

Mitochondrial Genetic Analyses Suggest Selection against Maternal Lineages in Bipolar Affective Disorder

Richard Kirk,^{1,*} Robert A. Furlong,^{1,*} William Amos,^{2,*} Gillian Cooper,² Judy S. Rubinsztein,³ Cathy Walsh,³ Eugene S. Paykel,³ and David C. Rubinsztein¹

¹Department of Medical Genetics, ²Department of Zoology, and ³Department of Psychiatry, University of Cambridge, Cambridge, United Kingdom

Summary

Previous reports of preferential transmission of bipolar affective disorder (BP) from the maternal versus the paternal lines in families suggested that this disorder may be caused by mitochondrial DNA mutations. We have sequenced the mitochondrial genome in 25 BP patients with family histories of psychiatric disorder that suggest matrilineal inheritance. No polymorphism identified more than once in this sequencing showed any significant association with BP in association studies using 94 cases and 94 controls. To determine whether our BP sample showed evidence of selection against the maternal lineage, we determined genetic distances between all possible pairwise comparisons within the BP and control groups, based on multilocus mitochondrial polymorphism haplotypes. These analyses revealed fewer closely related haplotypes in the BP group than in the matched control group, suggesting selection against maternal lineages in this disease. Such selection is compatible with recurrent mitochondrial mutations, which are associated with slightly decreased fitness. Although such mismatch distribution comparisons have been used previously for analyses of population histories, this is, as far as we are aware, the first report of this method being used to study disease.

Introduction

Compelling support for a major genetic influence in bipolar affective disorder (BP) comes from twin and family studies (Gershon 1990). Its prevalence is similar in males and females. Although the mode(s) of inheritance of BP are unclear, a number of groups have reported preferential transmission of BP through the maternal line. This may provide clues about the types of loci that are involved. Rosanoff et al. (1935) and Reich et al. (1969) suggested that X-linked loci may play a role in BP. A number of family studies have reported increased morbid risk to the mothers, relative to the fathers, of affected probands (Goetzl et al. 1974; Helzer and Winokur 1974; Dunner and Fieve 1975), or an excess of mother-offspring pairs (Smeraldi et al. 1977; Trzebiatowska-Trzeciak 1977; Angst et al. 1980).

More recently, McMahon et al. (1995) studied 31 families ascertained through probands treated for BP and selected for the presence of affected phenotypes in only one parental lineage. In the families in which a transmitting parent could be identified, there was a significant excess of transmitting mothers, compared with transmitting fathers. These families showed a 2.3–2.8-fold increased risk of illness for maternal relatives, compared with paternal relatives, and a 1.3–2.5-fold increased risk of illness for offspring of affected mothers, compared with affected fathers. Gershon et al. (1996) have replicated this observation by finding excess maternal transmission in their series of pedigrees with unilineal BP.

A number of genetic mechanisms can explain these phenomena. First, an X-linked locus can account for the excess inheritance from the maternal line. However, in seven large pedigrees from the series of McMahon et al. (1995), there was consistent failure of paternal transmission of the illness, whereas there were many cases of maternal transmission of the disease to both male and female children. There were many affected females in these pedigrees. Since males can pass X-linked genes to their daughters, McMahon et al. (1995) argued that an X-linked gene could not account for the complete failure

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Address for correspondence and reprints: Dr. David C. Rubinsztein, Department of Medical Genetics, University of Cambridge, Cambridge Institute for Medical Research, Wellcome/MRC Building, Addenbrooke's Hospital, Cambridge, CB2 2XY, United Kingdom. E-mail: dcr1000@cus.cam.ac.uk

*These authors contributed equally to this study.

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Table 1**Primers Used for Amplification of the Mitochondrial Genome for Sequence Analysis**

| Primer 1 | Primer 2 |
|---------------------------------------|---------------------------------------|
| M1561F 5'-CGTAACATGGTAAGTGTACTGG-3' | M3721R 5'-TTTGGGCTACTGCTCGC-3' |
| M3007F 5'-CCCGATGGTGACGCCG-3' | M5003R 5'-GATTTTGCCTAGCTGGGTCTGG-3' |
| M4470F 5'-ATTAATCCCCTGGCCCAACC-3' | M5918 5'-ACGGTCGGCGAACATCAGTG-3' |
| M5314F 5'-TAGCCACCATCACCCCTCC-3' | M7352 5'-TAGGACTTTTCGCTTCGAAG-3' |
| M6936F 5'-ATCTTTCTTTTCACCGTAGGTGGC-3' | M8865R 5'-CACTGTGCCCGCTCATAAGG-3' |
| M8265F 5'-TATAGCACCCCTCTACCC-3' | M10147R 5'-TAGCCGTTGAGTTGTGGTAGTC-3' |
| M9921F 5'-GCCTGATACTGGCATTGTAG-3' | M12367R 5'-TTAGGGTGGTTATAGTAGTGTGC-3' |
| M11582F 5'-ATCTGCCTACGACAAACAGACC-3' | M13644R 5'-GCGAGGTTGACCTGTTAGGG-3' |
| M13169F 5'-TATGCTTAGGCGCTATACC-3' | M14992R 5'-AAGGTAGCGGATGATTCAGC-3' |
| M14559F 5'-CGACCACACCGCTAACCAATC-3' | M16430R 5'-TGCGGGATATTGATTCACG-3' |
| M16205F 5'-CAAGTACAGCAATCAACCCTC-3' | M17966R 5'-TTTCCCTTGCGGTACTATATC-3' |

NOTE.—The 5' base of each primer is numbered according to Anderson et al. (1981).

of paternal transmission of the disease to at least 17 daughters in these pedigrees. Second, an imprinted locus can explain this phenomenon. However, we do not currently know about all of the imprinted regions of the human genome. Accordingly, this possibility is difficult to exclude. Third, mitochondrial inheritance can explain the excess maternal inheritance of this disease, since mitochondria are almost exclusively maternally inherited. There are a number of case reports of subjects with mitochondrial abnormalities who show features of depression, supporting the possibility that mutations in this genome may predispose to BP (McMahon et al. 1995; Kato et al. 1997; Miyaoka et al. 1997).

