Evidence for Linkage Disequilibrium Between the Alpha 7-Nicotinic Receptor Gene (CHRNA7) Locus and Schizophrenia in Azorean Families

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Recent studies have suggested that the alpha 7-nicotinic receptor gene (CHRNA7) may play a role in the pathogenesis of schizophrenia. The alpha 7-nicotinic receptor gene (CHRNA7) is involved in P50 auditory sensory gating deficits, and the genomic locus for this gene lies in the chromosome 15q13–14 regions. The human gene is partially duplicated (exons 5–10) with four novel upstream exons. The marker D15S1360 has been shown to be significantly linked with the phenotype of abnormal P50 suppression in schizophrenia families. The marker L76630 is 3 kb in the 3′ direction from the last exon of the CHRNA7 gene and is located in the duplicated region. The function of the two L76630 copies is unknown. We genotyped three polymorphic markers D15S1360, D15S165, and L76630 that are localized in a genomic fragment containing the CHRNA7 in 31 Azorean schizophrenia families/trios (including 41 schizophrenia individuals and 97 unaffected families members). An overall analysis utilizing the family-based association test revealed significant linkage disequilibrium between L76630 and schizophrenia ($P = 0.0004$). Using the extended transmission disequilibrium test and limiting the analysis to one triad per family, transmission disequilibrium of D15S1360 was near significance ($P = 0.078$). The 15q13 region overlaps with the location of two well-known genomically imprinted disorders: Angelman syndrome and Prader-Willi syndrome. Therefore, we investigated maternal and paternal meioses. We found significant transmission disequilibrium for D15S1360 through paternal transmission ($P = 0.0006$) in our schizophrenia families. The L76630 marker showed a significant disequilibrium in maternal transmissions ($P = 0.028$). No parent-of-origin effect was found in D15S165. Overall, our results suggest that the CHRNA7 may play a role in schizophrenia in these families. A parent of origin effect may be present and requires further study. Published 2001 Wiley-Liss, Inc.[†]

KEY WORDS: alpha 7-nicotinic receptor; exon duplication; schizophrenia; genetic linkage; polymorphism; Azores, Portugal; parent-of-origin effect

INTRODUCTION

Recent studies have suggested that the alpha 7-nicotinic receptor gene (CHRNA7) located in the 15q13–14 region may play a role in the pathogenesis of schizophrenia [Freedman et al., 1997; Faraone et al., 1998; Gejman et al., 1998; Owen et al., 1998]. Stronger genetic evidence shows that the alpha 7-nicotinic receptor gene (CHRNA7) is involved in the P50 auditory sensory gating deficit phenotype [Freedman et al., 1997]. CHRNA7 has a complicated genomic structure. The gene has 10 exons that are conserved across chicken and other mammalian species. The human gene is partially duplicated (exons 5–10) with four novel upstream exons. The duplication lies within

