mutations. Direct sequencing revealed *BRCA1* G2508T as a nonsense mutation resulting in Glu→stop codon and *BRCA2* 3295insA as an in-frame stop codon at position 1025. Mutation results were confirmed at a separate *BRCA1* and *BRCA2* testing facility in Toronto. These are novel mutations not previously reported in the Breast Cancer Information Core database.

This patient had a maternal and paternal history of breast cancer. The maternal side contained cases of postmenopausal breast cancer: her mother (patient 6) was diagnosed with bilateral breast cancer at ages 70 and 75 years, and her aunt (patient 5) was diagnosed at ages 68 and 72 years; her grandmother (patient 2) was diagnosed with breast cancer at age 72 years. The paternal side contained cases of premenopausal breast cancer: a cousin once removed (patient 35) was diagnosed with breast cancer at age 33 years, her grandmother (patient 20) was diagnosed at age 75 years, and a distant cousin (patient 59) was diagnosed at age 35 years. Interestingly, her mother did not have either mutation, suggesting that both *BRCA1* and *BRCA2* germ-line mutations originated from the father of the proband. This is consistent with the ages at onset of the women on the paternal side, indicating the presence of at least one of these mutations in the women diagnosed with premenopausal breast cancer. To date, no other family members are available for testing. For counseling of individuals identified as double heterozygotes for mutations in *BRCA1* and *BRCA2,* the risk of transmitting a breast cancer–susceptibility gene(s) to any offspring is 3/4.

The frequency of *BRCA1* and *BRCA2* mutations in the United Kingdom and Canada has been estimated at 1/850–1/500 individuals (Easton 1993; Ford et al. 1995); therefore, the likelihood of finding a double heterozygote in this population is between 1/700,000 and 1/250,000. It is estimated that, for individuals of Ashkenazi Jewish descent, the likelihood of being a carrier for one of three common *BRCA1* or *BRCA2* mutations is $\geqslant$1/50 (Roa 1996; Tonin et al. 1996); therefore, the likelihood of finding a double or compound heterozygote is ~1/2,500 for Ashkenazi Jewish families unselected for cancer.

This individual is the first example to date of a double heterozygote for the high-penetrance breast cancer–susceptibility genes, *BRCA1* and *BRCA2,* outside of the Ashkenazi Jewish population (Ramus et al. 1997). This finding is predictably rare, with a maximum frequency of 1/250,000. Our patient was diagnosed with early-onset breast cancer at age 35 years, an age typical of other *BRCA1/BRCA2*–associated breast cancers. Her case does not suggest a more severe presentation or younger age at diagnosis for women found to harbor germ-line mutations in both genes. We cannot generalize that complete screening of *BRCA1* and *BRCA2* in families with a previously identified mutation in either gene is necessary until other double heterozygotes are identified. However, if the family mutation is not found in women diagnosed with early-onset breast cancer or ovarian cancer, there is a basis for comprehensive screening of both genes.

Acknowledgments

We thank Eve Lopes and Karen Robb for technical support. We also thank Drs. Hilmi Ozcelik and Irene Andrulis for confirmational testing at the Mount Sinai Hospital, Ontario Cancer Genetics Network Laboratory, in Toronto.

Alexander Liede, Pauline Rehal, Danny Vesprini, Elaine Jack, John Abrahamson, and Steven A. Narod
University of Toronto and Women's College Hospital and The Ontario Cancer Genetics Network, Toronto

Electronic-Database Information

URLs for data in this article are as follows:

Breast cancer information core database, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

References

Ford D, Easton DF, Peto J (1995) Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 57:1457–1462

Easton DF, Ford D, Peto J (1993) Inherited susceptibility to breast cancer. Cancer Surv 18:1–17

Ramus SJ, Friedman LS, Gayther SA, Ponder BAJ (1997) A breast/ovarian cancer patient with germline mutations in both *BRCA1* and *BRCA2*. Nat Genet 15:14–15

Roa BB, Boyd AA, Vocik K, Richards CS (1996) Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2*. Nat Genet 14:185–187

Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, Neuhausen S, Godwin AK, et al (1996) Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. Nat Med 2:1179–1183