Current data do not allow one to confidently discriminate between these three possibilities. Two other problems need to be borne in mind. First, if one accepts that BP is genetically heterogeneous, it may be extremely difficult to resolve these models with segregation analyses. Second, the excess of maternally inherited disease observed by McMahon et al. (1995) and Gershon et al. (1996), was not seen by Gigoroiu-Serbanescu and colleagues (1998) in their collection of Bulgarian families affected by BP.

To address whether mitochondrial mutations play a role in BP, we first examined whether particular mitochondrial DNA variants were overrepresented in the patients with BP. We have sequenced the mitochondrial genome in 25 patients with BP and family histories of psychiatric disorder that suggest matrilineal inheritance. No polymorphism identified more than once in this sequencing showed any significant association with BP in association studies using 94 cases and 94 controls. However, analyses of all possible pairwise comparisons within the BP and control groups, based on multilocus mitochondrial polymorphism haplotypes, revealed a deficiency of closely related haplotypes in the BP group, compared with the matched control group, suggesting selection against maternal lineages in this disease. Such selection is compatible with recurrent mitochondrial mu-

tations, which are associated with slightly decreased fitness.

Subjects and Methods

Cases and Controls

Patients with BP type I were recruited from inpatient and outpatient clinics in East Anglia and were white and English in origin (i.e., both parents were English). The patients were assessed by trained clinicians using the SADS-L interview (Endicott and Spitzer 1978), supplemented by case note review, and met Research Diagnostic Criteria for BP type I (Spitzer et al. 1978).

We selected a subset of 25 patients for sequencing of the mitochondrial genome, comprising individuals with family histories that were suggestive of unilineal psychiatric disease inherited from the maternal side. The relevant affected relatives of these patients were reported to have manic-depressive disorder or unipolar depression. Family histories were based entirely on information from the affected probands.

Ninety-four patients (43 male, 51 female) with BP and 94 controls, matched for gender, age (within 24 months), and ethnic origin, were typed for 23 mitochondrial polymorphisms. Control DNA from anonymous unrelated individuals from an East Anglian population was obtained from the Molecular Genetics Laboratory (Addenbrooke's Hospital) DNA bank. Referral patterns to this laboratory suggest that at least 97% of controls are white, ~80% have parents who were East Anglian, and virtually all of the remainder are from the United Kingdom. The surnames of the controls were screened, to exclude individuals who were obviously of nonwhite origin. It is important to note that this region includes a large rural catchment area and is not characterized by the ethnic variation seen in many large cities. Ethical approval for genetic studies of BP were obtained from local ethics committees.

Table 2**Primers Used for the Detection of Mitochondrial Polymorphisms**

| | Primer 1 | Primer 2 | Primer 3 |
|------------------------------------|---|--|---|
| 295 MS-PCR | M392R 5'-AATCTGGTTAGGCTGGTG-TTAGGGTTC-3' | MSF268MUT 5'-CCACACAGACATCAT-AACAAAAAATTCT-3' | MSF248NOR 5'-AATGTCTGCACAGCCACTTTGGAC-ACAGACATCATAACAAAAAACTTC-3' |
| 309 MS-PCR | M392R 5'-AATCTGGTTAGGGCTGTGT-TTAGGGTTC-3' | MSF285MUT 5'-CAAAAAATTTCCACC-AAACCCCTCCC-3' | MSF264NOR 5'-CTTCCACACAGACATCATAAGTA-AAAATTTCCACCAAACCCCTCT-3' |
| 514 (CA) _n ^a | M449F 5'-TTATTTTCCCCTCCCACTCC-3' | M555R 5'-TTGGTTGGTTCGGGGTATG-3' | |
| 709 MS-PCR | M605F 5'-TACACTGAAAATGTTA-GACGGGCTC-3' | MSR757NOR 5'-TGCTTGCCCTTTG-ATCGTGTAGATTTAGAGGGTGAAC-TCACTGAAAC-3' | MSF736MUT 5'-GTGATTTAGAGGGTGAACACTCACTG-GAGT-3' |
| 1189 MS-PCR | M1279R 5'-GGTTTGCTGAAGATGGCG-GTATATAG-3' | MSR1859NOR 5'-TCATTATGCAGAAGG-TATAGCCGTTAGTCCTTGCTATAT-TATGCTCGGT-3' | MSR1839MUT 5'-GGGTTAGTCCTTGCTATATTATGC-TTGAC-3' |
| 1811 MS-PCR | M1707F 5'-CAGACAACCTTAGCCAAA-CCATTTACC-3' | MSR1859NOR 5'-TCATTATGCAGAAGG-TATAGCCGTTAGTCCTTGCTATAT-TATGCTCGGT-3' | MSR1839MUT 5'-GGGTTAGTCCTTGCTATATTATGC-TTGAC-3' |
| 1888 MS-PCR | M1765F 5'-CTGGCGCAATAGATATAG-TACCGCAAG-3' | MSR1912MUT 5'-TGTTTCGGGGGTCT-TAGCTTTGAT-3' | MSR1934NOR 5'-AGCTGTCTTAGGTAGCTCGTCAC-GTTTCGGGGGTCTTAGCTTCGGC-3' |
| 1888 <i>Cvi</i> II | M1786F 5'-CGCAAGGGAAAGATGAAA-AAT-3' | M1913R 5'-CTGGTTTCGGGGGTCT-TAG-3' | |
| 3010 MS-PCR | M2928F 5'-CCTAGGGATAACAGCGCA-ATCCTATTC-3' | MSR3057NOR 5'-GGACTTTAATCGTTG-AACAAACTCACCTTTAATAGCGGC-TGCACTATC-3' | MSR3035MUT 5'-GAACCTTTAATAGCGGCTGCACCA-CT-3' |
| 4216 <i>Nla</i> III | M4008F 5'-AAACACCCTCACCCTAC-AATC-3' | M4527R 5'-TGATGAGTGTGCCTGCAA-AG-3' | |
| 6261 <i>Hae</i> III | M6129F 5'-GGAGGCTTTGGCAACTGAC-3' | M6470R 5'-TAGGACGGATCAGACGAA-GAG-3' | |
| 8701 MS-PCR | M8599F 5'-CTATTTCCCCTCTATTG-ATCCCCAC-3' | MSR8726MUT 5'-GTTTCGTCCTTTAGTG-TTGTGTATGAC-3' | MSR8747NOR 5'-GATACTAGTATAAGAGATCAGCAT-CGTCCTTTAGTGTGTGTACGGT-3' |