0.5 cM of CHRNA7, and the duplicated sequence is expressed in the brain. CHRNA7 is also a reasonable candidate gene for schizophrenia because of the high incidence of smoking in schizophrenics [De Leon et al., 1995], with both nicotine [Adler et al., 1993] and clozapine [Nagamoto et al., 1996] ameliorating the sensory gating deficit. Freedman et al. [1997] reported an initial linkage for the neurophysiological phenotype associated with schizophrenia to the genetic marker D15S1360 at chromosome 15q13–14 in nine Utah families, generating a LOD of 5.3 when tested against the sensory gating phenotype and 1.33 when tested against schizophrenia. Data from South African Bantu families in a dense map of markers at 1 cM intervals around D15S1360 showed some evidence in support of this finding, with positive NPLZ results across the entire map [Riley et al., 2000]. Extended transmission disequilibrium testing (ETDT) [Sham and Curtis, 1995] gave an allele-wise and genotype-wise chi-square of 6.59 (2 and 3 df; $P = 0.037$) for D15S1360; analyses with TRANSMIT [Clayton, 1999; Clayton and Jones, 1999] gave evidence for haplotype transmission disequilibrium with a global chi-square of 10.647 (4 df; $P = 0.007$) and a maximum chi-square of 6.567 (1 df; $P = 0.004$) for marker D15S1360 [Riley et al., 2000]. Leonard et al. [1998] studied 29 families with at least one sibling pair concordant for schizophrenia, along with two parents or another affected relative outside the nuclear family. Sibling pair analysis revealed a significant proportion of D15S1360 alleles shared identical-by-descent ($P < 0.0024$). Leonard et al. [1998] reported a low positive score at D15S1360 in the NIMH Genetics Initiative Schizophrenia families ($Z = 1.5$; $\theta = 0.0$; $P < 0.002$). The NIMH Genetics Initiative investigators independently published a replication with a multipoint linkage score derived from markers in the region; the Z-score was higher for African American than for European American families in the sample [Kaufmann et al., 1998]. The study by Freedman et al. [2001] has failed to link D15S1360 to schizophrenia in their African American and European American samples. However, they showed significant genotype-wise transmission disequilibrium at D15S165 for the entire sample. Two studies have failed to find any evidence for involvement of this locus in five families from eastern Canada [Neves-Pereira et al., 1998] and 54 families from the Maryland sample [Curtis et al., 1999].

In view of these findings, we examined three polymorphic markers (D15S1360, D15S165, and L76630) that are localized in a genomic fragment containing the CHRNA7 in 31 schizophrenia families/trios (including 41 schizophrenia individuals and 97 unaffected families members) to investigate the potential association between the alpha 7-nicotinic receptor gene (CHRNA7) and schizophrenia in Azorean families.

MATERIALS AND METHODS

Ascertainment

The families meeting inclusion criteria had a minimum of two affected members. Probands were identified from the psychiatric services of the S. Miguel (Azores Islands) [Pato et al., 1997]. All probands and all available first- and second-degree relatives were administered the Diagnostic Interview for Genetic Studies (DIGS) (National Institute of Mental Health Molecular Genetics Initiative, 1992 [Nurnberger et al., 1994]) Portuguese version [Azevedo et al., 1993] by a clinician with extensive training in this interview. Data from the DIGS, for each subject, was compared with medical records and information from close relatives. All data were entered in the OPCRIT system. The OPCRIT facilitates a polydiagnostic approach to the diagnosis of major psychotic and mood disorders [McGuffin et al., 1991]. For each subject, two blind raters not involved in the assessment of these families produced the final diagnosis; if these are not concordant, the head of the diagnostic panel blindly reviews and serves as the tie breaker. Consensus was arrived at through a best-estimate diagnosis process.

Study Samples

Our group has been studying the genetics of psychotic disorders in a sample with reduced heterogeneity derived from mainland Portugal and Azores, Portugal [Pato et al., 1997]. Surname analysis of the population showed a 79-fold reduction in variability in surnames for the Azorean population and a 55-fold reduction in variability in surnames for the continental Portuguese population, in comparison to a U.S. urban population. These analyses are suggestive of a much higher degree of genetic homogeneity in the Portuguese population and especially in the geographically isolated Azorean and Madeiran population [Pato et al., 2000].

For this study, our overall sample consists of 31 schizophrenia families/trios, including 41 schizophrenia individuals and 97 unaffected family members from the Azorean families.

