THE GILLES DE LA TOURETTE SYNDROME
a psychiatric - genetic study

Het syndroom van Gilles de la Tourette
Een psychiatrisch - genetisch onderzoek

PROEFSCHRIFT

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The shock of the real. For a little while we are again able to see, as the child sees, a world of marvels. For a few moments we discover that nothing can be taken for granted, for if this ring of stone is marvelous then all which shaped it is marvelous, and our journey here on earth, able to see and touch and hear in the midst of tangible and mysterious things-in-themselves, is the most strange and daring of all adventures.

- Edward Abbey. *Desert Solitaire.* 1968.

To: Trees, Marleen, Liesbet & Fleur

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CONTENTS

Preface

The Gilles de la Tourette syndrome is a chronic neuropsychiatric disorder, usually with onset in childhood. The characteristic symptoms are motor, vocal, sensory and cognitive tics, often accompanied by echo- and coprophenomena, all in a characteristic waxing and waning course. In clinicial GTS populations obsessive-compulsive symptoms, attention deficit disorder with hyperkinesia, conduct disorder and self-injurious behaviors are frequently observed (1).

The estimated number of patients with GTS in the Netherlands is between 5,000 and 1,0000 according to the prevalence rates found in the first population-based epidemiological study, carried out in Israel (1). From a mental health policy point of view the GTS-problem may seem small by qualitative and quantitative standards. The fatality seems negligible. However, the occasionally devastating impact of the disorder on the perspective of a normal life, was one of the motivations to start a psychiatric-genetic study of GTS. The predominant idea was that finding clues for the (molecular) biologic mechanisms leading to the striking symptoms, might also open perspectives on other neuropsychiatric conditions.

The syndrome is named after Georges Gilles de la Tourette, who was a prominent clinician at the Hôpital de la Salpétrière in Paris at the end of the 19th century. He is described as a man of action - a man in constant motion, driven by unbridled curiosity and a consuming search for the unadorned truth, being inordinarely fond of history and literature (2). Besides publishing on hysteria, he formulated the concept of 'l'incoordination motrice', assuming a relation between tics and hyperekplexias, described by Beard in 'the jumping Frenchmen of Maine' (4,5). Shortly thereafter Charcot decided that 'l'incoordination motrice', was distinct from hyperekplexia and bestowed Gilles de la Tourette's name as an eponym on the disease (6). Recently, the gene for hyperekplexia has been mapped on chromosome 5q (7).

Neither Itard, who described the syndrome in 1825 as 'nevrose' and 'affection convulsive', nor Gilles de la Tourette, who named it 'l'incoordination motrice' have used the word tic, although the term tic was already used by Bell to describe 'spasmodic twittering' (4,8,9). The etymology of the word tic is unclear. In German the word 'ticken' means slapping, touching lightly. It has also been suggested that it originated from the description of sudden movements seen in goats (ticchio = goat [Italian]; Zichen = young goat [German]) (10). However, at the moment that physicians aspecifically started to use the term, as Meige and Feindel remarked, "wurde es ... ein wenig Mädchen für alles"

(10).

The severity of the GTS symptoms differs from person to person and may vary within an individual during different periods in his life following the characteristic waxing and waning course. Mild forms of GTS consist of just a few minor tics that frequently are considered by the affected person as "just a funny habit" rather than as a disease. Despite the rather rigid criteria for classification according to the DSM-III-R, the clinical picture can be quite variegated as described by Sacks (11):

> *"At one extreme is the stereotypic form with its simple motor tics, iterations, perseverations, and brief, explosive vocalisations. At the other extreme is an elaborate, innovatory, phantasmagoric form which is especially remarkable for its mimicry, antics, playfulness, extravagance, impudence, audacity, inventions, dramatisations, unexpected and sometimes surreal associations, intense and uninhibited affects, speed, 'go', vivid imagery and memory, hunger for stimuli and incontinent reactivity, and constant reaching into the inner and outer worlds for new material to Tourettise, to permute and transform".*

Many GTS patients excel in (rhythmic) musical performance and sports like jiujitsu, due to their quicksilver motor abilities. These positive aspects for few GTS patients must not distract from the distress and suffering of the patients with severe GTS. The dark side of the syndrome is characterized by social rejection, occupational and relational problems, depression, neuropathies caused by severe motor tics, debilitating self-injurious behaviors and, occasionally, suicide (12-15). The outcome of treatment, that consists of (supportive) counseling, pharmacotherapy with clonidine primarily in children, neuroleptic drugs (like pimozide, sulpiride or flupentixol), and selective serotonine reuptake inhibitors (like fluvoxamine and fluoxetine), and/or (behavioral) therapy, is too often not satisfactory, especially in the more severe cases (7). It may be expected that the elucidation of the genetic mechanisms underlying GTS will contribute significantly to a more fundamental insight into pathogenesis and eventually in therapy.

In this thesis the Dutch study on the genetics of the Gilles de la Tourette syndrome is described. The aim of the study was to identify GTS families in the Netherlands, to study the patterns of inheritance by formal genetic analysis and DNA analysis for linkage and mapping

studies of the responsible gene(s). A linkage analysis attempts to identify the chromosomal localization of (a) certain disease or trait related genes by studying the simultaneous segregation (cosegregation) of chromosomal specific polymorphic markers with the disease (or trait) genes. It is a first step in chromosomal localization and identification of the gene(s) themselves. Knowledge about the chromosomal localization would be the first step towards the elucidation of the etiology and pathogenesis of GTS. Started as a joint project of the Departments of Psychiatry and Clinical Genetics of the Erasmus University / University Hospital Rotterdam-Dijkzigt in 1987, it became part of an international collaborative study under the aegis of the American Tourette Syndrome Association. The collaborating groups are mentioned in Addendum 1. Collaboration is essential in efforts to map genes responsible for complex disorders.

As the search for the GTS gene(s) still continues, this thesis reports a 'state of the art' of the process. Part 1 is a review of the literature on the genetics of GTS. In Part 2 the clinical findings in the first ten families, nine of Dutch and one of Norwegian origin, are described. These families have been completely investigated. Two additional families are still in the process of being examined. The available data of these two incomplete families are incorporated in the simulation and linkage study, but not in the segregation analysis. Part 3 deals with statistical analysis of the family data to test various genetic models (Chapter 3.1) and the power to detect linkage in the Dutch families and the collaborative families (Chapter 3.2). In Part 4 examples are given of strategies to direct the random search for the global position of the genes on the human gene map, based on chromosomal abnormalities and on the assumption of candidate genes. A broader insight in the molecular-biological approach is given by P. Heutink in his companion thesis entitled 'Gene Mapping of Complex Disorders' . In Part 5 pilot studies to delineate the phenotype are reported. Particularly a differentation between obsessive-compulsive disorder and the specific nature of 'obsessive-compulsive symptoms' (OCS) in GTS is described. These observations may become fundamental to distinguish OCS in OCD and GTS-specific OCS with regard to the delineation of the GTS phenotype.

REFERENCES

1. Robertson MM. The Gilles de la Tourette syndrome: the current status. Brit J Psychiatry 1989; 154: 147-67.
2. Apter A, Pauls DL, Bleich A, Zohar AH, Kron S, Rateoni G, Dycian A, Kotler M, Weizman A, Cohen DJ. A population-based epidemiological study of Tourette Syndrome among adolescents in Israel. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 61-7.
3. Stevens H. Gilles de la Tourette and his syndrome by serendipity. Am J Psychiatry 1971; 128: 489-92.
4. Gilles de la Tourette GAEB. Etude sur une affection nerveuse caractérisée par l'incoördination motrice accompagnée d'écholalie et de coprolalie. Arch Neurol 1885; 9: 19-42, 158-200.
5. Beard GM. Remarks on 'jumpers or jumping Frenchmen'. J Nerv Ment Dis 1878; 5: 526.
6. Dordain G. Le concept de tic dans l'histoire des movements anormaux. Rev Neurol 1986; 142: 803-7.
7. Ryan SG, Sherman SL, Terry JC, Sparkes RS, Torres MC, Mackey RW. Startle disease, or hyperekplexia: Response tot clonazepam and assignment of the gene (STHE) to chromosome 5q by linkage analysis. Ann Neurol 1992: 31: 663-8.
8. Itard JMG. Mémoire sur quelques fonctions involontaires des appareils de la locomotion, de la préhension et de la voix. Arch Gen Med 1825; 8: 385-407.
9. Lees E. Tic and related disorders. Churchill Livingstone, Edinburgh 1985.
10. Meige H, Feindel E. Der Tic, sein Wesen und seine Behandlung. Deutiche, Leipzig 1903.
11. Sacks O. Tourette's syndrome and creativity. Brit Med J 1992; 305: 1515-6.
12. Robertson MM, Trimble MR, Lees AJ. The psychopathology of the Gilles de la Tourette syndrome: a phenomenological analysis. Brit J Psychiatry 1988; 152: 383-90.
13. Goetz C, Tanner C, Klawans H. Compressive neuropathies in Tourette Syndrome. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York, 1982; 345-7.
14. Robertson MM. Self-injurious behavior and Tourette syndrome. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 105-14.
15. Eapen V, Trimble MR, Wetering BJM van de, Robertson MM. Suicide in Gilles de la Tourette syndrome: A report of two cases. Submitted.

1. INTRODUCTION

1. The genetics of the Gilles de la Tourette syndrome: A review

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INTRODUCTION

The hallmarks of the syndrome described by Gilles de la Tourette were apparent from his 1885 paper: a neurological condition with uncoordinated movements associated with uncontrolled speech, like echolalia and coprolalia (1). This condition received his name as an eponym upon the suggestion of his teacher Charcot. Georges Gilles de la Tourette suggested in his first observations of 9 patients with motor and vocal tics a genetic etiology. He found a positive family history for neurologic disease in five and for tics in two families. However, in the subsequent 65 years psychologic theories predominated the clinical view on the Gilles de la Tourette syndrome (GTS) (2-8). Familial occurrence was mainly of interest from a family dynamic point of view (9). Arthur and Elaine Shapiro and their collaborators incited in over 50 papers and two books a renewed interest in neuro-biological mechanisms in GTS from the early fifties on (10-13). An excellent comprehensive review on the latest clinical and neuro-biological insights is given by Robertson (14).

Most GTS cases are idiopathic, i.e. without a known cause. The discovery by Seignot (1961) of the therapeutic effects of the dopamine receptor blocking agent haloperidol suggested a disturbance of the central dopaminergic system, especially the basal ganglia and the frontal cortex, in the pathogenesis of GTS (15). Now noradrenergic, serotonergic, GABA-ergic and endorphin systems in the brain are also suggested to be involved in the pathogenesis of GTS (13).

The diagnostic criteria for GTS (table 1) in the revised third edition of the Diagnostical and Statistical Manual of mental Disorders (DSM-III-R) exclude tics occurring during psychoactive substance intoxication or known central nervous diseases like Huntington's disease and postviral encephalitis (16).

Conditions mimicking GTS have been reported secondary to external or environmental events like neuroleptic treatment (17-19), carbon monoxide poisoning (20), gasoline inhalation (21), AIDS (13), post-encephalitic syndromes especially when treated with L-DOPA (22-24), head trauma (25), and angiographic complications (26). Though diffuse CNS damage was documented in these cases, involvement of the basal ganglia appeared to be the greatest common denominator. The initially alarming reports that GTS, tics and also obsessive-compulsive behaviors could be caused by neurostimulant drugs per se seem to be unwarranted (13,27-30). Whether biologic relatives of GTS patients have an increased risk to develop GTS after neurostimulant treatment is still unresolved (13,31-33).

TABLE 1

Diagnostic Criteria for the Gilles de la Tourette syndrome according to DSM-III-R (16)

A.	Both multiple motor and one or more vocal tics have been present at some time during the illness, allthough not necessarily concurrently.
B.	The tics occur many times a day (usually) in bouts, nearly every day or intermittently throughout a period of more than one year.
C.	The anatomic location, number, frequency, complexity, and severity of the tics change over time.
D.	Onset before age 21
E.	Occurrence not exclusively during Psychoactive Substance Intoxication or known central nervous system disease such as Huntington's chorea and postviral encephalitis

EPIDEMIOLOGY

Occurrence of GTS among populations

GTS with a positive family history for GTS and/or tics was initially described in Jewish, Italian and Northern-European families (11,34,35). However, subsequently it has been observed in nearly every population in the world, including western Europe and Russia, Korea (36), China (37,38), Japan (36,39), India and the Middle East (40,41).

Occurrence of tics among populations

The incidence of tics in relatives of GTS patients must be compared with the tic frequency in the population and 3% seems a minimal estimate (42). However, only limited population studies on tics are available. Simple, transient tics occur in 12 to 16% of American and European children at school age (43,44).

Prevalence of GTS and GTS & tics

The first estimates on the prevalence of Gilles de la Tourette syndrome were based on probably heterogeneous clinical samples. Koester (1899) reported 2 in 2500 outpatients and Ascher (1948) 4 in 59,000 in- and outpatients (45,46). Burd et al (1986) reported, based on questionnaires send to health professionals in North Dakota, prevalence rates of 1 per 10,000 girls and 9.3 per 10,000 boys at school age and much lower prevalence in adults: 0.22 per 10,000 for women and 0.77 per 10,000 for

men (47,48). The sex ratio of almost 10:1 in favor of the boys in childhood is much lower in adults (about 3.5:1). In a single school district in Los Angeles, Comings et al (1990) found prevalence rates of 1 in 95 for boys and 1 in 759 for girls (49). In a population based study among adolescents drafted for the army sex-specific prevalences of 4.90/10,000 for males and 3.10/10,000 females have been reported. The overall prevalence was estimated at 4.28/10,000 (50,51). Lucas et al (1982) calculated an incidence rate of 4.6/1,000,000 based on population data in an urban area in the USA (52).

These epidemiologic data have been difficult to interpret and compare for several reasons. GTS is considered to be a relatively rare condition and unfamiliarity may lead to underdiagnosis. Furthermore, large population samples need to be studied using proper diagnostic criteria to obtain reliable data. Moreover, the extent to which more or less severe cases are included will dramatically influence the prevalence rates.

Caine et al (1988) identified 41 Gilles de la Tourette cases from ± 140,000 children at school age in Monroe county (Rochester, NY, USA) using DSM-III criteria (53). Of these cases, 24 had already been diagnosed as GTS before and 17 new cases were diagnosed during the study. Of the 24 earlier diagnosed cases 19 (79%) had associated behavioral problems versus 1 (6%) of the new cases. Obviously more severe or more complicated cases will become diagnosed (and come to medical attention) earlier as compared to mild cases. Furthermore, in cases where associated behavioral problems are more striking than the tics, the medical sophistication of the patient's family and teachers may be key in determining whether an affected child is correctly diagnosed or merely considered to be delinquent. These considerations underscore the need to use proper diagnostic criteria, including attention for classification of mildly affected patients and correction for ascertainment bias, before the 'natural incidence' of tics and of GTS in population samples can be reliably established.

EARLY OBSERVATIONS ON FAMILIAL INCIDENCE

The genetics - environment (or nature - nurture) discussion: anecdotal and incidental reports

It took almost 80 years before Gilles de la Tourette's notion about genetic influences on the etiology of GTS became supported by other clinicians. At the onset of the GTS genetic research in the late sixties and

early seventies positive family histories have been found in about 10% of GTS cases (11,54,55). However, these studies were less systematic and relied on incidental clinical samples of GTS and family histories obtained during clinical work-up (31,55). Additional cases were not systematically evaluated or seen by the investigator, neither were systematic studies performed of all first and second degree relatives. The small recurrence risks in families suggested that "a family history of tics does not characterize patients with Tourette Syndrome" (11). Defense mechanisms consisting of partial introjection of an aggressive parent with tics and the identification with these behaviors have been suggested to explain familial occurrence rather than inheritance (56).

The first report of multiple diagnosed GTS cases in a nuclear family with 6 children concerned two sisters with childhood onset GTS and a formal diagnosis at the age of 28 and 34, respectively (57). These sisters had six children; four of them had definite and obvious multiple motor tics, only one of them had also vocal tics.

Frost et al (1976) did the first extended pedigree study in the family of a GTS patient with tics since age 6 (58). Fourteen of 17 maternal relatives were available and diagnosed according Feighner's Research Diagnostic Criteria using an extensive, structured psychiatric interview. None of the interviewed relatives had GTS, but 6 had tics (1 first degree, 2 second degree and 3 third degree relative(s)). Interestingly one paternal cousin had a temporary tic syndrome during 2 years.

The systematic evaluation of symptoms in relatives was a major methodological improvement and did increase the percentage of positive family histories in (selected) GTS samples to 60-80% (34,59,60). Tics were then found in about 10% of the relatives. In some families both parental lines included affected individuals (34). Several cautious hypotheses about the heredity of GTS emerged at this time, suggesting that GTS was very likely heterogeneous, some forms probably non-genetic, others either caused by autosomal recessive (59) or autosomal dominant inheritance with phenotypic variation and subclinical expression in some of the relatives (35,61).

These reports initiated the next phase in the search of the genetics of GTS, even while it was still impossible to utilize these data for quantitative analyses on patterns of heredity. Improved methodology now prescribed studies of randomly selected families in which all relatives and spouses could be systematically interviewed using standardized methods and reliable diagnostic schedules. Other evidence at that time for the involvement of genetic mechanisms in the etiology of GTS came from adoption and twin studies.

ADOPTION STUDIES

Adoption studies have been very relevant in studies about the 'nature - nurture' interaction, since they provide a way to analyze the expression of genetic factors in a new environment. There is only one study on the occurrence of tics among non-biological adoptive families of 22 adopted GTS patients, compared with the family histories of the biological families of 641 patients using the family history method (13). There was no history of tics in the adoptive families of GTS patients. From the 641 GTS patients 227 had a positive family history in at least one of their first degree relatives. The inclusion of second and third degree relatives raised this number to 302.

A limitation of this method is that precise data on the biological parents and other relatives of the adopted-away patients are generally not available. However the proportion of the differences observed (no GTS or tics in adoptive parents and 35% in the first degree relatives of GTS patients in general) is a strong argument for a genetic mechanism for GTS.

TWIN STUDIES

Monozygotic (MZ) twins are genetically identical and also have shared nearly the same prenatal environment. Dizygotic (DZ) twins differ from sibs only in the fact that they shared the same prenatal environment, genetically they are as different as sibs. If a disorder is purely genetically determined in a mendelian way, MZ twins are generally expected to be 100% concordant for this disorder.

There are anecdotal and incidental observations on DZ and MZ twins, with a larger than expected percentage of MZ twins (probably caused by reporting bias), with variations in age of onset, type and severity of symptoms between cotwins (34,58,59,62-70). These series of twins taken together, more or less rigorously studied for GTS and/or tics show a pairwise concordance of ± 50-70% for GTS and ± 75-90% for GTS and tics together in monozygotic twins (13,58). In the dizygotic twins the concordance rates for both condition were respectively ± 10% and ± 20% (13,58). Apparently, twin studies showed no 100% concordance rate for GTS and/or tics in MZ twins. Nongenetic circumstances like perinatal and postnatal factors may influence the expression of the GTS genetic vulnerability in individuals at risk (71,72). All the affected discordant MZ cotwins in one of these series had a lower birthweight as compared

to the unaffected cotwins (71). Moreover, in a series of MZ twins, concordant for GTS and tics, the cotwin with the lowest birth weight consistently had the most severe symptoms (73).

Twin studies may be very relevant to study the phenotypic relationships between GTS, tics and associated behavioral problems. Anecdotal concordance in GTS twins has been reported for obsessional traits and for attention deficit disorder (32,52,54,64,67,68,70). The delineation of the phenotype, that may possibly be more complex than initially was supposed, now became the most important issue in the diagnostic assessment of GTS families.

SYSTEMATIC FAMILY STUDIES

The aim of the systematic family studies that started around 1980 is to obtain insight in the mode of inheritance by means of segregation analysis and to collect family material for DNA linkage studies (74). Well documented, extended families are the cornerstone of this approach. Direct interviews of all available family members proved to be a more sensitive method to identify affected relatives and establish more reliably the momentary unaffected status of others (75). Supported by the American Tourette Syndrome Association groups in the USA and Europe have collected extended families in a collaborative effort to map the GTS gene. Consensus was reached that chronic and transient tics had to be considered as partial expressions of the gene (35,76-78). Clinical observations and the twin studies indicated that GTS patients have obsessive-compulsive symptoms much more frequently than expected according to the population prevalence for obsessive-compulsive disorder (OCD) (14). On the other hand children with OCD show tics much more often than their matched controls (79). This increased risk for OCD in GTS and for tics in OCD may very well reflect a variable expression of a common genetic diathesis for both conditions. Therefore it was decided to document systematically OCD and OC traits in the families that were part of the study. Other associated behavioral symptoms like attention deficit disorder with hyperkinesia (ADDH) and a variety of psychiatric conditions like anxiety disorders and drug abuse have also been suggested as part of the phenotype and are therefore also systematically recorded (80-85).

The early statistical analyses concluded that a single major gene could be responsible for the susceptibility for both GTS and chronic tics in families with a GTS proband (86-90). The mode of inheritance observed

in most of these studies is consistent with a single major autosomal dominant gene with incomplete penetrance and variable expression. The penetrance for GTS and tics in males is estimated about 0.99 and in females about 0.6 i.e. 99% of the males and 60% of the females with the gene will have at least chronic tics or full-blown GTS (90). When OCD is included as part of the phenotype, the penetrance is raised to 0.70 in females suggesting that OCD is the more frequent expression of the gene in females (91-94). Though ADDH was found to be increased in relatives of GTS probandi, an etiological relationship between ADDH and GTS could not be confirmed by family analysis (95,96).

By widening the GTS-phenotype with OCD, panic attacks, alcohol and drug abuse and behavioral problems, Comings and collaborators hypothesized a semi-dominant, semi-recessive model of inheritance with estimated penetrances of 0.6 for heterozygotes and 0.99 for homozygotes (equally for males and females) (97). Within this model, that assumes a much higher gene frequency than the afore mentioned model, heterozygotes have GTS or tics, while homozygotes have more severe forms of GTS or may show a wide variety of psychiatric syndromes ranging from chronic psychoses to simple phobias or conduct disorder. Such a model might explain, for example, the ≈20% incidence of diagnosible GTS in patients with Asperger's type pervasive developmental disorder (98).

LINKAGE ANALYSIS

The objective of linkage analysis is to correlate the inheritance of a distinct segment of DNA - a 'marker' with known chromosomal localization - with the inheritance of a disease (99). Recent developments in molecular biology have made it possible to clone and analyze a large number of disease genes. In most cases disease genes could be cloned because information was available about the biochemical defect i.e. the protein product or the function of the responsible gene. This strategy of identifying a disease gene is often referred to as 'functional cloning'. An alternative strategy, called 'positional cloning', is used to identify the genes responsible for diseases with unknown etiology, like the majority of neuropsychiatric disorders including GTS. Positional cloning starts with localizing a gene in a particular region of a chromosome. This candidate region is then narrowed down until the responsible gene is identified. Recent examples of genes mapped by positional cloning are those causing Duchenne muscular dystrophy, Huntington's chorea,

cystic fibrosis, neurofibromatosis type I and the fragile-X syndrome (100).

As the etiology of GTS is still unknown, no defective proteins or dysfunctional brain structures can be used to direct the search for the responsible gene. A consortium of groups in the USA and Europe under the aegis of the Tourette Syndrome Association collaborate in an effort of positional cloning the GTS gene. Several strategies can be used to determine the position of the GTS gene on the human genome:

1. Search for chromosomal abnormalities associated with the disease.

Most GTS patients have normal karyotypes. Incidental reports on chromosome aberrations in GTS patients have received interest, since the site of a chromosomal rearrangement might provide a clue to the localization of a GTS gene.

