American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 144B:351–354 (2007)

Brief Research Communication

Association of Adenomatous Polyposis coli (*APC*) Gene Polymorphisms With Autism Spectrum Disorder (ASD)

Xiao-Lei Zhou,[1] MaiBritt Giacobini,[1]* Britt-Marie Anderlid,[1] Henrik Anckarsäter,[2] Davood Omrani,[3] Christopher Gillberg,[4] Magnus Nordenskjöld,[1] and Annika Lindblom[1]

[1]*Department of Clinical Genetics and Department of Molecular Medicine, CMM, Karolinska University Hospital, Stockholm, Sweden*
[2]*Forensic Psychiatry Clinics, Malmö University Hospital, Malmö, Sweden*
[3]*Genetic Unit, Mottahary Hospital, Kashani AVE, Uromieh, Iran*
[4]*Department of Child and Adolescent Psychiatry, Göteborg University, Göteborg, Sweden*

We serendipitously identified a single nucleotide polymorphism (SNP), 8636C>A (rs1804197) in the 3′-untranslated region of the adenomatous polyposis coli (*APC*) gene to be associated with autism spectrum disorder (ASD). In order to gain further evidence for the association between the *APC* locus and ASD, we genotyped four additional adjacent common SNPs (rs2229992, rs42427, rs459552, and rs465899) in the coding regions within the *APC* gene in a set of Swedish ASDs and controls. One common haplotype TGAG was found to be associated with ASD after haplotype analysis using both Haploview v3.1.1 ($P = 0.006$) and COCAPHASE v2.403 ($P = 0.030$). This result is the first to suggest that the genomic locus at *APC* is associated with ASD, and that the *APC* gene itself is a good predisposing candidate to be evaluated in future studies due to its important role in neuronal development and function.

KEY WORDS: APC; SNP; haplotype; autism spectrum disorder (ASD); association

Please cite this article as follows: Zhou X-L, Giacobini M, Anderlid B-M, Anckarsäter H, Omrani D, Gillberg C, Nordenskjöld M, Lindblom A. 2007. Association of Adenomatous Polyposis Coli (*APC*) Gene Polymorphisms With Autism Spectrum Disorder (ASD). Am J Med Genet Part B 144B:351–354.

Mutations in the adenomatous polyposis coli (*APC*) gene are well known to predispose for colorectal cancer in Familial Adenomatous Polyposis and are of major importance for tumor development in sporadic colorectal cancer. We have previously studied this gene in sporadic colorectal cancer cases and controls to determine the relative risk of colorectal cancer related to various gene variants detected in a Swedish population [Zhou et al., 2004]. A single nucleotide polymorphism (SNP), 8636C>A, in the 3′-non-translated region, was the only one suggested to confer an increased risk of colorectal cancer. In this published association study on colorectal cancer we first used four different sets of patients without colorectal cancer as controls. One of these sets of controls differed from the other three by showing a higher frequency of the *APC* 8636C>A, similar to that found in the sporadic colorectal cancer cases. For that reason, we exchanged all four sets of controls for a new group of healthy Swedish controls collected for research purposes with the good representation of the general Swedish population. The comparison between these new controls and our colorectal cancer cases showed a trend towards overrepresentation of the *APC* variant in colorectal cancer cases [Zhou et al., 2004].

The original Swedish control set with a higher prevalence of the *APC* variant contained anonymous non-cancer patients undergoing genetic testing at the Department of Clinical Genetics, Karolinska Hospital. The most common genetic test performed at our department is for the Fragile-X syndrome as a routine investigation of children with mental retardation and/or autism spectrum disorder (ASD). ASD is a developmental disorder of the central nervous system with as yet unknown etiology. It encompasses a group of related but clinically heterogeneous behavioral disorders with considerable variations in symptomatology and degree of impairment. The triad of a reduced capacity for reciprocal social interaction, reciprocal communication, and a limited and repetitive behavioral pattern is shared by all individuals with ASD diagnoses [American Psychiatric Association, 1994]. The broad variation in phenotypes and severity within the autism spectrum suggests the involvement of multiple predisposing factors. A very strong genetic component is demonstrated by increased concordance in monozygotic as compared to dizygotic twins [Bailey et al., 1995], and a sibling recurrence risk is about 10 times greater than the population risk [Rutter et al., 1999]. The genetic basis of autism is well established, but the mode of genetic transmission remains unclear in the majority of cases [Volkmar and Pauls, 2003]. Several studies have indicated strong involvement of multigenic components in the etiology of autism, but linkage analyses and candidate gene search approaches so far have produced relatively few replicated findings [Muhle et al., 2004].