| | | | | |
|-------|--------------|--|--|--|
| 10398 | <i>Ddel</i> | M9969F 5'-TCCATCTATTGATGAGGG-TCTTAC-3' | M10635R 5'-CTAAGAGGGAGTGGGTGT-TG-3' | M10487MUT 5'-TATGTAAATGAGGGGCATTTGGTGG-3' |
| 10463 | MS-PCR | M10337F 5'-ATTAATCATCATCCTAGC-CCTAAGTC-3' | M10511NOR 5'-GATGGTAAATGCTAG-TATAATATTGTTGAAATGAGGGG-CATTTGATAA-3' | MSF12283MUT 5'-GCTATCCATTGGTCTTAGGCCCCA-GG-3' |
| 12308 | MS-PCR | M12404R 5'-AGGGTGGTAAG-GATGGGGGGAATTAG-3' | MSF12261NOR 5'-TCTCAAACCTTTTAAAG-GATAACACGTATCCATTGGTCTTA-GGCCCTAAA-3' | MSF12358NOR 5'-ACCACCCTAACCCCTGACATTCACAG-ATTCCCCCATCCTTACCGCCC-3' |
| 12403 | MS-PCR | M12476R 5'-CTGATAATAAAGGTGGAT-GCGACAATG-3' | MSF12379MUT 5'-CTAATTCCTCCCAATC-CTTACCACIT-3' | MSF12922MUT 5'-TGAGACCCCAACAATAAGCCCTT-CTAGC-3' |
| 12950 | MS-PCR | M13054R 5'GGCCTTCTATGGCTGAGG-GGAGTCAG-3' | MSF12902NOR 5'-TTATCCTACACTCCA-ACTCAACAGACCCCAACAATAAG-CCCTTCCAAA-3' | |
| 13708 | <i>SerFI</i> | M13466F 5'-GCCTAGCATTAGCAGGAA-TACC-3' | M13925R 5'-GGGTAGAATCCGAGTATG-TTG-3' | |
| 14110 | <i>EatI</i> | M13843F 5'-CCTCAACTACCTAACCAA-CAAAAC-3' | M14369R 5'-TGGTGCTGTGGGTGAAAAG-AG-3' | |
| 14766 | MS-PCR | MSF14718NOR 5'-ATCGTTGTATTTCAA-CTACAAAGATGACCAATGACCCCAA-TACGCAGAAT-3' | MSF14741MUT 5'-ACAGCAATGA-CCCCAATACGCAAAGC-3' | M14837R 5'-ATCATGCGGAGATGTTGGATGGGGTGG-3' |
| 14798 | MS-PCR | M14730F 5'-CAACTACAAGAACACCAA-TGACCCCAATACG-3' | MSR14823MUT 5'-TTGGATGGGGTGGG-AGGTCGATGGG-3' | MSR14845NOR 5'-GAAAGTTTCATCATGCGGAGATGGG-GGATGGGGTGGGGAGGTCGACGAA-3' |
| 15452 | MS-PCR | M15556R 5'-GGGCTTGATGTGGGG-AGGGGTGTTTAAAG-3' | MSF15407NOR 5'-CAGCCCTTACTA-CACAATCAACTACGCCCTCGGGCTT-ACTTCTCCTCC-3' | MSF15424MUT 5'-CAAAAGACGCCCTCGGGCTTACTTCT-CTTTA-3' |
| 15693 | <i>SspI</i> | M15149F 5'-TGAGGCCAAATATCATTC-TG-3' | M15857R 5'-GGGAGATAGTTGGTATTA-GGATTAG-3' | |
| 15928 | <i>MspI</i> | M15871F 5'-AATACTCAAATGGGCCTG-TC-3' | M16115R 5'-GGTGGCTGGCAGTAATGT-AC-3' | |

NOTE.—The 5' base of each primer is numbered according to Anderson et al. (1981).

^a Detected by use of ³²P-end labeled M449F primer.

