Am. J. Hum. Genet. 75:508–513, 2004

Report

Replication Study Supports Evidence for Linkage to 9p24 in Obsessive-Compulsive Disorder

Virginia L. Willour,[1] Yin Yao Shugart,[3] Jack Samuels,[1,5] Marco Grados,[1,6] Bernadette Cullen,[1] O. Joseph Bienvenu III,[1] Ying Wang,[1] Kung-Yee Liang,[4] David Valle,[2] Rudolf Hoehn-Saric,[1] Mark Riddle,[1,2] and Gerald Nestadt[1,5]

Departments of [1]Psychiatry and Behavioral Sciences and [2]Pediatrics, School of Medicine, and Departments of [3]Epidemiology, [4]Biostatistics, and [5]Mental Health, Bloomberg School of Public Health, The Johns Hopkins University, and [6]The Kennedy Krieger Institute, Baltimore

Obsessive-compulsive disorder (OCD) is a severe psychiatric illness that is characterized by intrusive and senseless thoughts and impulses (obsessions) and by repetitive behaviors (compulsions). Family, twin, and segregation studies support the presence of both genetic and environmental susceptibility factors, and the only published genome scan for OCD identified a candidate region on 9p24 at marker D9S288 that met criteria for suggestive significance (Hanna et al. 2002). In an attempt to replicate this finding, we genotyped 50 pedigrees with OCD, using microsatellite markers spanning the 9p24 candidate region, and analyzed the data, using parametric and nonparametric linkage analyses under both a narrow phenotype model (DSM-IV OCD definite; 41 affected sib pairs) and a broad phenotype model (DSM-IV OCD definite and probable; 50 affected sib pairs). Similar to what was described by Hanna et al. (2002), our strongest findings came with the dominant parameters and the narrow phenotype model: the parametric signal peaked at marker D9S1792 with an HLOD of 2.26 ($\alpha = 0.59$), and the nonparametric linkage signal (NPL) peaked at marker D9S1813 with an NPL of 2.52 ($P = .006$). These findings are striking in that D9S1813 and D9S1792 lie within 0.5 cM (<350 kb) of the original 9p24 linkage signal at D9S288; furthermore, pedigree-based association analyses also implicated the 9p24 candidate region by identifying two markers (D9S288 and GATA62F03) with modest evidence ($P = .046$ and .02, respectively) for association.

Obsessive-compulsive disorder (OCD [MIM 164230]) is a severe psychiatric illness that is characterized by intrusive and senseless thoughts and impulses (obsessions) and by repetitive behaviors (compulsions). OCD is estimated to affect 1%–3% of the population, and the World Health Organization ranks OCD as 1 of the 10 most disabling medical conditions worldwide (Murray and Lopez 1996). The concordance rate for OCD symptoms in MZ twins ranges from 80% to 87%, whereas the concordance rate in DZ twins ranges from 47% to 50%. Overall, family studies report OCD prevalence rates of 6.7%–15% in first-degree relatives of pediatric probands with OCD, and segregation analyses support the presence of a major gene with dominant or codominant transmission (for review, see Samuels and Nestadt [1997], Pato et al. [2001], and Grados et al. [2003]).

The only published genome scan for OCD studied seven pedigrees that have pediatric probands with OCD and at least two affected relatives (Hanna et al. 2002). The authors genotyped 56 subjects, using an 11.3-cM microsatellite-marker map, and followed up their initial findings on 2q, 9p, and 16q by genotyping additional markers in the original subjects plus 10 additional family members. The strongest finding was on 9p24, which originally had a dominant parametric LOD score of 2.25 at D9S288. After fine mapping with a 1.5-cM average microsatellite map, this LOD score fell slightly to 1.97 at D9S288. In an attempt to replicate the 9p24 finding, the Johns Hopkins OCD research group genotyped DNA samples from 50 pedigrees with OCD, using 13 microsatellite markers in the 9p24 region.

The 50 families in the study sample included all fam-

Received April 23, 2004; accepted for publication June 29, 2004; electronically published July 21, 2004.