Extraction of Genomic DNA

Blood samples were collected in anonymously identified 10 ml Vacutainer tubes (Becton Dickinson). DNA was prepared by a modified SDS/Proteinase K procedure [Gusella et al., 1979]. About 10 ml were diluted with three volumes of cold Miller RBCL (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA). Diluted blood was kept on ice for 30 min and centrifuged at 2,500 rpm for 15 min at 4°C. The pellet was well resuspended in 30 ml of cold Miller RBCL and centrifuged at the same speed. The pellet was well resuspended in 5 ml of SE buffer (75 mM NH_4Cl, 25 mM Na_2EDTA), 30 µl of proteinase K (20 mg/mL), 500 µl of 20% SDS was added, and the suspension was mixed until it appeared clear and viscous; the tube was incubated overnight at 42°C with gentle rocking. Next, the overnight suspension was added to 3 mL of 8 M ammonium acetate and centrifuge at 3,500 rpm for 10 min. The supernatant was transferred to a new Falcon tube, and 2 volumes of ice-cold absolute ethanol were added. The DNA was picked off, washed twice in 70% ethanol, partially air-dried, and slowly solubilized in

TABLE I. Oligonucleotide Primer Sequences and Annealing Temperature for D15S1360, L76630, and D15S165

Marker	Primer sequences	Annealing temperature
D15S1360	5′-GATCTTTGGTAGAAGC-3′ 5′-ACCACCACTACCATACAGAC-3′	50°C
L76630	5′-TCCAGCCATCTGGCTCACTCAT-3′ 5′-CCTGAGGCTGGAATGTCCTGAT-3′	62°C
D15S165	5′-GTTTACGCCTCATGGATTTA-3′ 5′-GGGCACACAGTCCCAA-3′	57°C

$1 \times$ TE with RNase at 4°C. Concentration and purity were evaluated with a spectrophotometer. A 260/280 nm ratio of 1.8 to 1.9 was usually obtained and the final concentration was adjusted to 100 μg/ml.

Genotyping

Three dinucleotide repeat polymorphic markers D15S1360, D15S165, and L76630, which are localized in a genomic fragment containing the CHRNA7 [Beckmann et al., 1993; Freedman et al., 1997], were tested. Oligonucleotide primer sequences for each polymorphism are shown in Table I. For PCR-based DNA markers, primer sets were synthesized in Roswell Park Cancer Institute Biopolymer Facility. The PCR was performed in a total volume of 20 μl containing the following: 40 ng genomic DNA; 200 μM each of dATP, dGTP, dTTP, and dCTP; 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 1.5 mmol $MgCl_2$; 10 pmol of each primer (for detection of radioactivity, one primer per pair was labeled with ^{32}P γ-ATP by using the T4 polynucleotide kinase forward reaction), and 0.5 units Taq polymerase. PCR condition is under an initial denaturation at 94°C for 5 min; 27 cycles were repeated with denaturation at 94°C for 30 sec, the different annealing temperature for respective marker (Table I) for 75 sec, extension at 75°C for 15 sec. A final synthesis step consists of 5 min at 72°C. After PCR, 5 μl of 95% formamide dye was added to each sample, which was then heated to 90°C for 5 min. The samples were then cooled on ice prior to loading on a 6% polyacrylamide/urea sequencing gel. The gel was run at 65 W for approximately 2–4 hr. After electrophoresis, the gel was dried and autoradiographed. Genotypes were confirmed by independent reading of the electropherograms by at least two individuals. Data were analyzed by family-based association test (FBAT) [Lake et al., 2000] and ETDT [Sham and Curtis, 1995].

Statistical Analysis

Our current sample includes both parents/proband trios and extended families, making the statistical analysis more complex. In fact, a simple and straightforward approach like the transmission/disequilibrium test is not applicable in extended families, since marker genotypes among siblings are correlated in linked regions. Thus, to detect a possible correlation between genotype and phenotype, we decided to use as primary analysis the FBAT procedure [Lake et al., 2000], which tests for association in the presence of linkage and generalizes the transmission disequilibrium test to any kind of family. The FBAT method is a valid test for the null hypothesis of no linkage and no association. However, we did not previously test for linkage to chromosome 15 and, given the cautionary statement of Lake et al. [2000], we also evaluated the presence of association with the ETDT program [Sham and Curtis, 1995] at the expense of reducing the power of our sample by analyzing only one parents/proband trio from each extended family.