Address for correspondence and reprints: Mr. Alexander Liede, University of Toronto and Women's College Hospital, 790 Bay Street, Suite 750A Toronto, Ontario M5G 1N8, Canada. E-mail: liede@ftn.net

0002-9297/98/6206-0035$02.00

Am. J. Hum. Genet. 62:1544–1548, 1998

Evidence of Founder Mutations in Finnish BRCA1 and BRCA2 Families

To the Editor:

Approximately 4%–10% of breast and ovarian cancer is thought to be inherited (Newman et al. 1988). Most hereditary ovarian cancer cases and a significant portion

of breast cancer cases are due to mutations in the BRCA1 gene (Miki et al. 1994). In addition, BRCA2 mutations account for a large fraction of hereditary breast cancer but for only a small number of cases of hereditary ovarian cancer (Wooster et al. 1995). Other cancer phenotypes associated with mutations in these genes are prostate cancer and colon carcinoma, for BRCA1 (Ford et al. 1994), and prostate cancer and pancreatic carcinoma, for BRCA2 (Wooster et al. 1995). Most of the identified mutations appear to be highly penetrant, conferring a remarkably elevated lifetime cancer risk (Easton 1997). The proportion of breast/ovarian cancer families whose disease is attributable to BRCA1 or BRCA2 mutations varies, depending on the population studied. Although there seems to be a wide spectrum of different germ-line mutations in most countries, some geographically or ethnically isolated populations, such as Icelanders and Ashkenazi Jews, have shown clusterings of specific mutations (Szabo and King 1997; also see Breast Cancer Information Core).

Population history explains much of the enrichment of certain disease-related gene defects in Finland. The concept of Finnish disease heritage currently includes >30 diseases that are more prevalent in Finland than in other populations (de la Chapelle 1993). The majority of these diseases are rare autosomal recessive disorders, but founder mutations can also be seen in the more common diseases, such as hereditary nonpolyposis colon cancer (HNPCC) (Nyström-Lahti et al. 1995). Therefore, we were interested to see whether we could find any mutational clustering in the BRCA1 and BRCA2 genes. Also, the phenotypic characteristics of the mutation carriers were determined.

Eighty-eight families from the Oulu University Hospital area (population 720,000), each with either at least two cases of breast and/or ovarian cancer in first-degree relatives or other characteristics of inherited susceptibility (e.g., bilateral/multiple primary tumors or age at diagnosis <40 years) were analyzed. Expression of breast and ovarian cancer in these families is shown in table 1. Pedigree data were obtained from the probands by means of a questionnaire and an interview. Permission for genetic testing was requested during genetic counseling. The study was approved by the Ethical Committee of the Oulu University Medical Faculty.

The protein-encoding and splice site–junction regions of BRCA1 and BRCA2 were examined mainly by conformation-sensitive gel electrophoresis, the mutation-detection sensitivity of which is estimated to be in the range of 60%–95% (Körkkö et al. 1998). Exon 11 of BRCA1 and exons 10 and 11 of BRCA2 were screened by use of protein-truncation test (Håkansson et al. 1997). For sequencing, we used Cyclist Exo$^-$ *Pfu* DNA Sequencing Kit (Stratagene). The primer sets and PCR conditions have been described elsewhere (Castilla et al. 1994; Friedman et al. 1994, 1997; Couch et al. 1996). The four microsatellite markers used to determine the disease-linked BRCA1 haplotypes were D17S846, D17S855, D17S1322, and D17S1323 (Genome Database).

