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Association between a common haplotype in the *COMT* gene region and psychiatric disorders in individuals with 22q11.2DS

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Abstract

The 22q11.2 deletion syndrome (22q11.2DS) is the most common hemizygous deletion syndrome in humans. In addition to a wide range of physical abnormalities 22q11.2DS subjects show high prevalence of several psychiatric disorders. In our previous study we showed that the low-activity allele (^{158}*Met*) of the *COMT* gene is a risk factor for attention deficit hyperactivity disorder (ADHD) and obsessive–compulsive disorder (OCD) in 22q11.2DS individuals. In the present study we have genotyped fifty-five 22q11.2DS individuals and 95 of their parents for eight SNPs in and around the *COMT* gene. A haplotype composed of three SNPs [rs2097603; rs4680 (^{158}*Val/Met*); rs165599] representing the major linkage disequilibrium blocks in *COMT* and previously implicated in functional variation, was found to be associated with ADHD and OCD in 22q11.2DS individuals. A common risk haplotype (G-A-A) was significantly associated with both ADHD (OR 3.13, $\chi^2 = 4.38$, $p = 0.036$) and OCD (OR 4.00, $\chi^2 = 6.41$, $p = 0.011$) in 22q11.2DS individuals. Interestingly, the same haplotype was recently found to be associated with efficient prefrontal performance in the general population. The risk haplotype was not found to be associated with IQ scores in our 22q11.2DS sample. Parental origin of the deletion did not affect the susceptibility to ADHD and OCD in the 22q11.2DS subjects. This study demonstrated the association of a particular *COMT* haplotype with susceptibility to both ADHD and OCD in 22q11.2DS and supports the hypothesis that *COMT* gene variations contribute to genetic predisposition to psychiatric disorders in the general population.

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Introduction

The 22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome (VCFS, OMIM #192430) and DiGeorge syndrome, is a genetic disorder caused by a hemizygous microdeletion of chromosome 22q11.2 (Carlson et al., 1997; Lindsay, 2001). In addition to a range of physical and behavioural abnormalities (Shprintzen, 2000) it is associated with high frequency of several psychiatric disorders, including schizophrenia, affective disorders, obsessive–compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD) (Feinstein et al., 2002; Gothelf et al., 2004a,b; Murphy et al., 1999; Murphy, 2002). Polymorphic variations in genes that reside in the deleted region, in the remaining chromosome, may contribute to differences in susceptibility to psychiatric disorders between patients with the same deletion. Such variations could affect the biological functions of the gene products and may be particularly important because the amount of mRNA and protein produced in 22q11.2DS individuals is

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expected to be reduced due to the deletion, as was demonstrated in a mouse model of 22q11.2DS (Meechan et al., 2006). The *COMT* gene (MIM #116790), coding for the enzyme catechol-*O*-methyltransferase (EC 2.1.1.6), is one of the prominent candidate genes for susceptibility to mental disorders located in this region. The COMT enzyme is responsible for catecholamine inactivation including brain neurotransmitters such as dopamine and norepinephrine. The ^{158}*Met* variant of the membrane-bound enzyme (MB-COMT) has substantially lower enzymatic activity in the brain compared to the ^{158}*Val* allele (Chen et al., 2004), thus these variants are often referred to as *COMT* high- and low-activity alleles. The ^{158}*Val/Met* variations in the *COMT* gene have been intensively investigated in relation to processes of prefrontal cortex (PFC) functions (Egan et al., 2001; Tunbridge et al., 2006) and predisposition to psychiatric disorders (Azzam and Mathews, 2003; Glatt et al., 2003; Qian et al., 2003). Since individuals with 22q11.2DS carry only a single copy of the gene they are of particular interest in studying the role of *COMT* variations in psychopathology. Recently, we analysed the association of the *COMT* ^{158}*Val/Met* polymorphism with ADHD, OCD and schizophrenia/schizoaffective (SZ/SZaff) disorders in 55 subjects with 22q11.2DS (Gothelf et al., 2006). The ^{158}*Met* allele was significantly more prevalent in 22q11.2DS subjects suffering from ADHD (73.9% vs. 33.3%, $p=0.005$) and OCD (78.6% vs. 33.3%, $p=0.007$) than in the control group (those without ADHD, OCD and SZ/SZaff). These findings suggested that *COMT* may be a susceptibility gene for 22q11.2DS-related ADHD and OCD. The fact that the ^{158}*Met* allele was shown to confer an increased genetic risk to develop these disorders suggested that hyperdopaminergic neurotransmission, as expected in individuals with a single low-activity allele, may be involved in the pathogenesis of these psychiatric disorders in 22q11.2DS individuals. One of the reasons that association studies of the ^{158}*Val/Met* polymorphism with schizophrenia, OCD, ADHD and other psychiatric disorders in both 22q11.2DS and in the general population yielded controversial results (Azzam and Mathews, 2003; Craddock et al., 2006; Glatt et al., 2003; Qian et al., 2003) may be that additional genetic variations in the coding sequence and/or regulatory regions contribute to the gene expression and enzymatic activity. Haplotypes including several polymorphic sites are much more powerful tools for studying the effect of genetic variations on biological functions and disease susceptibility (Hennah et al., 2004). Haplotypes encompassing several polymorphic sites at the *COMT* gene and part of the neighbouring *ARVCF* gene (armadillo repeat gene deleted in velocardiofacial syndrome) (MIM #602269) showed stronger association to psychiatric disorders than the ^{158}*Val/Met* alone as reported for schizophrenia (Li et al., 2000; Sanders et al., 2005; Shifman et al., 2002, 2004), panic disorder (Hamilton et al., 2002) and anorexia nervosa (Michaelovsky et al., 2005). Although no common haplotypes between disorders or different populations could be found, the consistent findings of statistically significant associations suggested that the *COMT* region is an important genetic locus for psychiatric disorders. Recently, statistical epistasis between *COMT* haplotypes and polymorphisms in schizophrenia candidate genes was shown (Nicodemus et al., 2007). *COMT* haplotypes were shown to affect mRNA amounts in the brain (Bray et al., 2003) and to modulate expression by altering mRNA secondary structure (Nackley et al., 2006). The effect of the ^{158}*Val/Met* polymorphism on working memory, executive functioning, attention-control and on brain functionality assessed by neuroimaging techniques has been demonstrated (Drabant et al., 2006; Egan et al., 2001; Heinz and Smolka, 2006; Tunbridge et al., 2006). Recently, it was found that the ^{158}*Val/Met* variant interacts with two functional polymorphisms in the *COMT* region, one from the P2 promoter region (rs2097603) and another in the 3′ (rs165599) region. A haplotype composed of the three SNPs predicted inefficient prefrontal working memory response in a normal population of Caucasians of European ancestry (Meyer-Lindenberg et al., 2006).