Table 3**Confirmed Mitochondrial Genome Polymorphisms in an East Anglian BP Group**

| Nucleotide Position | Nucleotide Change | Amino Acid Change | Locus | Frequency | Reference |
|---------------------|--------------------------------------|-------------------|----------|-----------|--|
| 73 | A→G | ... | att | 19/24 | Kogelnik et al. 1998 |
| 146 | T→C | ... | att; OH | 5/24 | Kogelnik et al. 1998 |
| 152 | T→C | ... | att; OH | 5/24 | Kogelnik et al. 1998 |
| 195 | T→C | ... | att; OH | 8/24 | Kogelnik et al. 1998 |
| 228 | G→A | ... | att; OH | 3/21 | Kogelnik et al. 1998 |
| 263 | A→G | ... | ... | 22/22 | Kogelnik et al. 1998 |
| 279 | T→C | ... | TFX; OH | 1/22 | |
| 295 | C→T | ... | TFX | 2/22 | Kogelnik et al. 1998 |
| 309 | ins C | ... | CSBII | 11/22 | Gill et al. 1994; Parsons et al. 1997 |
| 315 | ins C | ... | CSBII | 23/23 | Kogelnik et al. 1998 |
| 408 | T→A | ... | PL; OH | 1/22 | |
| 462 | C→T | ... | ... | 2/22 | Kogelnik et al. 1998 |
| 489 | T→C | ... | ... | 4/23 | Kogelnik et al. 1998 |
| 497 | C→T | ... | ... | 2/22 | Kogelnik et al. 1998 |
| 499 | G→A | ... | ... | 2/22 | Kogelnik et al. 1998 |
| 514 | (CA) ₄ -(CA) ₇ | ... | TFH | 2/22 | Kogelnik et al. 1998 |
| 573 | ins C | ... | ... | 1/22 | |
| 709 | G→A | ... | 12S | 4/23 | |
| 813 | A→G | ... | 12S | 1/23 | |
| 960 | ins C | ... | 12S | 1/24 | |
| 1189 | T→C | ... | 12S | 4/24 | Rieder et al. 1998 |
| 1438 | A→G | ... | 12S | 24/25 | Kogelnik et al. 1998 |
| 1811 | A→G | ... | 16S | 8/24 | Kogelnik et al. 1998 |
| 1888 | G→A | ... | 16S | 3/24 | Rieder et al. 1998 |
| 3010 | G→A | ... | 16S | 8/25 | Kogelnik et al. 1998 |
| 3107 | ΔC | ... | 16S rRNA | 25/25 | Kogelnik et al. 1998 |
| 4216 | T→C | Y→H | ND1 | 5/22 | |
| 4561 | T→C | V→A | ND2 | 1/23 | |
| 4564 | G→A | G→D | ND2 | 1/23 | |
| 4763 | C→A | I→M | ND2 | 1/23 | |
| 4936 | C→T | T→I | ND2 | 1/25 | |
| 4967 | T→C | synonymous | ND2 | 1/25 | |
| 5187 | C→T | synonymous | ND2 | 1/25 | |
| 5249 | T→C | synonymous | ND2 | 1/25 | |
| 6152 | T→C | synonymous | COI | 1/23 | |
| 6261 | G→A | A→T | COI | 2/24 | |
| 6293 | T→C | synonymous | COI | 1/24 | |
| 6473 | C→T | synonymous | COI | 1/24 | |
| 6554 | C→T | synonymous | COI | 1/24 | |
| 6570 | G→T | A→S | COI | 1/24 | |
| 6671 | T→C | synonymous | COI | 1/24 | |
| 7226 | G→A | synonymous | COI | 1/17 | |
| 7245 | A→G | T→A | COI | 1/17 | |
| 7768 | A→G | synonymous | COII | 1/24 | |
| 8098 | A→G | synonymous | COII | 1/24 | |
| 8496 | T→C | M→T | ATPase 8 | 1/23 | |
| 8512 | A→G | synonymous | ATPase 8 | 1/23 | |
| 8533 | G→A | synonymous | ATPase 8 | 1/23 | |
| 8701 | A→G | T→A | ATPase 6 | 2/23 | Kogelnik et al. 1998 |
| 8705 | T→C | M→T | ATPase 6 | 1/23 | |
| 8706 | A→G | synonymous | ATPase 6 | 1/23 | |
| 8860 | A→G | T→A | ATPase 6 | 23/23 | Kogelnik et al. 1998 |
| 9196 | G→A | D→N | ATPase6 | 1/23 | |
| 9425 | A→C | synonymous | COIII | 1/22 | |
| 9716 | T→C | synonymous | COIII | 1/22 | |
| 10398 | A→G | T→A | ND3 | 7/22 | Kogelnik et al. 1998 |
| 10463 | T→C | ... | R | 3/22 | |

(continued)

Table 3 (continued)