Table 1

Classification of 88 Cancer Families According to Occurrence of Breast and Ovarian Cancer in 1st-Degree Relatives

No. of Cases of Breast Cancer	No. of Families in Which No. of Cases of Ovarian Cancer =				
	0	1	2	3	4
0		1	2	1	1
1	13	9	2		
2	27	5	1		
3	14	1			
4	8				
5	1	1	1		

Altogether, five distinct mutations were observed—two in BRCA1 and three in BRCA2 (table 2). The proportion of disease-related BRCA1 and BRCA2 mutations in the population studied was 12.5% (11/88). The mutation prevalence of true high-risk families was 17% (6/36), and that for moderate-risk families was 10% (5/52), suggesting that a strong family history is not the only parameter that can predict mutations; early disease onset, tumor bilaterality, and multiple primary tumors should also be taken into account. Cancer phenotypes are shown in table 2. BRCA1 mutations were observed in 7% (6/88) of the studied kindreds: the 3745delT mutation leads to a premature protein-translation termination at codon 1209, and the A→G substitution at 4216-2nt generates an aberrant splice-acceptor recognition site. Both of these mutations were present in three families. The proportion of BRCA2 mutations was 6% (5/88): the 999del5 mutation leads to translation termination at codon 273, and the 6503delTT mutation, which also is a frameshift mutation, generates a stop codon 18 bp farther downstream. Each of these two mutations was found in one family. The A→G 9346-2nt splice-acceptor site mutation is predicted to result in the skipping of exon 24. Three families were found to carry this mutation. In addition to the disease-related mutations, other DNA alterations in the coding regions were seen (table 3). Furthermore, several polymorphisms were identified in the noncoding regions of BRCA1 (in introns 6, 9, 17, 18, and 21) and BRCA2 (in exon 2 and introns 8, 24, and 25).

Interestingly, four of the mutations identified in our study were found to be the same as those in another Finnish study, by Vehmanen et al. (1997*a*, 1997*b*). Combined data show the presence of the BRCA1 exon 11 3745delT mutation in six families, the BRCA1 exon 12 4216-2ntA→G mutation in five families, the BRCA2

exon 9 999del5 mutation in eight families, and the BRCA2 exon 24 9346-2ntA→G mutation in eight families, providing evidence of at least four different founder mutations in Finland. At present, the BRCA1 exon 12 and BRCA2 exon 24 splice-site mutations appear to be unique to the Finnish population, but the BRCA1 3745delT and BRCA2 999del5 mutations have also been observed in Sweden (Zelada-Hedman et al. 1997) and Iceland (Johannesdottir et al. 1996), respectively. The ancestors of all three families with the BRCA1 3745delT mutation have been traced back to the late 19th century and the same rural area south of Oulu. Families 014 and 016 share the same disease-linked haplotype for four BRCA1 markers, indicating a common origin of the mutation (data not shown). Interestingly, both families have relatives living in Stockholm, where the Swedish family with the BRCA1 3745delT mutations resides. Although extensive migration from Finland to Sweden has occurred for many centuries, there is no indication that the Swedish family is related to the Finnish families in our study. It is quite peculiar that the BRCA2 999del5 mutation, which occurs frequently in Iceland, appears to be a founder mutation in Finland also but, as yet, has not been reported in other Nordic countries. BRCA2 6503delTT is the only mutation with no present recurrence in the Finnish population. However, several other families with this mutation have been identified elsewhere (see Breast Cancer Information Core), and it is possible that this site represents a mutational hot spot. The observed phenotype of one breast cancer and two ovarian cancers in family 028 corresponds well to what was expected, since the mutation is located within the ovarian cancer cluster region. This family was negative for a polymorphic stop codon, lys3326ter, which has been detected in some individuals carrying the 6503delTT mutation (Gayther et al. 1997). Haplotype and genealogical studies to determine the age and possible common ancestors of the mutations that are now observed—and to elucidate their relationship to the same mutations observed in other populations—have been initiated.

The population history of Finland differs, in many respects, from that of most countries. The coastal areas were populated mainly during the 15th century, and it was not until the 17th century that the vast inland regions were gradually inhabited by a relatively small

Table 3

Polymorphisms and Unknown and Silent Variants Occurring in Exonic Regions

Gene and Exon	Alteration	Effect	Type (No. of Cases)[a]
BRCA1:			
9	C710T	Cys→Cys	S (2)
11	C3832T	Pro→Leu	UV (1)
13	T4427C	Ser→Ser	P (41)
15	G4654T	Ser→Ile	UV (1)
16	A4956G	Ser→Gly	P (27)
16	T5002C	Met→Thr	UV (3)
16	T5074C	Met→Thr	UV (1)
16	G5075A	Met→Ile	P (3)
BRCA2:			
25	T9639G	Thr→Thr	S (1)
27	A10462G	Ile→Val	UV (3)

[a] S = silent variant; UV = unknown variant; and P = polymorphism.