Our initial finding of an association of the *COMT* ^{158}*Met* allele with ADHD and OCD in 22q11.2DS subjects (Gothelf et al., 2006) encouraged us to conduct further analysis of the contribution of the *COMT* gene SNPs and haplotypes to the aetiology of these psychiatric disorders. In the present study we analysed the association between ADHD and OCD in 22q11.2DS individuals and eight polymorphic sites in the *COMT* region and haplotypes composed of these markers.

Another possible explanation for the variability in psychiatric manifestations in individuals with 22q11.2DS may be the phenomenon of genomic imprinting as illustrated in individuals with a 15q deletion where a different clinical phenotype (Prader–Willi or Angelman syndromes) is present depending on the parental origin of the deletion (Horsthemke and Buiting, 2006). Thus, we examined the possible role of parental origin of the deletion in predisposing 22q11.2DS individuals to ADHD and OCD.

Table 1. SNPs used in the study

Gene SNP	DbSNP	Allele location	Effect	Primers	Restriction enzyme	Genomic location[a]
COMT SNP-1	rs2097603	−1217A>G promoter		5′-TAGTAACAGACTGGGCACGAA-3′ 5′-GTTCAAAGGGCATTTATCATG-3′	*Hind*III(G)	18308092
COMT SNP-2	rs4633	386C >T Exon 3	62 His/His cac→cat	5′-TTGCTGGGCCTGGTGCTGCT-3′ 5′-ACGTTCATGGCCCACTCCTTC-3′	*Pml*I (C)	18330235
COMT SNP-3	rs4818	611C >G Exon 4	136 Leu/Leu ctc→ctg	5′-CCCTGCACAGGCAAGATCGTG-3′ 5′-GCATGCACACCTTGTCCTTCA-3′	*Bcl*I (G)	18331207
COMT SNP-4	rs4680	675G >A Exon 4	^{158}Val/Met gtg→atg	5′-ACTGTGGCTACTCAGCTGTG-3′ 5′-CCTTTTTCCAGGTCTGACAA-3′	*Nla*III (A)	18331271
COMT SNP-5	rs3838146	1029CIns/Del 3′UTR		5′-GACAACGTGATCTGCCCAGG-3′ 5′-GAGGTGTGCTTTGCATTTAG-3′	*Bgl*I (delC)	18336269
COMT-ARVCF SNP-6	rs165599	A>G Between *COMT* and *ARVCF*		5′-AACATTCAAAGCTCCCCTTG-3′ 5′-GCTGACTCCTCTTCGTTTCC-3′	*Hpa*II(G)	18336781
ARVCF SNP-7	rs2073748	929C>T Exon 5	220 Pro/Leu cca→cta	5′-ACGGTGACCACTCGGACAGTA-3′ 5′-GCTGCGCGTGTCATCCTCCAA-3′	*Bfa*I (T)	18348971
ARVCF SNP-8	rs2240717	794T>C Exon 5	175 Val/Ala gtg→gcg	5′-ACGGTGACCACTCGGACAGTA-3′ 5′-GCTGCGCGTGTCATCCTCCAA-3′	*Msc*I (T)	18349106

[a] Genomic location on chromosome 22q according to Build 36.1 of NCBI (www.ncbi.nlm.nih.gov/SNP).
All data refer to MB-COMT.