Address for correspondence and reprints: Dr. Virginia L. Willour, Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Meyer Building Room 4-132, 600 North Wolfe Street, Baltimore, MD 21287. E-mail: willour@jhmi.edu

0002-9297/2004/7503-0017$15.00

Table 1

Microsatellite Markers in 9p24 Candidate Region

Microsatellite Markers	Location		
	Genetic Map (cM)		Physical Map[a] (Mb)
	Present Study	Hanna et al. (2002)	
D9S1779	.00	.00	.51
D9S1858	.10	.10	.69
D9S129	2.90	2.90	1.85
D9S54	7.00	7.00	2.90
D9S288	9.90	9.90	3.94
D9S178	10.10	10.10	4.11
D9S1813	10.11	10.11	4.12
D9S1792	10.31	10.31	4.26
D9S199	15.32	15.32	4.50
GATA62F03	15.36	14.71	5.19
D9S1852	15.42	15.42	6.23
D9S281	16.02	16.02	6.85
D9S286	18.72	18.72	8.04

[a] Physical map locations were determined using UCSC Genome Browser July 2003 freeze and NCBI human genome map build 34.3.

ilies from the Johns Hopkins OCD Family Study (Nestadt et al. 2000) with an affected sibling pair for whom DNA was available, as well as additional opportunistic samples identified through Johns Hopkins clinics, OCD support groups, or the Internet. Probands met DSM-IV criteria for OCD (American Psychiatric Association 1994) and were clinically impaired, with a score >15 on the Yale-Brown Obsessive Compulsive Scale (Goodman et al. 1989). Probands with schizophrenia, mental retardation, dementia, Tourette disorder, and OCD limited to major depressive episodes were excluded from the study. The probands were assessed by psychiatrists and Ph.D. clinical psychologists using a number of diagnostic instruments, including the Schedule for Affective Disorders and Schizophrenia–Lifetime Anxiety version (Mannuzza et al. 1986), the Yale-Brown Obsessive Compulsive Scale (Goodman et al. 1989), and the Leyton Obsessional Inventory (Cooper 1970). Children were assessed using comparable age-appropriate instruments. Collateral information was gathered from knowledgeable informants, with the use of the Family Informant Schedule and Criteria (Mannuzza et al. 1985). The first-degree relatives were assessed in the same manner. In some cases, the first-degree relatives were unavailable for assessment or were unwilling to be assessed. In these cases, information was gathered from two knowledgeable informants. Best-estimate final diagnoses, with the use of all available information, were assigned independently by two psychiatrists expert in the diagnosis and treatment of OCD. All subjects signed institutional review board–approved informed consent forms prior to enrollment in the study.

DNA samples from the 50 pedigrees with OCD (193 subjects) were sent to the Australian Genome Research Facility for microsatellite-marker genotyping on an ABI PRISM 377 DNA Sequencer (Applied Biosystems). All samples were genotyped with the same 13 microsatellite markers used in the original study (Hanna et al. 2002). Prior to the analyses, the microsatellite-marker data was screened for Mendelian inconsistencies by the use of PedCheck version 1.1 (O'Connell and Weeks 1998) and Unknown version 5.23 from the Linkage analysis package (Lathrop and Lalouel 1984). Haplotypes were manually scanned for evidence of tight double recombinants (<5 cM), and haplotype and allele-sharing data were screened for evidence of previously unidentified identical twins (Kruglyak et al. 1996; McPeek and Sun 2000).

On the basis of these additional screening procedures, we determined that not all of the original 50 pedigrees with OCD were suitable for the 9p24 replication study. Prior to analyses, two of the pedigrees were removed because of poor sample amplification, and two pedigrees were removed because the only affected sib pairs in the pedigree were identical twins. In addition, some of the original 193 subjects were chosen for genotyping prior to the completion of the consensus diagnostic procedure. After the consensus diagnostic procedure was completed, 8 of the original 50 pedigrees did not contain an affected sib pair. Although these eight trio pedigrees were not useful as linkage pedigrees, they were included in the 9p24 association analyses (see below).