Comings and collaborators (1986) reported a 46,t(7;18)(q22;q22.1) balanced reciprocal translocation in six relatives suffering from GTS (101). This evidence suggested a localization of the GTS gene near the 18q22.1 breakpoint. Donnai (1987) reported a female patient with a mildly hypoplastic mid-face, tic-like movements, mild OCD, panic attacks and visual hallucinations (102). Cytogenetic analysis revealed a deletion of the long arm of chromosome 18 at 18q22.1, providing further evidence that this location might somehow be related to GTS. These findings led to the tentative assignment of the GTS gene to chromosome 18q22.1 by the chromosome 18 committee at Human Gene Mapping Conference IX. In linkage analyses using chromosome 18 probes on Dutch and Norwegian caucasian families, this linkage was refuted with high significance (103).

A de novo deletion, del(9)(qter→p2304:) in a Latin-American male GTS patient was reported by Taylor et al. (104). He was mildly-dysmorphic with microcephaly, prominent supraorbital ridges and slight mid-facial hypoplasia and a number of characteristics of the 9p deletion syndrome in the oral cavity and the fingers. Linkage analysis of chromosome 9p was hampered until now by a lack of polymorphic DNA markers in this region. Several markers on chromosome 9p have been tested by several groups without evidence for linkage. However, since only one of the tested markers maps into the region of the deletion, additional markers will have to be generated in order to obtain more definite results. A partial trisomy 16p has been found in a non-retarded male with GTS and autistic disorder (105).

2. Co-segregation of other inherited disease within GTS families.

For a complex disorder such as GTS, linkage analysis could be simplified if the disorder would cosegregate with another disorder that has already been localized or inherits in a mendelian way. In all the families currently being investigated for linkage analyses on GTS there are no diseases found cosegregating with GTS.

3. Candidate gene approach.

Candidate genes are genes that code for proteins (e.g. enzymes or receptors) that could be involved in the etiology of a hereditary disease. If the candidate gene is in fact the disease gene, it will cosegregate with the disease in the families studied. The candidate genes that have been studied for GTS are summarized in table 2. None of these genes showed linkage with GTS (106,107).

TABLE 2

Candidate genes that have been tested for GTS

Gene	Chromosomal Location
Dopamine receptor D1	5q
Dopamine receptor D2	11q22
Dopamine receptor D3	3q13.1
Dopamine receptor D4	11p15.5
Dopamine receptor D5	4p15.1-p15.3
Prodynorphin	20pter-p12
Pro-opiomelanocortin	2p23
Gastrin Releasing peptide	18q21
Tyrosine hydroxylase	11p15
Dopamine beta hydroxylase	9q34

Association studies are an alternative way of the candidate gene approach, in which the possible, often more remote cosegregation of a specific allele or a genetic marker and the disease is studied in patients as compared to matched controls. The hypothesis tested is whether the co-occurrence happens more frequently than can be expected by chance alone. Association is caused by the marker being located at or in the

near vicinity of the functional mutation in the gene. It should be noted that association can exist with a so-called modifier gene rather than with the gene primarily responsible for the trait under study; in GTS, for example, an association could be found with a gene which made behavioral features more striking and thus increased the likelihood of diagnosis. When modifier genes are involved the phenotype results from the interaction of alleles at more loci. Recently a possible association of the dopamine D_2 receptor locus and GTS was reported (108). Replication of the association study on well defined, large enough groups of GTS patients and also on groups of patients with associated behaviors are necessary. Linkage studies, which should provide more conclusive data, could not confirm this finding (106,107).

4. Systematic screening for polymorphic markers linked to the disease locus.

Until now about 600 markers on the autosomes have been tested trying to map the GTS gene. At the present time, no definite evidence for linkage has been obtained, although several markers produced positive lodscores in a subset of the families (109-111). These lodscores have not yet reached a significant level and need further attention in order to obtain more conclusive results. An exact calculation of the proportion of the human autosomes that have been excluded under homogeneity cannot be carried out for two reasons. Firstly, the exact length of the human autosomal map is not known. Secondly, a number of markers are not exactly localized and therefore it is unknown whether their exclusion zones overlap with those of well localized markers. A conservative estimate, based on only the well localized loci in the largest sex-specific maps, indicates an exclusion of at least 80% of the human autosomes as a possible site for the GTS gene(s). The remaining gaps in the gene map are mainly located on the chromosomes 3,4,6,9,14 and 16. The collaborative groups now focus on these regions. However, additional markers on these chromosomes need to be generated, since the number of well localized polymorphic markers on these chromosomes is still limited.

CURRENT STATUS

While it may be possible that the GTS susceptibility gene(s) is(are) located in the remaining 20% of the genome, it is necessary to consider

that the gene may have been overlooked in the screened part of the genome. It is essential that each step of the procedure, from the ascertainment of the families, the reading of polymorphic markers, and the estimates of the parameters used in the statistical analyses be carefully scrutinized.

The phenotype

The initial optimism that the phenotype was well defined and could be diagnosed objectively during a single observation of the patient has long been left behind. It is now well understood that tics may vary from severe to mild, even to the extent that the ticcing person is unaware of them. Tics may be suppressed consciously or unconsciously during the interviews, making it more difficult to document them. They may also be masked by their conscious or unconscious integration into apparently purposeful movements (for example, a hand-to-face tic may become smoothing the hair). Furthermore, compared to the rapid, sudden tic movements, the slow dystonic tics are often more difficult to detect. Moreover, a diagnosis of GTS of a currently symptom-free individual during examination based solely on a history of a small number of tics during early childhood is not as reliable as a diagnosis based on a variety of observed tics in addition to a positive history.

Complicating the situation even more is the fact that tics seem to be relatively frequent in the general population. It is not clear whether all tics arise from the same genetic diathesis responsible for the full GTS. If not, there is a fair chance that a proportion of the relatives with tics are phenocopies i.e. cases not due to the 'GTS' genes. Phenocopies in the linkage analyses are false-positive cases, and diminish greatly the power of the analyses to detect linkage. If, on the other hand, all tics share a common genetic diathesis the genetic models have to be revised taking into account higher gene frequencies, penetrance rates different than currently assumed and a considerably increased number of homozygotes (or a very high variation in expression).

The weight of the associated behavioral symptoms in the eventual diagnosis may be differently judged in different diagnostic schedules. An ever expanding phenotype makes segregation studies increasingly complex and diffuse. As long as there is no other biological marker for GTS, definite determination of the GTS phenotype will not be realised until linkage is established. Only then it will be possible to separate out accidentally comorbid conditions from the 'associated behaviors' that are an integral part of the syndrome and as such represent a variant expression of the susceptibility gene(s). This will also illuminate the

discussion as to whether the obsessive-compulsive behaviors in GTS are identical to primary OCD at an etiological and phenomenological level. Shapiro and coworkers question the hypothesis that there is an increased rate of OCD in GTS patients as compared to the general population (112). Instead of considering them to be part of an OCD spectrum, we suggest that these 'OCD-like' behaviors are a mental analogue of the motor and vocal tics, quite different from 'classic' OCD symptoms (113,114). Instead of being diagnosed as 'obsessions and compulsions' they may better be called 'impulsions' (i.e disturbances in the impulse control) as was suggested by Shapiro and coworkers (13).

The genetic models

In addition to the diagnostic issues the genetic models need to be reconsidered. As reliable epidemiologic data are still lacking, only rough estimates about the gene frequencies for GTS and tics are available. Assuming a reduced penetrance allows for the existence of asymptomatic gene carriers. In the linkage analyses they are considered false-negative cases that are not as detrimental to the power of the analyses as compared to false-positive cases (phenocopies). If the prevalence of GTS is indeed as high as is suggested in some studies, the autosomal dominant hypothesis will be challenged. Several families have been reported with two affected parents. This bilineality will be more likely in the case of a high gene frequency. But it may also be a function of ascertainment of highly loaded families. Many of the patients (a 25% probability in the offspring of such heterozygous parents) are then homozygous. Until now it is unclear whether the clinical profile of homozygous patients is different from the profile of heterozygous patients. Comings and coworkers reported in the pedigrees they studied the presence of tics and associated behaviors in 45% and associated behaviors alone in 35% on both parental sides (97). They also observed that the probands have more severe GTS symptoms than either parent. They suggest that the inheritance of GTS is more compatible with a semi-dominant, semi-recessive model of inheritance than with the autosomal dominant model.

For instance, if homozygotes have more severe tic symptoms and/or more associated behavioral problems, they tend to become more easily diagnosed than heterozygotes. Especially studies of samples originating from clinical populations including more severe or complicated cases due to referral bias, have to seriously consider such a possibility.

DNA linkage analyses: perspectives

There are several reasons why the current exclusion map of 80% of the genome is suspect. First, there are uncertainties about the definition of the phenotype that may lead to inaccurate estimates of prevalence rates and estimated gene frequencies. As these estimates are included as parameters in the linkage analyses, these inaccuracies may adversely affect linkage results. Second, the rapidly expanding number of new genetic markers will give us a more accurate and refined map of the human genome. Also the precise area's that have been excluded with the presently applied markers and the real gaps in the GTS exclusion maps will become clarified. Moreover, the current exclusion map is based on the assumption of genetic homogeneity i.e. all cases are based on one defective locus. However, in many human disorders genetic heterogeneity (both at the same locus or at different loci) has been found. The clinical picture of a single patient may not reveal which locus or mutation is involved. The different GTS families from the collaborative study must be analyzed both individually and combined to test for heterogeneity. The assumption is that the chance for genetic heterogeneity within one family is very small. Recently developed computer programs allow simulation of linkage to establish the power of the individual pedigrees for studying linkage (115). The most informative families can then be singled out for linkage studies. In addition to this, the influence of each individual in a single pedigree on the lodscore can be tested with simulation programs to reveal the impact of misdiagnoses.

The attempt to unravel the genetics of complex disorders, like GTS, has been compared to untying a gordian knot (116). Unfortunately, we only occasionally are delivered a chance observation that can serve as Alexander's sword! Nonetheless, the failure to date to establish linkage in GTS should not lead to pessimism about psychiatric genetic research. These observations have inspired the opening of new ways to proceed. Novel statistical approaches like sib-pair analysis and the affected pedigree member method are currently applied in addition to linkage analysis (117,118).

Once linkage will be found, the search to clone the GTS gene(s) will start. With the gene(s) in hands, the study on the interaction between the inherited vulnerability and environmental factors in the etiology will come to its ultimate challenge. These insights will provide the future key to a more rational treatment and optimal support for the GTS patient and his relatives at risk.

REFERENCES

1. Gilles de la Tourette GAEB. Etude sur une affection nerveuse caractérisée par l'incoördination motrice accompagnée d'écholalie et de coprolalie. Arch Neurol 1885; 9: 19-42, 158-200.
2. Meige H, Feindel E. Der Tic, sein Wesen und seine Behandlung. Deuticke, Leipzig-Wien, 1903.
3. Ferenczi S. Psycho-analytische Betrachtungen über den Tic. Int Z Psychoanalyse 1921; 7: 33-62.
4. Klein M. Zur Genese des Tics. Int Psychoanal 1925; 11: 332-49.
5. Mahler MS. Tics and impulsions in children: a study of motility. Psychoanalyt Q 1944; 13: 430-44.
6. Mahler MS. Psychoanalytic evaluation of tics: a sign and symptom in psychopathology. Psychoanal Study Child 1949; 3: 279-310.
7. Wilson SA. Tics and allied conditions. J Neurol Psychopath 1927; 8: 93-109.
8. Fenichel O. The psychoanalytical theory of neurosis. Norton Press, New York, 1945.
9. Dunlap JR. A case of Gilles de la Tourette's disease (maladie des tics): a study of intrafamily dynamics. J Nerv Ment Dis 1960; 130: 340-4.
10. Shapiro AK, Shapiro ES. Treatment of Gilles de la Tourette's syndrome with haloperidol. Brit J Psychiatry 1968; 114; 345-50.
11. Shapiro AK, Shapiro ES, Wayne H. Birth, developmental and demographic information in Tourette's syndrome. J Nerv Ment Dis 1972; 155: 335-44.
12. Shapiro AK, Shapiro ES. Tourette syndrome: history and present status. In: Friedhoff AJ, Chase TN (eds). Gilles de la Tourette Syndrome. New York: Raven Press, 1982: 17-23.
13. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
14. Robertson MM. The Gilles de la Tourette syndrome: the current status. Brit J Psychiatry 1989; 154: 147-67.
15. Seignot MJN. Un cas de maladie des tics de Gilles de la Tourette guéri par le R-165. Ann Med Psychol 1961; 119: 578-9.
16. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). American Psychiatric Press, Washington, 1987.
17. Devaugh-Geiss J. Tardive Tourette syndrome. Neurology 1980; 30: 562-3.
18. Klawans HL, Falk AK, Nausieda PA, Weiner WJ. Gilles de la Tourette syndrome after long-term chlorpromazine therapy. Neurology 1978; 28: 1064-6.
19. Mueller J, Aminoff MJ. Tourette-like syndrome after long-term neuroleptic drug treatment. Br J Psychiatry 1982; 141: 191-3.
20. Pulst S-M, Walshe TM, Romero JA. Carbon monoxide poisoning with features of Gilles de la Tourette syndrome. Arch Neurol 1983; 40: 443-4.
21. Koryani EK. Remarkable etiology in Gilles de la Tourette's disease. Psychiat J Univ Ottawa 1977; 1: 5-7.
22. Wohlfart G, Ingvar DH, Hellberg AM. Compulsory shouting (Benedek's 'Klazomania') associated with oculogyric spasms in chronic epidemic encephalitis. Acta Psychiat Scand 1961; 30: 369-77.

23. Sacks O. Acquired Tourettism in adult life. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 88-92.
24. Northam RS, Singer HS. Postencephalitic acquired Tourette-like syndrome in a child. Neurology 1991; 41: 592-3.
25. Fahn S. A case of post-traumatic tic syndrome. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 349-50.
26. Bleeker HE. Gilles de la Tourette's syndrome with direct evidence of organicity. Psychiatry Clin 1978; 11: 147-54.
27. Golden GS. Gilles de la Tourette syndrome following methylphenidate administration. Dev Med Child Neurol 1974; 16: 76-8.
28. Denckla MB, Bemporad JR, Mackay MC. Tics following methylphenidate administration. JAMA 1976; 235: 1349-51.
29. Lowe TL, Cohen DJ, Detlor J, Kremenitzer MW, Shaywitz BA. Stimulant medications precipitate Tourette's syndrome. JAMA 1982; 247: 1729-31.
30. Borcherding BG, Keysor CS, Rapoport JL, Elia J, Amass J. Motor/vocal tics and compulsive behaviors on stimulant drugs: is there a common vulnerability? Psych Res 1990; 33: 83-94.
31. Pollack MA, Cohen NL, Friedhoff AJ. Gilles de la Tourette's syndrome. Familial occurrence and precipitation by methylphenidate therapy. Arch Neurol 1977; 34: 630-2.
32. Bachman DS. Pemoline-induced Tourette's disorder: a case report. Am J Psychiatry 1981; 8: 1116-7.
33. Price RA, Leckman JF, Pauls DL, Cohen DJ, Kidd KK. Tics and central nervous stimulants in twins and non-twins with Tourette syndrome. Neurology 1986; 36: 232-7.
34. Eldridge R, Sweet R, Lake CR, Ziegler M, Shapiro AK. Gilles de la Tourette's syndrome: Clinical, genetic, psychologic and biochemical aspects in 21 selected families. Neurology 1977; 27: 115-24.
35. Golden GS. Tics and Tourette's: a continuum of symptoms? Ann Neurol 1978; 4: 145-8.
36. Nomura Y, Segawa M. Gilles de la Tourette syndrome in Oriental children. Brain Dev 1979; 1: 103-11.
37. Singer K. Gilles de la Tourette's disease. Am J Psychiatry 1963; 120: 80-1.
38. Lieh Mak F, Chung SY, Lee P, Chen S. Tourette syndrome in the Chinese: a follow-up of 15 cases. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 281-3.
39. Kondo K, Nomura Y. Tourette syndrome in Japan: etiologic considerations based on associated factors and familial clustering. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York 1982: 271-6.
40. El-Assra A. A case of Gilles de la Tourette's syndrome in Saudi Arabia. Brit J Psychiatry 1987; 151: 397-8.
41. Robertson MM, Trimble MR. Gilles de la Tourette in the Middle East. Brit J Psychiatry 1991; 158: 416-9.
42. Kurlan R. Preface. In: Kurlan R (ed). The handbook of Tourette's syndrome and associated tic and behavioral disorders. Marcel Dekker, New York, 1993.
43. La Pouse, Monk M. Behavior deviations in a representative sample of children: variation by sex, age race social class and family size. Am J Orthop-

sychiatry 1964; 34: 436-46.
44. Torup E. A follow-up study of children with tics. Acta Paediatr 1972; 51: 261-8.
45. Koester G. Über die maladie des tics impulsifs (mimische Krampfneurose). Deutsche Zeitschr Nervenheil 1899; 15: 147-59.
46. Ascher E. Psychodynamic considerations in Gilles de la Tourette's disease (maladie des tics). Am J Psychiatry 1948; 105: 267-76.
47. Burd L, Kerbeshian J, Wikenheiser M, Fisher W. A prevalence study of Gilles de la Tourette syndrome in North Dakota school-age children. J Am Acad Child Psychiatry 1986; 25: 552-3.
48. Burd L, Kerbeshian J, Wikenheiser M, Fisher W. Prevalence of Gilles de la Tourette's syndrome in North Dakota adults Am J Psychiatry 1986; 143: 787-8.
49. Comings DE, Himes JA, Comings BG. An epidemiological study of Tourette's syndrome in a single school district. J Clin Psychiatry 1990; 51: 463-9.
50. Apter A, Pauls DL, Bleich A, Zohar AH, Kron S, Ratzoni G, Dycian A, Kotler M, Weizman A, Cohen DJ. A population-based epidemiological study of Tourette Syndrome among adolescents in Israel. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 61-7.
51. Apter A, Pauls DL, Bleich A, Zohar AH, Kron S, Ratzoni G, Dycian A, Kotler M, Weizman A, Gadot N, Cohen DJ. An epidemiological study of Gilles de la Tourette's syndrome in Israel. Arch Gen Psychiatry 1993; in press.
52. Lucas AR, Beard CM, Rajput AH, Kurland LT. Tourette syndrome in Rochester, Minnesota, 1968-1979. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 267-9.
53. Caine ED, McBride MC, Chiverton P, Bamford KA, Rediess S, Shiao J. Tourette's syndrome in Monroe County school children. Neurology 1988; 38: 472-4.
54. Kelman DH. Gilles de la Tourette's disease in children: a review of the literature. J Child Psychol Psychiatry 1965; 6: 219-26.
55. Fernando SJM. Gilles de la Tourette's syndrome. A report on four cases and a review of published case reports. Brit J Psychiatry 1967; 113: 607-17.
56. Sanders DG. Familial occurrence of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1973; 28: 326-8.
57. Friel PB. Familial incidence of Gilles de la Tourette's disease, with observations on aetiology and treatment. Brit J Psychiatry 1973; 122: 655-8.
58. Frost N, Feighner J, Schuckit MA. A family study of Gilles de la Tourette syndrome. Dis Nerv System 1976; 37: 537-8.
59. Wassman ER, Eldridge R, Abuzzahab S, Nee L. Gilles de la Tourette syndrome: clinical and genetic studies in a midwestern city. Neurology 1978; 28: 304-7.
60. Wilson RS, Garron DC, Klawans HL. Significance of genetic factors in Gilles de la Tourette syndrome: a review. Behav Genet 1978; 8: 503-10.
61. Guggenheim MA. Familial Tourette syndrome. Ann Neurol 1979; 5: 104.
62. Ellison RM. Gilles de la Tourette's syndrome. Med J Aust 1964; 1: 153-5.
63. Escalar G, Majeron MA, Finavera L, Zamberletti P. Contributo alla conoscenza della sindrome di Gilles de la Tourette. Minerva Medica 1972; 63: 3517-22.
64. Jenkins RL, Ashby HB. Gilles de la Tourette's syndrome in identical

twins. Arch Neurol 1983; 40: 249-51.
65. Waserman J, Lal S, Gauthier S. Gilles de la Tourette's syndrome in monozygotic twins. J Neurol Neurosurg Psychiatry 1983; 46: 75-7.
66. Vieregge P, Schäfer C, Jörg J. Concordant Gilles de la Tourette's syndrome in monozygotic twins: a clinical, neurophysiological and CT-study. J Neurol 1988; 235: 366-7.
67. Walkup JT, Leckman JF, Price RA, Hardin M, Ort SI, Cohen DJ. The relationship between obsessive-compulsive disorder and Tourette's syndrome: a twin study. Psychopharm Bull 1988; 24: 375-9.
68. Goetz CG, Tanner CM. Gilles de la Tourette syndrome in Twins: clinical and neurochemical data. Ann Neurol 1988; 24: 152.
69. Segal NL, Dysken MW, Bouchard TJ, Pedersen NL, Eckert ED, Heston LL. Tourette's disorder in a set of reared-apart triplets: genetic and environmental influences. Am J Psychiatry 1990; 147: 196-9.
70. Price RA, Kidd KK, Cohen DJ, Pauls DL, Leckman JF. A twin study of Tourette syndrome. Arch Gen Psychiatry 1985; 42: 815-20.
71. Leckman JF, Price RA, Walkup JT, Ort S, Pauls DL, Cohen DJ. Nongenetic Factors in Gilles de la Tourette's syndrome. Arch Gen Psychiatry 1987; 44: 100.
72. Leckman JF, Dolnansky ES, Hardin MT, Clubb M, Walkup JT, Stevenson J, Pauls DL. Perinatal factors in the expression of Tourette's syndrome: an exploratory study. J Am Acad Child Adolesc Psychiatry 1990; 29: 220-6.
73. Hyde TM, Aaronson BA, Randolph C, Rickler KC, Weinberger DR. Relationship of birth weight to the phenotypic expression of Gilles de la Tourette's syndrome in monozygotic twins. Neurology 1992; 42: 652-8.
74. Nee LE, Caine ED, Polinsky RJ, Eldridge R, Ebert MH. Gilles de la Tourette syndrome: Clinical and family study of 50 cases. Ann Neurol 1980; 7: 41-9.
75. Pauls DL, Kruger SD, Leckman JF, Cohen DJ, Kidd KK. The risk of Tourette's syndrome and chronic multiple tics among relatives of Tourette's syndrome patients obtained by direct interview. J Am Acad Child Psychiatry 1984; 23: 134-7.
76. Kidd KK, Prusoff BA, Cohen DJ. Familial pattern of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1980; 37: 1336-9.
77. Pauls DL, Cohen DJ, Heimbuch R, Detlor J, Kidd KK. Familial pattern and transmission of Gilles de la Tourette syndrome and multiple tics. Arch Gen Psychiatry 1981; 38: 1091-3.
78. Kurlan R, Behr J, Medved L, Como P. Transient tic disorder and the spectrum of Tourette's syndrome. Arch Neurol 1988; 45: 1200-1.
79. Leonard HL, Llename MC, Swedo SE, Rettew DC, Gershon ES, Rapoport JL. Tic's and Tourette's disorder: a 2- to 7-year follow-up of 54 obsessive compulsive children. Am J Psychiatry 1992; 149: 1244-51.
80. Comings DE, Comings BG. A controlled study of Tourette syndrome.I. Attention-deficit disorder, learning disorders and school problems. Am J Hum Genet 1987; 41: 701-41.
81. Comings DE, Comings BG. A controlled study of Tourette syndrome.II. Conduct. Am J Hum Genet 1987; 41: 742-60.
82. Comings DE, Comings BG. A controlled study of Tourette syndrome.III.

