) and one affected male (8 years old). Likewise, family C included two affected members of 21 and 16 years of age and five healthy siblings from unrelated parents. Families D and G were from consanguineous marriages, the former represented a first-cousin marriage and comprised five siblings, two of whom were affected, whereas the latter included nine siblings, five of whom were affected. Family F had a total of six siblings from a consanguineous marriage with four affected sons. The pedigree spanned five generations with no previous history of the disorder. The last examined family in our cohort, family E, represented a nonconsanguineous couple and included four affected sons.

Loss of heterozygosity analysis
Using the homozygosity mapper software, we identified regions that were shared among the available affected members of each family (Table 1) and among the affected individuals across at least two families (Table 2).

Family A: five unique homozygous stretches mapped to four different chromosomes were linked to this family. The first stretch mapped to chromosome 3 (3p22.3–3p22.2), spanning 3 781 185 bp and included 34 genes; the second, spanning 2 753 826 bp, mapped to chromosome 6 (6p21) and included 46 genes; the third mapped to a larger region of 40 324 002 bp on chromosome 9 (9p21.2–9q21.12) and included a large set of 539 genes;
and the last two corresponded to areas on chromosome 10 (10p13 and 10q26.3), both spanning around 2 000 000 bp with 55 and 26 genes, respectively.

Family B: unique regions were identified in family B and were mapped to chromosome 6 (6p22.1, 6p22.1–6p21.33, and 6p21.32). These three areas spanned less than 1 000 000 bp each and included 2, 24, and 1 gene, respectively. Each interval consisted of at least one member of the major histocompatibility complex family (the human version of which is known as leukocyte antigen HLA).

Family C: a unique region was located on 6p22.1, spanning 301 071 bp and encompassing 44 genes. This region mapped to coordinates that fell very close to, but not within, areas implicated in family B. As with the intervals in family B, this area consisted of several members of the HLA gene family, strengthening the possibility of their involvement.

Family D: examination of chromosomal areas in this family identified six unique regions mapped to five different chromosomes: namely, chromosome 5 (5q11.2–5q12.2), chromosome 7 (7q21.11–7q21.3 and 7q31.1–7q31.32), chromosome 10 (10p11.22–10q22.1), chromosome 13 (13q33.3–13q34), and chromosome 17 (17q12–17q22). The region in chromosome 5 spanned 7 890 442 bp and 92 genes; the two stretches in chromosome 7 spanned 14 584 322 and 7 166 252 bp and encompassed 158 and 70 genes, respectively; the area covered in chromosome 10 spanned 37 738 913 bp and encompassed 501 genes, whereas that covered in chromosome 13 spanned 2 094 116 bp and included 22 genes. Finally, for chromosome 17, the reported region covered a total of 19 099 606 bp and included 643 genes.
Family G: this family had one unique region covering 2,636,508 bp with 21 genes and 2,372,343 bp with 38 genes. The coordinates of the former region fell close to but did not overlap with those of family D.

Family G: this family had one unique region corresponding to chromosome 18 (18p11.32–18p11.31) spanning 1,801,597 bp with 33 genes.

Besides unique regions found in single families, we were able to identify two that were shared among affected individuals across families.

Chromosome 8: the regions of homozygosity identified in families B and F were mapped to nonoverlapping areas on chromosome 8; however, further analysis revealed that both regions cover different parts of **CSMD1** (MIM# 608397; chr8: 2,935,353–4,994,972 bp).

Chromosome 17: family B included another homozygous stretch in chromosome 17 (17q21.33–17q22) that spanned 957,344 bp with 10 genes and fell within the chromosomal region indicated in family D mapping to chromosome 17. The 10 genes identified in family B were also found in the area implicated in family D and included **NME1**, **NME2**, **LOC105371828**, **HOMER1**, **HTR1A**, **ARSB**, **CFTR**, **CHAT**, **CLASP2**, **DPYSL4**, and **CA10**.

### Discussion

ADHD is a heritable neuropsychiatric condition that is characterized by inattention along with hyperactive and impulsive behaviors. Despite the numerous regions linked to ADHD through genome-wide linkage analysis, it is still difficult to replicate results mainly because of the absence of a well-defined Mendelian segregation model and the extreme variability in the severity of ADHD traits in the majority of families with affected individuals (Acosta et al., 2004).

In this study, we sought to map the chromosomal regions and to identify the genes contributing to ADHD in Saudi patients. We examined 41 individuals from seven unrelated multiplex Saudi families and identified homozygous regions in chromosomes either unique to single families or shared between affected members across families.