The 13 genotyped microsatellite markers spanned a distance of 19 cM, resulting in a 1.5-cM (average) microsatellite-marker density (table 1). The markers were

originally ordered as in Hanna et al. (2002). Prior to use, the locations of all markers were confirmed by cross-reference to published genetic maps (Broman et al. 1998; Kong et al. 2002) and physical maps (Kent et al. 2002; Wheeler et al. 2004). The physical-map information altered the location of one marker; GATA62F03 is now placed centromeric to D9S199. For the parametric linkage analysis, we used the same linkage parameters as Hanna et al. (2002). For both dominant and recessive models, the penetrance was set at 0.5, and the phenocopy rate was set at 0.0052. The disease allele frequency was set at 0.02 for the dominant model and at 0.20 for the recessive model. Because of the variability of the age at onset of OCD, unaffecteds were coded as unknown. Founders were used to determine allele frequencies. When founders were unavailable in the pedigree, a random sibling was chosen to represent the family.

Parametric and nonparametric linkage analyses were conducted using a narrow phenotype model (DSM-IV OCD definite) and a broad phenotype model (DSM-IV OCD definite and probable) and were implemented by GENEHUNTER version 2.1_r5 beta (Kruglyak et al. 1996). The narrow phenotype model included 35 pedigrees containing 138 total subjects, 91 subjects with definite OCD, and 41 affected sib pairs. The broad phenotype model included 38 pedigrees containing 147 total subjects, 95 subjects with definite OCD, 7 subjects with probable OCD, and 50 affected sib pairs.

Under the narrow phenotype model, the nonparametric linkage signal (NPL) peaked at marker D9S1813, with an NPL of 2.52 ($P = .006$). The parametric signal peaked nearby at marker D9S1792, with a dominant HLOD of 2.26 ($\alpha = 0.59$) (fig. 1). The broad phenotype model produced similar results; the NPL peaked at marker D9S1813, with an NPL of 2.37 ($P = .01$), and the parametric linkage signal peaked at marker D9S1792, with a dominant HLOD of 1.62 ($\alpha = 0.51$). All four linkage peaks spanned the entire 9p24 candidate region. Parametric recessive HLOD scores never exceeded 1.0 in any analysis.

We also tested the microsatellite-marker data for evidence of association, using the pedigree-disequilibrium test (PDT) (Martin et al. 2000). The PDT allows for the inclusion of both nuclear and trio families and tests for allelic and genotypic association with individual markers. The 46 pedigrees used in the association analyses included the 38 linkage pedigrees and the 8 trio pedigrees. In all, the association sample had 179 total subjects, including 105 subjects with definite OCD and 8 subjects with probable OCD.

The association analyses identified two markers (D9S288 and GATA62F03) with P values $<.05$ (table 2). No other markers had significant signals under either the narrow or the broad phenotype model. D9S288 (9.90 cM) and GATA62F03 (15.36 cM) are separated by 5.5 cM (1.25 Mb) on 9p24 and show no evidence of pairwise linkage disequilibrium. Because of the limited sample size, we did not perform haplotype-based association analysis.

This study aimed to test the hypothesis that 9p24 contains a susceptibility gene for OCD, by use of a set of 50 pedigrees with OCD collected by the Johns Hopkins OCD research group. This hypothesis was based on the findings of Hanna et al. (2002) that described evidence for suggestive linkage in seven pedigrees with OCD. Although the original finding did not meet genomewide significance, we believed that it was important to test whether the finding was reproducible because this is the only candidate region for OCD that meets suggestive linkage criteria (Lander and Kruglyak 1995).