Phobias and panic attacks. Am J Hum Genet 1987; 41: 761-81.
83. Comings DE, Comings BG. A controlled study of Tourette syndrome.IV. Obsessions, compulsions, and Schizoid behaviors. Am J Hum Genet 1987; 41: 782-803.
84. Comings DE, Comings BG. A controlled study of Tourette syndrome.V. Depression and mania. Am J Hum Genet 1987; 41: 804-21.
85. Comings DE, Comings BG. A controlled study of Tourette syndrome.VI. Summary: a common genetic disorder causing disinhibition of the limbic system. Am J Hum Genet 1987; 41: 839-66.
86. Baron M, Shapiro ES, Shapiro AK, Rainer JD. Genetic analysis of Tourette syndrome suggesting major gene effect. Am J Hum Genet 1981; 33: 767-75.
87. Kidd KK, Pauls DL. Genetic hypotheses for Tourette syndrome. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 243-9.
88. Comings DE, Comings BG, Devor EJ, Cloninger CR. Detection of a major gene for Gilles de la Tourette syndrome. Am J Hum Genet 1984; 36: 586-600.
89. Devor EJ. Complex segregation analysis of Gilles de la Tourette syndrome: Further evidence for a major locus mode of transmission. Am J Hum Genet 1984; 36: 704-9.
90. Price RA, Pauls DL, Kruger SD, Caine ED. Family data support a dominant major gene for Tourette syndrome. Psychiat Res 1988; 24: 251-61.
91. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. New Eng J Med 1986; 315: 993-7.
92. Pauls DL, Towbin KE, Leckman JF, Zahner GEP, Cohen DJ. Gilles de la Tourette's syndrome and obsessive-compulsive disorder: evidence supporting a genetic relationship. Arch Gen Psychiatry 1986; 43: 1180-2.
93. Pauls DL, Pakstis AJ, Kurlan R, Kidd KK, Leckman JF, Cohen DJ, Kidd JR, Como P, Sparkes R. Segregation and linkage analyses of Tourette's syndrome and related disorders. J Am Acad Child Adolesc Psychiatry 1990; 29: 195-203.
94. Pauls DL, Raymond CL, Stevenson JM, Leckman JF. A family study of Gilles de la Tourette syndrome. Am J Hum Genet 1991; 48: 154-63.
95. Comings DE, Comings BG. Tourette's syndrome and attention deficit disorder with hyperactivity: are they genetically related ? J Am Child Psychiatry 1984; 2: 138-46.
96. Pauls DL, Hurst CR, Kruger SD, Leckman JF, Kidd KK, Cohen DJ. Gilles de la Tourette's syndrome and attention deficit disorder with hyperactivity: evidence against a genetic relationship. Arch Gen Psychiatry 1986; 43: 1177-9.
97. Comings DE, Comings BG, Knell E. Homozygosity in Tourette syndrome. Am J Med Genet 1989; 34: 413-21.
98. Kerbeshian J, Burd L. Asperger's syndrome and Tourette syndrome: The case of the Pinball wizzard. Brit J Psychiatry 1986; 148: 731-6.
99. White R, Lalouel J-M. Chromosome mapping with DNA markers. Scient Am 1988; 258: 20-8.
100. Collins F. Positional cloning: let's not call it reverse anymore. Nature Genet 1992; 1: 3-6.
101. Comings DE, Comings BG, Dietz G, Muhleman D, Okada TA, Arinana F, Simmer R, Stock D. Evidence the Tourette syndrome gene is at 18q22.1. Proc

VIIth International Congress of Hum Gen, Berlin, 1986: 620.
102. Donnai D. Gene location in Tourette syndrome. Lancet 1987; i: 627.
103. Heutink P, Wetering BJM van de, Breedveld GJ, Weber J, Sandkuijl LA, Devor EJ, Heiberg A, Niermeijer MF, Oostra BA. No evidence for genetic linkage of Gilles de la Tourette syndrome on chromosome 7 and 18. J Med Genet 1990; 27: 433-6.
104. Taylor LD, Krizman DB, Jankovic J, Hayani a, Steuber PC, Greenberg F, Fenwick RG, Caskey CT. 9p Monosomy in a patient with Tourette syndrome. Neurol 1991; 41: 1513-5.
105. Hebebrand J, Martin M, Körner J, Roitzheim B, Schwanitz G, Werner W, Remschmidt H. Partial trisomy 16p in an adolescent with autistic disorder and Tourette syndrome. Submitted.
106. Devor EJ, Grandy DK, Civelli O, Litt M, Burgess AK, Isenberg KE, Wetering BJM van de, Oostra BA. Genetic linkage is excluded for the D_2-dopamine receptor lambdaHD2G1 and flanking loci on chromosome 11q22-q23 in Tourette syndrome. Hum Hered 1990; 40; 105-8.
107. Gelernter J, Pakstis AJ, Pauls DL, Chapell P, Kurlan R, Grandy DK, Bunzow J, Retief AE, Litt M, Civelli O, Kidd KK. Tourette syndrome is not linked to D_2 dopamine receptor. Arch Gen Psychiatry 1990; 47: 1073-7.
108. Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell, Kocsis, Baumgarten R, Kovacs BW, Levy DL, Smith M, Borison RL, Evans DD, Klein DN, MacMurray J, Tosk JM, Sverd J, Gysin R, Flanagan SD. The dopamine D_2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA 1991; 266: 1793-800.
109. Brett P, Curtis D, Gourdie A, Schnieder V, Jackson G, Holmes D, Robertson MM, Gurling H. Possible linkage of Tourette syndrome to markers on short arm of chromosome 3 (C3p21-14). Lancet 1990; 336: 1076.
110. Heutink P, Sandkuijl LA, Wetering BJM van de, Oostra BA, Weber J, Wilkie P, Devor EJ, Pakstis AJ, Pauls D, Kidd KK. Unjustified claim for linkage of Gilles de la Tourette syndrome. Lancet 1991; 337: 122-3.
111. Pakstis AJ, Heutink P, Pauls DL, Kurlan R, Wetering BJM van de, Weber J, Leckman JF, Sandkuijl LA, Kidd JR, Breedveld GJ, Castiglione CM, Sparkes RS, Cohen DJ, Kidd KK, Oostra BA. Progress in the search for genetic linkage with Tourette syndrome: an exclusion map covering more than 50% of the autosomal genome. Am J Hum Genet 1991; 48: 281-94.
112. Shapiro AK, Shapiro ES. Evaluation of reported association of obsessive compulsive symptoms or disorder with Tourette's disorder. Compr Psychiatry 1992; 33: 152-65.
113. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooymans HGM. Mental play in Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-5.
114. Cath DC, Hoogduin CAL, Wetering BJM van de, Woerkom TCAM van, Roos RAC, Rooymans HGM. Tourette syndrome and obsessive compulsive disorder. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 33-41.
115. Boehnke M. Estimating the power of a proposed linkage study: practical computer simulation approach. Am J Human Genet 1986; 39: 513-27.

116. Ott J. Cutting a Gordian knot in the linkage analysis of complex human traits. Am J Human Genet 1990; 46: 219-21.
117. Blackwelder WC, Elston RC. A comparison of sib-pair linkage tests for disease susceptibility loci. Genet Epidemiol 1985; 2: 85-97.
118. Weeks DE, Lange K. The affected-pedigree-member method of linkage analysis. Am J Hum Genet 1988; 42: 315-26.

2. THE FAMILY STUDY

2.1. The Dutch family study on Gilles de la Tourette syndrome: I. Tic disorders

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INTRODUCTION

The incidental observations of familial occurrence of the Gilles de la Tourette syndrome (GTS) since the early 1960's have evolved into systematic family studies in GTS families. After the development of advanced techniques in molecular biology, linkage studies have also been initiated (1). The majority of GTS cases appear to be familial. Sporadic cases do occur and are mainly viewed as secondary to traumatic, toxic, anoxic or infectious brain damage if suggested by the history (1), while either new mutations or combinations of polygenic factors from both parents cannot be excluded. The finding that dopamine receptor blockers can alleviate tics may indicate that several neurotransmitter systems, especially the dopaminergic system in the mid- and forebrain, are involved in their pathogenesis (2-4). However, the precise etiology of GTS is still unknown. Unravelling the genetic basis for GTS may contribute to the elucidation of the etiology and help find a cure.

The core symptoms of GTS, as described by George Gilles de la Tourette, are motor and vocal tics, which vary in complexity (5). Simple motor tics for example consist of increased eyeblinking, facial twitches and jerks (6). Simple vocal tics include throat clearing, yelps, squeals and snorts. In fact, vocal tics are the result of motor tics that involve movement of air in the pharynx. Rubbing, touching, dystonic movements, symmetry behavior and yelling words and phrases (including obscenities) are complex motor and vocal tics. More than being mere 'hyperkinetic' phenomena, these complex tics seem to implicate emotional and cognitive domains of cerebral function (7). Accordingly, it has become difficult to differentiate these from other behaviors, frequently observed in GTS patients, that have been designated as impulsions or obsessive-compulsive behaviors (7-10).

GTS is viewed by some as a spectrum disorder (11,12). Chronic multiple tics (CMT), simple tics and transient tics have been assumed accordingly to be variant (milder) symptoms expressing the same etiology, especially in biological relatives of GTS patients (12,14). The difference between chronic multiple (either motor or vocal) tic disorders and GTS is the occurrence of either motor or vocal tics alone in CMT instead of in combination. Tics differ from other hyperkinetic neurologic disturbances in that they are associated with sensory phenomena with either a global or focal character (15-17). The latter are called sensory tics (6,18). Thus the clinical spectrum of GTS includes a wide variety of overt simple and complex tics that could be related to often covert drives, emotions and cognitions.

The diagnosis of GTS is based primarily on observable clinical symptoms

and morbid history. The research criteria for GTS are defined in the DSM-III-R (19). The classification requires the occurrence of motor tics and at least one vocal tic over an one year period with a particular waxing and waning course. The criteria are based on overt tic behavior. Other symptoms in addition to the tics, like coprophenomena, 'symmetry' behavior, self-injurious behaviors, not mentioned in the DSM-III-R, are confirmatory. However, they are not obligatory i.e. the diagnosis can be made in their absence. For example, presence of coprolalia is considered as strong evidence for GTS. This occurs in only 20-30% of the patients (6).

The familial occurrence of GTS has been reported in Northern American, English and Mid Eastern families (20-25). As part of an international collaborative study under the aegis of the Tourette Syndrome Association a multidisciplinary group has started in Rotterdam, the Netherlands, a study on the genetics of GTS in the Netherlands. This project encompasses the collection of extended GTS families for a linkage study and of nuclear families for segregation analysis, the cytogenetical analysis of apparently sporadic GTS cases and the establishment of cDNA banks of brain tissue. In this paper we describe the occurrence of GTS and tics in the first 10 extended families from this ongoing study. Research questions to be answered are: 1. What is the frequency distribution of GTS and CMT in the biological relatives as compared to the non-biological relatives of GTS index patients in the Dutch study? 2. What is the interdependency of GTS and CMT for these male and female biological relatives?

METHODS

Subjects

Inclusion criteria for families with a GTS index case were: 1. availability of at least 3 generations; 2. at least one relative apart from the index patient with tics by family history.

All subjects above 18 years of age signed an informed consent. Of subjects younger than 18 years, one of the parents signed an informed consent. The study was approved of by the Medical Ethical Committee of the University Hospital Rotterdam - Dijkzigt.

Assessment

All biological and non-biological (married-in) relatives were interviewed by an experienced psychiatrist (BvdW) using the structured interview developed by Pauls & Hurst (26). This interview contains a detailed tic section, a general biographical section, a section on academic performance,

speech difficulties, a general psychiatric interview and the Yale-Brown Obsessive Compulsive scale. Each subject was asked to indicate which other family members they viewed as afflicted with tics. Subjects below age 16 were interviewed in the presence of at least one parent. All subjects above age 16 were asked to complete a translated Leyton questionnaire, validated for the Dutch population and a translated modified version of the questionnaire, developed to screen GTS related phenomena (27-29). The Norwegian family filled in the original English questionnaires.

Procedure for diagnostic classification

Written, precoded abstracts from interviews of each subject with any tic-like behavior were judged by a panel of 12 clinicians, all with ample clinical experience with GTS and other tic-syndromes. Each transcript was evaluated by two raters in order to establish best estimate classifications.

Based on the available data three levels of classification were distinguished for GTS and CMT in addition to the DSM-III-R criteria. A definite classification of GTS or CMT existed when tics were observed during the interview and reported in the history for a period of more than 1 year. The other levels were tics either by history or observed during the interview. This category is similar to the classification probable GTS or CMT as used by Pauls et al (1991) and by Robertson & Gourdie (1990) (21,24). A classification of possible GTS or CMT was not applied to this sample, as a conservative approach was preferred in order to minimize the number of false positive classifications.

RESULTS

The results of the first ten extended families that participated in the study are presented. Nine families were of Dutch origin, one family was Norwegian and was studied in Norway with help from Norwegian clinicians. The Norwegian family was brought to our attention by the American Tourette Syndrome Association. There were no cases of parental consanguinity in the ten index patients.

TABLE 1

Clinical profile of index patients with GTS from 10 families.

	Sex	Age	OCB	Associated behaviors
1	F	16	OCS	-
2	M	15	-	-
3	F	10	OCS	ADDH, stuttering
4	F	36	OCD	-
5	M	20	OCD	ADDH
6	M	32	OCD	-
7	M	10	-	ADDH, SE, CD
8	M	12	-	ADDH, CD
9	M	13	OCD	ADDH, SE, CD
10	M	8	-	-

Legend:
GTS : Gilles de la Tourette syndrome
OCB : Obsessive compulsive behaviors
OCD : Obsessive-compulsive disorder
OCS : Obsessive-compulsive symptoms
ADDH : Attention Deficit Disorder with Hyperkinesia
SE : Special Education
CD : Conduct Disorder

There were seven male and three female index patients (table 1). Their average age was 17.2 years (range 8 - 36). Six of the index patients had obsessive-compulsive behaviors, four of which met the DSM-IIIR criteria for obsessive-compulsive disorder. Five of them had attention deficit disorder with hyperkinesia (ADDH), two of these patients received special education. Half of the index patients (3 males, 2 females) had a parent with full GTS (table 2). Three male index patients had a father with GTS; one male and one female index patient had a GTS mother. The two other female index patients and two male index patients had an affected mother with chronic multiple tics (CMT). Index patient 8 had unaffected parents. However, there was a positive family history for GTS at the paternal side. His father was classified as having an obsessive-compulsive personality disorder (OCP) with phobia, his mother was phobic.

In addition to the index patients, 286 subjects (137 males, 149 females) have been interviewed. There were 233 biological relatives (109 males, 124 females) and 53 non-biological relatives (28 males, 25 females).

TABLE 2

Clinical characteristics of parents of 10 index patients with GTS.

	Sex	Father	Mother
1	F	anxiety	CMT, OCP, anxiety
2	M	OCP	CMT, OCD, dysthymia, anxiety
3	F	-	GTS, OPS, ADD-H, anxiety
4	F	-	CMT
5	M	-	GTS, stuttering
6	M	GTS, OCS	-
7	M	GTS, OCS	UPD, phobia
8	M	OCD, phobia[1)]	phobia
9	M	-	CMT, OCD, anxiety, stuttering
10	M	GTS, OCS, nervousness	-

[1)] Positive family history for GTS

Legend:
GTS : Gilles de la Tourette syndrome
CMT : Chronic multiple tics
ADD-H : Attention Deficit Disorder with Hyperkinesia
OCD : Obsessive-compulsive disorder
OCP : Obsessive-compulsive personality
OCS : Obsessive-compulsive symptoms
UPD : Unipolar Depression

First degree biological relatives

The percentages for first degree biological relatives both sexes combined were 36.6 (15:41) for 'All Tics', 19.5 (8:41) for GTS and 17.1 (7:41) for CMT (table 3). The male:female ratio's are for 'All Tics' 1:1.6 (6/21:9/20), for GTS 1.6:1 (5/21:3/20) and for CMT 1:6 (1/21:6/20).

Second degree (2D) biological relatives

The percentages for second degree biological relatives both sexes combined were 33.3 (17:51) for 'All Tics'; 13.7 (7:51) for GTS and 19.6 (10:51) for CMT (table 4). The male:female ratio's are 1:1.1 (7/20:10/31) for 'All Tics', for GTS 1:1.2 (3/20:4/31) and for CMT 1:1.0 (4/20:6/31).

TABLE 3

Frequency of GTS and CMT in first degree relatives (parents, sibs, children) of 10 GTS index patients.

	Male			Female			Total		
	GTS	CMT	Total	GTS	CMT	Total	GTS	CMT	Total
Parents	3/10	0/10	3/10	2/10	4/10	6/10	5/20	4/20	9/20
Sibs	2/11	1/11	3/11	0/ 8	2/ 8	2/ 8	2/19	3/19	5/19
Children	0	0	0	1/ 2	0/ 2	1/ 2	1/ 2	0/ 2	1/ 2
Total	5/21	1/21	6/21	3/20	6/20	9/20	8/41	7/41	15/41

Legend: GTS : Gilles de la Tourette syndrome
CMT : Chronic multiple tics

More distant (>2D) biological relatives

The percentages for more distant (>2D) biological relatives of both sexes combined were 26.2 (37:141) for 'All Tics'; 9.9 (14:141) for GTS and 16.3 (23:141) for CMT (table 4). The male:female ratio's are for 'All Tics' 1:1.1 (17/68:20/73), for GTS 1.4:1 (8/68:6/73) and for CMT 1:1.6 (9/68:14/73) (table 4). One of the (third degree) biological relatives with tics on observation and by history was the son of an unaffected uncle of the index patient, whose wife had obvious tics that were denied during the interview. They had four children, one of which had clear motor tics that had been noted by the parents.

'All Tics'

Of the 233 biological relatives, 69 (29.6%) were found to have chronic tics ('All Tics' = either GTS or CMT); 53 (76.8%) both by history and on observation; 12 (17,4%) on observation only (having both motor and/or vocal tics that have not been recognized by the individual) and 4 (5.8%) only by history (table 5). The male:female ratio is 1:1.1 (30/109:39/124) (table 6).

Among the non-biological relatives (53), only one female (1.9%) relatives had multiple tics on observation.

TABLE 4.

Frequency of GTS and CMT in first, second and more distant relatives of 10 GTS index patients.

	Male			Female			Total		
	GTS	CMT	Total	GTS	CMT	Total	GTS	CMT	Total (%)
first degree	5/ 21	1/ 21	6/ 21	3/ 30	6/ 20	9/ 20	8/ 41	7/ 41	15/ 41 (36.6)
second degree	3/ 20	4/ 20	7/ 20	4/ 31	6/ 31	10/ 31	7/ 51	10/ 51	17/ 51 (33.3)
more distant	8/ 68	9/ 68	17/ 68	6/ 73	14/ 73	20/ 73	14/141	23/141	37/141 (26.2)
Total	16/109	14/109	30/109	13/124	26/124	39/124	29/233	40/233	69/233

Legend: GTS : Gilles de la Tourette syndrome
CMT : Chronic multiple tics

TABLE 5

Interdependency of GTS and 'All Tics' for biological and non-biological relatives of 10 index patients with GTS.

		BIOLOGICAL RELATIVES			NON-BIOL. RELATIVES		
		GTS			GTS		
		+	-		+	-	
ALL	+	29	40	69	0	1	1
TICS	-		164	164		52	52
				233			53

TABLE 6

Interdependency of GTS and 'All Tics' for male and female biological relatives of 10 index patients with GTS.

		MALE			FEMALE		
		GTS (%)			GTS (%)		
		+	-		+	-	
ALL TICS	+	16 (14.7)	14 (12.8)	30 (27.5)	13 (10.5)	26 (21.0)	39 (31.5)
	-		79 (72.5)	79 (72.5)		85 (68.5)	85 (68.5)
				109 (100.0)			124 (100.0)

GTS

Of the 69 affected persons 29 (42%) were diagnosed as having GTS (Table 5); 23 (79.3%) both by history and on observation, 4 (13.8%) only on observation (having both motor and vocal tics that have not been recognized by the individual) and 2 (6.9%) by history. Of the 29 GTS subjects 16 (55.2%) were male and 13 (44.8%) were female (table 6). The male:female ratio is 1.4:1 (16/109:13/124).

CMT

Of the 69 affected persons 40, (58%) were diagnosed as having a chronic multiple tic syndrome (CMT: either chronic multiple motor or vocal tics) (table 5); 30 (75%) both by history and on observation, 8 (20%) only on observation (having motor or vocal tics that have not been recognized by the individual) and 2 (5%) by history. Of the 40 subjects with CMT 14 (35%) were male and 26 (65%) were female (table 6). The male:female ratio is 1:1.6 (14/109:26/124).

Odds ratio's

Odds for all males (biological relatives) with 'All Tics' on GTS+ versus

GTS- equals 16:14, whereas the odds for all females (biological relatives) with 'All Tics' on GTS+ versus GTS- equals 13:26 (table 6). As a consequence the ratio of the two odds equals 1.6 (95% confidence interval: 0.60 to 4.26), which is not significant.

DISCUSSION

This study in Dutch and Norwegian families confirms the finding that biological relatives of GTS patients have an increased risk for GTS and CMT as compared to non-biological relatives. The accuracy of diagnosis is critical for genetic or linkage studies. The judgements of the clinical raters resulted in (nearly) complete consensus. The judgements were based on precoded answers on the relevant questions from the questionnaire. It is of relevance whether these questions were perceived correctly during the interview. Misperceptions (mistaxations) of symptoms during examination is not prevented by this procedure. Simulation studies regarding inter- and intra-rater reliability might yield a better insight in the size of this methodological problem.

Four of the biological relatives reported a history of tics that in 2 cases fulfilled the criteria for GTS and in 2 cases for CMT including the presence for more than 1 year, while they did not show symptoms during the interview. One could argue whether these should be regarded as affected from a genetic point of view. They majority of tics reported in the general population disappears within one year (33). They are referred to as transient tics. It is not known whether they all point at the GTS diathesis. Tics existing longer than 1 year are more likely to be genetically related to GTS. Erenberg (1987) reported that about 70% of the GTS patients, examined during their childhood, reported that their tics had decreased markedly or had almost disappeared as they entered adolescence or early adulthood (30).

From a genetic point of view, the diagnosis of GTS and CMT means a lifelong classification as a "case", although tics have a tendency to diminish or disappear in later life (30). A patient once referred to GTS as a "cosmetic disease", indicating that the symptoms are "on the outside" and readily observable (31). However, considering the waxing and waning course, the possibility to suppress symptoms either consciously or automatically and the tendency for symptoms to almost disappear over time, the assignment of positive cases is not always a matter of course. The latter phenomenon might lead to the missing of cases, whenever an individual at later age does not remember possibly significant symptoms when interviewed

during a family study. This problem has been rarely addressed in genetic studies of GTS. However, the diagnosis of GTS is dichotomous, either present or absent regardless the age or severity of the symptoms. If there are symptoms reported by history or observed as a variety of tics including vocal tics, the classification as a 'case' seems to be justified. Strictly spoken a diagnosis of GTS by observation only is not possible according to the DSM-IIIR as the criterion of 1 year duration cannot be met. However, the findings during the interview can be so convincing that denying these might lead to underdiagnosis. The following case is an example of a subject denying any symptoms, but showing the full spectrum of GTS features.

Case *This man is 29 years old, married and without children. He is the son of a sister of the grandfather of the index patient. After above average results in primary and secondary school, he achieved a university grade in economics and has an advisory function in the government. He was never good in sports and was often teased because of his clumsiness. For several years during childhood he had interchanged r's and l's while speaking.*
At the interview he describes himself as very strict and stubborn. He considers himself as a perfectionist. He recalls counting distances in particular patterns (horse jumps in chess game). He frequently feels the urge to make sounds but replaces them by particular movements. Often he makes soft humming sounds. He also has the tendency to control things over and over both in the actuality and in thought. He also has unstoppable obsessive ideas/thoughts against his will about not wishing to see beloved persons (from age 15 on). He has a strong phobia (anticipation anxiety and avoidance behavior for spiders and insects). Until the age of 18 years old he feared and avoided eating in public places. He denies a history of motor and vocal tics. During the 90 minutes interview he can not sit still and showed increased eyeblinking, jerks of the arms and legs, repeated touchings of his hair and mouth and the soft humming sounds. We concluded that he has motor tics, one vocal tic, touching behavior, counting (mental play), obsessive-compulsive behavior and a history of phobia.

The ratio between males and females of the index patients is in agreement with the general finding of a male preponderance in the clinical GTS populations and in the scarce epidemiological studies of GTS (1). Whether the frequency of OCD and ADDH in the index patients (table 1) is related to the disorder, or the result of a selection bias and a small

sample or both is difficult to establish. Caine et al (1988) demonstrated that more severe and complicated cases tend to become referred more often for clinical evaluation than the milder cases (32).