RESULTS

We tested the relationship between CHRNA7 and schizophrenia using the family-based association test [Lake et al., 2000] and the extended transmission disequilibrium testing [Sham and Curtis, 1995] to look for distortions in the expected patterns of allele and genotype segregation to affected offspring. An overall analysis utilizing the FBAT revealed significant linkage disequilibrium between L76630 and schizophrenia ($P = 0.0004$). Results of ETDT analysis for the three markers D15S165, D15S1360, and L76630 in our schizophrenia families/trios are shown in Table II. These three markers did not exhibit linkage disequilibrium with each other. The marker D15S1360 is < 120 kb upstream from the first exon of the CHRNA7 gene, which Freedman et al. [1997] reported highly significant linkage with the P50 abnormality in schizophrenics. The analysis of the entire sample for marker D15S1360 shows that both the allele-wise chi-square and the genotype-wise chi-square were 6.81 (3 df; $P = 0.078$) for schizophrenia. For marker D15S165, the analysis of the entire sample shows the allele-wise chi-square was 2.56 (8 df; $P = 0.59$) and the genotype-wise chi-square was 13.49 (16 df; $P = 0.63$) for schizophrenia. For the duplicated marker L76630 of the CHRNA7 gene, the analysis of the entire sample shows that both the allele-wise and the genotype-wise chi-square were 1.99 (df 1; $P = 0.15$) for schizophrenia. Because of the strong evidence for imprinting in this region of 15q, we then ran the data looking at maternal and paternal meioses and found significant transmission disequilibrium for D15S1360 through the paternal

TABLE II. Extended Transmission Disequilibrium Test for D15S1360, D15S165, and L76630 in Schizophrenia

Marker	Sample	Transmission	Allele-wise	Genotype-wise
D15S1360	Schizophrenia	Maternal	6.03, df 3, $P = 0.11$	6.03, df 3, $P = 0.11$
		Paternal	17.54, df 3, $P = 0.0006$	17.55, df 3, $P = 0.00056$
		Combined	6.81, df 3, $P = 0.078$	6.81, df 3, $P = 0.078$
D15S165	Schizophrenia	Maternal	12.82, df 7, $P = 0.078$	12.81, df 9, $P = 0.17$
		Paternal	6.59, df 7, $P = 0.47$	12.81, df 9, $P = 0.17$
		Combined	2.56, df 8, $P = 0.59$	13.49, df 16, $P = 0.63$
L76630	Schizophrenia	Maternal	4.81, df 1, $P = 0.028$	4.81, df 1, $P = 0.028$
		Paternal	0.67, df 1, $P = 0.41$	0.67, df 1, $P = 0.41$
		Combined	1.99, df 1, $P = 0.15$	1.99, df 1, $P = 0.15$

transmission. The allele-wise chi-square was 17.54 (df 3; $P = 0.0006$), and the genotype-wise chi-square was 17.55 (df 3; $P = 0.00056$). Significant transmission disequilibrium for L76630 was found through maternal transmission, both the allele-wise and the genotype-wise chi-square were 4.81 (df 1; $P = 0.028$). No parent-of-origin effect was found for D15S165 in the entire sample of schizophrenia samples.

DISCUSSION

The FBAT was judged to be most appropriate for our sample because of its ability to extend traditional tests of transmission disequilibrium to a family of any structure and with multiple affecteds. Traditional linkage methods (parametric or nonparametric) would have little power to detect linkage in this pilot sample from a single Azorean island. We detected significant linkage disequilibrium between L76630 and narrowly defined schizophrenia ($P = 0.0004$). The other two markers did not show significant linkage disequilibrium with schizophrenia and there is no disequilibrium marker to marker across all three. This may be due to the evolutionary history of this island population, wherein, for example, the disequilibrium between the marker L76630 and schizophrenia may have been skewed by population selection at the time of island settlement. The basis for the significant disequilibrium detected between L76630 and schizophrenia but not detected with the other two markers may also be related in some way to the instability and duplication in the CHRNA7 gene area.