A Gut-Brain axis has long been discussed in relation to ASD [Wakefield, 2002] and a deletion of the *APC* gene and occurrence of adenomatous polyposis and rectal cancer were reported in a patient originally referred for autism [Barber et al., 1994]. It prompted us to focus on a possible association between the *APC* 8636C>A overrepresentation and the ASD symptoms shared by many individuals among the patients undergoing genetic testing for Fragile-X syndrome.

Grant sponsor: Swedish Cancer Society; Grant sponsor: Ragnar and Torsten Söderbergs stiftelse; Grant sponsor: Swedish Scientific Council.

*Correspondence to: MaiBritt Giacobini, Department of Clinical Genetics and Department of Molecular Medicine, CMM, Karolinska University Hospital, 171 76 Stockholm, Sweden.
E-mail: maj-britt.giacobini-arner@sll.se

Received 13 January 2006; Accepted 13 July 2006

DOI 10.1002/ajmg.b.30415

TABLE I. Characterization of 75 Swedish ASD Cases

	Number of subjects
Primary diagnosis	
Autism	26
Asperger syndrome	22
Atypical autism	21
Autistic traits	6
Comorbidity and associated handicaps	
Mental retardation	21
Chronic tics	14
ADHD (attention-deficit/hyperactivity disorder)	33
Bipolar disorder	4
Epilepsy	1
Country of origin	
Sweden	75

The 75 cases include 51 males and 24 females and the ages of whom range between 4 and 55 years old (median 25.0). The written consent was available from all these cases.

To test this hypothesis, we set up a retrospective case-control study where we used 177 patients with ASD symptoms as cases and the same set of 476 Swedish controls as used in our published study [Zhou et al., 2004]. The result seemed to support our hypothesis by showing a higher prevalence of the *APC* gene variant among the cases with ASD symptoms ($P = 0.065$, Chi-square test, data not shown). Next we recruited well-diagnosed ASD patients in the clinics to investigate their association with the *APC* 8636C>A variant. This study included 75 Swedish ASD patients compared with the same 476 Swedish controls as used in the case-control study where 177 patients with ASD symptoms were included. All ASD cases were assessed by the ADI-R and the DSM-IV criteria. In addition, for high-functioning autism/Asperger (HFA/AS) adults, Gillberg & Gillberg algorithm [Nylander and Gillberg, 2001] was employed on the basis of SCID-1 and -2 in most cases (Table I). The *APC* gene variant was found significantly overrepresented in ASD cases compared with normal controls ($P = 0.016$, Chi-square test, Table II).

Since the *APC* 8636C>A variant was located in the 3′ untranslating region of the *APC* gene, it might have a role in regulating messenger RNA (mRNA) expression. We therefore did single nucleotide primer extension (SNuPE) assay on mRNA extracted from EBV-transfected lymphocytes of variant carriers. However, the result excluded the possibility that this variant could relate to a differential mRNA expression between the two alleles (data not shown). So whether or not the *APC* 8636C>A has a causative role for ASD is still an open question, but the observed association of it with ASD did provide a clue that the *APC* locus might confer susceptibility to ASD.

The *APC* gene spans more than 100 kb. We chose four adjacent SNPs (rs2229992, rs42427, rs459552, and rs465899) for a haplotype analysis of the *APC* gene in 75 Swedish ASD patients and a new control group consisting of 280 healthy Swedish blood donors. These four SNPs cover over 10 kb in the genomic region and were selected on the basis of their high prevalence in the Swedish population [Zhou et al., 2004], and their location in the coding regions of the gene and thus do not cover all haplotypes of the gene. The genotypic distribution of all four SNPs in cases and controls were in Hardy–Weinberg equilibrium (HWE) and none of SNPs alone showed a genotype and allele association with ASD (Table III). The linkage disequilibrium (LD) between these four SNPs was analyzed using the software Haploview v3.1.1 [Barrett et al., 2005]. All four SNPs showed to be part of a single haplotype with D' values ranging from 0.86 to 0.98 (95% confidence). Among all the common individual haplotypes (with the frequencies >0.05) constructed from the four SNPs, one haplotype TGAG was found to be statistically associated with ASD using both the Haploview v3.1.1 ($P = 0.006$, Table IV) and the COCAPHASE module implemented in the UNPHASED program v2.403 [Dudbridge, 2003] ($P = 0.03$, Table IV). We also noticed that the rare allele of the *APC* C8636A was not on the haplotype of TGAG; however, this does not necessarily contradict the above-noticed association of the variant with ASD if it has arisen more recently and in LD with other haplotypes within the *APC* gene.