Table 2

Mutations and Cancers in BRCA1 and BRCA2 Families

Family	Gene	Exon	Mutation	Breast/Ovarian Cancer(s) in 1st- and 2d-Degree Relatives (Age [years] at Diagnosis)[a]	Other Cancers (No. of Cases)[a]
014	BRCA1	11	3745delT	Ov (46, 47, 50, 70[b])	Kid (1)
016	BRCA1	11	3745delT	Br (47, u)	Col (1), End (1), Lip (2), Liv (1)
062	BRCA1	11	3745delT	Ov (44, 50)	…
015	BRCA1	12	4216-2ntA→G	Br (28, 51, u), Ov (u)	Bt (1), Liv (1)
017	BRCA1	12	4216-2ntA→G	Br (29,[c] 36, 42, 52, u), Ov (u), Br+Ov (52)	Bt (1), Leu (1), Liv (1), Sto (1), Tes (1), Ton (1)
113	BRCA1	12	4216-2ntA→G	Br (62, u)	Col (1), Cx (1), Lu (1), Sto (1)
034	BRCA2	9	999delTCAAA	Bil Br (68/68[d])	Bo (1), Col (1), End (1), Lu (1), Sar (1)
028	BRCA2	11	6503delTT	Br (77), Ov (64, u)	End (2), Lu (1)
002	BRCA2	24	9346-2ntA→G	Br (45, 47, 48, u), Bil Br (62/64)	Csu (3), Liv (3), Pro (1), Sto (1), Thy (1)
005	BRCA2	24	9346-2ntA→G	Br (37, 38, 46, 68)	Csu (1), Leu (1)
087	BRCA2	24	9346-2ntA→G	Br (39)	Lu (1), Mel (1), Pan (1), Sto (2)

[a] Bas = basalioma; Bil Br = bilateral breast cancer; Bo = bone cancer; Br = breast cancer; Bt = brain cancer; Col = colon cancer; Csu = cancer site unknown; Cx = cervical cancer; End = endometrial cancer; Kid = kidney cancer; Leu = leukemia; Lip = lip cancer; Liv = liver cancer; Lu = lung cancer; Mel = melanoma; Ov = ovarian cancer; Pan = pancreatic cancer; Pro = prostate cancer; Sar = sarcoma; Sto = stomach cancer; Tes = testicular cancer; Thy = thyroid cancer; Ton = cancer of the tongue; and u = age unknown. Mutation carrier status is not defined for all individuals.

[b] Endometrial cancer also present.

[c] Liver cancer also present.

[d] Basalioma (at age 69 years) and colon cancer (at age 72 years) also present.

number of individuals. The presence of several regionally occurring BRCA1 and BRCA2 founder mutations could reflect this development, a situation similar to that for HNPCC. For instance, the four BRCA1 and BRCA2 Finnish founder mutations are responsible for 91% of the mutations in the families studied in Oulu but for only 45% of the mutations observed in the Helsinki study. None of the other 12 mutations identified in the study of individuals from southern Finland (Vehmanen et al. 1997*a,* 1997*b*) was seen in our material.

The clustering of mutations provides significant diagnostic advantages. In HNPCC, for example, two mutations have been found to account for a majority (63%) of the Finnish cases. It has therefore become important to design simple PCR-based tests for rapid mutation detection (Nyström-Lahti et al. 1995). Since the BRCA1 and BRCA2 founder mutations are currently seen in 55% (27/49) of the Finnish mutation-positive kindreds, it makes meaningful the development of similar diagnostic tests for breast and ovarian cancer.

Acknowledgments

This study was supported by the University of Oulu, Oulu University Hospital, the Finnish Cancer Society, the Cancer Foundation of Northern Finland, and the Finnish Breast Cancer Group. We also wish to thank Åke Borg, Arto Mannermaa, Jarmo Körkkö, Helena Rahja, and Kari Mononen.