It is important to remember that this region of 15q13–14 shows parent-of-origin effects in genetic transmission. To our knowledge, the results of significant transmission disequilibrium for D15S1360 through paternal transmission ($P = 0.0006$) and for L76630 through maternal transmission ($P = 0.028$) in our schizophrenia families provide the first evidence of the parent-of-origin effect for the CHRNA7 locus. Some suggestion of parent-of-origin effect has been previously noted for schizophrenia. Two studies [Ohara et al., 1997; Husted et al., 1998] found anticipation to be greater from paternal than maternal inheritance in schizophrenia families. Yaw et al. [1996], on the other hand, noticed a more severe symptomatology in maternally transmitted cases, whereas the age at onset was not significantly higher than that in paternally transmitted cases. McInnis et al. [1999] found anticipation in aunt-niece/nephew, but not in uncle-niece/nephew pairs in schizophrenia families. In addition, Schwab et al. [1998] found evidence for maternal transmission at the marker G-olf$_a$ on chromosome 18p in 12 schizophrenia families. In contrast, negative studies for parent-of-origin effect in schizophrenia have been reported by others [Asherson et al., 1994; Thibaut et al., 1995; Gorwood et al., 1996; Imamura et al., 1998; Valero et al., 1998; DeLisi et al., 2000].

Genomic imprinting is the process by which only the paternal or maternal allele of a gene is active in the offspring. Two well-known genomically imprinted disorders, Angelman syndrome (AS) and Prader-Willi syndrome (PWS), are located at 15q11–13 and are closely adjacent to CHRNA7. Angelman syndrome has both paternal uniparental disomy and deletion of the maternal 15q11–13 segments that lead to the different array of symptoms [Knoll et al., 1990]. Similarly, Prader-Willi syndrome is attributable to the exclusive expression of a maternally derived 15q11–13 chromosomal segment, resulting from either the deletion of that portion of the paternal chromosome or from maternal uniparental disomy [Nicholls et al., 1989; Lalande, 1997]. There is an unexpected high co-occurrence of schizophrenia and Prader-Willi syndrome [Clarke et al., 1993], perhaps because the deletion in some cases may involve CHRNA7. Recent findings have revealed that mutations of the imprinting center are different in Angelman syndrome and Prader-Willi syndrome patients. Both mutations are located in noncoding exons and differentially regulate the switch from maternal to paternal or paternal to maternal epigenotype on passage through the germline [Dittrich et al., 1996; Ferguson-Smith, 1996]. Thus, a mutation in the same gene is expressed in different ways, depending on whether the defect is transmitted maternally or paternally, and this in turn depends on whether there is differential parental methylation of the gene.

The CHRNA7 itself has a complicated genomic structure; the genomic locus for this gene lies in the chromosome 15q13–14 region [Freedman et al., 1997]. The gene has 10 exons that are conserved across chick and other mammalian species. However, the human

gene is part of a duplicated cassette of expressed sequences. Exons 5-10 of the alpha 7-nicotinic receptor gene are duplicated, which are located nearly adjacent to each other within approximately 1 Mb of DNA at chromosome 15q14. Two copies of exons 6 and 7 and the L76630 locus 3′ of exon 10 are detected in both a somatic cell chromosome 15 hybrid and in a single YAC (969b11) containing the full-length gene and duplicated CHRNA7 exons [Gault et al., 1998]. The marker L76630 is duplicated and therefore present in two copies, whereas markers D15S1360 and D15S165 are not duplicated. Since duplicated sequences that are expressed necessarily imply regulatory consequences, such replications have been implicated in several human genetic diseases [Lehrman et al., 1987; Den-Dunnen et al., 1989; Hu and Worton, 1992; Campbell et al., 1997]. If mutation is involved in the partial duplicated locus of CHRNA7, it may cause the parent-of-origin effect in schizophrenia by the same genetic imprinted mechanism as Angelman syndrome and Prader-Willi syndrome, because these three genes all are located in the same region of chromosome 15.

