Study of the Huntington’s disease (HD) gene CAG repeats in schizophrenic patients shows overlap of the normal and HD affected ranges but absence of correlation with schizophrenia

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Abstract
The CAG repeats in the Huntington’s disease gene were investigated in chromosomes from 71 unrelated schizophrenic persons and 18 patients with schizophrenia-affective disorder in order to determine if any of these patients had abnormal expansions. All of the probands had repeat sizes in the normal range (<35 repeats) and there was no significant difference between the allele distributions of these patients and the normal controls. The families of two patients with 32 repeats and one patient with 34 repeats were investigated further and showed no uniform segregation of the disease with the large repeat alleles. The proband with 34 repeats inherited a chromosome that originally had 36 repeats in her father. The presence of 36 CAG repeats in members of her family and in HD patients suggests that there is an overlap between the normal and Huntington’s disease CAG repeat size ranges. The more recently described CCG polymorphism in this gene was also examined in the schizophrenic and schizoaffective persons. All patients had alleles in the normal range.

Huntington’s disease (HD) is an autosomal dominant condition that usually presents with chorea and cognitive deterioration in middle age. However, not all patients present with chorea and one of the most frequent misdiagnoses of HD is schizophrenia. A number of people have proposed that schizophrenia may be a “forme fruste” of HD.

The gene that is mutated in HD has recently been cloned. At the 5’ end of its transcript is a stretch of CAG repeats that is abnormally expanded and unstable in HD chromosomes. Normal chromosomes are thought to have 34 or fewer repeats while the smallest reported CAG expansions in HD patients have 35 repeats. We and others have identified a trinucleotide length polymorphism in the CCG rich region of this gene immediately downstream of the CAG repeats that varies by at least six repeats. Although this polymorphism does not seem to have any pathogenic role in HD, it has not been investigated sufficiently to rule out variation at this site as a cause for other diseases.

A number of studies support the hypothesis that genetic factors predispose a person to schizophrenia. However, there have been no linkage studies that have been successfully replicated. Since there seems to be a certain degree of overlap in the features of HD and schizophrenia, we have tested the Huntington’s disease gene as a candidate gene in affected subjects from 89 unrelated families that each contained at least two sibs with either schizophrenia or schizoaffective disorder. Severity-one of the probands had schizophrenia and 18 were diagnosed as having schizoaffective disorder. The CAG repeats were measured in order to determine whether a significant proportion of patients with schizophrenic phenotypes had expansions and represented a variant form of HD. The CCG repeats were investigated to rule out the possibility that mutations at this site cause schizophrenia. The distribution of CAG repeats in these patients was similar to that found in 78 unrelated subjects from 22 normal CEPH families. However, two patients had 32 repeats and one patient had 34 repeats. The disease did not uniformly segregate with the expanded repeats in these families suggesting that the expanded CAG repeats do not play a role in the pathogenesis of schizophrenia. The proband with 34 repeats inherited a chromosome that originally had 36 repeats in her father. The presence of 36 CAG repeats in a number of persons in this family and in HD patients suggests that there is an overlap between the normal and Huntington’s disease size ranges. All of the schizophrenic and schizoaffective patients had CCG repeat lengths within the normal size range.

Methods
Families in which there were at least two sibs with either schizophrenia or schizoaffective disorder were identified from three main sources: (1) a cohort of cases receiving clinical care at a district hospital in north west London and its surrounding regions and other cases associated with the National Schizophrenia Fellowship; (2) a USA national registry of families identified from treatment centres within a NY county catchment area (Suffolk county), through clinician referrals, and advertisements throughout the USA, particularly with the aid of the National Alliance for the Mentally Ill; (3) families recruited in a similar manner by psychiatrists from the Institute of Psychiatry of the Uni-