| Nucleotide Position | Nucleotide Change | Amino Acid Change | Locus | Frequency | Reference |
|---------------------|-------------------|-------------------|--------|-----------|----------------------|
| 10907 | T→C | F→L | ND4 | 1/25 | |
| 11840 | C→T | synonymous | ND4 | 1/25 | |
| 11869 | C→A | synonymous | ND4 | 1/24 | |
| 12127 | G→A | synonymous | ND4 | 1/13 | |
| 12308 | A→G | ... | L(CUN) | 9/23 | Kogelnik et al. 1998 |
| 12346 | C→T | H→Y | ND5 | 1/23 | |
| 12397 | A→G | T→A | ND5 | 1/23 | |
| 12403 | C→T | L→F | ND5 | 2/23 | |
| 12714 | T→C | synonymous | ND5 | 1/24 | |
| 12738 | T→G | synonymous | ND5 | 1/24 | |
| 12771 | G→A | D→N | ND5 | 1/24 | |
| 12950 | A→C | N→T | ND5 | 2/24 | |
| 13056 | C→T | synonymous | ND5 | 1/24 | |
| 13111 | T→C | synonymous | ND5 | 1/24 | |
| 13708 | G→A | A→T | ND5 | 2/22 | |
| 13768 | T→C | F→L | ND5 | 1/22 | |
| 14037 | A→G | synonymous | ND5 | 1/25 | |
| 14110 | T→C | F→L | ND5 | 2/25 | |
| 14182 | T→C | synonymous | ND6 | 1/25 | |
| 14198 | G→A | T→M | ND6 | 1/25 | |
| 14766 | T→C | I→T | Cytb | 6/18 | Kogelnik et al. 1998 |
| 14798 | T→C | F→K | Cytb | 4/18 | Kogelnik et al. 1998 |
| 15244 | A→G | synonymous | Cytb | 1/21 | |
| 15247 | C→G | synonymous | Cytb | 1/19 | |
| 15262 | T→C | synonymous | Cytb | 1/19 | |
| 15326 | A→G | T→A | Cytb | 19/19 | Kogelnik et al. 1998 |
| 15452 | C→A | L→I | Cytb | 5/25 | Kogelnik et al. 1998 |
| 15575 | G→A | A→T | Cytb | 1/23 | |
| 15693 | T→C | M→T | Cytb | 2/23 | Kogelnik et al. 1998 |
| 15928 | G→A | ... | T; att | 2/20 | Kogelnik et al. 1998 |
| 16051 | A→G | ... | att | 1/12 | |
| 16224 | T→C | ... | att | 3/12 | Kogelnik et al. 1998 |
| 16311 | T→C | ... | att | 8/24 | Kogelnik et al. 1998 |
| 16519 | T→C | ... | att | 20/24 | Kogelnik et al. 1998 |

Differences from the sequence published by Anderson et al. (1981) in the East Anglian BP group. Five of the changes were observed in all the samples sequenced: at positions 263 A→G, 315 ins C, 3107 ΔC, 8860 A→G, and 15326 A→G. References for previously described variants are indicated.

Sequence Analysis of Mitochondrial Genome

The mitochondrial genome was amplified by use of 11 overlapping primer pairs. Fragments 1448–2446 bp in length were amplified with fragment overlaps 225–785 bp in length. The primer pairs used are shown in table 1.

PCR products were purified (High Pure PCR Product Purification Kit, Roche Diagnostics) and were sequenced (primer sequences available on request) by means of a dRhodamine Terminator Cycle Sequencing kit (PE Applied Biosystems). An ABI 377 semi-automated sequencer was used to produce overlapping sequence reads of the whole mitochondrial genome. Commonly, ≥22 of the 25 amplified products with a given primer from patients with BP produced unambiguous sequence reads of >400 bases (see table 3). Sequences were aligned by

means of Multalin (Corpet 1988), to identify polymorphisms. We confirmed novel polymorphisms, using an alternative sequencing primer, which was usually positioned on the complementary strand to the original sequencing primer.

Polymorphism Analyses of Mitochondrial DNA

Fifteen polymorphisms were typed by means of mutagenically separated PCR (MS-PCR) essentially as described by Rust et al. (1993), seven by PCR followed by restriction digestion analysis, and one microsatellite polymorphism by incorporation of ³²P into the PCR product followed by separation of alleles on a 6% sequencing gel and autoradiography. To compare MS-PCR and restriction digestion PCR, we typed one polymorphism (at position 1888), using both techniques; iden-

Table 4**Allele Frequencies of Each of the 23 Polymorphisms Tested in the BP Cases and Control Groups**

| NUCLEOTIDE POSITION | NUCLEOTIDE CHANGE | AMINO ACID CHANGE | LOCUS | REFERENCE | FREQUENCY | |
|---------------------|--------------------------------------|-------------------|----------|--|--|--------------------|
| | | | | | BP Cases | Control Groups |
| | | | | | Allele 1, Allele 2, Allele 3, Allele 4 | |
| 514 | (CA) ₇ -(CA) ₄ | ... | TFH | Kogelnik et al. 1998 | .03, .09, .79, .09 | .03, .03, .84, .10 |
| | | | | | Allele 1, Allele 2, Heteroplasmy | |
| 295 | C→T | ... | TFX | Kogelnik et al. 1998 | .89, .11, 0 | .88, .11, .01 |
| 309 | ins C | ... | CSBII | Gill et al. 1994; Parsons et al. 1997 | .03, .58, .39 | .05, .54, .40 |
| 514 | (CA) ₇ -(CA) ₄ | ... | TFH | Kogelnik et al. 1998 | .03, .09, .79, .09 | .03, .03, .84, .10 |
| 709 | G→A | ... | 12S | | .88, .12, 0 | .91, .09, 0 |
| 1189 | T→C | ... | 12S | Rieder et al. 1998 | .89, .11, 0 | .91, .09, 0 |
| 1811 | A→G | ... | 16S | Kogelnik et al. 1998 | .81, .19, 0 | .83, .17, 0 |
| 1888 | G→A | ... | 16S | Rieder et al. 1998 | .91, .09, 0 | .94, .06, 0 |
| 3010 | G→A | ... | 16S | Kogelnik et al. 1998 | .69, .28, .03 | .68, .31, .01 |
| 4216 | T→C | Y→H | ND1 | | .77, .23, 0 | .81, .18, .01 |
| 6261 | G→A | A→T | COI | | .03, .97, 0 | .01, .99, 0 |
| 8701 | A→G | T→A | ATPase 6 | Kogelnik et al. 1998 | .96, .04, 0 | 1, 0, 0 |
| 10398 | A→G | T→A | ND3 | Kogelnik et al. 1998 | .73, .27, 0 | .79, .21, 0 |
| 10463 | T→C | ... | R | | .91, .09, 0 | .94, .06, 0 |
| 12308 | A→G | ... | L(CUN) | Kogelnik et al. 1998 | .70, .30, 0 | .75, .25, 0 |
| 12403 | C→T | L→F | ND5 | | .98, .02, 0 | 1, 0, 0 |
| 12950 | A→C | N→T | ND5 | | .98, .02, 0 | 1, 0, 0 |
| 13708 | G→A | A→T | ND5 | | .13, .87, 0 | .13, .87, 0 |
| 14110 | T→C | F→L | ND5 | | .98, .02, 0 | 1, 0, 0 |
| 14766 | T→C | I→T | Cytb | Kogelnik et al. 1998 | .57, .43, 0 | .43, .57, 0 |
| 14798 | T→C | F→K | Cytb | Kogelnik et al. 1998 | .22, .78, 0 | .17, .83, 0 |
| 15452 | C→A | L→I | Cytb | Kogelnik et al. 1998 | .80, .20, 0 | .84, .15, .01 |
| 15693 | T→C | M→T | Cytb | Kogelnik et al. 1998 | .03, .97, 0 | .04, .96, 0 |
| 15928 | G→A | ... | T; att | Kogelnik et al. 1998 | .12, .88, 0 | .07, .93, 0 |