Summarizing our results of parent transmission with schizophrenia in the relatively homogeneous Portuguese population in the region of the CHRNA7 at chromosome 15q13−14 would suggest that imprinting may play a significant role in the transmission of schizophrenia, and the partially duplicated CHRNA7 may be a susceptibility gene for genomic imprinting in schizophrenia transmission. Since the sample of families was collected for a linkage study without the parent-of-origin effect in mind, there was no bias in selecting families demonstrating either maternal or paternal patterns of transmission. However, our sample size is not large enough to produce significant positive finding for D15S1360 and D15S165 when considered total transmission, which may limit the potential validity of our finding.

Overall, our results suggest that the CHRNA7 locus is involved in risk for the disorder. In the context of previous reports, we are seeing an increasing number of replications, thus more strongly implicating a role for this gene. Our study has the limitation of a relatively small sample size. However, the increased homogeneity of this island population reduces unwanted population genetic noise facilitating identification of genetic risk signals. If we consider this to be a replication study, our observed P value of 0.0004 can be judged as relatively robust support for CHRNA7 as a susceptibility locus for schizophrenia.

REFERENCES

Adler LE, Hoffer LD, Wiser A, Freedman R. 1993. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. Am J Psychiatry 150:1856−1861.

Asherson P, Walsh C, Williams J, Sargeant M, Taylor C, Clements A, et al. 1994. Imprinting and anticipation: are they relevant to genetic studies of schizophrenia? Br J Psychiatry 164:619−624.

Azevedo MH, Dourado A, Valente J, Macedo A, Coelho I, Pato M, Pato C. 1993. The Portuguese-language version of the Diagnostic Interview for Genetic Studies. Psychiatr Genet 3:189.

Beckmann JS, Tomfohrde J, Barnes RI, et al. 1993. A linkage map of human chromosome 15 with an average resolution of 2 cM and containing 55 polymorphic microsatellites. Hum Mol Genet 2:2019−2030.

Campbell L, Potter A, Ignatius J, Dubowitz D, Davies K. 1997. Genomic Variation and gene conversion in spinal muscular atrophy: implications for disease process and clinical phenotype. Am J Hum Genet 61:40−50.

Clarke DJ. 1993. Prader-Willi syndrome and psychoses. Br Psychiatry 163:680−684.

Clayton D. 1999. A generalization of the transmission/disequilibrium test for uncertain haplotype transmission. Am J Hum Genet 65:1170−1177.

Clayton D, Jones H. 1999. Transmission disequilibrium tests for extended marker haplotypes. Am J Hum Genet 65:1161−1169.

Curtis L, Blouin J-L, Radhakrishna U, Gehrig G, Lasseter VK, Wolyniec P, Nestadt G, Dombroski B, Kazazian HH, Pulver AE, Housman D, Bertrand D, Antonarakis SE. 1999. No evidence for linkage between schizophrenia and markers at chromosome 15q13−14. Am J Med Genet (Neuropsychiatr Genet) 88:109−112.

De Leon J, Dadvand M, Canuso C, White AO, Stanilla JK, Simpson GM. 1995. Schizophrenia and smoking an epidemiological survey in a state hospital. Am J Psychiatry 152:453−455.

DeLisi LE, Razi K, Stewart J, Shields G, Smith AB, Wellaman N, Larach VW, Loftus J, Vita A, Comazzi M, Crow TJ. 2000. No evidence for a parent-of-origin effect detected in the pattern of inheritance of schizophrenia. Biol Psychiatry 48:706−709.

