Letters to the Editor

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The D13S171 Marker, Misannotated to BRCA2, Links the AS3 Gene to Various Cancers

To the Editor:

Our comment concerns a recent letter to the *Journal,* in which de los Rios et al. (2001) reported the complete lack of BRCA2 mutations in 23 families with breast cancer and found only a single BRCA2 mutation in 66 Polish families with breast and ovarian cancer (Gorski et al. 2000). These data rule out BRCA2 from a portion of familial breast cancers, and they are reminiscent of data on the sporadic form of the disease, in which BRCA2 mutations are also absent (Miki et al. 1996; Teng et al. 1996; Brody et al. 1998).

A correlation of BRCA2 mutations and deletions with breast cancer is well established in a minority of families with breast cancer. At the biochemical level, BRCA2 is believed to function in DNA damage survey. The notion that BRCA2 is a tumor suppressor, however, is contradicted by its high expression in proliferating cells (Vaughn et al. 1996), its expression in sporadic breast cancer (Bieche et al. 1999), its positive correlation with a high mitotic index (Bieche et al. 1999), and the lack of mammary-gland cancer phenotype in BRCA2-truncated knockout mice (Connor et al. 1997). Moreover, in breast cancers with losses in the 13q12.3 region, the expression of the intact BRCA2 transcript (Bieche et al. 1999) and of the BRCA2 protein (Edwards et al. 1998) strongly suggest that the deletions targeted another gene in the area (a "cryptic" suppressor), a conclusion shared by others in the field.

Our search for genes involved in the repression of cell proliferation revealed a marker misannotation in the BRCA2 area. Our analysis shows that the correct annotation, in fact, identifies a new proliferation-arrest gene, known as "androgen-shutoff gene 3" (AS3), in this region and implicates it in cancer. Correct annotation also changes the interpretation of BRCA2 allelic imbalance data, which may ultimately explain the negative results reported by de los Rios et al. and others.

We mapped AS3 on chromosome 13 and found inconsistencies in the positions of critical markers in the databases of microsatellite markers (Cooperative Human Linkage Center, version 4), STS data (Unified Data Base, Integrated Chromosome 13 Map), radiation hybrid data (GeneMap99), and other sources. The nucleotide sequence and the individual contigs of the 13q12-13 area are now available through the Human Genome Project (*Homo sapiens* 13q12-q13 contig, 1,416,908 bp, accession number NT_000625). This allowed us to establish the precise nucleotide sequence–based positions, not only for AS3 (accession number NM_015928 or U95825) but for other markers and genes as well (see fig. 1)—for instance, the D13S260 (S260), D13S171 (S171), and D13S267 (S267) markers that have been the main focus of clinical studies. The S267 marker is telomeric to the sequence depicted in figure 1.

The assignment of the S171 marker (accession number Z17151) is of particular importance, because it has been annotated to BRCA2 and widely used as an intragenic marker of BRCA2 (accession number XM_007138). Allelic imbalance data directly linked the marker to a suppressor gene. Increased proliferation rates in invasive ductal breast carcinoma (Beckmann et al. 1996) and lung carcinoma (Gorgoulis et al. 2000), deletions in sporadic breast cancer (Cleton-Jansen et al. 1995), unfavorable prognosis in prostate cancer (Edwards et al. 1998), and lymph node metastases in esophageal carcinoma (Harada et al. 1999) were positively correlated with losses in the S171 marker. In a study of hepatocellular carcinoma, the smallest common deleted region (SCDR1) was also the S171 marker (Lin et al. 1999). These losses point to a critical gene in the S171 position, as documented in the studies of patients with cancer mentioned above.