Methods

Subjects

Fifty-five unrelated 22q11.2DS individuals referred to the Neurobehavioral Genetics Clinic of the Feinberg Child Study Center, Schneider Children's Medical Center of Israel, described in our previous study (Gothelf et al., 2006), and 95 of their parents were included in this study (ages 16.6±10.2 yr, 34 males, 21 females). Inclusion criteria were: a proven 22q11.2 deletion, confirmed by fluorescent in-situ hybridization (FISH) using a commercial probe (Vysis, Downers Grove, IL, USA), and age >6 years. Of the 55 individuals with 22q11.2DS 23 (41.8%) met DSM-IV criteria for ADHD, 14 (25.5%) for OCD and seven (12.7%) for both ADHD and OCD. Five subjects (9.1%) met DSM-IV criteria of SZ/SZaff disorder. Two of them had comorbid OCD and two had history of ADHD. Twenty-four 22q11.2DS subjects (45.4%) without ADHD, OCD or SZ/SZaff served as the control group for phenotypic and genetic analyses.

Since there is no information on haplotype frequency in different populations in Israel, only individuals of Jewish ethnic background were included. In families with several affected individuals, only the oldest patient from each family was included. In addition, 95 parents of 22q11.2DS subjects were included in the study in order to determine the origin of the 22q11.2 deletion and evaluation of Hardy–Weinberg equilibrium (HWE). The study protocol was approved by the Rabin Medical Center Review Board. Written informed consent was obtained from the study participants and their parents.

Psychiatric and IQ assessment

Description of diagnostic tools (K-SADS-PL; SCID-P structured interviews) and full-scale, verbal and performance IQ tests (FSIQ, VIQ and PIQ) applied to the 22q11.2DS participants is provided elsewhere (Gothelf et al., 2006). IQ score were obtained for 44 out of 55 22q11.2DS subjects.

SNPs used for genotyping

Eight previously reported SNPs (Table 1) were used in this study (Li et al., 2000; Shifman et al., 2002). Six SNPs are from the *COMT* gene region and two from *ARVCF*. The six *COMT* SNPs included: a polymorphic site from the P2 promotor of *COMT* (rs2097603) which is located 1217 bp upstream from the transcription

start, one from exon III (rs4633), two from exon IV (rs4818, rs4680) and one from the 3′ region of the gene (rs3838146). The ^{158}*Val/Met* polymorphism creating the ^{158}*Val* and ^{158}*Met* alleles is coded by rs4680. The rs165599, that is located downstream of *COMT*, was associated with schizophrenia in a large Ashkenazi Jewish population (Shifman et al., 2002) and was used for haplotype construction in several studies (Funke et al., 2005; Handoko et al., 2005; Meyer-Lindenberg et al., 2006; Turic et al., 2005). The two SNPs from the *ARVCF* gene represent sites with non-synonymous changes: ^{220}*Pro/Leu* (rs2073748) and ^{175}*Val/Ala* (rs2240717).

Genotyping of SNPs

DNA was extracted by a commercial kit (Roche Diagnostics, Mannheim, Germany) from 8 ml venous blood. Primers for all markers are described in Table 1. PCR reaction was performed in 15 μl containing: 20 ng DNA, 0.22 U *Taq* polymerase (Quantum-Appligene, Illkirch France), 0.5 μM of each primer, 0.2 mM of each dNTP and buffer recommended by the enzyme supplier. PCR protocol consisted of: 2 min at 95 °C followed by 35 cycles of 40 s at 95 °C, 40 s at the annealing temperature (55 °C for SNPs 1 and 4; 60 °C for SNP 6; 64 °C for SNPs 2, 3, 5, 7, 8) and 60 s at 72 °C. Termination was 5 min at 72 °C. PCR products were digested by the appropriate restriction enzymes (Table 1) (New England BioLabs, Beverly, MA, USA), separated on 1–4% agarose gels, stained with ethidium bromide and photographed under UV light.

Genotyping STRs in 22q11.2

The markers used to determine the parental origin of the deletion in subjects with 22q11.2DS were the short tandem repeats (STR) markers D22S1648 (location on 22q: 17787501-17787672) D22S941 (17789599-17789838) and D22S944 (17990406-17990571), which reside in 22q11.2, centromeric to the *COMT* and *ARVCF* genes (Morrow et al., 1995; Murphy, 2002; Perez and Sullivan, 2002). These highly polymorphic markers are of the CA repeat type and show heterozygosity in excess of 0.6. Primers were purchased from Research Genetics (Huntsville, AL, USA). PCR reactions were performed in 12.5 μl using 0.5 U *Taq* polymerase (Quantum-Appligene). Annealing temperature was 56 °C for all markers. PCR products were separated by electrophoresis on a 5–15% gradient polyacrylamide gel and visualized by staining with silver nitrate. Identification of alleles was aided by molecular markers (pBR322 DNA-*Msp*I digest; New England Biolabs). Patients and their parents were genotyped and this information, combined with the genotyping of the *COMT* region, enabled the analysis of the family trios to determine the parental origin of the deletion.

Statistical analysis

Haplotype and allelic distribution of the eight SNP loci were determined by direct counting in the hemizygote subjects. Possible differences in the frequency of haplotypes or alleles between the samples were calculated using the χ^2 test. The Arlequin software package (Excoffier et al., 2005) was used to assess pairwise linkage disequilibrium (LD) between the SNP markers (Slatkin, 1994), in the samples and to detect significant deviation from HWE (Guo and Thompson, 1992) in parents of the 22q11.2DS individuals. The Bonferroni correction was performed by the SISA online procedure (http://home.clara.net/sisa/bonfer.htm). The comparison of the IQ scores between carriers of 3-SNP haplotype (rs2097603-rs4680-rs165599) was performed by two-tailed *t* test.

