Y chromosome haplogroups in autistic subjects

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The male to female ratio in autism is 4:1 in the global autistic population, but increases to 23:1 in autistic subjects without physical or brain abnormalities.[1] Despite this well-recognised gender difference, male predisposition to autistic disorder remains unexplained and the role of sex chromosomes is still debated. Numerical and structural abnormalities of the sex chromosomes are among the most frequently reported chromosomal disorders associated with autism. However, genome scans have failed to detect linkage on the X chromosome[2-4] and this approach cannot study the non-recombining region of the Y chromosome. In this study, we searched for a specific Y chromosome effect in autistic subjects. Using informative Y-polymorphic markers, the Y chromosome haplotypes of 111 autistic subjects from France, Sweden and Norway were defined and compared with relevant control populations. No significant difference in Y-haplotype distribution between the affected and control groups was observed. Although this study cannot exclude the presence of a Y susceptibility gene, our results are not suggestive of a Y chromosome effect in autism.

A Y chromosome role in autism has been proposed since structural abnormalities[5-7] and aneuploidy[8-12] of the Y chromosome have been reported in boys with pervasive developmental disorders or autism. Furthermore, males with a 47, XYY karyotype exhibit a high incidence of cognitive, language, and behavioural deficits[13,14]. Taken together, these findings suggest a direct role of Y chromosome in autistic disorder.

Several Y-chromosome genes are expressed in the central nervous system (CNS)[15-18] and therefore are candidates for the establishment of the sexual dimorphism of the human brain. These include the transcription factors SRY (sex determining region Y) and ZFY (zinc finger protein, Y-linked), which are transcribed in the hypothalamus and in the cortex of the adult male human brain.[16] The protein kinase Y (PRKY) possesses a close mouse homologue, *Pka*, which is only expressed in brain and involved in neuronal differentiation.[15] In addition, the Y-linked protocadherin (PCDHY), expressed predominantly in the brain, is a member of the cadherin superfamily involved in cell-cell recognition.[17] Cadherins are also important in the regionalisation, morphogenesis and fibre tract formation in the CNS.[17] Both PRKY and PCDHY are localised on Yp11.2 with X homologues (PRKX and PCDHX) on Xp22.3 and Xq21.3, respectively.[15,17] PRKX and PRKY are candidates for autism since three cases of autistic females with overlapping deletions of the Xp22.3 region containing PRKX have been reported[19]. Furthermore, an X-Y homologous locus for a cerebral asymmetry gene within the non-recombining portion of the Y chromosome has been postulated on the basis of the deficits associated with sex chromosome aneuploidies.[20]

Most of the Y chromosome is made up of a long non-recombining region of ~60 megabases.[21] Polymorphic markers on the non-recombining region can determine Y chromosome lineages (or haplogroups) which are monophyletic groups of Y chromosomes defined by shared allelic states at slowly mutating binary markers.[22] Due to their non-recombining nature, these polymorphisms are in tight association with functional genetic variations associated with Y-linked phenotypes. Thus, if a Y-

linked genetic factor predisposing males to develop an autistic phenotype is present in a given population, two scenarios can be envisaged. First, a functional variation predisposing to autism could have appeared before the generation of the polymorphisms that define Y chromosome haplogroups. In this case, all Y chromosomes belonging to these haplogroups will contain the susceptibility variant. This situation is, however, highly unlikely since the incidence of autism should exhibit geographical variation among populations, which is not the case. The alternative possibility is that a predisposing variation arose on a single Y chromosome haplogroup background. In this case, due to the absence of recombination on the Y-chromosome, the susceptibility variant would be in strong association with the polymorphisms defining the haplogroup. As a consequence, the frequency of this haplogroup would be higher in the autistic subjects compared to the control population. Indeed, several positive associations have already been reported between Y-chromosome haplogroups and various phenotypes including predisposition to XX male sex reversal,[23] infertility,[24] and alcoholism.[25]