Our sequence-based mapping unambiguously demonstrated, however, that this gene is not BRCA2. Our data show that the S171 marker is not intragenic to BRCA2 (see fig. 1) and that losses in the marker cannot be directly interpreted as BRCA2 losses. The new gene, AS3, which is located in the S171 position, has recently been identified in prostate cancer cells undergoing androgen-induced proliferative arrest and shows the expression pattern of a negative regulator (Geck et al. 1997). Our sequence analysis identified the S171 microsatellite repeat as part of intron 10 of the genomic region of AS3. The 34 exons in a 200-kb area around the S171 marker code for a protein of ~1,400 residues with various consensus domains (Geck et al. 1999). The 165-kD

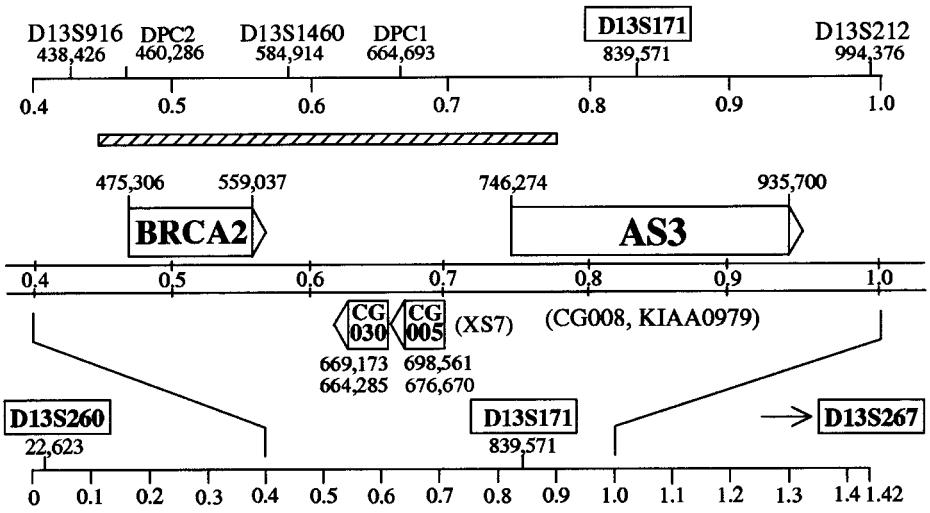

Figure 1 Map positions of markers and genes on the nucleotide sequence of the 13q12-13 contig. Boxes indicate markers that have been the main focus of clinical studies. *Top,* Microsatellite markers. Markers are shown with numbers that indicate the positions of the first nucleotides of the amplicons. The scale refers to that of the 13q12-13 contig. The hatched bar represents the homozygous deletion in a pancreatic adenocarcinoma that partially removed the AS3 coding region. The left border of the deletion is centromeric to DPC2 (at ~450,000 position), and its right border is telomeric to DPC1 (at ~780,000–830,000 positions) (Schutte et al. 1995). *Middle,* the identified coding sequences in the area. Sequences are shown on the same scale as in the top panel. The arrowheads indicate the directions of transcription. The names in parentheses indicate alternative names or alternative transcripts. *Bottom,* the nucleotide sequence of the 13q12-13 contig in 0.1-Mb units.

AS3 protein is localized in the cell nucleus. Expression of the sense and antisense AS3 sequence from a tetracycline-regulated retroviral construct showed that AS3 is a powerful negative regulator of cell proliferation (Geck et al. 2000). Recently, clinical studies have directly implicated AS3 in the development of esophageal cancer, through polymorphic variations and loss of heterozygosity that linked AS3 to lymph node metastases (Harada et al. 2001).

These data and the corrected map positions clearly indicate that (a) the exact location of the S171 marker is at the center of the 200-kb AS3 gene and is not intragenic to BRCA2, (b) the S171 microsatellite instability data link AS3 to a variety of cancers, (c) the AS3 gene product is a powerful negative regulator of cell proliferation, and (d) clinical data directly implicate AS3 in cancer. Unfortunately, on the basis of the public database annotations, the recent literature consistently assigns the S171 marker to BRCA2 (Edwards et al. 1998; Lin et al. 1999; Gorgoulis et al. 2000). In the light of our analysis, it is highly misleading to assign the S171 allelic instability data unconditionally and exclusively to BRCA2. Our results correct this misperception and reveal that AS3 is the real cognate gene of the marker, a prime candidate for a role in the negative regulation of cell proliferation.