Results

The study sample consisted of fifty-five 22q11.2DS individuals and was subdivided into the following diagnostic groups: ADHD, OCD, SZ/SZaff and control. A description of these individuals is provided in the Methods section and in our previous report (Gothelf et al., 2006). In the present study, in addition to the individual diagnostic groups, there is also a reference to all patients with any of the three psychiatric diagnoses ('cases'). The 'control' group is, as before, all 22q11.2DS subjects without ADHD, OCD or SZ/SZaff. In addition, available parents ($n = 95$) of these 22q11.2DS individuals were also included in order to determine the parental origin of the deletion.

The eight SNPs used in this study are described in Table 1. Possible deviation from HWE in the parents' sample was checked using the exact HWE test performed in Arlequin software (Guo and Thompson, 1992). No statistically significant deviations from HWE were found ($p > 0.13$). Table 2 shows the distribution of alleles in the eight SNPs in 22q11.2DS subjects with ADHD, OCD and SZ/SZaff compared to the 22q11.2DS control group. Significant associations were found between several SNPs and ADHD and OCD. ADHD: besides the ^{158}*Val/Met* (rs4860) all other SNPs in the *COMT* gene (rs2097603, rs4633, rs4818, rs3838146) showed significant association. In addition,

Table 2. Allele distribution of eight SNPs in 22q11.2DS diagnostic groups

SNP	Allele	Control (*n*)	ADHD			OCD			SZ/SZaff		
			n	$\chi^2(1)^a$	*p*	*n*	$\chi^2(1)^a$	*p*	*n*	$\chi^2(1)^a$	*p*
SNP 1	A	20	13	4.04	0.045	6	6.71	0.02	4	0.03	n.s.
(rs2097603)	G	4	10			8			1		
SNP 2	C	16	6	7.77	0.005*	3	7.24	0.007*	1	3.72	0.054
(rs4633)	T	8	17			11			4		
SNP 3	C	8	17	7.77	0.005*	11	7.24	0.007*	4	3.72	0.054
(rs4818)	G	16	6			3			1		
SNP 4	G	16	6	7.77	0.005*	3	7.24	0.007*	1	3.72	0.054
(rs4680)	A	8	17			11			4		
SNP 5	insC	16	22	6.37	0.012	13	3.36	0.067	4	0.34	n.s.
(rs3838146)	delC	8	1			1			1		
SNP 6	A	15	21	5.44	0.02	12	2.32	n.s.	3	0.01	n.s.
(rs165599)	G	9	2			2			2		
SNP 7	C	17	18	0.34	n.s.	7	1.65	n.s.	3	0.12	n.s.
(rs2073748)	T	7	5			7			2		
SNP 8	T	17	18	0.34	n.s.	7	1.65	n.s.	3	0.12	n.s.
(rs2240717)	C	7	5			7			2		

ADHD, Attention deficit hyperactivity disorder; OCD, obsessive–compulsive disorder; SZ/SZaff, schizophrenia/schizoaffective disorder.

[a] χ^2 test was performed for each SNP by comparison between diagnostic group and control.

* Remained significant following Bonferroni correction.

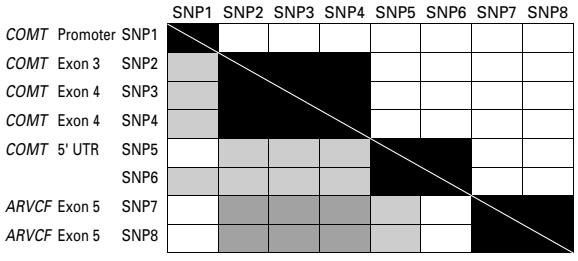

Figure 1. Linkage disequilibrium between SNPs in 22q11.2DS subjects. Above diagonal: 'Cases' – 22q11.2DS subjects with attention deficit hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), schizophrenia/schizoaffective disorder (SZ/SZaff). Below diagonal: 'Controls' – 22q11.2DS subjects without ADHD, OCD, SZ/SZaff. ■, $p < 0.0005$; ▩, $0.0005 < p < 0.005$; ▨, $0.005 < p < 0.05$; □, $p < 0.05$.

ADHD was also associated with rs165599. OCD was associated with four out of five SNPs in the *COMT* gene. Because SNPs were distributed into four tightly linked groups (Figure 1), according to Bonferroni correction α level was adjusted from 0.05 to 0.013. The association of three SNPs (rs4633, rs4818, rs4680) with both ADHD and OCD remained significant after Bonferroni correction. The two SNPs in the *ARVCF* gene (rs2073748, rs2240717) gave no significant association with either ADHD or OCD. Although the same tendency found in the other psychiatric disorders was observed for the SZ/SZaff group, the results did not reach significance, perhaps due to the small sample size.