The unilateral presence of GTS or CMT in parents of 9 out of 10 index cases (table 2) seems suggestive for unilateral rather than bilateral parental genetic influences. It is in accordance with a probable autosomal dominant gene of main influence in the disease; it is not in agreement with the bilineal model as proposed by Comings et al. (22). However, if GTS was a strictly dominant condition the expected rates in second and more distant degree relatives should drop with 50% in every succeeding degree of relationship (18.5 for second degree relatives, and less than 9.3 for the more distant relatives in this study).

The rate of GTS+CMT in the biological relatives is in agreement with Kurlan (1986), who found a rate of 37.8% (13). Calculating the odds ratio the conclusion is that GTS occurs significantly more among male biological relatives with tics as compared to female biological relatives with tics. Thus, looking at the occurrence of tics, males tend to have more frequently the full syndrome than females.

The rates of GTS and CMT for first degree relatives (resp. 19.5% and 17.1%) are slightly higher than the rates (8.3% and 16.3% respectively) reported by Pauls et al (1991) (21). This could be explained by the selection of families with a positive family history in our study.

Our sample is too small to calculate representative relative risks for biological relatives. For a more precise description of the model of genetic transmission, segregation analysis is needed, which will be the subject of a following paper. Our data support the hypothesis that tics occurring in families of GTS probands are expressions of an identical genetic susceptibility. That notion supports the possibility to use these families in DNA linkage studies, and also to refine the current diagnostic measures.

REFERENCES

1. Wetering BJM van de, Heutink P. The genetics of the Gilles de la Tourette syndrome: A review. J Lab Clin Med 1993; 121: in press.
2. Seignot MJN. Un cas de maladie des tics de Gilles de la Tourette guéri par le R1625. Ann Med Psychol 1961; 119: 578-9.
3. Devinsky O. Neuroanatomy of Gilles de la Tourette's syndrome: possible midbrain involvement. Arch Neurol 1983; 40: 508-14.
4. Haber SN, Lynd-Balta E. Basal ganglia-limbic interactions. In: Kurlan R (ed).

Handbook of Tourette's syndrome and related tic and behavioral disorders. Marcel Dekker, New York. 1993: 243-66.

5. Gilles de la Tourette GAEB. Etude sur une affection nerveuse caractérisée par l'incoördination motrice accompagnée d'écholalie et de coprolalie. Arch Neurol 1885; 9: 19-42, 158-200.
6. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
7. Shapiro AK, Shapiro ES. Evaluation of reported association of obsessive compulsive symptoms or disorder with Tourette's disorder. Compr Psychiatry 1992; 33: 152-65.
8. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooymans HGM. Mental play in Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-5.
9. Pitman RK, Green RC, Jenike MA, Mesulam MM. Clinical comparison of Tourette's disorder and obsessive-compulsive disorder. Am J Psychiatry 1987; 144: 1166-71.
10. Cummings JL, Frankel M. Gilles de la Tourette syndrome and the neurological basis of obsessions and compulsions. Biol Psychiatry 1985; 20: 117-26.
11. Golden GS. Tics and Tourette's: a continuum of symptoms? Ann Neurol 1978; 4: 145-8.
12. Kurlan R, Behr J, Medved L, Como P. Transient tic disorder and the clinical spectrum of Tourette's syndrome. Arch Neurol 1988; 45: 1200-1.
13. Kurlan R, Behr J, Medved L, Shoulson I, Pauls DL, Kidd JR, Kidd KK. Familial Tourette's syndrome: report of a large pedigree and potential for linkage analysis. Neurology 1986; 36: 772-6.
14. Pauls DL, Cohen DJ, Heimbuch R, Detlor J, Kidd KK. Familial pattern and transmission of Gilles de la Tourette syndrome and multiple tics. Arch Gen Psychiatry 1981; 38: 1091-3.
15. Bliss J. Sensory experiences of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1980; 37: 1343-7.
16. Bullen JG, Hemsley R. Sensory experience as a trigger in Gilles de la Tourette's syndrome. J Behav Ther Exp Psychiatry 1983; 14: 197-201.
17. Lang A. Patient perception of tics and other movement disorders. Neurology 1991; 41: 223-8.
18. Kurlan R, Lichter D, Hewitt D. Sensory tics in Tourette's syndrome. Neurology 1989; 39: 731-4.
19. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (3rd edition, revised). American Psychiatric Press, Washington, 1987.
20. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. New Engl J Med 1986; 315: 993-7.
21. Pauls DL, Raymond CL, Stevenson JM, Leckman JF. A family study of Gilles de la Tourette syndrome. Am J Hum Genet 1991; 48: 154-63.
22. Comings DE, Comings BG, Knell E. Homozygosity in Tourette syndrome. Am J Med Genet 1989; 34: 413-21.
23. McMahon WM, Leppert M, Filloux F, Wetering BJM van de, Hasstedt S. Tourette syndrome in 161 related family members. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment.

Raven Press, New York, 1992; 159-65.
24. Robertson MM, Gourdie A. Familial Tourette's syndrome in a large British pedigree. Brit J Psychiatry 1990; 156: 515-21.
25. Robertson MM, Trimble MR. Gilles de la Tourette in the Middle East. Brit J Psychiatry 1991;158:416-9.
26. Pauls DL, Hurst CR. Schedule for Tourette's syndrome and other behavioral syndromes. Yale University Child Study Center, 1987.
27. Cooper J. The Leyton Obsessional Inventory. Psychol Med 1970; 1: 48-64.
28. Kraaimaat FW, Dam van-Baggen CMJ. Ontwikkeling van een zelfbeoordelingslijst voor obsessief-compulsief gedrag. Ned T Psychologie 1976; 31: 201-11.
29. Frankel M, Cummings JL, Robertson MM, Trimble MR, Hill MA, Benson DF: Obsessions and compulsions in Gilles de la Tourette's syndrome. Neurology 1986; 36: 378-82.
30. Erenberg G, Cruse RP, Rothner AD. The natural history of Tourette syndrome: A follow up study. Ann Neurol 1987; 22: 383-5.
31. Handler L. Personal communication.
32. Caine ED, McBride MC, Chiverton P, Bamford KA, Rediess S, Shiao J. Tourette's syndrome in Monroe County school children. Neurology 1988; 38: 472-4.
33. Lees AJ. Tics and related disorders. Churchill Livingstone, London-New York, 1985.

2. THE FAMILY STUDY

2.2. The Dutch family study on Gilles de la Tourette syndrome: II. Obsessive-compulsive symptoms

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INTRODUCTION

The Gilles de la Tourette syndrome (GTS) is a chronic neuropsychiatric condition with childhood onset. It is characterized by a variety of motor and vocal tics, that follow a waxing and waning course, during at least 1 year (1). Tics have long been associated with obsessive-compulsive phenomena (2,3). Whether obsessive-compulsive symptoms (OCS) in general or obsessive-compulsive disorder (OCD) belong to the GTS behavioral spectrum, like chronic and transient tic syndromes, and should be considered as a variant expression of the GTS diathesis is one of the major topics in the current research on the phenomenology of GTS.

In a review of uncontrolled studies from 1965-1985 in GTS patients the reported occurrence of obsessive-compulsive symptoms, traits or illness appeared to vary enormously (11-80%) (4). Frankel et al., using a modified Leyton Obsessional Inventory, documented OCS-scores above the cut-off point in 50% of an Anglo-American GTS sample in contrast to 82% in an OCD sample (5). Comings et al. found unpleasant obsessive thoughts in 30.5% of GTS patients as compared to 6.25% in controls (6). As to the nature of OCD the patients with GTS had similar symptoms compared to patients with primary OCD (5-7). Their obsessions included forbidden sexual and aggressive images and impulses; their compulsions included checking, washing, counting and magical attempts to ward off imaginary feared events.

Heredity is of significant value in the etiology of either OCD or GTS (8-13). Moreover, twins concordant for Gilles de la Tourette syndrome, show a high concordance (86%) for obsessive-compulsive symptoms (14). Several family studies show that relatives of GTS patients have an increased risk for OC symptoms (11,15-17). In one study the autosomal dominant model for the inheritance of GTS showed the best goodness-of-fit when OCD was included in the behavioral spectrum of the GTS phenotype (11). This diagnostic scheme, that included GTS, chronic tics and OCD, resulted in a male to female ratio of 1:1 for affected cases (11). These family studies are considered the strongest support for the hypothesis that OCD is a component of the spectrum of behaviors associated with GTS.

The primary aim of the present clinical-empirical study was to estimate the occurrence of OCS in European GTS patients and their biological and non-biological relatives. In analogy of broadening the GTS concept by including other tic syndromes according to Pauls et al (17), we decided to include isolated obsessive-compulsive symptoms into a

new category of obsessive-compulsive symptoms (OCS). Secondary, the value of the Dutch version (18) of the Leyton Obsessional Inventory (LOI) (19) was studied in differentiating GTS from NON-GTS as well as OCS from NON-OCS, comparing biological and non-biological relatives, respectively. Specifically formulated, the research questions are: 1. Compared to the non-biological relatives do the biological relatives of GTS patients have a higher risk for tics and OCS separately? 2. Compared to the non-biological relatives do the biological relatives of GTS patients have a high risk for tics and OCS jointly? 3. Are there gender specific differences in occurrence of tics and OCS?

METHODS

Subjects

Ten families participated in the Dutch GTS family study that is part of an international collaborative study to map the gene(s) involved in the etiology of GTS. Nine families were ascertained in the Netherlands (see chapter 2.1). One Norwegian family was studied in Oslo. After the index patient was diagnosed as a definite GTS case, inclusion criteria for the family study were readiness of the index case (or their parents) to inform the extended family about the study, a family history of one other positive case and the availability of at least three living generations.

Assessment

All subjects were interviewed with a structured interview according to Pauls and Hurst (20). This schedule includes an extensive questionnaire about tics in addition to a standardized psychiatric interview. Furthermore, a Dutch version of the Leyton Obsessional Inventory was filled in by all Dutch subjects of 16 years and older.

GTS and other tic syndromes were diagnosed using the information from observation and the structured interview. OCS was assessed in two ways:

1) the method of structured interview and 2) Dutch version of the Leyton Obsessional Inventory (LOI).

Ad 1) *Structured interview.* The following sections from the structured interview by one of the investigators (BvdW) were utilized for this study: Family demographics, multiple tics and/or Tourette syndrome history and the section on obsessive-compulsive behaviors. All diagnoses were made according to the DSM-III-R (21). In addition to the

diagnosis obsessive-compulsive disorder (OCD) the category obsessive-compulsive symptoms (OCS) was used when subjects reported marked symptoms of this kind that were not sufficient to meet the DSM-III-R criteria for the two other categories.
Ad 2) *Dutch version of the Leyton Obsessional Inventory.* A Dutch version of the LOI, called "Inventarisatie Dagelijkse Bezigheden" (IDB) (18), comprising 32 five-point Likert-type items of the selfrating questionnaire, was administered only in subjects over age 15 in the Dutch families. The score range of the IDB varied between 32 and 160. The scores of the subjects have been dichotomized into low and high with a cut-off point at the average value plus one standard deviation (= 86) of the normal Dutch population (22). The rationale to dichotomize at this level was to differentiate the severe from the less severe cases.

Methods of statistical analysis
To detect possible differences in the association between tic syndromes and OCS for biological and non-biological relatives the odds ratio's are estimated including 95% confidence intervals. Zero values will be adjusted by adding 0.5.

RESULTS

General information
Ten extended families, comprising 286 relatives from 10 probands, participated in the study. All families were Dutch, except for one Norwegian family of 32 subjects.
There were four probands (all male, mean age 11 ± 2.6 years) without any OCS and six probands (three males, three females, mean age 21 ± 10.8 years) with OCS. The number of relatives for the ten families varied from 6 to 60.

OCS and IDB scores (table 1)
Of 233 biological relatives 51 (21.9%) were found to have OCS as 'clinical diagnosis' versus one (1.8) of the non-biological relatives. This difference is significant (odds ratio 11.60; confidence interval 1.57 - 85). There was a non-significant difference in occurrence of 'clinical' OCS among the 69 biological relatives with tics and the 164 biological relatives without tics (21 (30.4%) versus 30 (18.2%), respectively; odds ratio 1.66; confidence interval 0.87 - 3.18). There were no differences in the occurence of 'clinical OCS' between sexes and between biological

TABLE 1

Frequency of OCS according to clinical criteria ('clinical OCS') and of subjects with IDB score >86 in non-biological (non-BR) and biological relatives (BR), subdivided per sex and diagnosis of tics.

Relatives	'clinical' OCS			IDB≥86		
	n	+	-	n	+	-
Non-BR	53	1	52 *	45	3	42 ↕
BR (total)	233	51	182 *	172	14	158 ↕
BR + any tics	69	21	48	55	12	43 §
BR - any tics	164	30	134	117	2	115 §
♂ BR + any tics	30	10	20	22	8	14
♀ BR + any tics	39	11	28	33	4	29
♂ BR - any tics	79	16	63	60	1	59
♀ BR - any tics	85	14	71	57	1	56
♂ BR + GTS	16	10	6	12	3	9
♂ BR + CMT	14	0	14	10	5	5
♀ BR + GTS	13	4	9	12	1	11
♀ BR + CMT	26	7	19	21	3	18

* odds ratio 11.60; confidence interval 1.57 - 85.97: significant.
↕ odds ratio 1.22; confidence interval 0.34 - 4.45: not significant.
§ odds ratio 12.76; confidence interval 2.74 - 59.38: significant.
Any tics : either GTS or CMT.
GTS : Gilles de la Tourette Syndrome.
CMT : Chronic Multiple Tics.

relatives with GTS and CMT. In comparing sexes males with GTS had relatively more 'clinical' OCS' than females and females with CMT more than males with CMT, though none of these differences were significant.

Three non-biological relatives, not including the one with the 'clinical OCS', had a score ≥ 86 on the IDB, versus 15 of the 172 biological relatives that had completed the IDB. This difference is not significant (odds ratio 1.22; confidence interval 0.34 - 4.45). However, the difference in high IDB-scores between biological relatives with tics (n=55) differred significantly from the biological relatives without tics (n=117): 12 (27.9%) versus 2 (1.7%), respectively (odds ratio 12.76; confidence interval 2.74 - 59.38).

TABLE 2

Frequency of obsessive-compulsive disorder and obsessive-compulsive symptoms according to clinical criteria in subjects with IDB scores ≥86 and <86.

Relatives	n	IDB ≥ 86			IDB <86		
		n	OCD	OCS[c]	n	OCD	OCS[c]
Non-BR	45	3	0	0	42	1	0
BR (total)	172	14	3	4	158	11	21
BR + any tics	55	12	3	2	43	6	10
BR - any tics	117	2	0	2	115	5	11
♂ BR + any tics	22	8	3	2	14	3	4
♀ BR + any tics	33	4	0	0	29	3	6
♂ BR - any tics	60	1	0	1	59	1	8
♀ BR - any tics	57	1	0	1	56	4	3
♂ BR + GTS	12	3	2	0	9	3	4
♂ BR + CMT	10	5	1	2	5	0	0
♀ BR + GTS	12	1	0	0	11	0	3
♀ BR + CMT	21	3	0	0	18	3	3

IDB : 'Inventarisatie Dagelijkse Bezigheden' (Dutch version of Leyton Obsessional Inventory).
OCD : Obsessive-compulsive disorder according to DSM-III-R.
OCS[c] : Obsessive-compulsive symptoms: clinical diagnosis.

Discriminative value of IDB (table 2)

Of the three non-biological relatives with high IDB scores, none had a clinical diagnosis of OCD and OCS. Of the 12 biological relatives with tics and a high IDB score only three had a clinical diagnosis of OCD and two of OCS, while six of of the biological relatives with tics and a low IDB score were diagnosed as having OCD and 10 as having OCS. The two biological relatives without tics and with a high IDB score were diagnosed as having OCS versus 11 of the 115 biological relatives without tics and with a low IDB score. Five of this latter group were diagnosed as having OCD. The agreement between the clinical diagnoses and the IDB scores was only complete in the male biological relati-

ves with high IDB scores with and without tics and in the male biological relatives with CMT and low IDB scores.

DISCUSSION

The results emphasize the significantly elevated frequency of tics in biological relatives as compared to the non-biological relatives of GTS patients as has been found in the USA and the UK studies (8-11, 23). A diagnosis of OCS as assessed in a structured interview by a psychiatrist (BvdW) in addition to the diagnosis tics contributed even more to this distinction. Apparently, OCS in GTS families could be a variant expression of the genetic GTS diathesis (16). In contrast with this finding, OCS as assessed by the IDB scale, a selfrating questionnaire derived from the LOI, appeared to give much lesser discriminative power in this study.

In a GTS sample from the USA and UK a modified LOI was found to give a wide range of scores, ranging from normal to 'typical OCD' (5). It was concluded that 51% of the GTS patients also had OCD. That is considerably higher than the 16.7% found with the Dutch version of the LOI (3 males and 1 female out of the 24 GTS patients that completed the IDB). Such differences do not seem to be explained by the modifications implemented by Frankel et al. only, nor by different cut-off points in both studies. In the USA/UK study younger GTS patients endorsed more items related to impulse control whereas older GTS patients were more concerned with checking, arranging and fear of contamination. In our study the IDB did discriminate between biological relatives with and without any tic syndrome, but not between males and females and also not between GTS and CMT. Moreover, the IDB scores did not parallel the clinical diagnosis of OCD and OCS in our study. This might suggest that the LOI/IDB is less suitable for measuring obsessive-compulsive phenomena in GTS. The most plausible explanation is that the 'OCS' in GTS are of a different character than OCS in primary OCD.

Obsessive-compulsive disorder (OCD) is nowadays considered to be a form of anxiety disorder. Of crucial importance seems to be anxiety and discomfort that is either increased or decreased by the obsessions or rituals (24). OCD occurs in 0.5-4% of the general population (25-27). The clinical classification of OCD is not circumscript and describes a vast multitude of symptoms without any reference to the etiology. The criteria according to the DSM-III-R are generally applied (21). The average age of onset of OCD is in late adolescence or early twenties (27), for males the onset is about three years earlier than for females

(27,28). OCS, however, tend to change over time (29).

The obsessions and compulsions in OCD are considered to function as to ward of danger or anxiety. Phenomenological analysis of OCD in GTS indicates that the 'OCS' in GTS are not related to anxiety but rather to non-anxiety provoking stimuli (30,31). They mostly seem to have the character of a passtime. 'OCS-like' symptoms in GTS have accordingly been proposed as to reflect disturbed impulse control (1,30,31). Hence forward impulse control might be the basic mechanism underlying GTS, being reflected on a motor level into motor and vocal tics, at the sensory level into sensory tics and at the cognitive level into symptoms that might better be designated as 'cognitive tics' rather than OCD or the vague description of 'OCS'.

REFERENCES

1. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
2. Gilles de la Tourette GAEB. Etude sur une affection nerveuse caractérisée par l'incoördination motrice accompagnée d'écholalie et de coprolalie. Arch Neurol 1885; 9: 19-42, 158-200.
3. Janet PMJ. Les obsessions et la psychasthénie I. Alcan F, Paris, 1903.
4. Robertson MM. The Gilles de la Tourette syndrome: The current status. Brit J Psychiatry 1989; 154: 147-69.
5. Frankel M, Cummings JL, Robertson MM, Trimble MR, Hill MA, Benson DF. Obsessions and compulsions in Gilles de la Tourette's syndrome. Neurology 1986; 36: 378-82.
6. Comings DE, Comings BG. A controlled study of Tourette syndrome. IV. Obsessions, compulsions, and schizoid behaviors. Am J Hum Genet 1987; 41: 782-803.
7. Pitman RK, Green RC, Jenike MA, Mesulam MM. Clinical comparison of Tourette's disorder and obsessive-compulsive disorder. Am J Psychiatry 1987; 144: 1166-71.
8. Kidd KK, Pauls DL. Genetic hypotheses for Tourette syndrome. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York 1982: 243-9.
9. Comings DE, Comings BG, Dietz G, Muhleman D, Okada TA, Sarinana F, Simmer R, Sparkes R, Crist M, Stock D. Linkage studies in Tourette syndrome. Am J Hum Genet 1986; 39: 447.
10. Pauls DL, Kruger SD, Leckman JF, Cohen DJ, Kidd KK. The risk of Tourette's syndrome and chronic multiple tics among relatives of Tourette's syndrome patients obtained by direct interview. J Am Acad Child Psychiatry 1984; 23: 134-7.
11. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome

and associated behaviors. N Engl J Med 1986; 315: 993-7.
12. Carey G, Gottesman IJ. Twin and family studies of anxiety, phobic, and obsessive disorders. In: Klein DF, Rabkin J (eds). Anxiety: New research and changing concepts. Raven Press, New York 1981: 117-36.
13. Lenane MC, Swedo SE, Leonard H, Pauls DL, Sceery W, Rapoport J. Psychiatric disorders in first degree relatives of children and adolescents with obsessive compulsive disorder. J Am Acad Child Adolesc Psychiatry 1990; 29: 407-12.
14. Walkup JT, Leckman JF, Price RA, Hardin M, Ort SI, Cohen DJ. The relationship between obsessive-compulsive disorder and Tourette's syndrome: A twin study. Psychopharm Bull 1988; 24: 375-9.
15. Comings DE, Comings BG. Hereditary agoraphobia with panic attacks and hereditary obsessive-compulsive behavior in relatives of patients with Tourette Syndrome. Brit J Psychiatry 1985; 151: 195-9.
16. Pauls DL, Leckman JF, Towbin KE, Zahner GEP, Cohen DJ. A possible genetic relationship exists between Tourette's syndrome and obsessive-compulsive disorder. Psychopharm Bull 1986; 22: 730-3.
17. Pauls DL, Towbin KE, Leckman JF, Zahner GEP, Cohen DJ. Gilles de la Tourette's syndrome and obsessive-compulsive disorder: Evidence supporting a genetic relationship. Arch Gen Psychiatry 1986; 43: 1180-2.
18. Kraaimaat FW, Dam van-Baggen CMJ. Ontwikkeling van een zelf-beoordelingslijst voor obsessief-compulsief gedrag. Ned T Psychol 1976; 31: 201-11.
19. Cooper J. The Leyton Obsessional Inventory. Psychol Med 1970; 1: 48-64.
20. Pauls DL, Hurst DR. Schedule for Tourette's Syndrome and other behavioral syndromes. Yale University Child Study Center, 1982.
21. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). American Psychiatric Press, Washington 1987.
22. Arts W, Severeijns R, Hoogduin CAL, Haan E de, Schaap C. Inventarisatielijst Dagelijkse Bezigheden (IDB); een replicatieonderzoek naar betrouwbaarheid en validiteit. Gedragsther 1990; 23: 17-27.
23. Robertson MM, Gourdie A. Familial Tourette's syndrome in a large British pedigree. Brit J Psychiatry 1990; 156: 515-21.
24. Jenike MA, Baer L, Minichello We (eds). Obsessive Compulsive Disorders. PSG Publishing Company, Littleton, Mass 1986.
25. Robins LN, Helzer JE, Weissman MM, Orvaschel H, Gruenberg E, Burke JD, Regier DA. Lifetime prevalence of specific psychiatric disorders in three sites. Arch Gen Psychiatry 1984; 41: 949-58.
26. Karno M, Golding JM, Sorenson SB, Burnam MA. The epidemiology of obsessive-compulsive disorder in five U.S. communities. Arch Gen Psychiatry 1988; 45: 1094-9.
27. Flament M, Whitaker A, Rapoport J et al. Obsessive-compulsive disorder in adolescence: An epidemiological study. J Am Acad Child Adolesc Psychiatry 1988; 27: 764-71.
28. Rasmussen SA, Eisen JL. Epidemiology of obsessive-compulsive disorder. J Clin Psychiatry 1990; 51(S): 10-4.
29. Mavissakalian M. Functional classification of obsessive-compulsive

phenomenona. J Behav Assessment 1980; 1: 271-4.
30. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooijmans HGM. Mental play in Gilles de la Tourette syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-45.
31. Cath DC, Hoogduin CAL, Wetering BJM van de, Woerkom TCAM van, Roos RAC, Rooymans HGM. Tourette syndrome and obsessive compulsive disorder. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 33-41.