As in the Hanna et al. (2002) study, the narrow phenotype model combined with dominant parameters and a penetrance of 0.5 gave the strongest parametric findings in the present study (HLOD = 2.26; $\alpha = 0.59$). Our strongest nonparametric finding also came from the narrow phenotype model (NPL of 2.52; $P = .006$). In the original linkage report, the dominant parametric linkage score peaked at marker D9S288 with a LOD score of 1.97 (Hanna et al. 2002). Together, these findings are striking in that they agree very well, especially when it is noted that markers D9S1813, D9S1792, and D9S288 lie within 0.5 cM (<350 kb) of each other (UCSC July 2003 freeze). This confirmatory finding has significant implications, especially in psychiatric genetics, because replication studies frequently generate localizations that vary substantially from the original (Roberts et al. 1999).

Neither phenotype model produced a linkage score that met criteria for significant linkage (Lander and Kruglyak 1995). Nevertheless, our positive linkage findings strengthen support for the original Hanna et al. (2002) finding. A collaborative effort is now underway to collect almost 300 sib-pair families and multiplex families with OCD, which will provide the opportunity to test this finding in a larger sample. However, even with this relatively small sample of pedigrees, there is evidence of interfamilial heterogeneity: only 59% of the pedigrees showed evidence for linkage with the strongest linkage signal. Incorporation of age at onset and other OCD clinical characteristics in further linkage analyses may be useful in identifying a subset of pedigrees with even stronger linkage to 9p24.

The present study diverged in design from the Hanna et al. (2002) study in two potentially important ways. First, the present study ascertained small nuclear pedigrees, whereas the initial study ascertained multigenerational multiplex pedigrees. Second, the present study excluded probands with Tourette disorder, whereas the initial study contained three probands with a history of Tourette disorder. Nevertheless, both samples implicated

9p24. Our ability to replicate the 9p24 signal may have been enhanced by the fact that 93% of the probands in this study have an early age at onset (≤16 years old), which is comparable to the early age at onset (≤14 years old) of probands in the Hanna et al. pediatric sample.

It is important to note that the entire 9p24 candidate region spans 7.5 Mb and contains dozens of known and predicted genes (UCSC July 2003 freeze). As a result of the very broad 9p24 linkage signals, all of these genes are legitimate OCD positional candidate genes. One of them, encoding the neuronal and epithelial glutamate transporter EAAC1, is located ~350 kb centromeric to our NPL peak and has already been tested as a candidate gene for OCD. Veenstra-VanderWeele et al. (2001) conducted an *EAAC1* mutation screen in the seven pediatric

Table 2

Family-Based Association Signals Identified Using the PDT

	P for Phenotype Model			
	Narrow		Broad	
Marker	Sum	Average	Sum	Average
GATA62F03	.0233	.0196	.0218	.0196
D9S288	.0578	.0455	.0576	.0455

Note.—Both the sum statistics, which give more weight to larger families, and average statistics, which give equal weight to all families, were computed for each marker. These *P* values were not corrected for the tests at multiple alleles.