LD between SNPs in the COMT *region in 22q11.2DS individuals*

Association data presented in Table 2 suggested that high degree of LD exists between some of the SNPs used. Figure 1 shows a plot of the calculated LD between SNPs in 22q11.2DS subjects. Analysis was performed for 'cases' (22q11.2DS subjects with ADHD, OCD, and SZ/SZaff) and 'controls' (subjects without these disorders). Four haplotype blocks could be distinguished (SNPs 1, 2-4-3, 5-6 and 7-8) in a region that spans 41 kb on 22q11.2 (genomic location on 22q: 18308092-18349106) including the entire *COMT* gene and part (about 25%) of the 3′ region of the *ARVCF* gene. Both the cases and controls share the same four blocks of tightly linked SNPs ($p < 0.0005$ by the exact LD test) (Slatkin and Excoffier, 1996). However, in the cases the blocks are 'isolated' (LD between them was

Table 3. Distribution of the 8-SNP haplotype in 22q11.2DS diagnostic groups

Haplotype	Control (*n*)	ADHD				OCD			
		n	OR	$\chi^2(1)^a$	*p*	*n*	OR	$\chi^2(1)^a$	*p*
G-T-C-A-$_{ins}$C-A-C-T	1	7	7.30	5.74	0.017	4	6.86	4.61	0.032
G-T-C-A-$_{ins}$C-A-T-C	2	2	1.04	0	n.s.	3	2.57	1.33	n.s.
A-T-C-A-$_{ins}$C-A-C-T	1	4	4.17	2.16	n.s.	0	0.60	1.58	n.s.
G-T-C-A-$_{ins}$C-A-T-C	4	3	0.78	0.12	n.s.	3	1.29	0.13	n.s.
A-C-G-G-$_{ins}$C-A-C-T	6	4	0.70	0.41	n.s.	1	0.29	1.88	n.s.
A-C-G-G-$_{del}$C-G-C-T	8	1	0.13	6.37	0.012	1	0.21	3.36	0.067
Others[b]	2	2	1.04	0	n.s.	2	1.71	0.33	n.s.

ADHD, Attention deficit hyperactivity disorder; OCD, obsessive–compulsive disorder; OR, odds ratio.

χ^2 test for the overall haplotype distributions: ADHD vs. control [$\chi^2(6) = 12.27$, $p = 0.056$]; OCD vs. control [$\chi^2(6) = 10.24$, $p = 0.115$].

Haplotype blocks are denoted by frames.

[a] χ^2 test was performed for each haplotype in diagnostic group vs. control.

[b] Rare haplotypes that were present only once in the total sample.

not significant) while the controls show varying degrees of LD between the blocks. The three LD blocks in the *COMT* gene that were identified in this study coincide with the LD blocks suggested by the HapMap (www.hapmap.org) for the *COMT* gene in Caucasians. HapMap shows an additional block between block III of *COMT* and block IV of the 3′ region of the *ARVCF*gene (Figure 1) which was not detected by our SNPs.

Association analysis of COMT *region haplotypes with ADHD and OCD in 22q11.2DS*

8-SNP COMT-ARVCF *haplotype*

Since the haplotype structure of individuals with 22q11.2DS is directly inferred from the genotyping results of the individual SNPs, it was straightforward to analyse the haplotypes of the 22q11DS subjects. Distribution of 8-SNP haplotypes in ADHD, OCD and the control groups is presented in Table 3. The haplotype analysis for SZ/SZaff patients was not performed due to the small sample size ($n = 5$). In our population we have observed six main haplotypes out of the 256 possible combinations (Figure 1). Both ADHD and OCD were associated with the same risk haplotype (G-T-C-A-insC-A-C-T) (OR 7.30, $p = 0.017$ and OR 6.86, $p = 0.032$ for ADHD and OCD, respectively), while haplotype (A-C-G-G-delC-G-C-T) was associated with decreased risk for ADHD (OR 0.13, $p = 0.012$) and the same trend was observed for OCD (OR 0.21, $p = 0.067$).

3-SNP COMT *haplotype*

Three SNPs (rs2097603, rs4680, rs165599) representing the three LD blocks identified in the *COMT* gene (Figure 1) were chosen for intra-gene haplotype analysis. Analysis of the 3-SNP haplotypes in the 22q11.2DS diagnostic groups (Table 4) showed that the overall differences in distribution were significantly associated with both ADHD [ADHD vs. control; $\chi^2(5) = 11.12$, $p = 0.049$] and OCD [OCD vs. control; $\chi^2(5) = 11.22$, $p = 0.047$].

Frequency of the 3-SNP haplotype in the 22q11.2DS diagnostic groups is illustrated in Figure 2. The same haplotype allele (G-A-A) was associated with increased genetic risk to both ADHD and OCD. This haplotype was carried by 39.1% (9/23) of ADHD and 50% (7/14) of OCD patients compared with only 12.5% (3/24) in the control group (OR 3.13, $p = 0.036$ for ADHD, and OR 6.41, $p = 0.011$ for OCD). Another haplotype allele (A-G-G) was associated with a protective effect for two these disorders (OR 0.12, $p = 0.006$ for ADHD, and OR 0.19, $p = 0.04$ for OCD).