In the present study, we have found statistical evidence for an association between the *APC* locus and ASD. This association could provide some insight into the complex molecular mechanisms underlying the development of autism. Several studies on the *APC* gene localization, expression, and function point to an involvement of the *APC* gene product in the central nervous system in animals. The APC protein is found localized in many cell types including neurons in the adult rodent brain [Brakeman et al., 1999]. A high level of expression of the *APC* mRNA is observed during brain development [Bhat et al., 1994] and associated with neuronal differentiation of PC12 cells in rats [Dobashi et al., 1996]. APC can interact with Discs-Large 20 and beta-catenin proteins involved in regulating structural aspects of synaptic contacts. Defective synaptogenesis has been postulated as an etiological mechanism for

TABLE II. Genotypes and Allele Frequencies of the *APC* 8636C>A in 75 Swedish ASD Patients and 476 Healthy Swedish Controls

	Swedish ASD patients	Healthy Swedish controls	Odds ratio[b]	
	(n = 75)	(n = 476)	(95% CI[c])	*P*-value[d]
Genotypes[a]				
C/C	70 (93.3%)	464 (97.5%)	1.0	0.016
C/A	4 (5.3%)	12 (2.5%)	2.2 (0.7–7.0)	
A/A	1 (1.3%)	0 (0)	NA[e]	
Alleles				
C	144 (96.0%)	940 (98.7%)	1.0	0.014
A	6 (4.0%)	12 (1.3%)	3.4 (1.2–8.9)	

[a]Genotyping was performed by the combined use of denaturing high-performance liquid chromatography analysis (DHPLC) and direct sequencing analysis. Information about PCR primers and experimental procedures are available upon request.
[b]Odds ratios was calculated against the most common genotype, C/C, or the most common allele, C.
[c]95% CI, 95% confidence interval.
[d]*P*-value was calculated according to the Chi-square test.
[e]NA, not applicable.

TABLE III. Genotype and Allele Frequencies of Four Common APC SNPs in Cases and Controls

SNPs	Study group	Genotypes (%)				Alleles (%)		
		1/1	1/2	2/2	*P*-value	1	2	*P*-value
rs2229992	Cases	31.6	48.7	19.7	0.46	55.9	44.1	0.30
	Controls	35.1	50.9	14.0		60.5	39.5	
rs42427	Cases	38.1	46.1	15.8	0.47	61.2	38.8	0.27
	Controls	42.9	46.3	10.8		66.0	34.0	
rs459552	Cases	60.5	36.9	2.6	0.80	78.9	21.1	0.81
	Controls	60.4	35.3	4.3		78.1	21.9	
rs465899	Cases	37.7	44.2	18.1	0.23	59.7	40.3	0.20
	Controls	41.6	47.5	10.9		65.3	34.7	

Genotyping was performed by pyrosequencing. Information about PCR and pyrosequencing primers and experimental procedures are available upon request. rs2229992, 1 = C, 2 = T; rs42427, 1 = A, 2 = G; rs459552, 1 = A, 2 = T; rs465899, 1 = A, 2 = G. Common allele is 1 and the less common allele is 2. *P*-value was calculated according to the Chi-square test.

TABLE IV. Results of Testing Association Between Major Haplotypes (With Frequencies >0.05) at the APC Locus and ASD Using Haploview v3.1.1 and COCAPHASE v2.403

Haplotypes rs2229992-rs42427-rs459552-rs465899	Frequency in cases (%)	Frequency in controls (%)	*P*-value (Haploview)	*P*-value (COCAPHASE)
CAAA	52.4	56.7	0.337	0.360
TGTG	18.7	20.2	0.678	0.856
TGAG	19.2	11.0	**0.006**	**0.030**
TAAA	5.4	7.4	0.387	0.396

Significant differences are denoted in bold.

autism. Jamain et al. [2003] have shown that mutations in neuroligins NLGN3 and NLGN4, which encode cell adhesion molecules present at the postsynaptic side of the synapse, are associated with autism [Jamain et al., 2003]. In addition, two components, Dvl1 and WNT2, of the Wnt pathway of which APC is also involved have been linked with neurodevelopmental and behavior disorder. Lijam et al. [1997] reported that in mice lacking Dvl1, there are social interaction and sensorimotor gating abnormalities; and Wassink et al. [2001] reported association of WNT2 as a candidate susceptibility gene for autism, though this was not replicated [McCoy et al., 2002; Li et al., 2004]. It is worthwhile to note that in a very recent study, *APC* was claimed to be associated with susceptibility to schizophrenia [Cui et al., 2005], providing additional line of evidence for the possible general and important role that American Psychiatric (APC) has in the development of neurodevelopmental disorders including ASD.