Pia Huusko,[1] Kati Pääkkönen,[1] Virpi Launonen,[1] Minna Pöyhönen,[1] Guillermo Blanco,[2] Antti Kauppila,[3] Ulla Puistola,[3] Heikki Kiviniemi,[4] Marika Kujala,[1] Jaakko Leisti,[1] and Robert Winqvist[1, 2]

Departments of [1]Clinical Genetics, [2]Oncology and Radiotherapy, [3]Obstetrics and Gynecology, and [4]Surgery, Oulu University Hospital, University of Oulu, Oulu, Finland

Electronic-Database Information

URLs for data in this article are as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/
Genome Database, http://gdbwww.gdb.org/

References

Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE, Boyd J, et al (1994) Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. Nat Genet 8:387–391

Couch FJ, Farid LM, DeShano ML, Tavtigian SV, Calzone K, Campeau L, Peng Y, et al (1996) BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 13:123–125

de la Chapelle A (1993) Disease gene mapping in isolated human populations: the example of Finland. J Med Genet 30:857–865

Easton D (1997) Breast cancer genes—what are the real risks? Nat Genet 16:210–211

Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE, Breast Linkage Consortium (1994) Risks of cancer in BRCA1-mutation carriers. Lancet 343:692–695

Friedman LS, Gayther SA, Kurosaki T, Gordon D, Noble B, Casey G, Ponder BAJ, et al (1997) Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population. Am J Hum Genet 60:313–319

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE, King MC (1994) Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 8:399–404

Gayther SA, Mangion J, Russell P, Seal S, Barfoot R, Ponder BAJ, Stratton MR, et al (1997) Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. Nat Genet 15:103–105

Håkansson S, Johannsson O, Johansson U, Sellberg G, Loman N, Gerdes A-M, Holmberg E, et al (1997) Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. Am J Hum Genet 60: 1068–1078

Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, Johannsson OT, et al (1996) High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. Cancer Res 56: 3663–3665

Körkkö J, Annunen S, Pihlajamaa T, Prockop DJ, Ala-Kokko L (1998) Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 95:1681–1685

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71

Newman B, Austin MA, Lee M, King MC (1988) Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. Proc Natl Acad Sci USA 85:3044–3048

Nyström-Lahti M, Kristo P, Nicolaides NC, Chang SY, Aaltonen LA, Moisio AL, Järvinen HJ, et al (1995) Founding mutations and Alu-mediated recombination in hereditary colon cancer. Nat Med 1:1203–1206

Szabo CI, King M-C (1997) Population genetics of BRCA1 and BRCA2. Am J Hum Genet 60:1013–1020

Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, et al (1997*a*) Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. Hum Mol Genet 6:2309–2315

Vehmanen P, Friedman LS, Eerola H, Sarantaus L, Pyrhönen S, Ponder BAJ, Muhonen T, et al (1997*b*) A low proportion of BRCA2 mutations in Finnish breast cancer families. Am J Hum Genet 60:1050–1058

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792

Zelada-Hedman M, Wasteson Arver B, Claro A, Chen J, Werelius B, Kok H, Sandelin K, et al (1997) A screening for BRCA1 mutations in breast and breast-ovarian cancer families from the Stockholm region. Cancer Res 57:2474–2477

Address for correspondence and reprints: Dr. Robert Winqvist or Dr. Pia Huusko, Department of Clinical Genetics, Oulu University Hospital Kajaanintie 50, FIN-90220 Oulu, Finland. E-mail: robert.winqvist@oulu.fi

0002-9297/98/6206-0036$02.00

Am. J. Hum. Genet. 62:1548–1551, 1998

A Triplet Repeat on 17q Accounts for Most Expansions Detected by the Repeat-Expansion–Detection Technique

To the Editor:

Eight hereditary neurogenerative disorders have been identified that result from expansions of CAG trinucleotide repeats (Gilles et al. 1997). Thus, there has been great incentive to develop techniques to efficiently screen for repeat sequences in specific patient populations. The repeat-expansion detection (RED) is a widely used technique that screens for trinucleotide expansions without a requirement of prior knowledge of the disease locus (Schalling et al. 1993; Zander et al. 1997). The method uses genomic human DNA as a template, specific-repeat oligonucleotide primers, and a thermostable ligase to generate oligomers of the primer. However, there are limitations to the technique. Non–disease-related expansions occur frequently in the population, which complicate the interpretation of disease-association studies. For a repeat to be detected by the RED, it must stand out in size, and smaller expansions causing disease can be missed. Also, once an expanded repeat is found, there is no information about chromosomal localization.