The rs2097603-rs4680-rs165599 (G-A-A) haplotype and IQ scores

IQ scores were available for 44 out of fifty-five 22q11.2DS subjects. The full-scale, verbal, and performance IQ (FSIQ, VIQ, PIQ) scores of carriers of the G-A-A haplotype ($n = 15$) did not differ significantly ($p > 0.80$) from that of those not carrying it ($n = 29$) [FIQ ($\pm$S.E.), 75.00 ± 3.73 vs. 77 ± 2.67; VIQ ($\pm$S.E.),

Table 4. Association of the 3-SNP haplotype (rs2097603-rs4680-rs165599) with 22q11.2DS diagnostic groups

Haplotype	Control (*n*)	ADHD				OCD			
		n	OR[a]	$\chi^2(1)$[b]	*p*	*n*	OR[a]	$\chi^2(1)$[b]	*p*
G-A-A	**3**	**9**	**3.13**	**4.38**	**0.036**	**7**	**4.00**	**6.41**	**0.011**
A-A-G	0	1		1.07	n.s.	1		1.64	n.s.
A-A-A	5	7	1.46	0.57	n.s.	3	1.03	0	n.s.
G-G-A	1	1	1.04	0	n.s.	1	1.71	0.16	n.s.
A-G-A	6	4	0.70	0.41	n.s.	1	0.29	1.88	n.s.
A-G-G	**9**	**1**	**0.12**	**7.71**	**0.006**	**1**	**0.19**	**4.2**	**0.04**

ADHD, Attention deficit hyperactivity disorder; OCD, obsessive–compulsive disorder; OR, odds ratio.
χ^2 test for the overall haplotype distributions: ADHD vs. control [$\chi^2(5) = 11.12$, $p = 0.049$]; OCD vs. control [$\chi^2(5) = 11.22$, $p = 0.047$].
[a] Odds ratio value is defined only for haplotypes that are present in the control sample.
[b] χ^2 test was performed for each haplotype in diagnostic group vs. control.

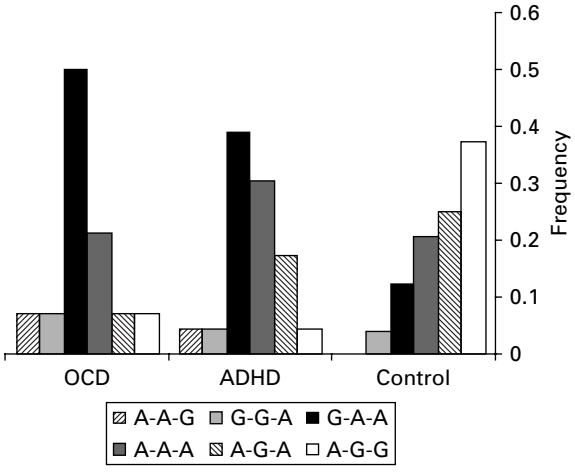

Figure 2. Frequency of the 3-SNP haplotype (rs2097603-rs4680-rs165599) in the 22q11.2DS diagnostic groups. ADHD, Attention deficit hyperactivity disorder; OCD, obsessive–compulsive disorder.

76.14 ± 3.56 vs. 78.55 ± 2.59; PIQ (± S.E.), 76.79 ± 4.00 vs. 78.90 ± 2.70].

Parental origin of the deletion in 22q11.2DS subjects and psychiatric morbidity

All fifty-five 22q11.2DS subjects had a de-novo deletion. The parental origin of the deleted chromosome was identified by analysis of the genotypes of subjects and their parents. In addition to the eight SNPs in the *COMT* region (Table 1) three polymorphic STR markers (D22S941, D22S944, D22S1648) from the 22q11.2 deletion were used. The parental origin of the deletion could be unequivocally determined in 42 individuals who were included in the analysis. Table 5 shows the parental origin of the deletion in ADHD, OCD and control subjects. Excess of maternal origin was found in ADHD and OCD patients (Table 5), but the same trend was also obtained in the control group and the differences were not statistically significant. Interestingly, when all 22q11.2DS subjects were analysed together an over-representation (69%) of maternal origin was observed (29 cases of maternal and 13 of paternal origin). This difference was statistically

Table 5. Parental origin of the deletion and psychiatric morbidity in 22q11.2DS subjects[a]

Parental origin	Control		ADHD		OCD	
	n	%	*n*	%	*n*	%
Maternal	13	72	13	72	7	64
Paternal	5	28	5	28	4	36

ADHD, Attention deficit hyperactivity disorder; OCD, obsessive–compulsive disorder.
[a] Only subjects for whom the parent of origin could be determined.

significant [maternal: 29 cases vs. expected 21 (half of 42); $\chi^2(1) = 6.09$, $p = 0.014$].

Discussion

In our previous study (Gothelf et al., 2006) we found an association of ADHD and OCD with the ^{158}*Val/Met* *COMT* polymorphism in individuals with 22q11.2DS, and the low-activity ^{158}*Met* allele was identified as the risk allele. In the present study we showed that in addition to the ^{158}*Val/Met*, there are several other SNPs in the *COMT* gene region that were associated with ADHD and OCD. The calculation of linkage disequilibrium between these SNPs allowed us to delineate LD blocks in the 22q11.2DS population and study association of the above psychiatric disorders with haplotypes composed of representative SNPs. Since all 22q11.2DS individuals have only a single copy of the *COMT* gene region they offer a unique opportunity to study the effect of particular *COMT* haplotypes on susceptibility to psychiatric disorders. Another advantage of this molecular status is that the structure of haplotypes is identified unequivocally from genotyping of the individual SNPs.