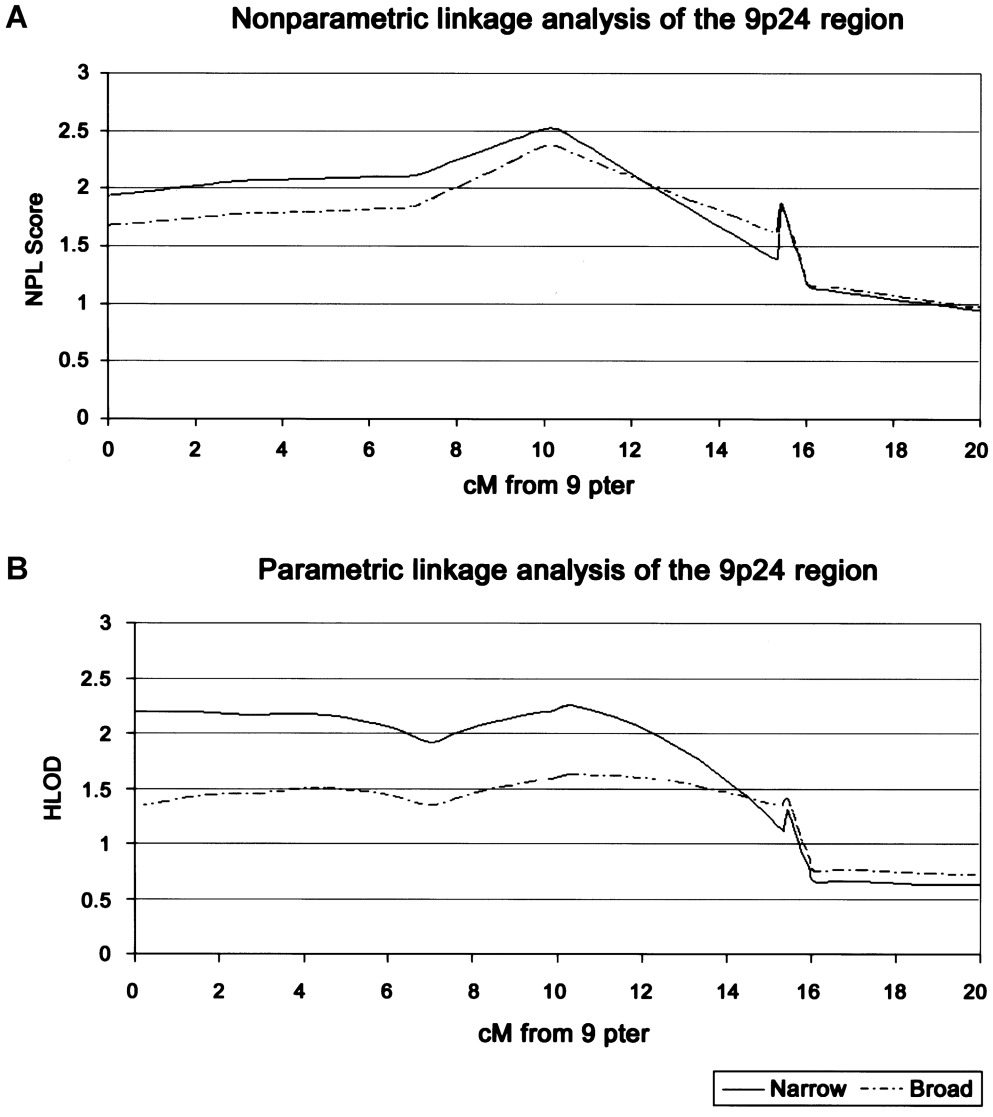

Figure 1 Multipoint nonparametric and parametric linkage analysis results for the 9p24 candidate region. Chromosomal positions are listed in Kosambi cM.

probands with OCD from the Hanna et al. (2002) pedigrees. Veenstra-VanderWeele et al. identified two SNP polymorphisms within exons that did not change the amino acid sequence (synonymous cSNPs) and six intronic polymorphisms that did not appear to affect predicted splice sites. A family-based test of association with the use of two of the intronic SNPs did not identify evidence of association with OCD. The Illumina Corporation is currently genotyping chromosome 9 as part of the HapMap project (International HapMap Consortium 2003). The identification of haplotype tag SNPs in the *EAAC1* gene and in the rest of the 9p24 candidate region will enable a more systematic screening for OCD-associated markers and haplotypes.

In summary, this initial 9p24 replication study provides promising additional evidence for an OCD susceptibility gene in this region. Although it is possible that this 9p24 signal is due to chance, support for this finding is strengthened by the fact that the 9p24 signal is observed in both parametric and nonparametric linkage analyses, as well as by the tight colocalization with the original 9p24 linkage finding.