The shared regions within families spread over several chromosomes: namely, chromosomes 3, 5, 6, 7, 8, 9, 10, 13, 17, and 18. The largest homozygous stretch of 40,324,002 bp was detected in family A, whereas the smallest of 38,783 bp was found in family B, mapping to chromosomes 9 and 8, respectively. Concurrently, the largest consecutive set of shared genes was found in chromosome 17 of family D, whereas the smallest set (of only a single gene) was observed in chromosomes 6 and 8 of family B. Only two regions were found to be

### Table 1  Regions of homozygosity detected in seven different families

| Family ID | Chromosome number | Chromosomal region | Chromosomal coordinates | Region size (bp) | Number of genes found | Top three genes prioritized by ToppGene |
|-----------|-------------------|---------------------|--------------------------|-----------------|-----------------------|---------------------------------------|
| A         | 3                 | 3p22.3–3p22.2       | 32 704 825–36 486 010    | 3,781,185       | 34                    | **CCR4**, **GLB1**, **CLASP2**        |
| 6         | 6p21              | 106 374 058–109 127 884 | 2,753,826               | 46              |                       | **NRZ1**, **PDS2**, **CD24**          |
| 9         | 9p21.1–9q21.12    | 30 872 374–71 196 376 | 40,324,002               | 539             |                       | **SIGMAR1**, **UNC13B**, **VCBP**     |
| 10        | 10p13             | 13 029 221–15 269 846 | 2,240,625               | 55              |                       | **SUV39H2**, **PHNY**, **OPTN**       |
| 10        | 10q26.3           | 130 840 798–132 931 819 | 1,991,021               | 26              |                       | **NX6-2**, **BNIIP3**, **DPYSL4**     |
| B         | 6                 | 6p22.1              | 30 361 558–30 429 383    | 68,025          | 2                     | **MBTC**, **UBQLN1P1**                |
| 6         | 6p22.1–6p21.33    | 30 430 520–30 766 211 | 335,691                 | 24              |                       | **FLOT1**, **HLA-E**, **ATA1**        |
| 6         | 6p21.32           | 33 019 688–33 064 598 | 44,912                  | 1               |                       | **HLA-DPA1**                          |
| 8         | 8p23.2            | 2,955 341–2,994 124  | 38,783                  | 1               |                       | **CSMD1**                             |
| 17        | 17q21.33–17q22    | 51 156 755–52 114 089 | 957,334                 | 10              |                       | **NME1**, **NME2**, **MBTD1**         |
| C         | 6                 | 6p22.1              | 29 732 813–30 033 884    | 301,071         | 44                    | **HLA-A**, **HLA-G**, **HLA-F**       |
| 7         | 7q11.2–7q11.2     | 55 918 530–63 808 972 | 7,890,442               | 92              |                       | **PDE4D**, **IL6ST**, **NDUFAP2**     |
| 10        | 10p11.22–10q22.1  | 83 085 832–97 670 154 | 14,584,322              | 158             |                       | **SGCE**, **GRM5**, **ABCBI**         |
| 12        | 12q13.3–12q34     | 114 865 172–122 031 424 | 7,166,252              | 70              |                       | **KCN2**, **C4V**, **CFTR**          |
| 13        | 13q33.3–13q34     | 34 156 833–71 897 746 | 37,738,913              | 501             |                       | **SLC1A8**, **ARN**, **CHAT**         |
| 14        | 14q23.3–14q32     | 108 013 711–110 107 827 | 2,094,116              | 22              |                       | **LIG4**, **IR5**, **MYO16**          |
| 15        | 15q21.2–15q22     | 36 131 437–55 231 043 | 19,099,606              | 643             |                       | **PMNT**, **MAPT**, **CRHR1**         |
| E         | 5                 | 5q11.1–5q11.2       | 50 162 948–52 799 456   | 2,638,508       | 21                    | **ISL1**, **ITGA1**, **EMB**          |
| 5         | 5q14.1            | 78 110 532–80 482 875 | 2,372,343               | 38              |                       | **HOMER1**, **BHMT**, **ARSB**        |
| F         | 8                 | 8p23.2              | 4 070 495–6 021 203     | 1,950,708       | 5                     | **CSMD1**, **RPL23AP54**, **LOC392180** |
| G         | 18                | 18p11.32–18p11.31   | 2,644 571–4,446 168     | 1,801,597       | 33                    | **DLGAP1**, **LPIN2**, **TGF1**       |

### Table 2  Chromosomal regions and genes found in more than one family

| Family ID | Chromosome number | Shared chromosome regions | Shared genes |
|-----------|-------------------|---------------------------|--------------|
| B and F   | 8                 | 8p23.2                    | **CSMD1**    |
| B and D   | 17                | 17q21.33–17q22            | **NME1–NME2**, **NME1**, **NME2**, **MBTD1**, **UTP18**, **LOC101927274**, **LOC440446**, **LOC105371828**, **RPL7P48** and **CA10** |
shared across families, encompassing 11 genes altogether. One region was identified on chromosome 8 that was shared between families B and F and the other was mapped to chromosome 17 and shared between families B and D.