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Electronic-Database Information

URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://lpg.nci.nih.gov/CHLC/ (for databases of microsatellite markers)
GeneMap99, http://www.ncbi.nlm.nih.gov/genemap99/ (for radiation-hybrid data)
Human Genome Project, http://research.marshfield.org/genetics (for 13q12-q13 contig)
Unified Data Base, http://bioinformatics.weizmann.ac.il/udb/ (for STS data)

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The Autism Genetic Resource Exchange: A Resource for the Study of Autism and Related Neuropsychiatric Conditions

To the Editor:

In this letter, we describe the Autism Genetic Resource Exchange (AGRE), a resource for the study of autism and pervasive developmental disorder (PDD). Autism presents within the first 3 years of life, is characterized by qualitative impairments in communication and social interaction—in the presence of restricted repetitive and stereotyped patterns of behavior, interests, and activities—and is part of a spectrum of disorders that includes Asperger syndrome and PDD (American Psychiatric Association 1994). Estimates of the prevalence of autism in the general population ranges from 0.04% to >0.1% (Bryson et al. 1988; Gillberg et al. 1991). Twin and family studies have demonstrated that the genetic contribution to autism and PDD is significant, with an MZ-twin concordance of 60%–90% and a 45- to 150-fold increase in risk to siblings (Ritvo et al. 1989; Jorde et al. 1990; Bailey et al. 1995). Thus, molecular genetic studies of autism-spectrum disorders are likely to contribute significantly to our understanding of this condition, as the recent results of several independent genome scans suggest (International Molecular Genetic Study of Autism Consortium 1998; Barrett et al. 1999; Philippe et al. 1999; Risch et al. 1999).

AGRE has been developed as a joint effort of the Cure Autism Now (CAN) Foundation and the Human Biological Data Interchange (HBDI), to facilitate collaborative genetic research into the etiology of autism and PDD and to make biomaterials from well-characterized families with autism widely available to the scientific community, so as to accelerate research. Since genetic studies of complex neuropsychiatric conditions are limited by the large sample sizes needed to attain adequate power (Lander and Kruglyak 1995; Risch and Merikangas 1996), the consolidation of large numbers of families into one collection that is made available to researchers at a fraction of the cost originally incurred

in their ascertainment and collection is of great value to the community.

One unique feature of AGRE, which has enabled the rapid ascertainment of large numbers of families, has been the development of a protocol and the infrastructure to conduct the majority of the evaluations and blood draws in the families' homes. This process may prove useful for more-rapid family ascertainment in studies of other neuropsychiatric conditions (AGRE Web site). To date, ~400 multiplex families with autism and PDD are in various stages of clinical evaluation, with DNA collection completed (table 1). Both an online and a hard-copy catalogue are available, containing the pedigrees in the collection, with notations of affectation status and basic phenotypic features, such as language delay. A sample pedigree is depicted in figure 1. Biomaterials from 343 of these completed families are currently available to the scientific research community, and this resource continues to expand, with the goal of 500 families by the end of the year 2001. Family biomaterials for the AGRE program are housed at the HBDI Repository at Rutgers University, under the direction of Jay Tischfield. Quality-controlled samples, including immortalized cell lines (1×10^6 cells/ampoule), 20-μg aliquots of DNA, and 50-μl aliquots of sera, are available to the research community by simple application, which requires proof of institutional review board (IRB) approval. Samples are available to academia and industry, and significant discounts, as well as limited grants to support academic use of the resource, are available to academic researchers through the CAN Foundation. To facilitate collaboration, free samples are available to researchers who deposit their collections in AGRE through the Sharing Researcher Program.

Scientific oversight for the program is provided by a Steering Committee, which includes researchers from the fields of genetics and autism. Human subjects protection oversight is provided by the IRB at the University of Pennsylvania School of Medicine. In addition to providing researchers with biomaterials, a major effort has been undertaken to develop a state-of-the-art, Internet-accessible database of detailed clinical information. Phenotypic assessment is ongoing and includes the two examinations that are completed by all of the NIH autism collaborative groups: the Autism Diagnostic Interview–Revised (ADI-R) (Lord et al. 1994) and the Autism Diagnostic Observational Schedule (ADOS) (Lord et al. 2000). All ADI and ADOS raters undergo ongoing reliability checks to prevent any drift in diagnosis (Lord et al. 1994, 2000). In addition, photographic dysmorphology, physical and neurological examination, and medical and family history are being collected by pediatric neurologists. Probands with possible secondary autism resulting from perinatal trauma, from an identified genetic syndrome, or from other medical causes are noted, although this is only a small percentage of cases. Currently, the ADI data and a subset of the ADOS data, both coded for confidentiality,