Den-Dunnen JT, Grootscholten PM, Bakker E, Blonden LA, Ginjaar HB, Wapenaar MC, van PH, van Broeckhoven C, Pearson PL, van Ommen GJ. 1989. Topography of the Duchenne muscular dystrophy (DMD) gene: FIGE and cDNA analysis of 194 cases reveals 115 deletions and 13 duplications. Am J Hum Genet 45:835−847.

Dittrich B, Buiting K, Kom B, Rickard S, Buxton J, Saitoh S, Nicholls RD, Pouska A, Winterpacht A, Zabel B, Horsthemke B. 1996. Imprint switching on human chromosome 15 may involve alternative transcripts of the SNPPN gene. Nat Genet 14:163−170.

Faraone SV, Matise T, Svrakic D, et al. 1998. Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. Am J Med Genet 81:290−295.

Ferguson-Smith AC. 1996. Imprinting moves to the center. Nat Genet 14:119−121.

Freedman R, Coon H, Myles-Worsley M, et al. 1997. Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. Proc Natl Acad Sci USA 94:587−592.

Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA, Tsuang MT, Farone SV, Malaspina D, Svrakic DM, Sanders A, Gejman P. 2001. Linkage disequilibrium for schizophrenia at the chromosome 15q13−14 locus of the alpha 7-Nicotinic acetylcholine receptor subunit gene (CHRNA7). Am J Med Genet 105:20−22.

Gault J, Robinson M, Berger R, Drebing C, Logel J, Hopkins J, Moore T, Jacobs S, Merriwether J, Choi MJ, Kim EL, Walton K, Buiting K, Davis A, Breese C, Freedman R, Lenard S. 1998. Genomic organization and partial duplication of the human 7 neuronal nicotinic acetylcholine receptor gene. Genomics 52:173−185.

Gejman PV, Gershon ES, Zhang J, et al. 1998. Progress of genome scan of the NIMH Intramuron schizophrenia collection. Am J Med Genet 81:453.

Gorwood P, Leboyer M, Falissard B, Jay M, Rouillon F, Feingold J. 1996. Anticipation in schizophrenia: new light on a controversial problem. Am J Psychiatry 153:1173−1177.

Gusella J, Varsanyi-Breiner A, Kao FT, Jones C, Puck TT, Keys C, Orkin S, Housman D. 1979. Precise localization of human beta-globin gene coplex on chromosome 11. Proc Natl Acad Sci USA 76:5239−5242.

Hu X, Worton RG. 1992. Partial gene duplication as a cause of human disease. Hum Mutat 1:3−12.

Husted J, Scutt LE, Bassett AS. 1998. Paternal transmission and anticipation in schizophrenia. Am J Med Genet 81:156−162.

Imamura A, Honda S, Nakane Y, Okazaki Y. 1998. Anticipation in Japanese families with schizophrenia. J Hum Genet 43:217–223.

Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD, Meyer J, Zambuto CT, Schmitt K, Matise TC, Harkavy FJ, Jampe C, Lee H, Shore D, Wynne D, Faraone SV, Tsuan MT, Cloninger CR. 1998. NIMH genetics initiative millennium schizophrenia consortium: linkage analysis of African-American pedigrees. Am J Med Genet 81:282–289.

Knoll JHM, Nicholls RD, Magenis RE, Glatt K, Graham JM Jr, Kaplan L, Lalande M. 1990. Angelman syndrome: three molecular classes identified with chromosome 15q11–q13-specific DNA markers. Am J Hum Genet 47:149–155.

Lake SL, Blacker D, Laird NM. 2000. Family-based tests of association in the presence of linkage. Am J Hum Genet 67:1515–1525.

Lalande M. 1997. Parental imprinting and human disease. Ann Rev Genet 30:173–195.

Lehrman MA, Goldstein JL, Russell DW, Brown MS. 1987. Duplication of seven exons in the LDL receptor gene caused by Alu-Alu recombination in a subject with familial hypercholesterolemia. Cell 48:827–835.