In conclusion, we reported an association between the *APC* locus and ASD, and suggested the *APC* gene itself to be a good candidate gene predisposing to ASD. Future mutation screening and association studies in larger datasets from different populations are needed to tell whether it is genetic variants in the *APC* gene, or variants in a different gene in LD with *APC*, which confer the increased risk for ASD.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

ACKNOWLEDGMENTS

We are grateful to the patients and their families for their cooperation and to Lennart Iselius for valuable discussions. The study was supported by the Swedish Cancer Society (grant for AL), and Ragnar and Torsten Söderbergs stiftelse (grant for MBG), and the Swedish Scientific Council (grant for CG).

APPENDICIES

Ethical Approval

The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and has been granted the ethics approvals, Dnr 02-145, UHRB8210/07-12-2003 and Ö 586-99, issued by Karolinska Institutet, Urmia University of Medical Sciences, and Göteborgs University, respectively.

REFERENCES

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders, 4th ed. Washington DC: American Psychiatric Association.

Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. 1995. Autism as a strongly genetic disorder: Evidence from a British twin study. Psychol Med 25(1):63–77.

Barber JC, Ellis KH, Bowles LV, Delhanty JD, Ede RF, Male BM, Eccles DM. 1994. Adenomatous polyposis coli and a cytogenetic deletion of chromosome 5 resulting from a maternal intrachromosomal insertion. J Med Genet 31(4):312–316.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265.

Bhat RV, Baraban JM, Johnson RC, Eipper BA, Mains RE. 1994. High levels of expression of the tumor suppressor gene APC during development of the rat central nervous system. J Neurosci 14(5 Pt 2):3059–3071.

Brakeman JS, Gu SH, Wang XB, Dolin G, Baraban JM. 1999. Neuronal localization of the Adenomatous polyposis coli tumor suppressor protein. Neuroscience 91(2):661–672.

Cui DH, Jiang KD, Jiang SD, Xu YF, Yao H. 2005. The tumor suppressor adenomatous polyposis coli gene is associated with susceptibility to schizophrenia. Mol Psychiatry 10(7):669–677.

Dobashi Y, Bhattacharjee RN, Toyoshima K, Akiyama T. 1996. Upregulation of the APC gene product during neuronal differentiation of rat pheochromocytoma PC12 cells. Biochem Biophys Res Commun 224(2):479–483.

Dudbridge F. 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25(2):115–121.

Jamain S, Quach H, Betancur C, Rastam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, et al. 2003. Mutations of

the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet 34(1):27–29.

Li J, Nguyen L, Gleason C, Lotspeich L, Spiker D, Risch N, Myers RM. 2004. Lack of evidence for an association between WNT2 and RELN polymorphisms and autism. Am J Med Genet Part B Neuropsychiatr Genet 126B(1):51–57.

Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, et al. 1997. Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. Cell 90(5):895–905.

McCoy PA, Shao Y, Wolpert CM, Donnelly SL, Ashley-Koch A, Abel HL, Ravan SA, Abramson RK, Wright HH, DeLong GR, et al., 2002. No association between the WNT2 gene and autistic disorder. Am J Med Genet 114(1):106–109.

Muhle R, Trentacoste SV, Rapin I. 2004. The genetics of autism. Pediatrics 113(5):e472–e486.

Nylander L, Gillberg C. 2001. Screening for autism spectrum disorders in adult psychiatric out-patients: A preliminary report. Acta Psychiatr Scand 103(6):428–434.

Rutter M, Silberg J, O'Connor T, Simonoff E. 1999. Genetics and child psychiatry: II Empirical research findings. J Child Psychol Psychiatry 40(1):19–55.

Volkmar FR, Pauls D. 2003. Autism. Lancet 362(9390):1133–1141.

Wakefield AJ. 2002. The gut-brain axis in childhood developmental disorders. J Pediatr Gastroenterol Nutr 34(Suppl 1):S14–S17.

Wassink TH, Piven J, Vieland VJ, Huang J, Swiderski RE, Pietila J, Braun T, Beck G, Folstein SE, Haines JL, et al. 2001. Evidence supporting WNT2 as an autism susceptibility gene. Am J Med Genet 105(5):406–413.

Zhou XL, Eriksson U, Werelius B, Kressner U, Sun XF, Lindblom A. 2004. Definition of candidate low risk APC alleles in a Swedish population. Int J Cancer 110(4):550–557.