3. STATISTICAL ANALYSES

3.1. Segregation analysis of familial Gilles de la Tourette syndrome: A state of the art report

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INTRODUCTION

The Gilles de la Tourette syndrome is a chronic neuropsychiatric condition characterized by multiple motor and vocal tics (1). Onset is usually in childhood. The severity of the symptoms varies between patients and fluctuates over time. The clinical picture may be complicated by various associated symptoms including obsessive-compulsive behavior, attention disturbances, conduct disorder and self-injury (2). The disorder is more common in males than in females. Occasional reports of the familial occurrence of GTS have appeared since the beginning of the century, but systematic family studies were initiated only recently (3). Further indications for the involvement of genetic mechanisms in the etiology of GTS came from adoption and twin studies. In monozygotic twins the pairwise concordance rates for GTS and for GTS and tics combined are 50-70% and 75-90%, respectively; in the dizygotic twins about 10% and 20% (2).

The first study to quantify the increased risk for GTS and multiple tics to relatives of GTS probands was reported by Kidd et al. in 1980 (4). Further analysis of their data, extended with family history data from consecutive admissions, suggested that GTS and chronic multiple tics (CMT) should be considered as variant expressions of the same underlying liability (5). Both GTS and CMT were transmitted from parent to child, with multiple occurrences of male to male transmission excluding X-linked inheritance.

Pauls recently reviewed the five early studies that tested models of inheritance in nuclear and extended families with diagnostic classifications based on the family history method (table 1) (6-11). A genetic model with a single major gene provided a good explanation for the familial occurrence of GTS and CMT, but a multifactorial-polygenic model could not be rejected in two studies (8,9). Confirmation of a single gene model came from Pauls et al. (12,13). Three studies favored a dominant gene, with a penetrance in heterozygote gene carriers (f_1) that was (almost) identical to the penetrance in the homozygous genotype (f_2). The other studies, particularly the study by Devor, favored a more additive action (f_1 much smaller than f_2).

Evidence from segregation analysis for the existence of a single major gene is a starting point for linkage analysis. Linkage analysis with polymorphic DNA markers has proven successful in finding the chromosomal localization of an increasing number of monogenic disorders (14). Segregation analysis also provides the estimates for penetrance and gene frequency that are required in the most common statistical

TABLE 1

Genetic Analyses of Family History data: Sex-specific penetrances for GTS, GTS+CMT and GTS+CMT+OCS

Phenotype	Study	Subjects	q	Kp		f_2		f_1		f_0	
				♂	♀	♂	♀	♂	♀	♂	♀
GTS+CMT	Baron et al (1981)	123 N^1	0.003	0.023	0.008	0.953	0.875	0.812	0.640	0.018	0.004
	Kidd & Pauls (1982)	188 N^1	0.03	0.0172	0.0018	0.933	0.744	0.309	0.106	0.000	0.000
	Comings et al (1984)	242 E^1	0.005	0.0127	0.0059	0.987	0.893	0.677	0.297	0.006	0.006
	Devor (1984)	35 E^1	0.0149	0.012	0.003	0.999	0.999	0.125	0.015	0.001	0.000
	Price et al (1988)	50 E^1	0.004	0.0106	0.0094	0.789	0.763	0.789	0.763	0.004	0.003
	Pauls & Leckman (1986)	27 N^2	0.003	0.01	0.003	0.99	0.56	0.99	0.56	0.01	0.00
	Pauls et al (1990)	1 E^2	0.003	0.01	0.003	0.99	0.60	0.99	0.60	0.01	0.00
GTS+CMT +OCS	Pauls & Leckman (1986)	27 N^2	0.006	0.0125	0.0075	1.00	0.71	1.00	0.71	0.00	0.00
	Pauls et al (1990)	1 E^2	0.006	0.0125	0.0075	0.99	0.70	0.99	0.70	0.00	0.00
GTS	Pauls & Leckman (1986)	27 N^2	0.0004	0.0005	0.00015	0.45	0.17	0.45	0.17	0.00	0.00
	Pauls et al (1990)	1 E^2	0.0002	0.0005	0.00015	0.81	0.31	0.81	0.31	0.00	0.00

GTS = Gilles de la Tourette syndrome
CMT = Chronic multiple tics
OCS = Obsessive-compulsive symptoms
q = estimated allele frequency for abnormal allele
Kp = population prevalence for males (♂) and females (♀)
f_2 = penetrance for the homozygous genotype
f_1 = penetrance for the heterozygous genotype
f_0 = penetrance for the genotype wth no susceptibility alleles

N = nuclear family
E = extended family
1 = family history
2 = direct interview

method of linkage analysis, the lod score calculation. The accuracy of these parameter estimates influences the outcome of the linkage study (15-17).

Considering the differences in the reported parameter estimates (table 1), we decided to perform a segregation analysis in order to determine the mode of inheritance and the parameter estimates (gene frequency, penetrance rates for males and females) for our own linkage sample which is included in the data set currently analyzed by the international GTS-gene mapping consortium. Both the results for the Dutch sample and for a combined Dutch-American-Canadian sample will be described.

METHODS

Family material

In this study we used pedigree information and diagnostic classifications on 24 GTS families ascertained by four different research groups: University Hospital Rotterdam-Dijkzigt and Erasmus University, Rotterdam, The Netherlands (10 families), University of Iowa, Iowa City, Iowa, USA (16 families), Marshfield Medical Research Foundation, Marshfield, Wisconsin, USA (1 family) and Hospital for sick Children, Toronto, Canada (1 family). These families were included in linkage and simulation studies as described elsewhere (13,18,19). All diagnosed subjects were interviewed by investigators from the contributing centers using structured interviews. The diagnostic procedure applied in the Dutch families will be described in detail elsewhere. Segregation analysis was performed using the diagnostic classifications from first interviews; follow-up information has not been included systematically at this stage.

Ascertainment

Families were selected for suitability for linkage analysis. The inclusion criteria for Dutch families with a GTS index case were: availability of at least 3 generations, and presence of tics in at least one relative of the index patient, as determined via family history. In addition to the nuclear families consisting of proband, parents and siblings, extended family data were used in the analysis by including all available second, third, fourth and fifth degree relatives as respective nuclear families ascertained through the proband. After separation of the extended families into nuclear families, the Dutch sample included 93 nuclear

families, and the total sample amounted to 122 nuclear families.

Segregation analysis

Segregation analysis was performed using the computer program POINTER (20). This program implements the so-called UNIFIED model in which an autosomal di-allelic locus with alleles *TS* and *ts* is assumed to be in Hardy-Weinberg equilibrium. Let q denote the frequency of allele *ts*, then the frequencies of the three genotypes *TSTS*, *TSts* and *tsts* are $(1-q)^2$, $2q(1-q)$ and q^2. The effects of variation at the major locus are parametrized in terms of gene frequency (q), the distance (t) between the mean liability of the two homozygotes and a dominance parameter (d). The distance between the genotypic means of *TSTS* and *TSts* individuals is equal to dt. In addition the model includes a parameter H, the proportion of background variation due to an additive polygenic component.

The analyses were performed separately using four diagnostic schemes 1) GTS, 2) GTS and CMT, 3) GTS, CMT and OCS and 4) GTS and OCS, respectively. The diagnostic criteria for GTS and CMT were according to the DSM-III-R (21). OCS included both obsessive-compulsive disorder according to DSM-III-R and marked repetitive behaviors that did not necessarily cause distress (22).

In POINTER the population prevalence of the disorder (Kp) needs to be specified. The population prevalence is a function of the allele frequency q and of the penetrances f_2, f_1 and f_0. Specifically, $Kp = f_2*[\text{freq}(TSTS)] + f_1*[\text{freq}(TSts)] + f_0*[\text{freq}(tsts)]$ (13). Thus, by specifying the population prevalence it is possible to obtain maximum likelihood solutions for q, f_2, f_1 and f_0 (13).

RESULTS

Comparison of segregation models

For the Dutch sample the analyses were carried out using three levels of population prevalences per diagnostic category. The population prevalences for category 1 (GTS) and 2 (GTS + CMT) were identical to those used by Pauls and coworkers (12,13). For category 3 (GTS + CMT + OCS) a population prevalence of 0.01 for females was incorporated instead of 0.0075 as used by Pauls and coworkers. A range of hypotheses about models of transmission were tested (see table 2). All tests were carried out in a hierarchical fashion. First, the null hypothesis of no transmission (NTR) was compared to an alternative hypothesis

TABLE 2

Comparison of segregation models for diagnostic category GTS in the Dutch Sample, male prevalence (Kpm) is 0.0005, female prevalence (Kpf) is 0.00015.

GTS	Kpm = 0.0005		Kpf = 0.00015			
Model	D	T	Q	H	Tau_2	-2ln(L) + c
NTR	[0]	[0]	[0]	[0]	[0]	370.60
REC	[0]	9.2	0.02246	[0]	[0.5]	112.45
POLY	[0]	[0]	[0]	0.9999	[0.5]	83.04
DOM	[1]	6.1	0.00036	[0]	[0.5]	0
MEN	1.0	6.0	0.00036	[0]	[0.5]	0
MIX	0.54	11.1	0.00037	0.0556	[0.5]	-0.08
TAU_2	0.76	4.0	0.00043	[0]	0	-26.87

Note. Fixed parameter values are shown in brackets, e.g. [0] and [0.5]

NTR : No transmission
REC : Recessive
POLY : Polygenic
DOM : Dominant
MEN : Mendelian
MIX : Mixed
TAU_2 : Transmission probability for the heterozygote

Parameter labels in POINTER
D : Dominance
T : Displacement
Q : Gene frequency
H : Heretability

that transmission was due to a single major genetic locus with polygenic background (MIX: the so-called mixed model hypothesis). If there was significant evidence for transmission (i.e. the null hypothesis could be rejected), the maximum likelihood of the mixed model was compared to the maximum likelihoods of the hypotheses of: i) polygenic inheritance (POLY: no single major locus), and ii) single gene inheritance (MEN: no polygenic background).

The single gene hypothesis specifies that transmission is due to segregation of different alleles at one locus. If the polygenic hypothesis could be rejected and the single gene hypothesis could not, then the

Mendelian hypotheses were compared to the general single gene hypothesis. Specifically, the hypotheses of autosomal dominant (DOM) and recessive inheritance (REC) were tested. Segregation analyses for the total sample were carried out for the four different phenotypic classifications using only one prevalence value for each diagnostic scheme.

For each phenotypic classification and each prevalence value, the model of no transmission (NTR) could be rejected in favor of the mixed model (MIX: a single major gene with polygenic background) (See table 2). Statistical evidence against the model of no transmission was always highly significant, with chi^2_4 (with four degrees of freedom) ranging from 156.06 ($p<10^{-16}$) to 861.5 for the Dutch data set and from 216.95 to 1101.32 for the combined data set.

Similarly, when the data were fitted to a polygenic model (POLY), this model could be rejected in favor of the mixed model for all analyses. Significance was high, with p values smaller than 0.0005 for all assumptions, except when the GTS phenotype alone was taken to be very frequent (prevalence 0.01 among males, 0.003 for females), which led to a chi^2_3 of 11.86, with a corresponding $p<0.01$.

Subsequently, the data were fitted to both recessive (REC) and dominant (DOM) models. These models can be regarded as constrained forms of the mixed model. The recessive model could be rejected when compared to the full mixed model for all diagnostic schemes and all prevalences (chi^2_2 of at least 39.7, with a corresponding p<10-8 for the Dutch data, and at least chi^2_2 of 58.03 for the combined data). The dominant model, however, could never be rejected when compared to the mixed model, or with the monogenic model. Evidence in favor of the two latter models never exceeded $chi^2 = 3.37$, and frequently the mixed and monogenic models yielded results identical to the dominant model.

Finally, we tested whether transmission probabilities for heterozygotes (tau_2 model) could be different from the Mendelian ratio of 0.5. This model provided better fit than any of the previous models (chi^2 varying between 6.45 and 143.61!).

Penetrance rates

From the parameter estimates obtained in the segregation analysis, gender specific penetrance values could be calculated for the different diagnostic schemes. As the dominant model provided the most parsimonious single gene explanation for all diagnostic schemes and

TABLE 3

Gender specific genetic model parameters (gene frequencies, prevalences and penetrance rates) for four different prevalence rates with regard to the diagnostic classifications 'GTS' and 'GTS + CMT + OCS' as estimated by segregation analysis in 10 Dutch extended GTS families.

Phenotype	Sex	q	Prevalence	f_2	f_1	f_0
GTS	♂	0.0014	0.0002	0.7174	0.7174	0.0000
	♀		0.00005	0.1801	0.1801	0.0000
	♂	0.00753	0.01	0.6754	0.6754	0.00004
	♀		0.003	0.2019	0.2019	0.0000
GTS+CMT+OCS	♂	0.00338	0.0125	0.8426	0.8426	0.00685
	♀		0.01	0.8065	0.8065	0.00459
	♂	0.00578	0.25	0.8988	0.8988	0.19352
	♀		0.2	0.8610	0.8610	0.14571

GTS = Gilles de la Tourette syndrome
CMT = Chronic multiple tics
OCS = Obsessive-compulsive symptoms
q = estimated allele frequency for abnormal allele
f_2 = penetrance for the homozygous genotype
f_1 = penetrance for the heterozygous genotype
f_0 = penetrance for the genotype with no susceptibility alleles

prevalences, penetrances were only calculated for this model. For the most restricted phenotype (GTS only), penetrance rates differed markedly between males and females, with little variation over a wide range of prevalence values ($f_2 = f_1 = 0.7174$ for males and $f_2 = f_1 = 0.1801$ for females at $Kp_m = 0.0002$ and $Kp_f = 0.00005$, see table 3). In the two schemes which include OCS in the phenotype, penetrances reached approximately equal values for males and females (($f_2 = f_1 = 0.8426$ for males and $f_2 = f_1 = 0.8065$ for females at $Kp_m = 0.0125$ and $Kp_f = 0.01$, see table 3). Even for high prevalences, the estimated gene frequency for GTS remained low, with the single exception of the broadest phenotype (GTS+CMT+OCS) in the highest prevalence (0.25 in males, 0.2 in females) (table 3). Identical patterns were observed in the analysis of the Dutch data and of the combined data set (table 4).

TABLE 4

Gender specific genetic model parameters (gene frequency, prevalence and penetrance rates) for four different diagnostic classes as estimated by segregation analysis of Dutch and Norwegian families and a combined data set of Dutch, Norwegian, Canadian and American families.

Phenotype	Study	q	Kp		f_2		f_1		f_0	
			♂	♀	♂	♀	♂	♀	♂	♀
GTS	Dutch	0.0036	0.0005	0.00015	0.6967	0.2104	0.6967	0.2104	0.0000	0.0000
	Total	0.0036	0.0005	0.00015	0.6932	0.2104	0.6932	0.2104	0.0000	0.0000
GTS + CMT	Dutch	0.00367	0.01	0.003	0.7808	0.3935	0.7808	0.3935	0.00433	0.00012
	Total	0.00454	0.01	0.003	0.7658	0.3246	0.7658	0.3246	0.00321	0.00005
GTS + CMT + OCS	Dutch	0.00338	0.0125	0.01	0.8426	0.8065	0.8426	0.8065	0.00685	0.00459
	Total	0.00238	0.0125	0.01	0.9215	0.9023	0.9215	0.9023	0.00809	0.00578
GTS + OCS	Dutch	0.00028	0.0005	0.0005	0.6932	0.6932	0.6932	0.6932	0.00022	0.00022
	Total	0.00028	0.0005	0.0005	0.6754	0.6754	0.6754	0.6754	0.00012	0.00012

GTS = Gilles de la Tourette syndrome
CMT = Chronic multiple tics
OCS = Obsessive-compulsive symptoms
q = estimated allele frequency for abnormal allele
Kp = population prevalence for males (♂) and females (♀)
f_2 = penetrance for the homozygous genotype
f_1 = penetrance for the heterozygous genotype
f_0 = penetrance for the genotype with no susceptibility alleles

DISCUSSION

The results of our segregation analysis support the hypothesis that the clinical GTS spectrum is caused by a single, dominantly acting gene. Evidence for additional polygenic effects was minimal. Penetrance of this gene was highly gender-specific in the more restricted diagnostic schemes. The observation that penetrances leveled when OCS was included in the phenotype suggests that OCS in females is a variant expression of the GTS susceptibility gene, but this observation cannot be regarded as formal evidence.

A direct comparison between our study and some of the studies summarized in table 1 is hampered by differences in statistical methodology and clinical assessment. In the two earliest studies, segregation analysis was carried out according to Kidd (4), while later studies have applied the Mixed Model as proposed by Morton and MacLean (23).

Segregation analysis is generally used to distinguish between monogenic and polygenic contributions in the etiology of inherited traits and diseases. In the Mixed Model, it is possible to obtain estimates of the gene frequency, the penetrances for a single major gene, and the heritability of a polygenic background. The approach of Morton and MacLean allows comparisons between dominant ($f_2 = f_1$), recessive ($f_1 = f_0$) and intermediate ($f_2 > f_1 > f_0$) models. When applied to a spectrum of clinically related disorders, the method may also be used in a less formal way to separate phenotypes that are likely to have a common genetic etiology from those that only have clinical resemblance. Such analyses can be carried out both in nuclear and in extended families. When extended pedigrees are used, it is important that the diagnostic information is equally reliable for distant relatives as for the proband and the first degree relatives.

The initial optimism about the sensitivity of the family history method in psychiatric-genetic research diminished, when it was shown that it tended to underestimate the number of affected relatives due to reporting bias (24,25). This might have a pronounced impact in segregation studies of extended families, probably biasing the conclusions towards an additive or even recessive model. Indeed, in the study of Devor, segregation analysis in nuclear families pointed towards a dominant mode of inheritance for GTS, while in the extended families an additive model was favored. In their recent GTS studies, Pauls and co-workers have shown the direct structured interview to be a more sensitive tool to detect affection status in relatives (26). The two segregation studies which made use of direct interviews consistently favored a single,

dominantly acting GTS gene with incomplete penetrance and variable expression. Our results confirm the conclusions of the latter studies.

While the prevalence of GTS is estimated to be relatively low, reliable data concerning the prevalences of the milder phenotypes is not available. The results of our analyses showed that penetrances and gene frequency for the GTS gene remained fairly stable over a wide range of prevalences values. Increases in prevalence were mainly reflected in the numbers of phenocopies (table 3). When a prevalence in males of 0.25 was assumed for the broadest phenotype, 72% of all cases can be estimated to be phenocopies (calculations based on data in table 3).

A remarkable finding was the good performance of the tau_2 model, which is regarded as a general test for (non-)Mendelian inheritance. Several explanations can be given. Firstly, the large difference in prevalence of GTS and CMT between males and females that was reported earlier is not entirely supported in our data. Thirty male relatives of probands had either GTS or CMT, against 39 female relatives (data not shown). By fixing prevalence rates for males and females at values that were not in good agreement with the data, we may have introduced a bias towards a non-Mendelian mode of inheritance. Secondly, it should be realized that our family sample was selected specifically for the purpose of a linkage study, which can be expected to introduce a bias towards large, multiply affected families. The effect of this selective ascertainment scheme on the results of segregation analysis is not known. Thirdly, it has been suggested that the implementation of non-Mendelian transmission probabilities in the POINTER program might be incorrect (27). Clerget-Darpoux et al. (15) obtained correct results with the POINTER program when testing non-Mendelian transmission in nuclear families, but we are not aware of any systematic verification in extended families. Finally, it has been argued that rejection of a simple Mendelian model in favor of a non-Mendelian model should not be regarded as conclusive evidence against a prominent role for a single gene. When, for instance, the effect of a major gene is modulated by one or several modifier genes, the strict assumptions of the single major locus model of segregation analysis may already be violated sufficiently to lead to rejection of that model (28). In this context, it is important to realize that the difference in penetrance between males and females might point to an X-linked modifier gene. It is not clear whether the results obtained with the tau_2 model reflect a true biologic phenomenon in GTS, or rather are an artifact of ascertainment or of a too stringent statistical approach. The possibility that the inheritance of GTS is not consistent with a Men-

delian pattern should not be discarded, but other explanations need to be investigated before a definite conclusion can be made.

Our results can be regarded as a reassurance for the ongoing linkage mapping of GTS. The lack of positive findings in tests of more than 600 DNA markers by the GTS linkage consortium (19) has raised doubts about the appropriateness of the diagnostic and genetic models used in the linkage analysis. This study yields support for the penetrance estimates previously adopted in our linkage studies. It also showed that a dominant model provides a plausible explanation for the observed inheritance for all diagnostic classifications. Segregation analysis, however, will not provide clues about locus heterogeneity, which conceals existence of linkage.

REFERENCES

1. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
2. Robertson MM. The Gilles de la Tourette syndrome: the current status. Brit J Psychiatry 1989; 154: 147-67.
3. Wetering BJM van de, Heutink P. The genetics of the Gilles de la Tourette syndrome: a review. J Lab Clin Med 1993; 121: in press.
4. Kidd KK, Prusoff BA, Cohen DJ. Familial pattern of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1980; 37: 1336-9.
5. Pauls DL, Cohen DJ, Heimbuch R, Detlor J, Kidd KK. Familial pattern and transmission of Gilles de la Tourette syndrome and multiple tics. Arch Gen Psychiatry 1981; 38: 1091-3.
6. Pauls DL. The inheritance pattern. In: Kurlan R (ed). Handbook of Tourette's syndrome and related tic and behavioral disorders. Marcel Dekker, New York. 1993: 307-15.
7. Baron M, Shapiro ES, Shapiro AK, Rainer JD. Genetic analyses of Tourette syndrome suggesting major gene effect. Am J Hum Genet 1981; 33: 767-75.
8. Kidd KK, Pauls DL. Genetic hypotheses for Tourette syndrome. In: Friedhoff AJ & Chase TN (eds). Gilles de la Tourette Syndrome. Raven Press, New York. 1982: 243-9.
9. Comings DE, Comings BG, Devor EJ, Cloninger CR. Detection of a major gene for Gilles de la Tourette syndrome. Am J Hum Genet 1984; 36: 586-600.
10. Devor EJ. Complex segregation analyses of Gilles de la Tourette syndrome: Further evidence for a major locus mode of transmission. Am J Hum Genet 1984; 36: 704-9.
11. Price RA, Pauls DL, Kruger SD, Caine ED. Family data support a dominant major gene for Tourette syndrome. Psychiat Res 1988; 24: 251-61.
12. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. N Engl J Med 1986; 315: 993-7.

13. Pauls DL, Pakstis AJ, Kurlan R, Kidd KK, Leckman JF, Cohen DJ, Kidd JR, Como P, Sparkes R. Segregation and linkage analyses of Tourette's syndrome and related disorders. J Am Acad Child Adolesc Psychiatry 1990; 29: 195-203.
14. Collins F. Positional cloning: Let's not call it reverse anymore. Nature Genet 1992; 1: 3-6.
15. Clerget-Darpoux, Bonaiti-Pelli C, Hochez J. Effects of misspecifying genetic parameters in lod score analyses. Biometrics 1986; 42: 393-9.
16. Greenberg DA, Hodge SE. Linkage analyses under 'random' and 'genetic' reduced penetrance. Genet Epidemiol 1989; 6: 259-64.
17. Cox NJ, Hodge SE, Marazita ML, Spence MA, Kidd KK. Some effects of selection strategies on linkage analyses. Genet Epidemiol 1988; 5: 289-97.
18. Devor EJ, Grandy DK, Civelli O, Litt M, Burgess AK, Isenberg KE, Wetering BJM van de, Oostra BA. Genetic linkage is excluded for the D_2-dopamine receptor lambdaHD2G1 and flanking loci on chromosome 11q22-q23 in Tourette syndrome. Hum Hered 1990; 40; 105-8.
19. Heutink P, Breedveld GJ, Niermeijer MF, Wetering BJM van de, Oostra BA. Progress in gene localization. In: Kurlan R (ed). The handbook of Tourette's syndrome and associated tic and behavioral disorders. Marcel Dekker, New York, 1993: 317-35.
20. Lalouel JM, Morton NE. Complex segregation analyses with pointers. Hum Hered 1981; 31: 312-21.
21. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). American Psychiatric Press, Washington, 1987.
22. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooymans HGM. Mental play in Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-5.
23. Morton NE, MacLean CJ. Analysis of family resemblance. III. Complex segregation of quantitative traits. Am J Hum Genet 1974; 26: 489-503.
24. Andreasen NC, Endicott J, Spitzer RL, Winokur G. The family history method using diagnotic criteria: reliability and validity. Arch Gen Psychiatry 1977; 34: 1229-35.
25. Silverman JM, Breitner JCS, Mohs RC, Davis KL. Reliability of the family history method in genetic studies of Alzheimer's disease and related dementia's. Am J Psychiatry 1986; 143: 1279-82.
26. Pauls DL, Kruger SD, Leckman JF, Cohen DJ, Kidd KK. The risk of Tourette's syndrome and chronic multiple tics among relatives of Tourette's syndrome patients obtained by direct interview. J Am Acad Child Psychiatry 1984; 23: 134-7.
27. Iselius L, Morton NE. Transmission probabilities are not correctly implemented in the computer program POINTER. Am J Hum Genet 1991; 49: 459.
28. Iselius L, Littler M, Morton NE. Transmission of breast cancer - a controversy resolved. Clin Genet 1992; 41: 211-7.