NOTE.—With the exception of the microsatellite at position 514, all polymorphisms were biallelic. A high degree of heteroplasmy was noted in both groups for the insC polymorphism at position 309. PCRs were repeated on a subsample of 48 cases of BP and 48 controls and confirmed the original genotyping.

tical results were obtained. The position of each polymorphism within the mitochondrial genome and the primers used are shown in table 2.

Haplotype Mismatch Distribution

A matrix of the number of mismatches between each pair of haplotypes was constructed, with the microsatellite locus (ID) treated as a simple nucleotide, with no account taken of the magnitude of the length difference between haplotypes. The relative frequencies of pairs differing by i nucleotide sites, where $i = 0, 1, 2, \dots, n$ (where $n =$ number of loci in haplotype), were tabulated and plotted.

Results

We set out to sequence the 16,569-bp mitochondrial genome in a panel of 25 patients selected because their family histories suggested unilineal psychiatric disease inherited from the maternal side. These BP patients reported that their affected relatives had manic-depressive disorder or unipolar depression. Our initial screen at-

tempted to generate sequences for overlapping fragments of the mitochondrial genome for these 25 cases. All cases were sequenced once in the initial screen (see table 3 for confirmed sequence variants).

Our subsequent analyses focused on any sequence variant from the Cambridge sequence (Anderson et al. 1981), found in two or more patients with BP, which was of possible functional significance. We included variants resulting in amino acid substitutions in proteins, changes in rRNAs and tRNAs, and variations in the membrane-attachment sites and in replication origin. Previously unreported variants were confirmed as polymorphisms by sequencing with another primer. Ultimately, our polymorphism analyses (see table 4) confirmed these variants.

Primers were designed to detect these 23 variants, and the allele frequencies at each site were determined in panels of patients with BP and regionally matched controls (see table 4). Allele frequencies were not significantly different for each of the sites (χ^2 tests, $P > .05$). In addition, haplotype frequencies did not differ in the two groups (χ^2 test, $P > .05$).