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Figure 1 Pedigree of family 3 and autoradiograph of a gel showing the number of CAG repeats in each person's huntingtin gene. The number of CAG repeats is indicated. This is a family of western European descent with three generations of schizophrenia illness, unilineal paternal inheritance, and an equal number of males and females affected. Mild mental retardation, possibly related to familial hypothyroidism has also been inherited from the maternal side and overlaps, but does not seem to segregate with schizophrenia in the families. All persons had psychotic symptoms suppressed with standard neuroleptic medication. None has evidence of movement disorder. The following descriptions pertain to the persons whose CAG repeats have been measured. The samples that are next to that of subject K are from two Huntington's disease patients who both have 36 CAG repeats in their larger alleles. Sizes were confirmed by comparison to a sequence ladder. A (37): psychosis not otherwise specified may be alcohol induced, paranoid personality disorder, alcohol abuse. B (71): mild mental retardation, hypothyroidism. C (42): schizophrenia, chronic undifferentiated (first hospitalised aged 19). Symptoms include multiple delusions, disorganised and bizarre behaviour, auditory hallucinations, questionable history of seizure disorder. D (46): schizotypal personality disorder, major depression, mental retardation. E (15): psychosis not otherwise specified, mental retardation. F (37): schizophrenia, chronic undifferentiated. Symptoms of recurrent depression also present. Has multiple delusions, probable thought disorder, learning difficulty, alcoholism. G (48): major depression, recurrent. Has had episodes of nocturnal hallucinations (visual). Also has asthma, migraine, rheumatoid arthritis. She had a cleft palate and right facial hypoplasia that required operations in childhood. Had Jacksonian epilepsy since childhood. She has had tremors in right facial muscles since she was very young. H (24): mental retardation, autism. I (30): depression, not otherwise specified. J (46): mental retardation, unipolar depression, recurrent. K (21): chronic undifferentiated schizophrenia, mild mental retardation. Symptoms of schizophrenia include multiple delusions, auditory hallucinations, formal thought disorder. Generalised seizure disorder, unknown type. Inherited platelet disorder according to family history.

Figure 2 Population distributions of the numbers of CAG repeats in affected subjects from 89 unrelated families that contained at least two sibs with either schizophrenia or schizoaffective disorder and 78 normal controls. Seventy-one of the affected probands had schizophrenia and 18 had schizoaffective disorder. Pairwise analysis of the allele sizes in these two populations (using Friedman's \( \chi^2 \) test) showed no significant difference (\( \chi^2 = 0.615, p = 0.433 \)).

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versity of Milan, Italy, from surrounding northern Italian communities. Blood samples were collected and DNA prepared from all available first degree relatives and affected subjects from these predominantly nuclear families. Ethical approval has been granted for the use of these samples for linkage analysis and for the investigation of candidate genes.

All of these persons were evaluated by a trained clinician. Subjects were interviewed using a modified SADS structured format (Schedule for Affective Disorders and Schizophrenia, Spitzer and Endicott, 1978). Records from previous admissions to hospital and of psychiatric treatment were obtained, and further information was collected on each person from reliable family members. Diagnoses were
made, based on information from these multiple sources using DSM-III-R (APA, 1987) criteria for major psychiatric and schizophrenia spectrum personality disorders. We investigated 71 probands with schizophrenia and 18 probands with schizoaffective disorder. None of them had any movement disorder. DNA from family 1 and two members of family 2 was extracted from lymphoblastoid lines but DNA from families 3 and 4 was extracted from whole blood. DNA was analysed for the CCG repeats as described previously. Any unusual or ambiguous results for the CCG polymorphism were confirmed by using both of the above methods. The CAG repeats were analysed as described exception that $x^{2}$P-dCTP and a 3:1 ratio of 7-deaza-dGTP to dGTP was used. DNA from two HD patients with 36 CAG repeats was kindly provided by Dr John MacMillan. A detailed clinical history of family 3 can be found in the legend to fig 1. The accuracy of our CAG repeat sizing has been kindly confirmed by Dr Jon Warner to be within one trinucleotide repeat length of their sizing. Statistical analysis was performed using SPSS/PC+ software, SSPC Inc, Chicago, Illinois.

Results

The CAG allele size distributions in the 89 affected probands from families with schizophrenia or schizoaffective disorder and 78 normal controls are shown in fig 2. The overall shapes of the curves are similar and pairwise analysis of the allele sizes in the two populations (using Friedman’s $x^{2}$ test) showed no significant difference ($x^{2}=0.615$, $p=0.433$). No significant differences emerged when these patients were subdivided into schizoaffective (18 persons) and schizophrenic (71 persons) groups and compared in the same way with the normal controls ($x^{2}=1.885$, $p=0.170$ and $x^{2}=0.000$, $p=1.000$, respectively). Although there was no significant difference between the patient and control CAG size distributions and all of the probands’ chromosomes were in the normal size range (34 or fewer repeats), the presence of three alleles with 32 or more repeats in the patient group was unusual. The possibility that these large alleles were of pathogenic significance was considered by investigating the families (figs 1, 3, 4). In family 1 it was notable that each son inherited different large alleles from their mother (fig 3). In family 2 (fig 4) there are both unaffected subjects with 32 CAGs and affected subjects with more typically normal repeat numbers suggesting that the large normal CAG repeats are not causing the disease in this family. The chromosome of the proband (subject C) in family 3 (fig 1) that has 34 repeats is derived from her father’s chromosome with 36 repeats that lost two repeats through meiotic instability. Non-paternity is unlikely in this family as we have never detected an allele with 34–36 repeats in more than 450 normal chromosomes that have been typed. In addition, the patient with the new CAG allele had the identical X chromosome alleles to her father at the six informative sites that were analysed (T Crow, R Lothhouse, unpublished data). Although there are no unaffected subjects with 36 CAG repeats in this family, there are affected subjects with typically normal repeat numbers. Thus, it is unlikely that the disease in these families is caused by the CAG expansions unless there are modifying factors that alter penetrance of the disease.