In family A, five homozygous regions (3p22.3–3p22.2, 6p21, 9p21.2–9q21.12, 10p13, and 10q26.3) were uniquely shared between the affected sons (V.1 and V.2). Likewise, common homozygous regions (6p22.1, 6p22.1–6p21.33, and 6p21.32) in family B were shared between an affected daughter and son (II.1 and II.2). In family C, only one region, 6p22.1, was shared between an affected son and daughter (II.2 and II.6). Six unique homozygous regions (5q11.2–5q12.2, 7q21.11–7q21.3, 7q31.1–7q31.32, 10p11.22–10q22.1, 13q33.3–13q34, and 17q12–17q22) were shared between two affected brothers (IV.2 and IV.4) in family D, whereas all affected sons (II.1, II.2, II.3, and II.4) of family E shared two homozygous regions, 5q11.1–5q11.2 and 5q14.1. In family F, a single region, 8p23.2, was shared between four affected sons (V.1, V.3, V.4, and V.5), and finally, in family G, one region, 18p11.32–18p11.31, was shared between two affected brothers and their sister (II.3, II.5, and II.7). All the previously mentioned regions were found to be homozygous only in our affected patients; the remaining unaffected family members (family A.III.2 and A.IV.1; family B.I.1, B.I.2, B.II.3, and B.II.4; family C.I.1 and C.I.2; family D.III.1, D.III.2, D.IV.1, and D.IV.3; family E.I.1 and E.I.2; family F.III.1, F.IV.1, F.V.2, and F.V.6; and family G.I.1, G.I.2, G.II.6, and G.II.8) were either heterozygous or wild type.

Some of the candidate regions of homozygosity identified in this study were previously associated with certain neuropsychological abnormalities observed in ADHD patients. For instance, the 3p24.3 locus was involved in visuospatial working memory, the 9p21.2 locus in motor tasking (Rommelse et al., 2008), and the 6p21.3 locus in ADHD susceptibility and reading disability (Willcutt et al., 2002). Rare ADHD-related CNVs were also mapped to regions on chromosomes 7, 10, and 13 (Elia et al., 2010). In addition, the 5p15.32–5q14.3 region was linked to ADHD (Zhou et al., 2008) and its distal region (5p13) covered one of the highest logarithms of the odds scores for ADHD (Friedel et al., 2007). Besides these regions, distinct novel linkage loci across families with ADHD were mapped to chromosome 5 (Romanos et al., 2008). Certain members of the major histocompatibility complex gene family (identified in families B and C) are known to be positively associated with ADHD pathology; these gene families are located between 6p22.1 and 6p21.3 in humans (Aureli et al., 2008). Similarly, inherited CNV in the intronic region of CSMD1 (found in families B and F) was reported in an ADHD case (Elia et al., 2010). The band 17q22 was also reported as a candidate site for inherited CNVs in children with ADHD (Elia et al., 2010), and therefore the genes found shared between families B and D on chromosome 17 close to this area may harbor interesting pathological variants.

The regions identified in this study were further analyzed using the ToppGene candidate-gene prioritization tool. This analysis yielded a diverse set of genes encoding different classes of proteins, including, but not limited to, kinases (NME1, NME2, and RET), transferases (BMT), receptors (GRM3 and SIGMAR1), and transcription factors and regulators (ISL1 and TGIF1, respectively) (Table 1). Among the top-ranking genes were members of the HLA family (HLA-A, HLA-DPA1, and MHC) as well as genes encoding for proteins involved in acetylcholine transport and synthesis (SLC18A3 and CHAT). A growing number of studies suggest a role for the HLA family of genes in different psychiatric disorders such as schizophrenia, autism, and depression (Chien et al., 2012; Sullivan et al., 2012; Al-Hakbay et al., 2014; Kodavali et al., 2014; Morgan et al., 2016). However, although this report recalls the potential involvement of the HLA family in ADHD, conflicting results exist regarding this matter (Orell et al., 1997; Payton et al., 2003; Aureli et al., 2008).

Moreover, cholinergic dysfunction has been proposed to contribute to the attention and motor control impairment typical of ADHD individuals on the basis of the following: (a) the effectiveness of nicotine and nicotine agonists in alleviating the symptoms of ADHD in adult patients, and (b) the association of specific genetic variants in two cholinergic genes (CHRNA4 and SLC5A7, a presynaptic acetylcholine transporter similar to SLC18A3 identified here) (Todd et al., 2003; English et al., 2009).

Identification of different loss of heterozygosity regions suggests the possibility of locating homozygous causative variants within each respective area; however, the existence of such variants must be further investigated. The assumption of a single causative gene is best suited for nuclear families with multigenerational pedigrees affected with ADHD and genetically isolated for long periods of time, hence providing exceptionally effective power to detect linkage (Arcos-Burgos et al., 2002). The families that we recruited for this study were from a population with a high rate of consanguinity and endogamy, but a single causative gene may be hard to uncover as we are dealing with a multifactorial and heterogeneous disorder. On the other hand, difficulty in defining the phenotype remains the major obstacle in describing the ADHD cohort. Inclusion of participants with lower IQs can negatively impact the generalizability of the findings, thereby conferring a possibility of environmental contribution to the genetic risk for ADHD in our participants with intellectual disability (Biederman et al., 2012; Owen 2012).

Taken together, this work identified candidate regions that are suitable for exploring genetic causes of ADHD in the Saudi population. The specific genetic causes of ADHD remain unknown and need to be further
investigated in a larger cohort to develop a complete molecular characterization of ADHD in Saudi Arabia.

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Conflicts of interest
There are no conflicts of interest.

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