3. STATISTICAL ANALYSES

3.2. Linkage studies on Gilles de la Tourette syndrome: Which families and phenotypes should be analyzed further?

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Submitted.

INTRODUCTION

Gilles de la Tourette syndrome (GTS) is a chronic neuropsychiatric disorder with unknown etiology. The syndrome is characterized by multiple, intermittent motor and vocal tics. Affected individuals frequently display associated behaviors like obsessive compulsive symptoms, attention deficit and hyperactivity disorder, coprolalia and echolalia (1). Expression of the phenotype follows a waxing and waning course and is influenced by sex and age. Patients are often capable of suppressing tics for limited periods of time.

Analysis of family data is consistent with an autosomal dominant mode of inheritance with incomplete penetrance according to the most important studies available until recently (2-4). It has been suggested that a number of milder behavioral problems should be considered as variant expressions of the presumed genetic defect responsible for GTS. The chronic multiple tic syndrome (CMT) is generally agreed to be a variant phenotype of GTS. There is also evidence for a genetic relationship between GTS and obsessive compulsive symptoms (OCS).

In recent years six research groups have initiated a collaboration, under the auspices of the American Tourette Syndrome Association, in order to localize the gene(s) responsible for GTS. Among the human genes that have recently been characterized no obvious candidate genes for GTS have been identified. Chromosomal regions 18q22.1 and 9p23-pter, implicated by structural rearrangements in GTS patients have failed to generate positive evidence for linkage (5,6). In a systematic global genome search the collaborating research groups have tested more then 600 genetic markers (5-12). No strong and definite evidence for linkage was obtained. Assuming locus homogeneity, and considering CMT as a variant phenotype of GTS, an exclusion map based on a well localized subset of markers shows exclusion of at least 80% of the human autosomes.

In linkage studies for bipolar disorder and schizophrenia promising findings could not be confirmed or supported (13-15). The failure to localize genes for psychiatric disorders through linkage analysis has generated a broad discussion, not only about the appropriateness of single-gene assumptions for complex disorders (16-24), but also on the question how genetic disease entities might be delineated in the intricate diagnostic classification schemes of today's psychiatry. Some authors propose to include only the most extreme phenotypes in the linkage analysis, assuming that these phenotypes are most likely based on genetic factors (25,26). Others have carried out multiple analyses,

gradually broadening the phenotype definition to include milder or less specific diagnoses (27). The former suggestion might lead to severe loss of information, while the practice of multiple testing will undoubtedly give rise to an increased frequency of false positive linkage findings if no statistical corrections for multiple testing are made. Decisions on the strategy of choice have been made arbitrarily.

Linkage studies on GTS are subject to the same complexities as those for other psychiatric disorders. The question arises whether GTS should be considered as yet another example of a complex disease where linkage analysis will fail to produce dependable conclusions. Alternatively, the accumulated family material might simply be not large enough to produce convincing evidence for linkage.

In an attempt to characterize the available family material more accurately with respect to size, diagnostic uncertainties and sensitivity to clinical and genetic assumptions, we have carried out an extensive simulation study. The use of computer simulations to evaluate the adequacy in size of a linkage sample is becoming more common (28); its use has also been suggested for the assessment of the expected frequency of false positive findings in a multiple test situation (29). Terwilliger and Ott proposed efficient procedures to reduce the time needed for this analysis (30).

In this simulation study we are addressing three major questions concerning the collaborative data set of GTS families. Firstly, what is the probability of generating conclusive evidence for linkage in these combined families assuming locus homogeneity? Secondly, which of these families contains sufficient linkage information for mapping under extensive locus heterogeneity? Finally, what approach should be taken in the analysis with respect to the spectrum disorders, CMT and OCS? We systematically investigated whether a narrowly defined phenotype would give better probabilities to detect linkage compared to a strategy where three diagnostic categories with a broadening in the spectrum of included clinical characteristics was used.

Our findings are of relevance to researchers involved in the mapping of psychiatric disorders, but the approaches presented here are also applicable in mapping projects of other complex disorders.

MATERIAL AND METHODS

Family material

In the study reported here we used pedigree information and diagnostic

data on twenty-six GTS families ascertained by six different research groups: Erasmus University Rotterdam, The Netherlands (12 families), Yale University School of Medicine, New Haven, CT, USA (2 families), Marshfield Medical Research Foundation, Marshfield, WI, USA (1 family), Hospital for Sick Children, Toronto, Canada (1 family), University of Iowa, Iowa City, Iowa, USA (16 families).

All families have been previously included in linkage studies, as described elsewhere (4,10-18,31) except for the family that was contributed by the research group from Toronto. This set of families was also used in a segregation analysis which will be reported separately. A detailed description of the pedigrees is available upon request.

All diagnosed subjects were personally interviewed by investigators from the contributing centers. For diagnostic assessment a structured questionnaire was used with a section on GTS and CMT (32). A separate questionnaire was used for OCS. Diagnoses were confirmed by independent clinical investigators who had no prior knowledge of family history.

Statistical analyses

The assumptions concerning the mode of inheritance of GTS made in the analyses presented here, were identical to those adopted in our previous linkage studies (9,11-18). GTS was taken to be caused by an autosomal dominant mutation, incompletely penetrant, with a population frequency of 0.003. Probabilities to express the complete GTS phenotype were 0.81 and 0.31 for male and female gene carriers respectively, and 0.005 and 0.003 for males and females without this genetic defect (phenocopy frequency). Persons with CMT or OCS were treated in various ways, depending on the diagnostic model chosen. Three different diagnostic models were applied in these analyses: a broad, intermediate, and narrow model. In the broad model, it was assumed that the GTS gene also predisposes to development of both the CMT and the OCS phenotypes. Probabilities to manifest these phenotypes were 0.9 and 0.6 for CMT (males and females respectively), and 0.9 and 0.71 for OCS. In the broad model, phenocopy rates for both phenotypes were fixed at 0.01 for males and 0.006 for females. In the intermediate model, subjects with OCS were treated as 'phenotype unknown', which implies that they did not contribute directly to the linkage analysis, although their marker genotypes may have aided in the reconstruction of marker genotypes for unavailable persons. In the narrow diagnostic model, subjects with CMT were also treated as 'phenotype unknown'.

Penetrances for persons homozygous for the abnormal GTS allele were

kept identical to those for heterozygous individuals.
Male and female recombination fractions were assumed to be equal.

The computer simulations that we carried out will be described in three separate steps: i) the construction of marker data for a hypothetical marker either closely linked or unlinked to the GTS gene, ii) the linkage analysis of those marker results as if it concerned real marker data, and iii) the evaluation of the resulting lod scores.

Marker data were generated for all family members for whom DNA was available in reality, using the computer program SLINK (33). Markers of various informativeness (2, 4, and 8 alleles), with a polymorphism information content (PIC value), of 0.375, 0.70 and 0.86 respectively, were simulated to be either linked to the GTS gene with 5% recombination, or unlinked. For each family 100 or 400 distinct replicates were prepared depending on the size of the family. Simulations were carried out separately for each of the three diagnostic models (for the linked marker only; in the absence of linkage, the choice of the diagnostic model will not influence the construction of the hypothetical marker data).

Analyses of the resulting data were carried out for each replicate of each family separately, with a slightly modified version of the MLINK option of the LINKAGE package, version 5.03 (33). All simulated data were analyzed under the diagnostic scheme used for simulation, but also under the other diagnostic models. Lod scores for each replicate were calculated for recombination fractions ranging from 0.0 to 0.5 in steps of 0.01. The resulting lod score lists were manipulated via the computer programs SIMSUM and SIMCOMP (unpublished programs by L.A. Sandkuijl) to yield expected lod scores for individual families and for sets of families. Expected lod scores for individual families were calculated for each marker and each model. For each replicate of a given family, the maximum lod score was identified. The mean of those maxima (over 100 or 400 replicates) was taken to represent the expected lod score in that family.

The expected maximum lod score in a set of families was obtained via a bootstrap procedure, as proposed by Terwilliger and Ott (34). For each family of a given set, a replicate was selected at random. Lod scores for the selected replicates were summed for each value of the recombination fraction, and the resulting lod score curve was taken to represent one simulated replicate of that set of families. Unless otherwise specified, analyses of sets of families were based on 10,000 bootstrapped

replicates.

Corrections for multiple testing were as proposed by Risch (35): lod score threshold = 3 + LOG(t) where t represents the number of tests carried out.

RESULTS

Individual families

Expected lod scores varied widely between the twenty-six families, and, for a given family, between different diagnostic models. With a four-allele marker thirteen of the families yielded mean lod scores of 0.5 or more under at least one of the diagnostic models (figure 1). For these families, the broadest diagnostic model yielded the highest lod score, but differences between the broad model and the narrower ones showed marked variation between families. In family S18 all linkage information appeared to be based upon persons with tics, while in family S14 information depended almost exclusively on GTS patients. Family S14 was the most informative family under all diagnostic models. Under the narrow model, and with only a two-allele marker, this family already generated an average lod score of 2.4. With the more informative markers, this average lod score increased to 4.8 for a four-allele marker and to 6.0 for an eight-allele marker. Kindreds S8, S10, and S13 also yielded mean lod scores close to (S8), or over 3.0 with a four-allele marker, but only for the broader diagnostic models. For the thirteen most informative families, use of a two-allele marker led to an average loss in lod score of 50.6% when compared to results for a four-allele system, while lod scores increased by an average 30.9% when the most informative marker was used.

Combinations of families

In order to assess the probability to map a GTS gene for each of the research groups contributing to the collaborative GTS mapping effort, the results of the simulations were grouped by contributing center. In each of these sets of families, the average expected lod score was calculated (figure 2). The family sets from Rotterdam and Rochester/New Haven could already generate significant lod scores with a two-allele marker under the most restrictive diagnostic model. When more informative markers and the broadest diagnostic scheme were applied, the expected maximum lod score rose to well above the generally accepted threshold for significance; the mean Z_{max} amounted

Figure 1 Mean lod scores per family under three diagnostic models for a four-allele marker, PIC=0.7.

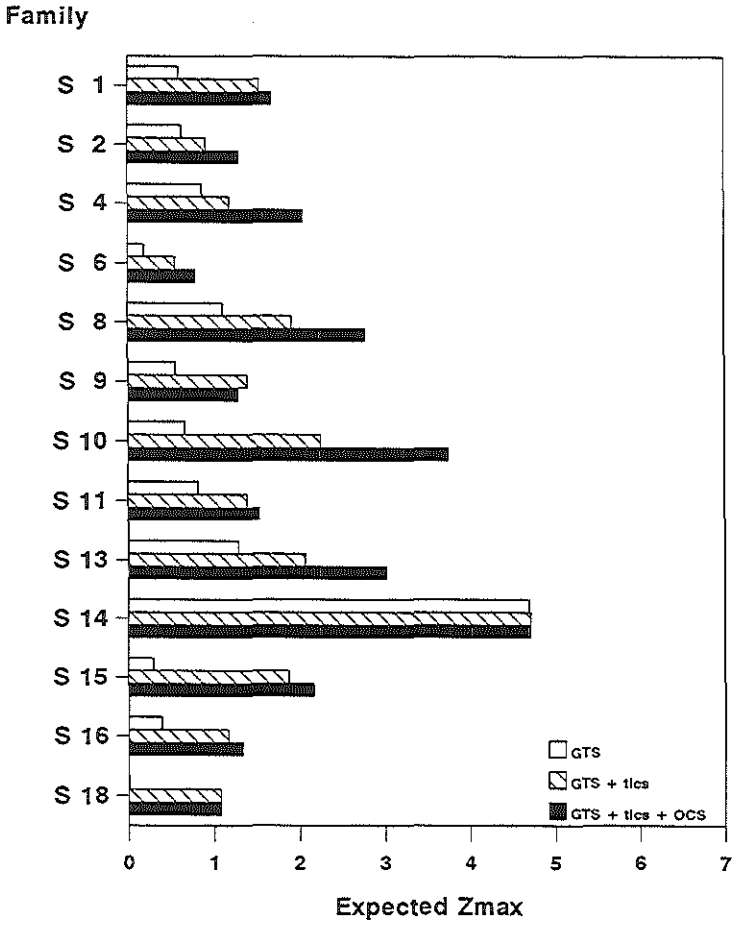
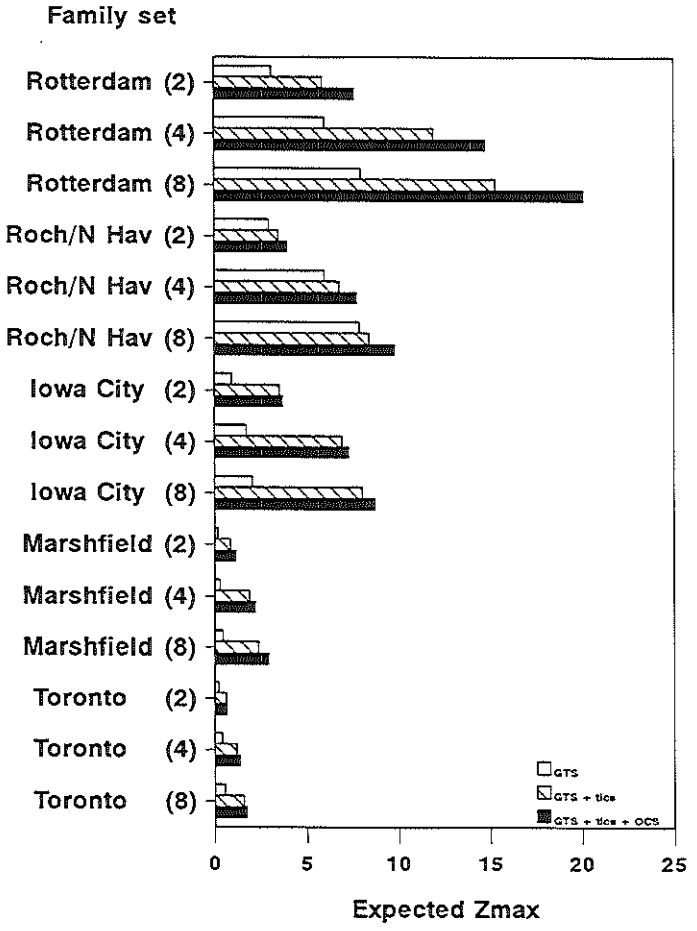


Figure 2 *Mean lod scores under genetic homogeneity for three diagnostic models. Results are presented per family set ascertained at the six collaborative research centers. Values between brackets indicate the informativety of the simulated marker. (2) Two-allele marker with PIC value of 0.375. (4) Four-allele marker with PIC value of 0.7. and (8) Eight-allele marker with PIC value of 0.86.*



to 20.1 and 9.8 for data sets from Rotterdam and Rochester/New Haven respectively.

Use of incorrect model for analysis

The results presented so far were obtained by generating marker data for a given diagnostic model, and analyzing the data under the identical model. We have also analyzed the data for the Dutch families under different, 'incorrect' models, thereby obtaining an indication for the loss of statistical power when incorrect diagnostic models are used.

Ten thousand different combinations of the simulated data for the Dutch families were analyzed with the broad, intermediate and narrow diagnostic models seperately. For each model, the frequency of a lod score above three was scored. In addition, we applied the three analysis models simultaneously on 10,000 simulated family sets, and scored how frequently a lod score above 3.477 was obtained under at least one of the diagnostic models. In this latter analysis, a higher lod score threshold was chosen, in order to compensate for the increased probability of false positive linkage findings due to multiple tests (35).

Results are presented in table 1. For marker data generated under the broadest model, the analyses yielded almost always significant results with the broader models, but with the narrow model only in approximately 50 % of cases. Using the broad diagnostic model in the analysis when the simulated, 'true', model was narrow resulted in a dramatic loss of power: significant results were obtained in only 3.2 % of all replicates.

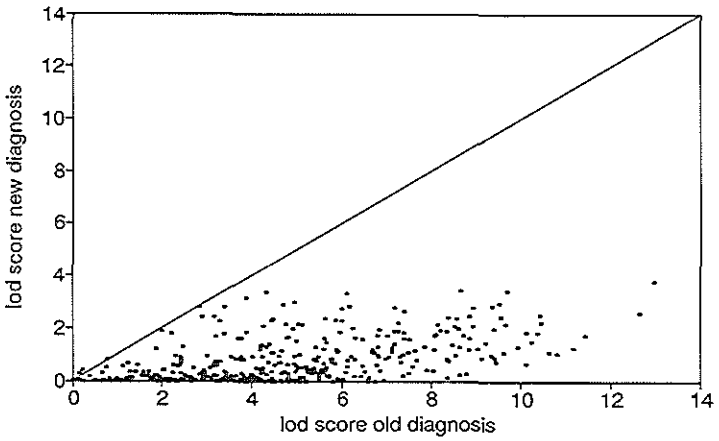
Table 1

Expected frequency of lod scores over 3.0 in the Dutch data set for various models of simulation and analysis.

Model of simulation	Model of analysis			
	Broad	Interm.	Narrow	Combined*
Broad	99.9 %	98.2 %	48.0 %	99.9 %
Intermediate	84.4 %	99.7 %	73.2 %	99.5 %
Narrow	3.2 %	13.0 %	78.4 %	70.5 %
Unlinked	$19/0.5x10^6$	$22/0.5x10^6$	$25/0.5x10^6$	$20/0.5x10^6$

* For the combined tests, a lod score threshold of 3.477 was applied.

Figure 3 The effect of changed diagnosis in family S14. Simulation of a four-allele marker (PIC=0.7) under the intermediate diagnostic model with new diagnoses. Analysis under the intermediate model with the old diagnoses (X axis) and the new diagnoses (Y axis).



Similar analyses were carried out on data simulated under the absence of linkage. Here, as many as 500,000 replicates were evaluated, in order to count the rare occurrences of false positive linkage findings. The frequency of incorrect linkage findings was remarkably low: between 19 and 25 per 500,000 (table 1).

Impact of diagnostic instability

For one of the families, family S14, the diagnoses have been re-evaluated via repeated interviews. Diagnoses have changed for several family members, and additional diagnoses have been obtained for subjects who had not been interviewed previously. For ten subjects, hitherto regarded as unaffected, the diagnosis of GTS was established upon their second interview. For three subjects, previously regarded as

unaffected, the diagnosis of CMT was established in the second interview. Diagnosis for one subject changed from GTS to unaffected and diagnosis of one subject changed from CMT to unaffected.

In order to evaluate the possible impact of diagnostic instability, we carried out simulations in these families under the assumption that the recently updated diagnoses are correct. Subsequent lod score calculations were carried out once with the updated diagnoses, and once with the older diagnostic information. The intermediate diagnostic model was used in both the simulations and the analyses. In these analyses not a single lod score exceeded the threshold of three in the analyses with the older diagnoses (figure 3). The average loss in peak lod score due to use of the older diagnoses was 84.5%. In an attempt to reduce this damaging effect of diagnostic instability, we carried out additional analysis under the narrow diagnostic model. An approximately equal reduction in lod score of 86.3%. was obtained (data not shown).

DISCUSSION

In planning a linkage study, the use of computer simulations for evaluation of the adequacy in size of a linkage sample is becoming more common. In our ongoing collaborative effort to localize the gene(s) responsible for GTS simulation studies had not been applied. Now that more than 600 markers have been tested without evidence for linkage, two questions arise: Is the set of available families adequate to detect linkage and, what is the effect of diagnostic uncertainties on the power to detect linkage in this family set?

Our current results show that when locus homogeneity is assumed the power of the family set to detect linkage is very high with all three diagnostic models. Even when a two-allele marker is used, significant results can be obtained (figure 2). From the increase in the expected lod scores it can be concluded that an important part of the available information was derived from patients with the associated spectrum disorders, CMT and OCS.

In order to have a family set informative enough to detect linkage in each collaborating center family material was shared between different groups so that all centers have access to adequate family material for their linkage studies.

By pooling all the available linkage data on more than 600 markers from the six research centers more than 80% of the genome can be

excluded as a site for the GTS gene, assuming that CMT is variant expression of the GTS gene defect. Most markers, however, have been tested in a subset of the families. Therefore we have to consider the possibility of locus heterogeneity. In our simulations we studied the power of each separate family. One family was informative enough to detect linkage under all diagnostic models, while three other families yielded evidence for linkage under the two broader diagnostic models. We conclude that even in the case of the most extensive genetic heterogeneity the family set is informative enough to detect linkage.

Other explanations for the failure to detect linkage are the diagnostic uncertainties for GTS. Associated behavioral problems as CMT and OCS are likely to be variant expressions of the GTS gene defect but are more difficult to diagnose. Furthermore, GTS shows a waxing and waning course of expression and patients are often able to suppress symptoms for limited time. This may lead to misdiagnosis or inclusion of phenocopies. Both the role of spectrum disorders and of uncertainty of diagnosis were studied in our simulation studies by the testing of three diagnostic models and the testing of the impact of diagnostic instability on the statistical power to detect linkage. As a remedy against false positive linkage findings it has been proposed to include only the most narrow phenotypes in a linkage analysis. This will result in a loss of information but it seems more likeley that the phenotype is the result of a genetic factor (table 1). This method does not protect us, however, against the consequences of diagnostic instabilities. An alternative solution that has been proposed is to define different models with a broadening in diagnostic criteria. This approach has been used by the GTS consortium research centers in their linkage studies. There is an increased risk on false positive results which can be circumvented by a correction for multiple testing. Risch (35) proposed to increase the lod score threshold of significance of 3.0 with a factor equal to the logarithm of the number of tested models. In this case three genetic models were tested implying an increase in lod score to 3.477. In the simulated data of the Dutch families we investigated whether the use of three diagnostic models would protect us better against false negative findings than the use of a single model that carries the risk of mis-specification.

If CMT and OCS are variant expressions of the GTS gene defect, analysis of the two broadest models was very powerful. In more than 98% of the replicates linkage was detected (table 1). Analysis with the narrow model showed a considerable loss in information, the power to detect linkage was lowered to 48% as linkage information from individuals expressing CMT or OCS was lost. If OCS is not part of the

GTS spectrum (intermediate model) the probabilities to detect linkage using three analysis models are high, between 73% and 99.7%, but a dramatically reduction is observed when the most narrow model is correct. If analyzed correctly the probabilities of reaching a threshold of three are 78.4% but they drop to 3.2% if analyzed under the broadest model.

These results can be explained by the fact that individuals with CMT and OCS will be classified inappropriately as gene carrier, and frequently will be scored as recombinants in the analysis. The results indicate that choosing a single genetic model will lead to a dramatic loss of information except if by chance the correct model is chosen. As a remedy the application of three diagnostic models is very effective (table 1). Whatever diagnostic model is correct, the probability of detecting linkage always exceeded 70%.