All of the schizophrenic and schizoaffective probands had CCG repeat lengths within the previously reported normal size ranges of 7–12 repeats (CCG13,110 chromosomes, CCG8, four chromosomes, CCG5, six chromosomes, CCG11, seven chromosomes, CCG10, 56 chromosomes, CCG12, one chromosome). The CCG12 chromosomes are very rare as only one such chromosome out of 205 was detected by Andrew et al, and we have not seen a chromosome with 12 CCG repeats in more than 220 normal persons (D C Rubinstein et al, manuscript in preparation). However, the presence of the CCG12 allele in the unaffected father in family 4 (fig 5) suggests that it is not pathogenic. Furthermore, we have recently identified a chromosome with CCG2 repeats in a normal subject, which suggests that 12 CCG repeats is within the range of normal variation.

Discussion

The results of this study suggest that few, if any, persons with schizophrenia or schizoaffective disorder have expanded CAG repeats in their huntingtin genes. The presence of 34 and 36 CAG repeats in affected subjects in family 3 (fig 1) cannot be ignored, since previous reports have suggested that normal persons have up to 34 repeats and HD patients have 35 or more repeats. (Two HD patients with 36 repeats are shown in fig 1 for comparison.) It is possible that this family could have inherited genes for behavioural disorder(s) in addition to HD (that was either misdiagnosed or masked by another illness or has yet to present). It is unlikely that HD will present in subject A who has 36 repeats, as he was 87 at the time of sampling and was free of any atypical movements. This might represent the overlap of the HD and normal ranges that was previously observed by Snell et al who determined the CAG repeat number with a PCR assay that encompassed both the CAG repeats and the CCG polymorphism. It is possible that at least some of the overlap that was observed by Snell et al was artefactual and was the result of a mis-calculation of the CAG repeat sizes owing to the incorrect assumption that the CCG rich region immediately downstream of the CAG repeats was of invariant length. Unfortunately, most of the major studies (including references 11, 12, and 13 but not 4 or 6) that have examined the CAG repeats in the Huntington’s disease gene have used PCR assays that contain the polymorphic CCG repeats and are likely to have inaccurate sizing for many of their chromosomes. Further detailed studies will be needed to determine the pathological significance of alleles with 32–36 repeats and whether there is indeed an overlap of the normal and HD ranges. A possible con-
founding factor will be the later onset and milder phenotype that correlate with lower numbers of CAG repeats in the abnormal range. 6-8 Such persons might not always be ascertained as having HD. Greater certainty of the precise CAG repeat size ranges in normals and HD patients is of obvious diagnostic importance. Confirmation of the presence of an overlap between these two size ranges by other workers will strengthen any arguments that there may be other factors in addition to the CAG repeats that affect the development of HD.

Meiotic instability of the CAG repeats was detected in family 3. This family provides possibly the first and only “prospective” study of the stability of a large normal allele so far. It is notable that the 36 CAG repeats are transmitted unaltered six out of seven times and two of the transmissions occurred consecutively through the male line. The only evidence of meiotic instability was a size reduction of two CAG repeats. This may suggest that 36 repeats are neither as unstable nor as prone to mutation as previous studies that examined de novo HD mutations have suggested. 9-11 This underlines the difficulties involved in interpreting retrospective studies that incorporate selection bias.

The CCG repeat sizes in the patients with schizophrenia and schizoaffective disorder were all in the normal range. The CCG12 allele found in family 4 is certainly very rare as it has only been detected once previously. 8 However, it is unlikely to be pathogenetic as we have discovered a CCG1, chromosome in an apparently normal control and the CCG12 chromosome was found in a normal subject in family 4.

In general, we conclude that abnormalities in the HD gene are not associated with schizophrenia or schizoaffective disorder. The finding of 36 repeats in members of family 3 suggests either that this family has a variant form of HD in addition to other behavioural mutations or that there is a real overlap in the repeat sizes found in HD and normal subjects.

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