The main finding of the present study was that the distribution of haplotypes composed of three functional SNPs in the *COMT* gene (rs2097603-rs4680-rs165599) was significantly associated with both ADHD and OCD in 22q11.2DS subjects.

Interestingly, the same haplotype allele G-A-A was found to increase the genetic risk of ADHD and OCD, while haplotype A-G-G had a protective effect for these disorders.

The effect of the risk haplotype on disease susceptibility may be attributed to variations in function of the individual SNPs and/or to additional effects of the entire haplotype. The G-A-A risk haplotype is composed of the ^{158}*Met* allele and two other SNPs, one in the P2 promotor of the membrane-bound *COMT* (rs2097603) and one from the 3′ region of the gene (rs165599). Each of the three polymorphic sites was previously implicated in modulation of COMT function. The ^{158}*Met* allele, coding for a relatively heat-labile enzyme variant, was found to cause a marked reduction in COMT enzymatic activity in peripheral blood cells (Lachman et al., 1996; Weinshilboum and Dunnette, 1981) as well as in protein abundance and enzymatic activity in post-mortem dorsolateral PFC tissue (Chen et al., 2004). In the latter study, the P2 promotor SNP was shown to further reduce ^{158}Met COMT enzyme activity in human lymphocytes and the same trend was observed in the PFC. The 3′ SNP, previously found to be highly associated with schizophrenia in Ashkenazi Jews (Shifman et al., 2002), was shown to affect *COMT* mRNA expression in post-mortem brain tissue (Bray et al., 2003). In addition, the risk haplotype might contain other variations whose functions have hitherto not been investigated. For example, synonymous change in the coding region of *COMT* (rs4818) has recently been found to affect secondary mRNA structure and consequently protein quantity and enzymatic activity (Nackley et al., 2006).

The functional consequences of *COMT* haplotypes composed of the above-mentioned three SNPs were recently investigated in relationship to their effects on human brain function in normal subjects (Meyer-Lindenberg et al., 2006). Studying 126 healthy Caucasian subjects of European ancestry during working-memory paradigms it was found that ^{158}*Val/Met* interacted with the P2 promoter and the 3′ region polymorphisms in predicting inefficient prefrontal working-memory response. Moreover, out of several combinations of haplotypes examined they found that the same haplotype combination as the one that we investigated (rs2097603-rs4680-rs165599) showed the statistically strongest impact on prefrontal function. Interestingly the G-A-A haplotype, found by us to be a risk factor for ADHD and OCD in 22q11.2DS, exhibited the smallest activation of right ventrolateral PFC during the performance of working-memory tasks, indicating more efficient PFC function. Conversely, haplotype A-G-G, identified by us as protective, revealed the lowest efficiency. The comparison of our study to that of Meyer-Lindenberg et al. (2006) is relevant since we observed the same four main haplotypes in our population and the distributions in the two studies were not statistically different.

Based on its effect on brain processes described above and the findings for the individual SNPs, we speculate that the G-A-A haplotype, containing the ^{158}*Met* variant, has lower enzymatic activity than the ^{158}*Met* allele alone. The relationship between cortical dopaminergic levels and working-memory performance was proposed to follow an inverted U-shaped curve (Goldman-Rakic et al., 2000; Tunbridge et al., 2006). According to this hypothesis, 22q11.2DS individuals carrying a single 'super-low' activity G-A-A haplotype with the resulting high dopamine levels are expected to show a serious impairment in PFC-related cognitive functions and brain processes. In addition, variations in the *COMT* gene have also been suggested to entail repercussions on dopamine pathway in other areas of the brain, e.g. the striatum (Akil et al., 2003) and midbrain (Meyer-Lindenberg et al.,

2005). We hypothesize that the existence of a single G-A-A haplotype in our cohort of 22q11.2DS subjects predisposes them to ADHD and OCD via an extreme PFC-related dopaminergic neurotransmission and possibly limbic-striatal dopamine pathway dysregulation. Additionally, a recent fMRI study in 22q11.2DS subjects indicates a possible alternative or complementary role for *COMT* gene variations in the regulation of parietal and cingulated cortex activation in a task requiring response inhibition in 22q11.2DS (Gothelf et al., 2007b).

Converging evidence from animal and human studies implicate the dysregulation of frontal-subcortical-cerebellar catecholaminergic circuits in the pathophysiology of ADHD, (Biederman and Faraone, 2005; Fallgatter et al., 2005) and both hypo- and hyper-functioning models of dopaminergic transmission were suggested as possible contributors (Pliszka, 2005). A role for the dopamine system was also proposed for the pathophysiology of OCD (in addition to the well-established serotonergic theory) and is supported by clinical and preclinical evidence (Goodman et al., 1990; Stein, 2002).

The fact that the G-A-A haplotype was found as a risk factor for both ADHD and OCD in 22q11.2DS subjects is interesting and may hint at a common genetic risk factor or intermediate phenotype (endophenotype) of altered prefrontal brain function mediated by dysfunctional dopaminergic transmission (Fallgatter and Lesch, 2006). Interestingly, Geller et al. (2007) provided evidence for co-segregation between paediatric OCD and ADHD by a familial risk analysis, indicating the possibility of common underlying genetic risk factors. Our results cannot be extrapolated directly to ADHD and OCD in the general population since 22q11.2DS subjects represent a particular and unique case.