To investigate the selection against the maternal line-

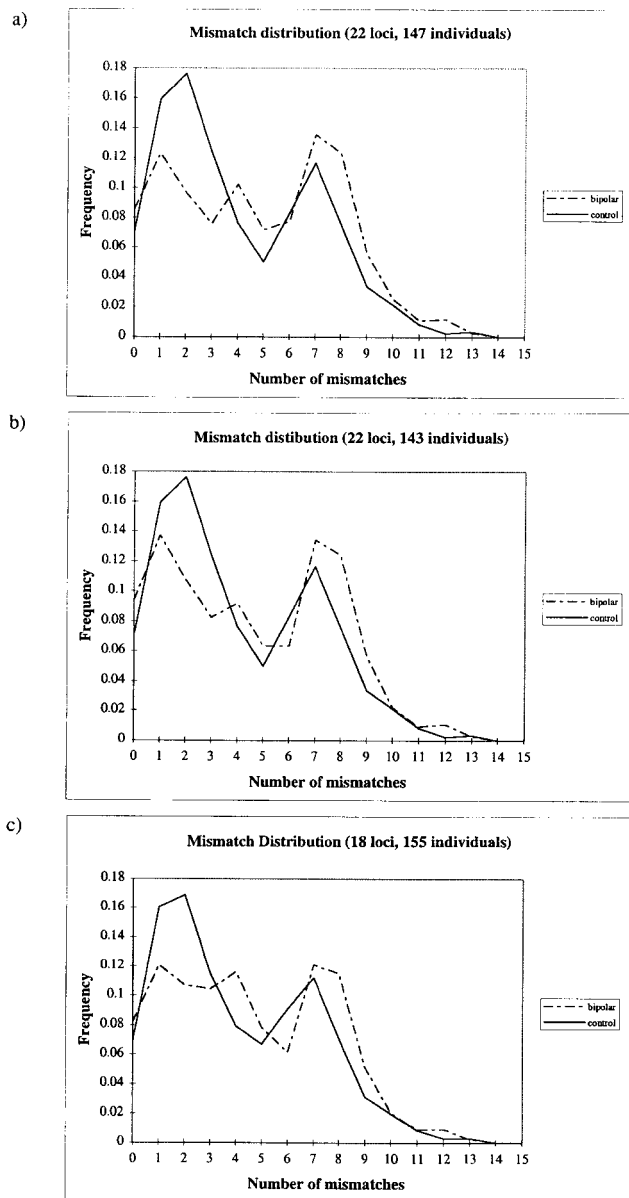


Figure 1 a, Mismatch distributions within BP and control groups, determined by use of 22 polymorphic loci. b, Mismatch analysis within BP and control groups after removal of the individuals with atypical alleles at positions 8701, 12403, 12950, and 14110 (18 variable loci, 71 individuals with BP, 72 controls, and 51 different haplotypes). c, Mismatch analysis within BP and control groups after elimination of all data from positions 8701, 12403, 12950, and 14110 from the analysis (18 variable loci, leaving 77 individuals with BP, 78 controls, and 57 different haplotypes).

age in BP, we determined mitochondrial genetic-distance distributions between all possible pairs of individuals in BP and control groups separately. One locus (insertion at position 309), which exhibited very high levels of heteroplasmy (35 BP cases and 37 control cases), was discarded from further analysis. In addition, all individuals who had an untyped locus or exhibited hetero-

plasmly at one or more of the remaining loci were removed from the data set. This left 75 individuals with BP and 72 controls, which together comprise a total of 53 different 22-locus haplotypes.

For any given pair of haplotypes, similarity can be expressed in terms of the total number of polymorphic sites out of 22 at which they differ. Such mismatch genetic distances were determined for all possible pairs of haplotypes in the BP group alone and in the control group alone, and the resulting data were plotted as mismatch frequency distributions (fig. 1a [Slatkin and Hudson 1991; Rogers and Harpending 1992]). The distributions have both similarities and differences. To examine the significance of the mismatch distribution differences, we used 1,000 bootstrap resamplings to establish confidence limits for the difference between the two distributions at each point along the profile. Differences between BP and control mismatch distributions at specific mismatch scores, which lie outside the 95% confidence limits of the bootstrap simulation, were considered to be significant. The BP group shows, compared with the control group, significant fewer pairs of individuals with haplotypes that differ at two or three loci

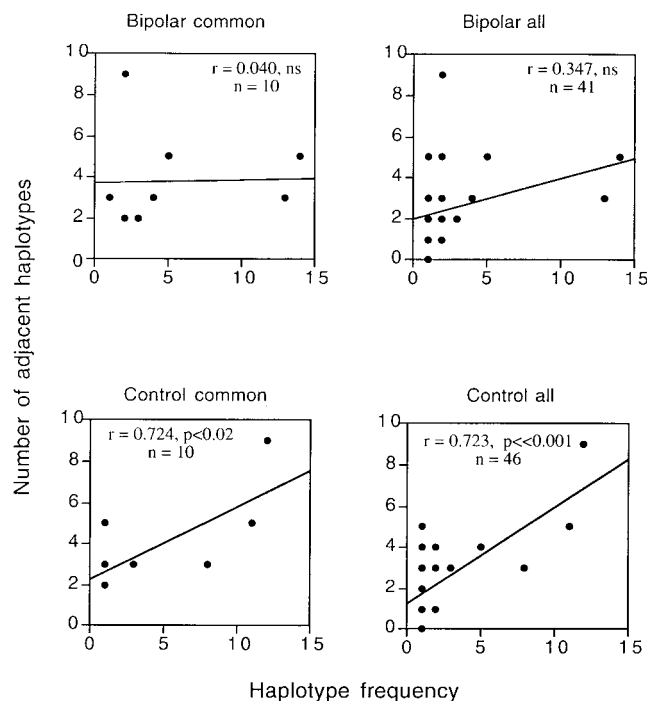


Figure 2 Relationship between mitochondrial haplotype frequency and the number of haplotypes that differ by one mutation from the relevant haplotype. BP and control groups were analyzed separately. The first analysis was performed on all haplotypes after removal of loci (8701, 12403, 12950, and 14110) that were not polymorphic in both the control and BP samples (labeled as “bipolar all” and “control all”). A second, very stringent analysis was also performed, using only those haplotypes present in both cases and controls (labeled as “bipolar common” and “control common”).

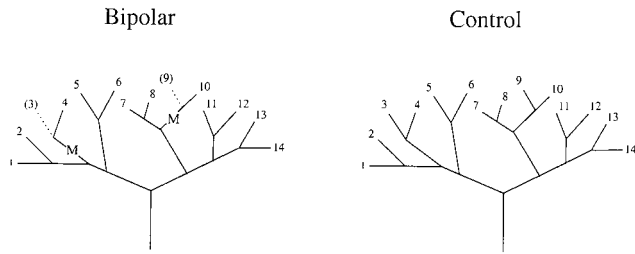


Figure 3 Pictorial description of hypothetical mitochondrial phylogenies showing how weak selection against multiple mitochondrial mutations can distort mismatch analyses without resulting in a detectable change in allele or haplotype frequencies. These panels represent idealized haplotype trees. Haplotypes are labeled 1–14. In the BP tree, different mutations have occurred on distinct branches (labeled M). Some BP patients with haplotypes 4 and 10 have inherited the mitochondrial mutations. Because there has been weak selection against BP in the maternal lineage because of these mutations, there are fewer BP cases with closely related haplotypes (e.g., 3 and 9).

and a significant excess of individuals with differences at eight loci.