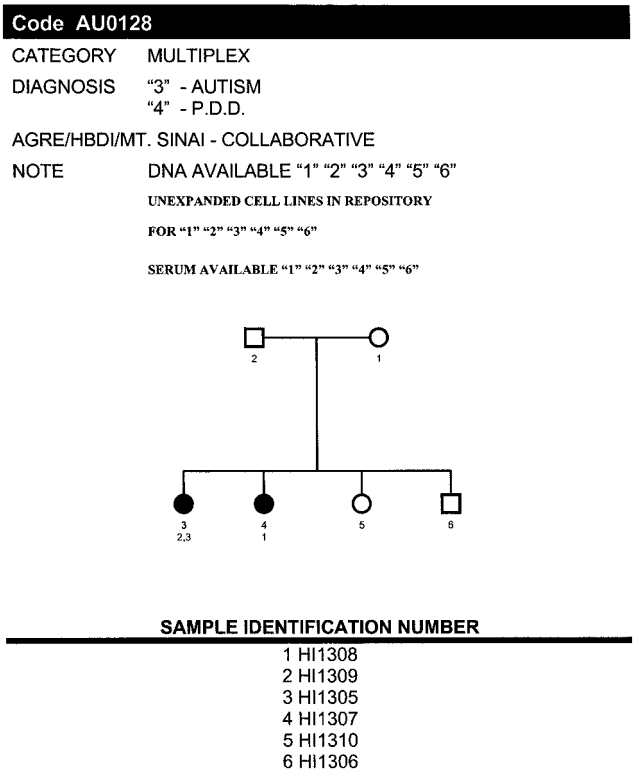

Figure 1 Sample AGRE pedigree. A typical AGRE pedigree is depicted, with the identifying numbers given directly underneath the individuals. The second number refers to the following key, as defined by ADI scores: 1 = verbal; 2 = nonverbal; 3 = regression; and 4 = late onset. The HI numbers are actual coded database numbers that uniquely identify each individual. The availability of biomaterials is also indicated if the family was corecruited with another academic group—in this case, Mt. Sinai (AGRE Web site).

Table 1

Patient Recruitment and Availability

Status	Current	Projected 2001
Recruited:[a]		
Families	428	500
Individuals	1,978	2,250
Completed:[b]		
Families	343	420
Individuals	1,595	1,890
In process:[c]		
Families	85	80
Individuals	496	360

[a] Consented and scheduled for ADI and blood draw.

[b] ADI complete, biomaterials complete, quality controlled and available for distribution.

[c] Incomplete biomaterials or ADI.

are available online for researcher access through the AGRE Web site. Online phenotypic databases for the remainder of the data collected are being developed and will be available in ≤6 mo. More information on the timeline of data and material collection is available at the Web site. We are striving to improve the utility of AGRE, and user feedback is an important element in this process.

Fragile-X testing is conducted in all families (Brown et al. 1986), and cytogenetic analysis—including FISH for 15q and telomere screening—is commencing. Of 220 families tested, 3 have subjects that carry a fragile-X expansion (1.3%; W. T. Brown, unpublished data). A genome scan at an average 10-cM resolution has been completed on the first 132 families in the collection (T. C. Gilliam, personal communication), and genotype data from 188 families are available online at the AGRE Web site. As genome scans on additional families are completed, these data will be updated regularly, and investigators accessing these data will be notified of the updates automatically. More information regarding this resource, including pricing of samples and access to the resource, can be obtained from the CAN Foundation and AGRE Web sites, or by contacting the authors.

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Electronic-Database Information

The URLs for data in this article are as follows:

Autism Genetic Resource Exchange, http://www.agre.org/
Cure Autism Now, http://www.canfoundation.org/

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