Other techniques for identifying trinucleotide expansions have recently been developed (Sanpei et al. 1996; Koob et al. 1998). The direct identification of repeat expansion and cloning technique (DIRECT) was designed to enable the localization and cloning of expanded-repeat regions (Sanpei et al. 1996). By means of the DIRECT technique, a novel, long, and unstable CAG/CTG trinucleotide repeat (Dir I) was identified and localized to chromosome 17q (Ikeuchi et al. 1998). This repeat is highly polymorphic, ranging in size from 10 to 92 repeat copies (30–276 bp) in normal individuals (Ikeuchi et al. 1998). Independently, a second group identified this expanded repeat by cloning the gene fragment from RED positive DNAs (Nakamoto et al. 1997).

DNA samples, obtained with informed consent from psychiatric patients with childhood onset of disease, were studied specifically to detect increased numbers of triplet repeats by use of the RED technique (Burgess et al. 1998). Since children and adolescents with psychiatric disorders appear to have functional brain abnormalities (McKenna et al. 1994), these patients are a valuable resource for such studies. Although expansions of trinucleotide repeats have been associated with several disorders affecting the brain and nervous system, their involvement in the etiology of psychiatric disorders has not been clearly demonstrated (Lindblad et al. 1995; Morris et al. 1995; O'Donovan et al. 1995). Since expanded repeats can be associated with genetic anticipation, patients with an early onset of disease are especially good candidates to evaluate.

The RED technique was used to identify trinucleotide expansions in 227 individuals, including 36 patients diagnosed with childhood-onset schizophrenia (COS); 21 diagnosed with atypical psychosis, termed by us as "multidimensionally impaired" (MDI) (Kumra et al. 1998); 46 patients with attention-deficit hyperactivity disorder (ADHD); 51 screened controls; and 73 relatives of probands. Patients were diagnosed according to standard *Diagnostic and Statistical Manual of Mental Disorders* definitions with standardized interviews as described elsewhere (Gordon et al. 1994; Castellanos et al. 1996). Diagnostic criteria for the MDI group has been discussed elsewhere (Kumra et al. 1998). The RED analysis was performed with a CTG_{10} oligonucleotide in the RED reaction, producing a repeat-size representation at 30-nucleotide intervals (Lindblad et al. 1996; Zander et al. 1997). The same samples were then analyzed for expansions of the polymorphic CAG/CTG Dir I trinucleotide repeat on chromosome 17q, by use of PCR conditions as described elsewhere (Ikeuchi et al. 1998; see fig. 1).

RED expansions of $\geqslant$180 nucleotides were detected in a total of 99 (44%) of the 227 individuals screened, with the distribution of RED scores shown in table 1. When diagnoses were evaluated separately, RED scores of $\geqslant$180 nucleotides were seen in 41% of COS patients ($n = 36$), 43% of MDI patients ($n = 21$), 43% of ADHD patients ($n = 24$), and 29% of the controls ($n = 51$) (fig. 2*A*).

In analyzing the CAG/CTG repeat on chromosome 17q, we scored the allele in each individual with the largest repeat size. A total of 81 (36%) of the 227 individuals screened had a chromosome 17q Dir I repeat expansion of >150 bp. Interestingly, 80 of the 81 individuals with a repeat size of >150 bp on chromosome 17q had RED scores of $\geqslant$180 nucleotides. Thus, the RED technique appeared to detect this expansion reliably. There was also a strong correlation between the size of the Dir I expansion and the size of the expansion detected by RED (table 1).

Dir I repeats in excess of 50 copies (150 bp) were