Acknowledgments

Our research was supported by grant R01 MH50214 from the National Institute of Mental Health (to G.N.); by grant RR00052 from the Division of Research Resources General Clinical Research Centers, National Institutes of Health; by funds from the Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University (to Y.Y.S.); and by a grant from the National Alliance for Research on Schizophrenia and Depression (to V.L.W.). We thank the many families for participating in the study; J. Raymond DePaulo, Jr. (Department of Psychiatry and Behavioral Sciences at Johns Hopkins University), for financial support of V.L.W.; Edwin Cook, Gregory Hanna, and Jeremy Veenstra-VanderWeele for providing access to their primer sequences; Jennifer Hahn, Malgorzata Lamacz, Karan Lamb, Yung-Mei Leong, Tracey Lichner, James Luke, Dan McLeod, David Wellen, and Ruth Zitner for conducting clinical evaluations; Laura Eisen, Sandra Hensley, and Krista Vermillion for their efforts in coordinating the study; and Tyra Diggs, Jennifer Chellis, Yuqing Huo, and Amanda Rebert for technical assistance.

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

NCBI Database, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for OCD)
UCSC Genome Bioinformatics, http://genome.UCSC.edu/

References

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders. 4th ed. American Psychiatric Association, Washington, DC

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL (1998) Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63:861–869

Cooper J (1970) The Leyton Obsessional Inventory. Psychol Med 1:48–64

Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, Heninger GR, Charney DS (1989) The Yale-Brown Obsessive Compulsive Scale. I. Development, use, and reliability. Arch Gen Psychiatry 46:1006–1011

Grados MA, Walkup J, Walford S (2003) Genetics of obsessive-compulsive disorders: new findings and challenges. Brain Dev Suppl 25:S55–S61

Hanna GL, Veenstra-VanderWeele J, Cox NJ, Boehnke M, Himle JA, Curtis GC, Leventhal BL, Cook EH Jr (2002) Genome-wide linkage analysis of families with obsessive-compulsive disorder ascertained through pediatric probands. Am J Med Genet 114:541–552

International HapMap Consortium (2003) The International HapMap Project. Nature 426:789–796

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D (2002) The human genome browser at UCSC. Genome Res 12:996–1006

Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K (2002) A high-resolution recombination map of the human genome. Nat Genet 31:241–247

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247

Lathrop GM, Lalouel JM (1984) Easy calculations of LOD scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Mannuzza S, Fyer AJ, Endicott J, Klein DF (1985) Family Informant Schedule and Criteria. Anxiety Family Study Unit, New York State Psychiatric Institute, New York

Mannuzza S, Fyer AJ, Klein DF, Endicott J (1986) Schedule for Affective Disorders and Schizophrenia–Lifetime Version modified for the study of anxiety disorders (SADS-LA): rationale and conceptual development. J Psychiatr Res 20:317–325

Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: the pedigree disequilibrium test. Am J Hum Genet 67:146–154

McPeek MS, Sun L (2000) Statistical tests for detection of misspecified relationships by use of genome-screen data. Am J Hum Genet 66:1076–1094

Murray CL, Lopez AD (1996) The global burden of disease: a comprehensive assessment of mortality and disability from

diseases, injuries, and risk factors in 1990 and projected. Harvard University Press, Cambridge, MA

Nestadt G, Samuels J, Riddle M, Bienvenu OJ III, Liang KY, LaBuda M, Walkup J, Grados M, Hoehn-Saric R (2000) A family study of obsessive-compulsive disorder. Arch Gen Psychiatry 57:358–363

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266

Pato MT, Schindler KM, Pato CN (2001) The genetics of obsessive-compulsive disorder. Curr Psychiatry Rep 3:163–168

Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS (1999) Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65:876–884

Samuels J, Nestadt G (1997) Epidemiology and genetics of obsessive-compulsive disorder. Int Rev Psychiatry 9:61–71

Veenstra-VanderWeele J, Kim SJ, Gonen D, Hanna GL, Leventhal BL, Cook EH Jr (2001) Genomic organization of the *SLC1A1/EAAC1* gene and mutation screening in early-onset obsessive-compulsive disorder. Mol Psychiatry 6:160–167

Wheeler DL, Church DM, Edgar R, Federhen S, Helmberg W, Madden TL, Pontius JU, Schuler GD, Schriml LM, Sequeira E, Suzek TO, Tatusova TA, Wagner L (2004) Database resources of the National Center for Biotechnology Information: update. Nucleic Acids Res 32:D35–D40