A potential ascertainment bias was created by selection of loci that showed variation in the subsample of patients with BP who were sequenced. Such loci, by definition, are likely to be more variable in individuals with BP, and they could cause a slight inflation of BP mismatch scores. Four loci were invariant within the control group (8701, 12403, 12950, and 14110) but reveal alternative alleles in four individuals with BP (two different haplotypes). Two approaches could be used to eliminate the effects of these loci: (1) removal of the individuals with atypical alleles at any of the four loci (fig. 1*b*), or (2) removal of all information for these loci (fig. 1*c*). Both approaches leave 18 variable loci; the former leaves 71 individuals with BP, 72 controls, and 51 different haplotypes (fig. 1*b*), whereas the latter leaves 77 individuals with BP, 78 controls, and 57 different haplotypes (fig. 1*c*). In approach 1, bootstrap significance was achieved at two mismatches (depleted in BP) and at eight mismatches (depleted in controls), whereas, in approach 2, bootstrapping revealed significant differences at two mismatches only (depleted in BP). Thus, for each of the test conditions used, the mitochondrial phylogeny of the BP group appears to have a deficiency of terminal branches that are closely related to one another.

For normal mitochondrial phylogenies, the prevalence of a particular haplotype should correlate with the number of neighboring haplotypes that differ by one mutation, since common haplotypes tend to be old haplotypes that have had time to mutate to adjacent states. Negative selection would be expected to disrupt this relationship. We find a positive correlation between haplotype frequency and the numbers of one-step neighbors

in the control haplotypes, but not in the BP haplotypes (fig. 2). To remove the ascertainment bias mentioned above, the analysis was first performed on all haplotypes after the removal of loci that were not polymorphic, in both the control and BP samples (positions 8701, 12403, 12950, and 14110 were excluded). A second, very stringent analysis was also performed, using only those haplotypes that were present in both cases and controls ($n = 10$). The resulting correlation remains significantly positive in the control sample but, again, is not apparent in the BP group. This pattern supports our conclusion, from the mismatch distribution differences, that BP haplotypes appear to leave fewer descendent states.

Discussion

In an attempt to elucidate the role of the mitochondrial genome in BP, we have used the mismatch distribution approach. This method has been used previously to study population history (Rogers 1995) but, to our knowledge, has not been used as a test for the effects of negative selection acting against particular haplotypes. This approach is best suited to studies of the mitochondrial genome and nonrecombining parts of the Y chromosome. It is unlikely to be of general use for studies of autosomal loci, unless one is analyzing a small cluster of tightly linked markers.

We have analyzed pairwise genetic distances between mitochondrial haplotypes within the BP and matched control groups, and we have found fewer individuals who were closely related to each other in the BP population. This pattern is also reflected in the expected relationship between haplotype frequency and absolute number of nearest-neighbor haplotypes, which is a feature of the control haplotypes but not the BP haplotypes.

Broadly speaking, the mismatch profile can be separated into two components. First, comparisons between haplotypes that lie on different primary branches of the mitochondrial tree give rise to a wide range of mismatch values dominated by larger scores. Second, just as old graveyards often bear witness to the domination of a small number of locally successful families, so some mitochondrial branches also proliferate more than others, giving rise to clusters of closely related haplotypes. Multiple sampling of haplotypes from within these clusters thus contributes the large number of small mismatch scores that dominate the left end of the mismatch profile.

The action of natural selection will distort the shape of the mitochondrial tree, and this will be reflected in the resulting mismatch distribution. In the case of deleterious mutations, most of the effect on the mismatch profile will be seen in the component associated with local clustering. This is because any mitochondrial mutation that lowers the fitness of females who carry it will act to reduce twig production on the branch on which

it arose. A hypothetical model of this situation is shown in figure 3. Under strong selection, such haplotypes disappear as quickly as they arise. Under weak selection, lower rates of lineage survival act to curtail the formation of local clusters of related haplotypes, reducing the relative frequency of small mismatch scores. In this way, detrimental haplotypes can be identified by a deficit of small mismatch scores.

In theory, the exact position of the maximal deficit in the mismatch distribution should be informative about the strength of selection; a large deficit beginning at or near zero suggests strong selection (with local clusters forming rarely, if ever), whereas weaker troughs peaking around one, two, or three mismatches are more compatible with weak selection (with some small clusters forming). Some selection against BP is likely, since patients with "affective psychosis" appear to have decreased fertility: about ~70% of the levels in the general population (Vogel 1979). If certain BP-associated haplotype lineages do suffer reduced longevity because of negative selection, the existence of many large mismatch scores and the absence of a gross difference in mitochondrial haplotype distributions would be compatible with a number of independent origins.

In conclusion, our data show no significant differences in frequency between BP and control mitochondrial haplotypes, but they do show significant differences in the underlying patterns of relatedness of these haplotypes within BP and control groups. East Anglian BP maternal lineages appear deficient in near neighbors, a state that strongly suggests weak negative selection against the maternal line. A number of genetic models can account for this. Such a process can occur either with imprinted genes or with X-linked inheritance. However, in both of these scenarios, the causative mutation is rapidly dissociated from the mitochondrial lineage by passage through the male line. A more parsimonious explanation for our findings is that BP involves low-penetrance mitochondrial DNA variants. Such variants are often successfully transmitted through the maternal line, as seen in the McMahon et al. (1995) and Gershon et al. (1996) families, but, ultimately, their reduced maternal fecundity increases the chance of lineage extinction. The result is a characteristic deficiency of closely related haplotypes, left as a footprint on the mitochondrial tree.

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