This situation does not necessarily apply to other disorders. In this specific case a large amount of the information is obtained from associated behaviors of GTS. For other disorders the gain in information using broader models might not be enough to compensate for the raise in the lod score threshold.

When multiple models are tested, an increased lod score threshold should be adhered to due to of an increased probability of false positives. We evaluated the appropriateness of the correction proposed by Risch. When only a single model was tested, the frequency of false positive findings was extremely low (less then 1 in 20,000, see table 1). When all three tests were applied simultaneously with a threshold of 3.0 each, an approximate threefold increase in false positive findings was observed (1 in 7812, data not shown). The proposed correction dealt appropriately with this (only 1 in 25,000 cases the lod score threshold of 3.477 was exceeded).

Testing three diagnostic models is not a remedy against diagnostic instability. The waxing and waning course of phenotype expression and the possibility that patients suppress tics lead to diagnostic instability. This can result in a dramatic loss in lod score as we observed due to the updated diagnoses in one of the families (figure 3). Diagnostic instability could very well vary between different families, but could also be the result of the long time span between subsequent interviews. The time between the two series of diagnoses in this family was approximately five years. Recently, a second family was completely re-diagnosed after a period of only eighteen months without any changes in diagnosis. In order to account for the diagnostic uncertainties we propose to establish a lifetime diagnosis via repeated interviews. Individuals that have been

diagnosed as affected will remain affected in the analyses even if in subsequent series of diagnosis no disease phenotype is observed. Linkage information from unaffected individuals could be omitted in the analysis by applying alternative analytic methods as affected only analysis and sib-pair analysis.

In conclusion, the available family set is informative enough to detect linkage even in the case of extensive heterogeneity. In order to increase the chances of detecting linkage the four most informative families will be shared between all the members within the consortium. The consequences of diagnostic instability can be dramatic. As a remedy the members of the consortium agreed to re-investigate the available families periodically and to perform sib-pair analysis on a large set of sib-pairs.

By using simulation techniques the strategy of choice for a linkage study can be determined systematically instead of being made arbitrarily. In the case of GTS the simultaneous testing of three diagnostic models was shown to give the best probabilities on detecting linkage.

REFERENCES

1. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
2. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. N Engl J Med 1986; 315: 993-7.
3. Pauls DL, Towbin KE, Leckman JF, Zahner GEP, Cohen DJ. Gilles de la Tourette's syndrome and obsessive-compulsive disorder: evidence supporting a genetic relationship. Arch Gen Psychiatry 1986; 43: 1180-2.
4. Pauls DL, Pakstis AJ, Kurlan R, Kidd KK, Leckman JF, Cohen DJ, Kidd JR, Como P, Sparkes R. Segregation and linkage analyses of Tourette's syndrome and related disorders. J Am Acad Child Adolesc Psychiatry 1990; 29: 195-203.
5. Heutink P, Wetering BJM van de, Breedveld GJ, Weber J, Sandkuijl LA, Devor EJ, Heiberg A, Niermeijer MF, Oostra BA. No evidence for genetic linkage of Gilles de la Tourette syndrome on chromosome 7 and 18. J Med Genet 1990; 27: 433-6.
6. Heutink P, Breedveld GJ, Niermeijer MF, Wetering BJM van de, Oostra BA. Progress in gene localization. In: Kurlan R (ed). The handbook of Tourette's syndrome and associated tic and behavioral disorders. Marcel Dekker, New York, 1993: 317-35.
7. Devor EJ, Grandy DK, Civelli O, Litt M, Burgess AK, Isenberg KE, Wetering BJM van de, Oostra BA. Genetic linkage is excluded for the D_2-dopamine receptor lambdaHD2G1 and flanking loci on chromosome 11q22-q23 in

Tourette syndrome. Hum Hered 1990; 40: 105-8.
8. Gelernter J, Pakstis AJ, Pauls DL, Chapell P, Kurlan R, Grandy DK, Bunzow J, Retief AE, Litt M, Civelli O, Kidd KK. Tourette syndrome is not linked to D_2 dopamine receptor. Arch Gen Psychiatry 1990; 47: 1073-7.
9. Pakstis AJ, Heutink P, Pauls DL, Kurlan R, Wetering BJM van de, Weber J, Leckman JF, Sandkuijl LA, Kidd JR, Breedveld GJ, Castiglione CM, Sparkes RS, Cohen DJ, Kidd KK, Oostra BA. Progress in the search for genetic linkage with Tourette syndrome: an exclusion map covering more than 50% of the autosomal genome. Am J Hum Genet 1991; 48: 281-94.
10. Heutink P, Wetering BJM van de, Breedveld GJ, Oostra BA. Genetic study on Tourette syndrome in the Netherlands.In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 167-72.
11. Wilkie PJ, Ahmahn PA, Hardacre J, Laplant RJ, Hiner BC, Weber JL. Application of microsatellite DNA polymorphisms to linkage mapping of Tourette syndrome gene(s). In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 173-80.
12. Devor EJ. Linkage studies in 16 St. Louis families: Present status and pursuit of an adjunct strategy. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York,19-92: 181-8.
13. Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, Long RT, Kidd KK, Conte G, Housman DE, Paul SM. Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish. Nature 1989; 342: 238-43.
14. Kennedy JL, Giuffra LA, Moises HW Cavalli-Sforza LL, Pakstis AJ, Kidd JR, Castiglione CM, Sjögren B, Wetterberg L, Kidd KK. Evidence against linkage of schizophrenia to markers of chromosome 5 in a Northern Swedish pedigree. Nature 1988; 336: 167-70.
15. Baron M, Freimer NF, Risch N, Lerer B, Alexander JR, Straub RE, Asokan S, Das K, Peterson A, Amps J, Endicott J, Ott J, Gilliam TC. Diminished support for linkage between manic depressive illness and X-chromosome markers in three Israeli pedigrees. Nature Genet 1993; 3: 49-55.
16. Risch N. Genetic linkage and complex diseases, with special reference to psychiatric disorders. Genet Epidem 1990; 7: 3-16.
17. Elston RC, Wilson AF. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 17-20.
18. Gershon RC. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 21-4.
19. Green P. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 25-8.
20. Matthyse S. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 29-32.
21. Morton NE. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 33-4.
22. Ott J. Genetic linkage and complex diseases: A comment. Genet Epidem 1990;

7: 35-6.
23. Suarez BK, Reich T, Rice JP, Cloninger CR. Genetic linkage and complex diseases: A comment. Genet Epidem 1990; 7: 37-40.
24. Risch N. Genetic linkage and complex diseases: A response. Genetic Epidem 1990; 7: 41-5.
25. Baron M. Genetic linkage in mental illness. Nature 1990; 346: 618.
26. Byerley WF. Genetic linkage revisited. Nature 1989; 340: 340-1.
27. Sherrington R, Brynjolfsson J, Petursson H, Potter M, Dudleston K, Barraclough B, Wasmuth J, Dobbs M, Gurling H. Localization of a susceptibility locus for schizophrenia on chromosome 5. Nature 1988; 336: 164-7.
28. Boehnke M. Estimating the power of a proposed linkage study: Apractical computer simulation approach. Am J Hum Genet 1986; 39: 513-27.
29. Weeks DE, Lehner T, Squires-Wheeler E, Kaufman C, Ott J. Measuring the inflation of the lod score due to its maximization over model parameter values in human linkage analysis. Genet Epidemiol 1990; 7: 237-43.
30. Terwilliger JD, Ott J. A multisample bootstrap approach to the estimation of maximized-over-models lod score distributions. Genetic Analysis Workshop 7: Issues in gene mapping and detection of major genes. In: MacCluer JW, Chakravarti A, Cox D, Bishop DT, Bale SJ, Skolnick MH (eds). Cytogenet Cell Genet 1992; 59: 142-4.
31. Kurlan R, Behr J, Medved L, Shoulson I, Pauls DL, Kidd JR, Kidd KK. Familial Tourette syndrome: report of a large pedigree and potential for linkage analysis. Neurology 1986; 36: 772-6.
32. Pauls DL, Hurst CR. Schedule for Tourette's syndrome and other behavioral syndromes. Yale University Child Study Center, 1987.
33. Weeks DE, Ott J, Lathrop GM. Slink: A general simulation program for linkage analysis. Am J Hum Genet 1990; 47 (A204):1428.
34. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on a small computer. Am J Hum Genet 1989; 44: 388-96.
35. Rish N. A note on multiple testing procedures in linkage analysis. Am J Hum Genet 1991; 48: 1058-64.

4. LINKAGE STUDIES: *CHROMOSOMAL ABNORMALITIES*

4.1. No evidence for genetic linkage of Gilles de la Tourette syndrome on chromosome 7 and 18.

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INTRODUCTION

Gilles de la Tourette Syndrome (GTS) is a neuropsychiatric disorder with an unknown etiology. The syndrome is characterized by recurrent, involuntary, repetitive multiple motor- and vocal tics. In many patients associated behavioral problems like obsessive compulsive behavior and copro- and echophenomena are observed (1).

There is strong evidence that GTS is genetically determined. The exact mode of inheritance, however, is still a matter of discussion. Both single major locus and multifactorial models have been proposed (1-6). The most widely held hypothesis states that a single autosomal dominant gene with reduced penetrance is involved (6). Still there is discussion which associated behavioral symptoms should be considered as variant expressions of the presumed genetic defect responsible for GTS. There is general agreement that the chronic tic syndromes, according to DSM--III-R criteria, in families afflicted with GTS are variant phenotypes of the GTS gene defect (2,7).

Comings et al. presented evidence for the localisation of the GTS gene (8). They reported a 46,t(7;18)(q22;q22.1) reciprocal translocation in six relatives suffering from GTS. No support for linkage was found on the breakpoint on chromosome 7q22 with the COLA1 locus at 7q21.3-q22.1, suggesting a localization of the GTS gene near the 18q22.1 breakpoint. Donnai reported a GTS patient with a deletion of the long arm of chromosome 18 at 18q22.2 (9). These findings led to the tentative assignment of the GTS gene to chromosome 18q22.1. At 18q21 a candidate gene, the Gastrin Releasing Peptide (GRP), has been localised coding for a neuropeptide like protein (10,11).

In order to determine the chromosomal location of the GTS gene, we have started genetic linkage studies in five families of Dutch origin and in one family of Norwegian origin. In our present study we found no support for linkage, on either chromosome 18 or chromosome 7, including the COLA1 locus (7q21.3-q22.1) near the translocation breakpoint.

MATERIAL AND METHODS

Family material

Clinical and genetic studies were performed in five Dutch families and one Norwegian family. Complete pedigree data and methods of ascertainment on these data will be published elsewhere and are briefly summarized here (see chapters 2.1 and 2.2).

DNA analysis

DNA was isolated from peripheral blood lymphocytes of family members as described by Miller et al. (12). Chromosomal DNA was digested with various restriction enzymes (Boehringer, Pharmarcia, BRL) according to the manufacturers instructions. Gel electrophoresis of 8µg DNA samples on 0.7% agarose gels, and DNA immobilization by alkaline blotting onto nylon membranes (Gene Screen plus) were done according to standard procedures (13). Hybridisation conditions were as described by Maniatis (13), washing was performed at 65°C at 0.1 x SSC final stringency. DNA was labelled by random hexamer priming according to Feinberg & Vogelstein (14).

Markers B74 (D18S3), OLVIIA8 (D18S7), OS-4 (D18S5), pHF12-62 (D18S1) and pERT25 (D18S11) were used as reference points for chromosome 18 as they had been mapped previously by physical methods and were used for the construction of a continuous linkage map for chromosome 18 (15). They were kindly provided by Drs. J.L. Mandel, H. Olek, H. Tateishi, R. White and U. Müller, respectively. Markers OLVIIE10 and GRP have previously been described (16,17) and shown to map to chromosome 18.

Markers pJ2(TCRB), Cgamma (TCRG), pMetH (MET), NJ3 (COLA1), pTHH28 (D7S371), pRMU7.4 (D7S370), pYNB3.1R (D7S372) C33 (D7S1-26), TM102L (D7S135) and pB79A (D7S13) were previously mapped on chromosome 7 and used in a linkage map (18). In our present study they were used as reference points for chromosome 7. These markers were kindly provided by Drs. T.W. Mak, R. White, P. Tsipouras, Y. Nakamura, L. Tsui and J. Schmidtke, respectively. Markers pXV-2C (D7S23) and TN127 (D7S144) have been described previously and shown to map to chromosome 7 (19,20).

Linkage analysis

Linkage analysis was performed using the Linkage package program version 5.03 (21,22). The GTS gene frequency was estimated to be 0.003, with a male penetrance of 0.999 and a female penetrance of 0.56. The correction for possible phenocopies was 0.0002 (6). Two point linkage analysis was performed with the MLINK program and multipoint analysis with the LINKMAP program using Haldane's mapping function for interference. No allowance was made for spontaneous mutations. In the multipoint analysis we assumed a constant sex ratio for crossing over of 2.1 (female/male) for chromosome 18 (15). For chromosome 7 we assumed a constant sex ratio for crossing over of 2.0 (18). A lod score of at least 3.0 was considered as evidence for genetic linkage, a

lod score of -2.0 was considered as evidence for exclusion of linkage, for the assumed model of a single dominant gene with reduced penetrance (23).

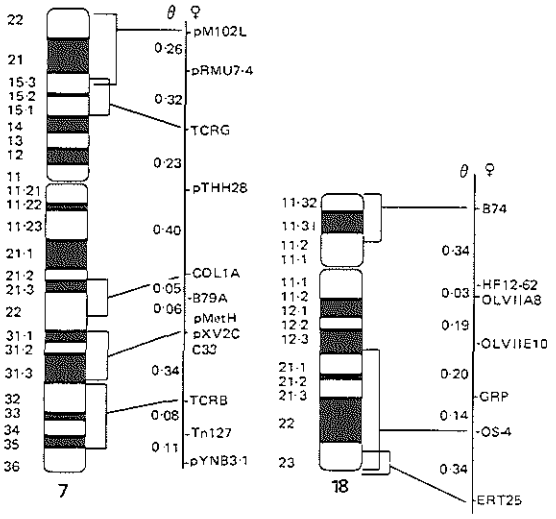

Figure 1 Female specific genetic maps of chromosomes 7 and 18. Physical locations are indicated where known. Marker order and map distances are based on the linkage maps of O'Connell et al[15, 18] and mapping studies with our family material. Map distances are presented as θ, assuming a constant female/male crossing over ratio of 2·0 for chromosome 7 and 2·1 for chromosome 18.

RESULTS AND DISCUSSION

Chromosome 18 markers were tested in six extended families and a linkage map was constructed using the continuous linkage map of O'Connell et al. as a basis (15). Mapping the genetic distances of corresponding markers B74, pHF12-62, OLVIIA8, OS-4 and pERT25 with our own material differred only marginally from the O'Connell mapping distances. Additional markers OLVIIE10 and GRP were mapped, with our family material, into the fixed O'Connell linkage map.

Comings et al. and Donnai postulated that the gene responsible for GTS resides on chromosome 18q22.1 (8,9). Markers OLVIIE10 at 18q21.3, GRP at 18q21, OS-4 at 18q21-qter and pERT25 at 18q23 map into the region surrounding the t(7:18)(q22:q22.1) translocation breakpoint (24). Given the linkage map and the cytogenetic maps (15,24,fig 1.) the most

TABLE 1

Two point linkage data for chromosome 18.

	Recombination fraction (Θ)							A.
Locus	.0	.05	.1	.15	.2	.3	.4	
B74	-23.46	-6.68	-4.24	-2.86	-1.96	-0.85	-0.26	25
pHF12-62	-15.89	-3.92	-2.18	-1.26	-0.69	-0.11	0.07	11
OLVIIA8	-14.33	-3.92	-2.22	-1.32	-0.77	-0.21	-0.02	11
OLVII10	-13.88	-5.39	-3.34	-2.20	-1.46	-0.62	-0.24	18
GRP	-10.26	-2.76	-1.84	-1.30	-0.91	-0.40	-0.11	5
OS4	-12.77	-4.32	-2.83	-1.93	-1.31	-0.51	-0.11	17
pERT25	-25.07	-8.04	-4.47	-2.61	-1.50	-0.44	-0.11	18

A.: Centimorgans definitely excluded on either site of the tested marker, using Haldane's mapping function (23).
A lodscore of -2 or less was assumed as definite proof for exclusion.

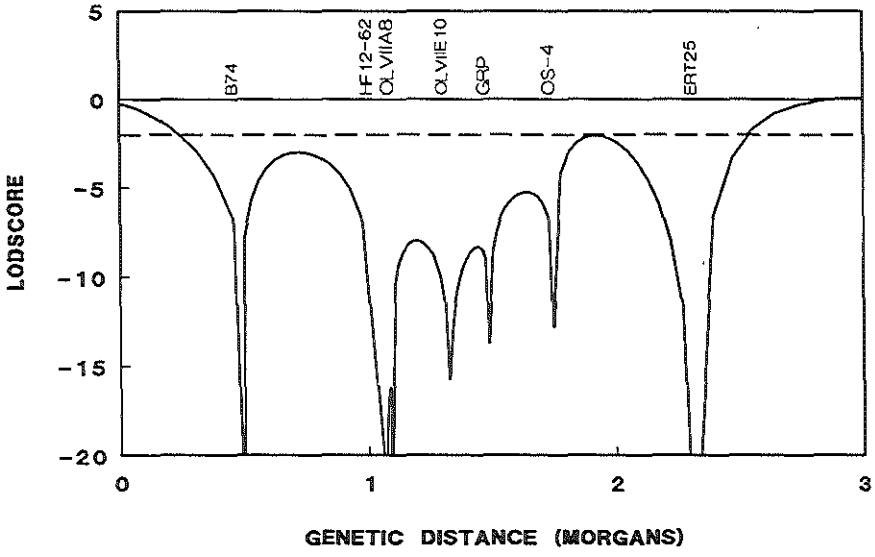

Figure 2a Multipoint linkage analysis showing the exclusion of the GTS locus from chromosome 18. Dotted line represents a value of -2.

TABLE 2

Two point linkage data for chromosome 7.

	Recombination fraction (Θ)							A.
Locus	.0	.05	.1	.15	.2	.3	.4	
Tm102L	-22.62	-5.09	-2.48	-1.17	-0.43	0.19	0.22	11
TCRG	-12.68	-4.90	-3.29	-2.29	-1.60	-0.73	-0.26	18
pRMU7.4	-21.01	-5.06	-2.92	-1.76	-1.04	-0.58	-0.30	11
pTHH28	-11.04	-2.78	-1.43	-0.75	-0.36	-0.01	0.07	5
NJ3	-13.62	-2.73	-1.26	-0.54	-0.13	0.23	0.23	5
pB79A	-12.28	-3.28	-1.75	-0.93	-0.44	0.02	0.10	5
pMetH	-17.36	-3.91	-1.95	-1.00	-0.47	-0.01	0.06	11
pXV2C	-25.28	-6.47	-3.46	-1.93	-1.02	-0.13	0.11	17
C33	- 1.54	0.04	0.20	0.23	0.21	0.12	0.03	0
TCRB	-21.18	-6.15	-3.63	-2.20	-1.28	-0.30	0.04	18
Tn127	-15.73	-4.98	-3.05	-1.99	-1.31	-0.52	-0.14	18
pYNB3.1	- 7.28	-2.63	-1.45	-0.82	-0.43	-0.05	0.05	5

A.: Centimorgans definitely excluded on either site of the tested marker, using Haldane's mapping function (23).
A lodscore of -2 or less was assumed as definite proof for exclusion.

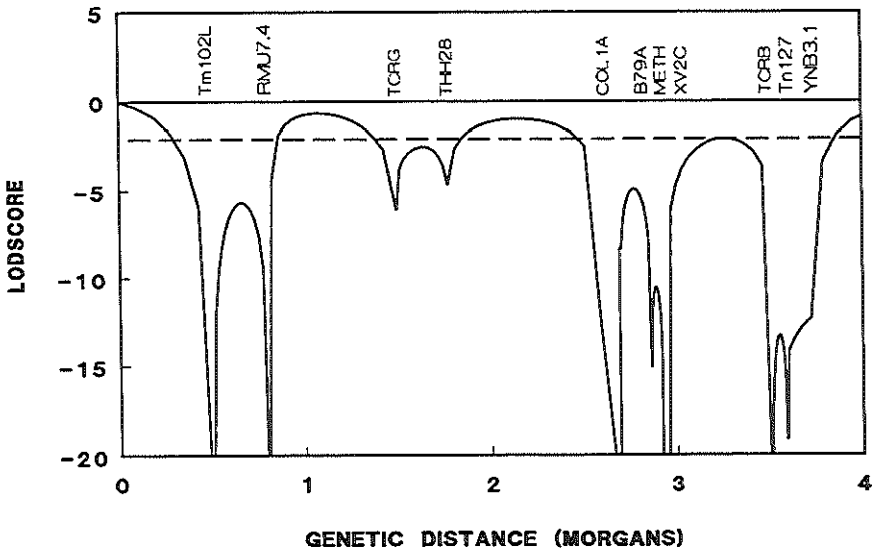

Figure 2b Multipoint linkage analysis showing the exclusion of the GTS locus from chromosome 7q21.3-qter. Dotted line represents a value of -2.

likely localization for the translocation breakpoint is between markers OLVIIE10 and OS-4.

Lod scores for the two point analysis of chromosome 18 between GTS and marker loci are shown in table 1. Only individuals presenting GTS or Tic Syndromes were included as affected in our present study. None of the markers tested showed evidence for linkage and excluded linkage for the genetic distance mentioned in table 1. Using multipoint analysis (fig 2), we obtained lod scores of at least -5 for the translocation breakpoint region between markers OLVIIE10 and OS-4. A lodscore of at least -2 was obtained for the complete linkage map of chromosome 18. This value is generally accepted as evidence for the exclusion of linkage (23). We therefore conclude that chromosome 18 can be excluded as a site for the GTS gene. These results are not in agreement with the findings of Comings et al. and Donnai (8,9).

Another possible site for the GTS gene would be the breakpoint of the translocation on chromosome 7q22 reported by Comings et al (8). We have tested several RFLP markers on chromosome 7 with our family material, and a linkage map was constructed. Comparison of our linkage map with the primary linkage map of O'Connell gave marginal differences only except for marker C33, which we mapped at a theta of 0.01 telomeric of pMetH instead of theta 0.11 in the O'Connell map (18). Our results are consistent with the findings of Rommens et al. (19). To avoid possible mapping errors we did not include marker C33 in the multipoint analysis. Marker TN127, not on the primary linkage map of O'Connell et al., was mapped using our family material and added to the combined linkage map (fig 1) (18).

For none of the markers tested on chromosome 7 we found evidence for linkage, including the COLA1 locus which is located proximal of the EPO locus at 7q21 and thus must be located proximal of the presumed breakpoint of the translocation (24). With multipoint analysis we have been able to exclude part of chromosome 7p and the 7q21.3-qter region including the translocation breakpoint region. The region around marker C33 is excluded by flanking markers pMetH, pXV2C and TCRB in the two point analysis as well as in the multipoint analysis, even if we assume that marker C33 is localised at $\Theta = 0.11$ of pMetH.

With the exclusion of chromosome 18 as a possible site for the GTS gene and the exclusion of the 7q21.3-qter region of chromosome 7, we conclude that the 46,t(7;18)(q22;q22.1) reciprocal translocation is not linked to the gene responsible for the Gilles de la Tourette Syndrome. However, genetic heterogeneity could mask a positive result. In this study, all families contributed to the negative lod scores on both chromosomes.

Currently, we are performing collaborative genetic linkage studies on other parts of the genome in order to find the location of the GTS gene (25).

Chromosomal rearrangements in families suffering from GTS could facilitate the localisation of the GTS gene but should be followed by extensive linkage studies, in order to obtain definite proof for genetic linkage.