In this study, we searched for a specific Y chromosome effect in autistic subjects. To test this hypothesis, we defined Y-chromosome haplogroups in 111 autistic subjects from France, Norway, and Sweden and compared their frequency distribution to the relevant control populations. A set of 10 polymorphic markers, defining 12 Y-chromosome haplogroups in European populations, was analysed. The frequency distribution of Y chromosome haplogroups in autistic males is indicated in Table 1, together with those of relevant control groups. The most frequent haplogroups in the French autistic and control males were hg1 (47% *vs* 50%), hg2 (25% *vs* 25%), and at lower frequencies, hg9 (13% *vs* 5%) and hg21 (9% *vs* 7.5%). For statistical comparisons, the Scandinavian control population was constructed respecting the same geographic origin ratio of the autistic sample (Sweden:Norway, 2:1). The most frequent haplogroups in the Scandinavian autistic and control males were hg2 (39% *vs* 39%), hg3 (22% *vs* 25%), hg1 (22% *vs* 26%), and hg16 (8% *vs* 6%).

The Y-haplogroup distribution among autistic males is consistent with the haplogroup distribution in the general European population. Hg1 is the most represented in western European populations, showing an increased frequency towards Western Europe[25]. Hg2 is widely distributed across the whole European landscape and hg3 has a maximum frequency in Eastern Europe, with a decreasing frequency towards the south-east and south-west[25].

Overall, there is no significant difference in haplogroup distribution between the autistic subjects and the relevant general population. An analysis was performed on samples divided by geographic region using Fisher exact test. This indicated no significant differences between autistic and control males in any of the two populations (French, $P = 0.54 \pm 0.003$; Scandinavian, $P = 0.27 \pm 0.003$). Moreover, French and Scandinavian autistic populations were significantly different ($P < 0.0005$), as were their control populations ($P < 0.0005$), indicating that, within the population of subjects affected with autism, males shared less similitude among them than they did when compared with their own relevant control populations.

Taken together, these results indicate that there is no specific Y chromosome haplogroup in association with autism. However, a direct role of one or more Y chromosomal genes in the

predisposition to the syndrome cannot be excluded. Indeed, the Y chromosome has a relatively high frequency of *de novo* point mutations or deletions compared to other chromosomes, so the appearance of neo-mutations leading to predisposition to autism would not be detected by a simple definition of haplogroups.

In conclusion, within the limits of association studies, this investigation supports the absence of a specific Y chromosome effect in autism but analysis of candidate genes may be necessary to exclude a direct role of the Y chromosome in autistic disorder.

Methods

Subjects

Families with one or more autistic children were recruited at specialized clinical centers in France, Norway, and Sweden, as part of the Paris Autism Research International Sibpair (PARIS) study. All autistic subjects fulfilled the DSM IV criteria for autistic disorder and the Autism Diagnostic Interview Revised (ADI-R) algorithm for ICD-10 childhood autism. Subjects were included only after thorough clinical, neuropsychological and neurological examination described elsewhere.[3] Cases diagnosed with associated organic conditions or other established chromosomal disorders were excluded. The study was approved by the ethical committees of the collaborating institutions. Informed consents were obtained from the parents of each child included in the study.

DNA and statistical analyses

DNA was extracted from whole blood or lymphoblastoid cell lines. A total of 10 biallelic markers were used in this study and were chosen on the basis of their polymorphic status in European populations[25]. Analysis of these markers was performed as already described.[26] Analysis of these markers was performed as already described.[27] Comparison between autistic and control populations was performed using the ARLEQUIN software.[28] The test of population differentiation assays significant departures from the null hypothesis of random distribution of alleles (or haplogroups) among population pairs.[29] A Markov chain of 100 000 steps was used and the significance level of the test was set at 5%.

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Table 1 Y chromosome haplogroups in autistic subjects and control populations

	Haplogroups (%)											
	1	2	3	4	7	8	9	12	16	21	22	26
French autistic subjects N= 75	35 (47)	19 (25)	2 (3)	0	0	0	10 (13)	0	1 (1)	7 (9)	1 (1)	0
French controls N= 40	20 (50)	10 (25)	2 (5)	0	0	1 (2.5)	2 (5)	0	0	3 (7.5)	2 (5)	0
Scandinavian autistic subjects N= 36	8 (22)	14 (39)	8 (22)	0	0	0	0	2 (6)	3 (8)	0	1 (3)	0
Scandinavian controls N= 100	26 (26)	39 (39)	25 (25)	0	0	0	2 (2)	0	6 (6)	2 (2)	0	0