The hyper-dopaminergic hypothesis of schizophrenia is well-established (for review see Sawa and Snyder, 2002) and schizophrenia is considered to be one of the major psychiatric disorder associated with 22q11.2DS (Murphy, 2002). It would have been very relevant and interesting to investigate the association of the *COMT* risk haplotype with schizophrenia among our 22q11.2DS subjects. Unfortunately, the number of SZ/SZaff patients in our cohort was very small ($n=5$) and could not afford proper haplotype analysis. This might be explained by the relatively young age of the 22q11.2DS participants in our study (16.6 ± 10.2 yr) compared to other studies (e.g. 33.8 ± 10.1 yr; in Bassett et al., 2007). It is possible that when our sample will advance in age more individuals will develop schizophrenia and the proportion of schizophrenia patients will increase. A most interesting question is whether ADHD and OCD in the 22q11.2DS cohort represent a prodrome for later development of schizophrenia and schizophrenia-spectrum disorders. A recent longitudinal study found that all four children with 22q11.2DS and OCD developed a psychotic disorder at follow-up, 5 yr later, during late adolescence-young adulthood (Gothelf et al., 2007a).

The G-A-A haplotype may not be the only genetic risk factor and it probably interacts with other genes in the deletion area and in other genes relevant for psychiatric disorders. For example it was found that in 22q11.2DS subjects, the ^{158}*Met* allele interacted with hyper-prolinaemia, caused by mild to severe mutations in the *PRODH* gene, to give an increased risk to psychosis (Raux et al., 2007). Recently, a haplotype composed of the same SNPs examined by us (rs2097603, rs4680 and rs165599) showed an epistatic interaction with polymorphisms in other schizophrenia candidate genes, most notably *RGS4*, to increase genetic susceptibility to schizophrenia in the general population (Nicodemus et al., 2007). Presently, we are in the course of investigating the interaction between the *COMT* risk haplotype identified in this study with polymorphisms and putative mutations in the *PRODH* gene. The question whether the psychiatric phenotype in our 22q11.2DS sample is influenced by the parental origin of the deleted chromosome is very interesting in view of the known influence of genomic imprinting on the clinical phenotype of individuals with the 15q hemizygous deletion (for review see Horsthemke and Buiting, 2006). Association was found between maternal inheritance of 22q11.2 deletion and a significant reduction (9%) in the total volume of cerebral grey matter (Eliez et al., 2001), and also with increased severity of deficits in language skills (Glaser et al., 2002). Genotyping 22q11.2DS individuals in our sample and their parents for eight *COMT* SNPs and three STR markers enabled us to unequivocally determine the parental origin of the deleted chromosome in 42 of the participants. Comparing the frequency of individuals with paternal and maternal deletion in ADHD and OCD patients to the control group in our cohort, we found no evidence for an association between the parental origin of the deletion and the risk to develop ADHD and OCD. This result seems to indicate that there is no strong effect of parental origin on susceptibility to OCD and ADHD, although the existence of subtle effects can not be ruled out. However, when all 22q11.2DS participants were considered, there was a statistically significant excess (69%, 29 vs. 13) of maternal deletion. Other

studies, having smaller sample size ($n = 8$–21) (Chung et al., 2001; Demczuk et al., 1995; Fokstuen et al., 1998; Lu et al., 1999), also reported a tendency towards an excess maternal transmission (56–78%) but with no statistical significance while one study ($n = 8$) found an excess (62%) of paternal transmission (Vittorini et al., 2001). Assessment of these findings should probably await an independent replication in larger samples. Our study found no association between cognitive functioning (e.g. VIQ scores) and the G-A-A *COMT* haplotype. Cross-sectional studies of the effect of the *COMT* ^{158}*Val/Met* polymorphism on cognition and neuropsychological performance in 22q11.2DS individuals revealed mixed results (Baker et al., 2005; Glaser et al., 2006; Kates et al., 2006; Shashi et al., 2006). Of note, a longitudinal study of 22q11.2DS adolescents showed that carriers of the ^{158}*Met* allele had a more robust decline with age in VIQ and language skills (Gothelf et al., 2005). This finding may suggests that the effect of *COMT* genotype on cognitive development is most prominent during adolescence, in parallel with the developmental increase of prefrontal dopaminergic tone (Lambe et al., 2000). Longitudinal studies with adolescents with 22q11.2DS using more specific dopamine-dependent prefrontal cognitive tests are required to further investigate the role of *COMT* haplotypes on cognitive development in 22q11.2DS. In addition, future studies should assess the relationship between the *COMT* risk haplotype reported in the present study and endophenotypes that might be common to ADHD and OCD in the general population. These endophenotypes may include deficits in response inhibition and other cognitive impairments mediated by dysfunction of prefrontal brain areas (Fallgatter and Lesch, 2006).

In conclusion, this study demonstrated the association of a particular *COMT* haplotype with susceptibility to both ADHD and OCD in 22q11.2DS individuals and supports the hypothesis connecting *COMT* gene variations and neuropsychiatric disorders in 22q11.2DS.

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Statement of Interest

None.

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