Leonard S, Gault J, Moore T, et al. 1998. Further investigation of a chromosome 15 locus in schizophrenia: analysis of affected sibpairs from the NIMH Genetics Initiative. Am J Med Genet 81:308–312.

McGuffin P, Frarmer A, Harvey I. 1991. A polydiagnostic application of operational criteria in studies of psychotic illness: development and reliability of the OPCRIT system. Arch Gen Psychiatry 8:764–770.

McInnis MG, McMahon FJ, Crow TJ, Ross CA, DeLisi LE. 1999. Anticipation in schizophrenia: a review and consideration. Am J Med Genet 88:686–693.

Nagamoto HT, Adler LE, Hea RA, Griffith JM, McRae KA, Freedman R. 1996. Gating of auditory P50 in schizophrenics: unique effects of clozapine. Biol Psychiatry 40:181–188.

Neves-Pereira M, Bassett AS, Honer WG, Lang D, King NA, Kennedy JL. 1998. No evidence for linkage of the CHRNA7 gene region in Canadian schizophrenia families. Am J Med Genet (Neuropsychiatr Genet) 81:361–363.

Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M. 1989. Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 342:281–285.

Nurnberger J, Blehar M, Kaufmann C, Yok-cooler C, Simpson S, Harkavy-Friedman J, Severe J, Malaspina D, Reich T. 1994. Diagnostic interview for genetic studies: rationale, unique features and training. Arch Gen Psychiatry 51:849–859.

Ohara K, Xu HD, Mori N, Suzuki Y, Xu DS, Ohara K, Wang ZC. 1997. Anticipation and imprinting in schizophrenia. Biol Psychiatry 42:760–766.

Owen MJ, Craddock N. 1998. Chromosome 11 workshop. Psychiatr Genet 8:89–92.

Pato CN, Azevedo MH, Pato MT, Kennedy JL, Coelho I, Paz Ferreira C, Dourado A, Macedo A, Valente J, Madeira J, Gago da Camara J, Moniz M, Correia C. 1997. Selection of homogeneous populations for genetic study: the Portugal Genetics of Psychosis Project. Am J Med Genet (Neuropsychiatr Genet) 74:286–288.

Pato CN, Macedo A, Ambrosio A, Vincent J, Bauer A, Schindler K, Xu J, Coelho I, Dourado A, Valente J, Azevedo MH, Kennedy J, Pato M. 2000. Detection of expansion regions in Portuguese bipolar families. Am J Med Genet (Neuropsychiatr Genet) 96:854–857.

Riley BP, Makoff A, Mogudi-Carter M, Jenkins T, Williamson R, Collier D, Murray R. 2000. Haplotype transmission disequilibrium and evidence for linkage of the CHRNA7 gene region to schizophrenia in Southern African Bantu families. Am J Med Genet 96:196–201.

Schwab SG, Hallmayer J, Lerer B, Albus M, Borrmann M, Hönig S, Strau M, Segman R, Lichtermann D, Knapp M, Trixler M, Maier W, Wildenauer DB. 1998. Support for a chromosome 18p locus conferring susceptibility to functional psychoses in families with schizophrenia, by association and linkage analysis. Am J Hum Genet 63:1139–1152.

Sham PC, Curtis D. 1995. An extended transmission/disequilibrium test (TDT) for multi-allele marker loci. Am Hum Genet 59:323–336.

Thibaut F, Martinez M, Petit M, Jay M, Campion D. 1995. Further evidence for anticipation in schizophrenia, Psychiatry Res 59:25–33.

Valero J, Martorell L, Marine J, Vilella E, Labad A. 1998. Anticipation and imprinting in Spanish families with schizophrenia. Acta Psychiatr Scand 97:343–350.

Yaw J, Myles-Worsley M, Hoff M, Holik J, Freedman R, Byerley W, Coon H. 1996. Anticipation in mutiplex schizophrenia pedigrees. Psychiatr Genet 6:7–11.