Analysis of shared homozygosity regions in Saudi siblings with attention deficit hyperactivity disorder

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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 child-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 between 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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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;

Biederman *et al.*, 1990; Faraone and Biederman, 1998; Sprich *et al.*, 2000; Faraone *et al.*, 2005).

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 meta-analysis (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. A few 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 to 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 serotoninergic 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*, *5HTT*, *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 (KFSH&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. KFSH&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 KFSH&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 (Adie *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, *TSPAN7*, *DLGAP2*, *APBA2*, *TJP1*, *NDNL2*, *PRODH*, *DGCR6*, *NRXN1*, *DOC2A*, *MAPT*, *SHANK3*, *GABRA5*, *GABRB3*, *GABRG3*, *GRIA3*, *CHRNA7*, *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

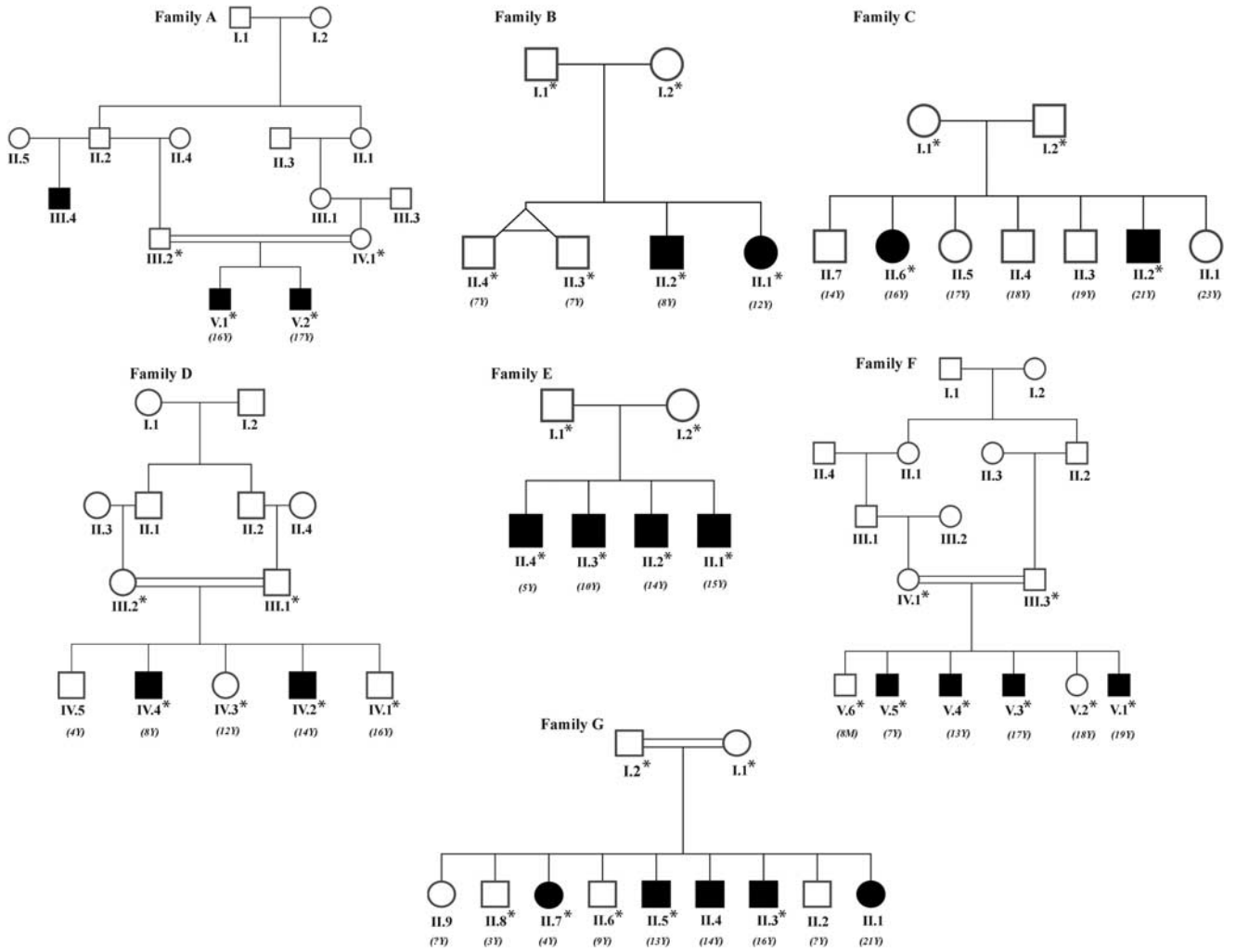
In total, 41 individuals from seven multiplex families were examined in this study (Fig. 1). Family A comprised two affected sons of a consanguineous marriage who were aged 17 and 16 years. The pedigree spanned five generations, and the parents were unaffected. Family B comprised four siblings from unrelated 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;

Fig. 1



Multiplex Saudi attention deficit hyperactivity disorder families recruited for this study. Families A, D, F, and G represent consanguineous families, whereas families B, C, and E represent nonconsanguineous families. Individuals from which DNA was available are marked with an asterisk.

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.

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 in region (from NCBI)	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	6q21	106 374 058–109 127 884	2 753 826	46	*NR2E1*, *PDSS2*, *CD24*
	9	9p21.1–9q21.12	30 872 374–71 196 376	40 324 002	539	*SIGMAR1*, *UNC13B*, *VCP*
	10	10p13	13 029 221–15 269 846	2 240 625	55	*SUV39H2*, *PHYH*, *OPTN*
	10	10q26.3	130 840 798–132 831 819	1 991 021	26	*NKX6–2*, *BNIP3*, *DPYSL4*
B	6	6p22.1	30 361 358–30 429 383	68 025	2	*MICC*, *UBQLN1P1*
	6	6p22.1–6p21.33	30 430 520–30 766 211	335 691	24	*FLOT1*, *HLA-E*, *ATAT1*
	6	6p21.32	33 019 686–33 064 598	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*
D	5	5q11.2–5q12.2	55 918 530–63 808 972	7 890 442	92	*PDE4D*, *IL6ST*, *NDUFAF2*
	7	7q21.11–7q21.3	83 085 832–97 670 154	14 584 322	158	*SGCE*, *GRM3*, *ABCB1*
	7	7q31.1–7q31.32	114 865 172–122 031 424	7 166 252	70	*KCND2*, *CAV1*, *CFTR*
	10	10p11.22–10q22.1	34 156 833–71 895 746	37 738 913	501	*SLC18A3*, *RET*, *CHAT*
	13	13q33.3–13q34	108 013 711–110 107 827	2 094 116	22	*LIG4*, *IRS2*, *MYO16*
	17	17q12–17q22	36 131 437–55 231 043	19 099 606	643	*PNMT*, *MAPT*, *CRHR1*
E	5	5q11.1–5q11.2	50 162 948–52 799 456	2 636 508	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*, *TGIF1*

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*

Family E: two small areas in this family were mapped to chromosome 5 (5q11.1–5q11.2 and 5q14.1), spanning 2 636 508 bp with 21 genes and 2 372 343 bp with 38 genes, respectively. 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*, *NME1*, *NME2*, *MBTD1*, *UTP18*, *LOC101927274*, *LOC440446*, *LOC105371828*, *RPL7P48*, 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

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.3, 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 logarithm 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 (*BHMT*), 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 *MICC*) 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-Hakbany *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 (Odell *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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