REFERENCES

1. Shapiro AK, Shapiro ES, Young JG, Feinberg FE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
2. Kidd KK, Pauls DL. Genetic hypotheses for Tourette syndrome. In: Friedhoff AJ, Chase TN (eds). Advances in Neurology. Raven Press, New York 1982; 35: 243-9.
3. Baron M, Shapiro ES, Shapiro AK, Rainer JD. Genetic analysis of Tourette Syndrome suggesting major gene defect. Am J Hum Genet 1981; 36: 767-75.
4. Comings DE, Comings BG, Devor EJ, Cloninger CR. Detection of a major gene for Gilles de la Tourette. Am J Hum Genet 1984; 36: 586-600.
5. Devor EJ. Complex segregation analysis of Gilles de la Tourette Syndrome: further evidence for a major locus mode of transmission. Am J Hum Genet 1984; 36: 704-9.
6. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's Syndrome and associated behaviours: Evidence for autosomal dominant transmission. N Eng J Med 1986; 315: 993-7.
7. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd ed, revised). APA, Washington DC, 1987.
8. Comings DE, Comings BG, Dietz G, Muhleman D, Okada TA, Sarinana F, Simmer R, Sparkes R, Crist M, Stock D. Linkage studies in Tourette Syndrome. Am J Hum Genet 1986; 39: 447.
9. Donnai D. Gene Location in Tourette Syndrome. Lancet 1987; i: 627.
10. Shaw D, Eiberg H. Report of the committee for chromosomes 17, 18, and 19. Cytogen Cell Genet 1987; 46: 242-56.
11. Spindel ER, Chin WW, Price J, Rees LH, Besser GM, Habener JF. Cloning and characterization of cDNAs encoding human gastrin-releasing peptide. Proc Nat Acad Sci USA 1984; 81: 5699-703.
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 1988; 16: 1214.
13. Maniatis T, Fritsch EF, Sambrook J. Molecular Cloning: A laboratory Manual. Cold Spring Harbor Laboratory. 1982.
14. Feinberg A, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983; 132: 6-13.
15. O'Connell P, Lathrop GM, Leppert M, Nakamura Y, Müller U, Lalouel JM, White R. Twelve loci form a continuous linkage map for human chromosome 18. Genomics 1988; 3: 367-72.
16. Marlkens F, Delattre O, Bernard A, Olschwang S, Dutrillaux B, Thomas G. RFLP identified by the anonymous DNA segment OLVIIE10 at 18q21.3.

Nucleic Acid Res 1987; 15: 1348.
17. Detera-Wadleigh SD, Anderson S, Spindel ER. A frequent PvuII RFLP of the Human Gastrin Releasing gene. Nucleic Acid Res 1987; 15: 375.
18. O'Connell P, Lathrop GM, Leppert M, Nakamura Y, Tsui LC, Lalouel JM, White R. A primary genetic linkage map of chromosome 7. Cytogen Cell Genet 1987; 46: 672.
19. Rommens JM, Zengerling S, Burns J, Melmen G, Keren B, Lavsic N, Zsiga M, Kennedy D, Markiewicz D, Rozmanel R, Riordan JR, Buchwald M, Tsui L. Identification and regional localisation of DNA markers on chromosome 7 for the cloning of the Cystic Fibrosis gene. Am J Hum Genet 1988; 43: 645-63.
20. Estivill X, Farrall M, Scambler PJ, Bell GM, Hawley KMF, Lench NJ, Bates GP, Kruyer HC, Frederick PA, Stanier P, Watson EK, Williamson R, Wainwright BJ. A candidate for the Cystic Fibrosis locus isolated by selection for methylation free islands. Nature 1987; 326: 840-45.
21. Lathrop GM, Lalouel JM. Easy calculations of lodscores and genetic risks on a small computer. Am J Hum Genet 1984; 36: 460-5.
22. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. Proc Nat Acad Sci USA 1984; 81: 3443-6.
23. Conneally PM, Rivas ML. Linkage analysis in Man. In: Harris H, Hirschhorn K (eds). Advances in Human Genetics. Plenum Press, New York & London, 1980; 10: 209-61.
24. Tsui L.-C, Farrall M, Donis-Keller H. Report of the committee on the genetic constitution of chromosome 7 and 8. Cytogen Cell Genet 1989; 51: 166-201.
25. Devor EJ, Grandy DK, Civelli O, Litt M, Burgess AK, Isenberg KE, Wetering BJM van de, Oostra B. Genetic linkage is excluded for the D2-dopamine receptor lambda HD2G1 and flanking loci on chromosome 11q22-q23 in Tourette syndrome. Hum Hered 1990; 40: 105-8.

4. LINKAGE STUDIES: *CANDIDATE GENES*

4.2. Genetic linkage is excluded for the D_2-dopamine receptor lambda-HD2G1 and flanking loci on chromosome 11q22-q23 in Gilles de la Tourette syndrome.

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Adapted from:
Human Heredity 1990; 40: 105-8.

INTRODUCTION

Familial/genetic studies of Gilles de la Tourette syndrome (GTS) have demonstrated transmission via a single autosomal dominant gene (1-3). Several independent lines of evidence including analogous, trauma-induced conditions, animal models, and the successful therapeutic use of haloperidol (4-6) suggest that the dopamine system of the midbrain and its various projections may be the locus of action of the GTS gene. Specifically, the D_2-dopamine receptor is a prime candidate locus (7).

We have used the D_2-dopamine receptor clone HD2G1, which has been localized to human chromosome 11q22-q23, to screen a sample of fifteen families (n = 166) ascertained through GTS probands (8). In addition, the flanking probes p2-7-1D6 (D11S84), O 2-11-2.2 (D11S34), and L7 (D11S29) have been screened. In all cases tight linkage to GTS is excluded. These data, along with those from two other laboratories, permit unequivocal exclusion of the entire region 11q22-q23 in GTS.

MATERIALS AND METHODS

High molecular weight genomic DNA was extracted from either whole bloods or transformed cell lines via standard methods (9,10). Restriction endonuclease digestions were performed at 5 U/µg using manufacturer-recommended conditions (Boehringer-Mannheim Biochemicals). Following size fractionation by agarose gel electrophoresis, restriction fragments were transferred to charged nylon membranes (Zeta-Bind) using the alkaline modification of the method of Southern (11). Probes were labeled with Ó-^{32}P dCTP by primer extension (12). Hybridizations and washes were at conventional stringencies.

Linkage analyses were performed pairwise with a version of LIPED incorporating sex-specific penetrances at the trait locus (13,14). Following Pauls and Leckman both GTS and chronic motor tics were considered as affected, a dominant major locus was assumed with a male penetrance of 0.999 and female penetrance of 0.561, and the frequency of the trait allele was set at 0.3% (3).

RESULTS AND DISCUSSION

Linkage analysis results for the four chromosome 11q probes are presented in table 1. Tight linkage is excluded for all loci. For the DRD2 locus

Table 1

Lod scores for linkage analysis of chromosome 11 markers against GTS, $\Theta_m=\Theta_f$

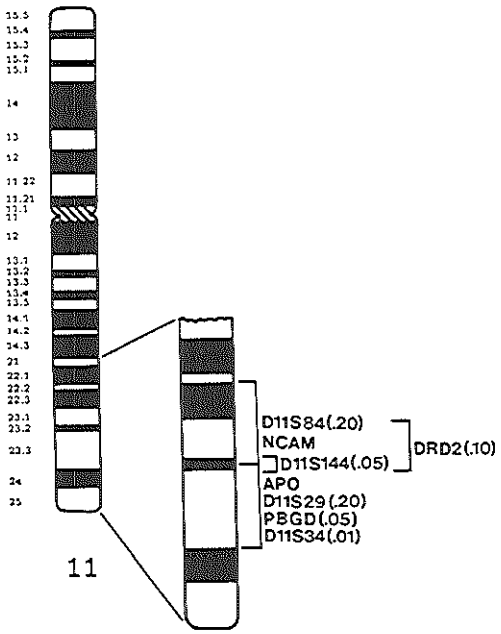
Locus/(probe)	Recombination fraction (Θ)						
	0.00	0.05	0.10	0.20	0.30	0.40	0.50
DRD2(HD2G1)	-8.85	-2.68	-1.37	-0.35	-0.07	-0.03	0
D11S84(p2-7-1D6)	-9.92	-5.06	-3.36	-1.69	-0.81	-0.30	0
D11S29(L7)	-4.93	-1.33	-0.77	-0.29	-0.09	-0.02	0
D11S34(O2-11-22)	-2.77	-0.02	0.50	0.65	0.44	0.13	0

the region of exclusion extends nearly to ± 7% recombination. The greatest region of exclusion is seen for the anonymous locus D11S84, this region extending well beyond ± 15% recombination. The least amount of information was obtained for the locus D11S34 for which tight linkage only (± 1% recombination) could be excluded. Recent linkage data presented by Gelernter et al. suggest that the locus D11S29 is tightly linked to DRD2 and lies qter of that locus (15). The locus D11S84 is located some 15 cM centromeric of DRD2. These and other primary mapping data are presented in figure 1. The exact location of D11S34 is not shown but it does lie toward the qter end of the chromosome beyond PBGD (16).

Also shown in figure 1 are the maximum exclusions obtained for several of the mapped markers studied in TS families. DRD2, D11S29, and D11S84 each studied by us as well as by the laboratories of Dr. K. Kidd at Yale University and Dr. B. Oostra at Erasmus University in Rotterdam. In addition, we have studied D11S23, Dr. Oostra has added D11S144, Drs. Kidd and Oostra have both added PBGD [unpubl. data]. Accepting the 15-cM distance between DRD2 and D11S84 as a standard the region of combined exclusion extends some 35 cM beyond DRD2 in the centromeric direction and and 20-25 cM beyond DRD2 in the qter direction. Thise distances are sufficient to exclude 11q22-q23.

The results shown here permit the conclusion that the potential candidate gene/region DRD2/11q22-q23 is not linked to GTS. This exclusion is especially strengthened by the addition of independently obtained linkage results from other laboratories. However, while we suggest that the D_2-dopamine receptor gene/region is not directly involved in GTS, we do not suggest that a dopaminergic model of this disorder is to be abandoned.

Figure 1 *Primary gene map of chromosome 11q22-q23 indicating the positions and best order of marker loci spanning the region. DRD2 refers to the D_2-dopamine receptor, NCAM is the neural cell adhesion molecule, APO is the cluster of apo-liproteins, and PBGD is porphobilinogen deaminase. The other loci are anonymous clones. The numbers associated with several of the markers indicate the maximum region of exlusion against GTS as ± percent recombination (Θ), where Θ = 0.01 10^6bp.*



Indeed, the possibility of the role of regulatory genes and/or other interactions in the dopamine system remains in spite of these results. Moreover, there is the likelihood that other dopamine receptors genes exist as the family of G-protein coupled receptors continues to grow (17).

REFERENCES

1. Comings DE, Comings BG, Devor EJ, Cloninger CR. Detection of a major gene for Gilles de la Tourette Syndrome. Am J Hum Genet 1984; 36: 586-600.
2. Devor EJ. Complex segregation analysis of Gilles de la Tourette syndrome: Further evidence for a major locus mode of transmission. Am J Hum Genet 1984; 36: 704-9.
3. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. N Engl J Med 1986; 315: 993-7.
4. Devinsky O. Neuroanatomy of Gilles de la Tourette's syndrome: possible midbrain involvement. Arch Neurol 1983; 40: 508-14.
5. Gayley D, Simon H, Le Moal M. Behavioral effects of lesions in the A10 dopaminergic area of the rat. Brain Res 1977; 124: 83-97.
6. Shapiro AK, Shapiro ES, Yong JG, Feinberg TE. Gilles de la Tourette syndrome. 2nd ed. Raven Press, New York 1988.
7. Devor EJ. Untying the Gordian Knot: The genetics of Tourette syndrome. J Nerv Ment Dis 1990; 178: 669-79.
8. Grandy DK, Litt M, Allen LJ, Bunzow J, Magenis RE, Civelli O. Dopamine D_2 receptor maps to human chromosome 11q22-q23 and identifies a TaqI RFLP. Cytogenet Cell Genet 1989; 51: 1007.
9. Kunkel LM, Smith KD, Boyer SH, Borgoanker DS, Wachtel SS, Miller OJ, Breg WR, Jones HW Jr, Rary JM. Analysis of human Y-chromosome-specific reiterated DNA in chromosomal variants. Proc Nat Acad Sci USA 1977; 74: 1245-9.
10. Bell GI, Karam JH, Rutter WJ. Polymorphic DNA region adjacent to the 5' end of the human insulin gene. Proc Nat Acad Sci USA 1981; 78: 5759-63.
11. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Molec Biol 1975; 98: 503-17.
12. Feinberg AP, Vogelstein B. A technique for radio-labeling DNA restriction endonuclease fragments to high specific activity. Analyt Biochem 1983; 132: 6-13.
13. Ott J. Analysis of human genetic linkage. John Hopkins University Press Baltimore, 1985.
14. Van Eerdewegh P. Linkage analysis with cohort effets: An application to X-linkage. Genet Epidemiol 1989; 6: 271-6.
15. Gelernter J, Grandcy DK, Bunzow J, Pakstis AJ, Civelli O, Retief AE, Litt M, Kidd KK. D_2 dopamine receptor locus (probe hD2G1) maps close to D11S29 (probe PBGD0.9) and D11S84 (probe p-2-7-1D6) on 11q. Cytogenet Cell Genet 1989; 51: 1002.
16. Junien C, McBride OW. Report of the committee on the genetic constitution of chromosome 11. Cytogenet Cell Genet 1989; 51: 226-58.
17. Barnard EA. Separating receptor subtypes from their shadows. Nature 1988; 335: 301-2.

5. REFLECTIONS ON THE PHENOTYPE

5.1. No specific ophthalmologic abnormalities in Gilles de la Tourette syndrome

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INTRODUCTION

The Gilles de la Tourette syndrome (GTS) is a chronic neuropsychiatric disorder characterized by motor and vocal tics (1). In the majority of cases the onset is in childhood with tics in the facial area; in about one-third of the cases as increased eye-blinking (1). Gradually the motor tics spread in rostrocaudal direction over the body, following a waxing and waning course in severity. In addition to the tics a variety of other symptoms like obsessive-compulsive behavior, attention deficit disorder with hyperkinesia, conduct disorder, sleep disturbances and self-injurious behavior has been documented in many GTS patients (2). In the absence of specific, confirmatory laboratory examinations or imaging techniques, the diagnosis is made primarily on the presence of both motor and vocal tics in the history and/or during examination. As tics can be suppressed, consciously or unconsciously, a definite diagnosis is often difficult to make.

With regard to the etiology disturbances in dopaminergic, noradrenergic, serotonergic, cholinergic, GABA-ergic and opioid circuits in the fore- and midbrain have been hypothesized (2). However, a primary structural or functional disturbance of the brain has not yet been demonstrated. The treatment is symptomatic and consists of supportive counselling, medication (clonidine, dopamine receptor blocking agents, serotonin re-uptake blockers) and/or (behavioral) psychotherapy (3). In severe cases the results of treatment are often limited.

In his 1885 paper Gilles de la Tourette already noted the familial occurrence of tics in some of his patients (4). Succeedingly, twin studies, adoption studies and systematic family studies have confirmed his observation (5). The most generally accepted hypothesis about the genetic model is consistent with an autosomal dominant transmission of a single major gene with incomplete penetrance and a variable expression (5). Chronic multiple tics (either motor or vocal) and obsessive-compulsive behaviors are suggested to belong to the phenotype as part of a continuum of symptoms (6,7). Currently an extensive collaborative linkage study is undertaken to map the GTS-susceptibility gene(s) (8).

Linkage studies are highly dependent on the quality of diagnostic procedures in defining the phenotype. In addition to a clinical diagnosis, the existence of biological markers is invaluable for the identification of the affected individuals in the family. For GTS no neuro-anatomical, -chemical or -physiological marker has been found, yet.

Enoch and associates have reported a series of visual field defects, which they consider to be specific for GTS (9-12). They include nasal and temporal step-like defects, enlargement of the blind spot, nerve fibre bundle-like defects anomalies, baring of the blind spot and fatigue effects (i.e. reduced sensitivity over time) (9-12). The visual field defects were found to be fluctuant over time, both progressive and remissive in 50% (16). About 50% of the earlier demonstrated field defects remained stable over time. The number of steps in the visual fields and the areas of sensitivity difference at the horizontal meridian appeared to be reduced by neuroleptic treatment, given for the tics (11). Interestingly, the field defects appeared to exist in biologic relatives of GTS patients, but not in a normal control group (12). If indeed, these field defects are linked to tic disorders in biological relatives of GTS probands, then they would provide the much wanted biological marker for GTS.

As part of our genetic GTS research program we decided to perform a pilot study in a series of GTS patients to replicate the findings of Enoch and associates to decide whether visual field studies should be performed in the extended GTS pedigrees under study.

MATERIALS AND METHODS

Ten consecutive outpatients with a diagnosis of GTS according to DSM-III-R criteria participated in this study after having given informed consent (13). Sexes turned out to be equally represented. Ages ranged from 16 to 54, the average age was 28.7 years. In two cases subjects were first-degree relatives (mother and son, brother and sister).

The ophthalmic examinations performed by ophthalmologists are given in table 1. Goldmann perimetry was performed twice with a delay of about one year by a technician with 10 years of experience in automated and kinetic (Goldman) perimetry. Before the first examination session she was not told which visual field abnormalities had previously been described in GTS. For the second test, she was especially instructed to look for the abnormalities as described by Enoch et al. (9-12).

RESULTS

The results are summarized in Table 2. By chance half of the patients used neuroleptic drugs and half of the patients had no drug treatment. On routine ophthalmologic examination no gross abnormalities were

TABLE 1

Ophthalmologic examination in 10 subjects with GTS

Best refracted visual acuity
Ocular alignment and versions
15 Diopter prism base temporal cover test
Direct and consensual pupillary reactions on light
Pupillary reactions on accommodation
Checking for afferent pupillary defects
Applanation tonometry
Slitlamp examination before and after pupillary dilatation
Checking for media opacities like cataract
Direct and indirect ophthalmoscopy in mydriasis
Repeated kinetic Goldmann perimetry;
V-4, I-4, I-3, I-2, I-1 isopters.
Target 1/4mm^2. Background luminance 31.5 asb.
Electrophysiological testing:
Flash electroretinography (ERG) with eye lid electrodes
Electro-oculography (EOG) with eye lid electrodes
Pattern or flash visually evoked cortical potentials (VEP)

found. Anisocoria with a pear-shaped pupil was found in case 8, a mild convergent strabismus in case 4 and a tilted optic disc in case 6. Case 7, who suffered from severe self-injurious tics involving hitting his head, ears and eyes with his fists, did not show any traumatic eye abnormalities. Only in case 2 a visual field abnormality has been found during the first perimetric examination. He showed a concentric restriction that was most likely related to diminished cooperation. At the second examination no abnormalities were found. Case 8 refused the second perimetry examination.
All electrophysiologic parameters failed to show any abnormalities. The EOG was not performed in two patients because of the interference of frequent eyemovements by one of them, the other did not keep his appointment. In one case the technical quality of the EOG was poor, but the results seemed to be normal.

DISCUSSION

Routine ophthalmologic examination revealed abnormalities in nine of the 10 patients, though only one (case 4) had consulted an ophthal-

TABLE 2

Characteristics and results of ophthalmologic examinations of 10 consecutive GTS patients.

Nr	sex	age	medication	Routine ophthalmologic examination	Kinetic Goldmann perimetry	ERG	EOG	VECP
1	♂	22	no	slight exophoria	1. NA 2. NA	NA	NA	NA
2	♂	42	no	R visual acuity 0.8 (amblyopia)	1. slight concentric narrowing R I-4 isopter 2. NA	NA	-[1]	NA
3	♂	25	no	R congenital ptosis	1. OU marked concentric constriction 2. NA	NA	NA	NA
4	♀	54	no	L convergent squint; slight ptosis	1. NA 2. NA	NA	-[2]	NA
5	♀	19	no	slight exophoria slightly hypopigmented choroid	1. slightly enlarged blind spot 2. NA	NA	NA	NA
6	♂	22	pimozide	slightly tilted disc	1. NA 2. NA	NA	NA	NA
7	♂	20	pimozide diazepam	slight esophoria	1. NA 2. slightly enlarged blind spot	NA	NA	NA
8	♀	16	pimozide	Slight anisocoria L pupil > R	1. NA 2. refused	NA	-[3]	NA
9	♀	38	pimozide	normal	1. R nasal lower slight constriction V-4 isopter 2. NA	NA	NA	NA
10	♀	29	sulpiride	slight iris stroma atrophy	1. NA 2. NA	NA	NA	NA

ERG : Electroretinogram
EOG : Electro-oculogram
VEP : Visual Evoked Cortical Potential
NA : No abnormalities
R : right eye
L : left eye
OU : oculus uterque, each eye
1. : First examination
2. : Second examination
1) not performed
2) technically not optimal, results appeared normal
3) not made because of frequent eye-movements

mologist before. These abnormalities showed a wide variation within this group. Esophoria and exophoria are for example quite common in the normal population. Amblyopia is present in 0.6-4.3% of the population. All other signs such as (congenital) ptosis, convergent squint, choroidal hypopigmetation, tilted disc, anisocoria, and iris stroma atrophy are rather often encountered in a regular ophthalmologic out-patient population, although their exact prevalences could not be found in the literature. We also found a slight enlargement of the blind spot in two cases but this can be explained by the kinetic disturbances and difficulty in fixation of our subjects.

The visual field abnormalities as described by Enoch and associates, have not been confirmed in our sample. As the reported defects are subtle and rather subjective, a design was chosen in which the technician was kept uninformed about the diagnosis and the reported visual field defects at the first examination, while the second time she was asked to look for the nasal steps and fatigue effects in this sample of GTS patients. In both conditions the overall findings were negative. Enoch and associates reported visual field defects in 95% of GTS patients and in 81% of 111 relatives that had been studied (12).

There is a wide fluctuation in results from visual field testing in normal persons comparable to the findings by Enoch and associates in GTS. In the absence of an age and sex matched control group, their findings may fall within normal variation. It is unknown how much variation exists in a normal population, when critical points in the visual fields are repeatedly tested. Moreover, the significance of a nasal or temporal step in the visual field of 5 degrees, as often found in Enoch's patients, is unclear. The same holds for the subtle enlargement of the blind spot as often demonstrated in their visual field plots. The arcuate scotomas, described in most of their patients, were not obvious from the visual fields shown. Finally, they did not describe whether the perimetrist was aware of the diagnostic status of the subjects, the family relationship and the findings to expect.

Enoch and associates did not perform automated perimetry on their GTS patients because of the modest anomalies recorded in paracentral isopters. However, it is generally acepted that automated perimetry is more suitable for quantitative perimetry in repeated sessions than kinetic Goldmann perimetry. The motor disturbances of GTS patients do not seem to justify the preference for Goldmann perimetry instead of automated perimetry. Recently it has been suggested that automated perimetry was more sensitive in visual field testing in GTS than kinetic perimetry (14). In this study the rates of visual field defects in GTS

patients and in a normal population did not differ. Moreover, no other ocular abnormalities except two cases with esotropia were found (14).

The nasal step in the visual field testing is a well-known sign in glaucoma explained by changes of the retinal nerve fibres that meet in a horizontal raphe in the temporal area of the retina. An asymmetric loss of nerve fibres in the upper and lower temporal retina can cause a nasal step in the visual field. Enoch et al. have not only been unable to explain why such a difference in nerve fibre loss would occur in the temporal retinal field, but they also admitted that such an explanation would not fit the temporal step in visual field testing. They only mention that the defect must be in the retina and hypothesize that these defects are due to dopaminergic involvement. To our knowledge, no additional ophthalmologic testing like electrophysiology or focal perimetry of specified areas in the nasal and temporal retina has been performed. This seems to be the obvious next step considering the unexplained findings.

In most of our GTS patients the course of tic development appeared to start in the facial area, most frequently as increased eyeblinking. Neuro-ophthalmologic disturbances reported in GTS include clonic bilateral symmetric or alternating or unilateral blepharospasm, forced staring and involuntary gaze deviation (15). These behaviors have not been exclusively reported in GTS but also in parkinsonian syndromes due to encephalitis and manganese intoxication (15). It is argued that this analogy in symptomatology points at involvement of the basal ganglia in GTS. Systematic abnormalities in eyemovement could not be demonstrated in GTS (16). In few patients sun-gazing has been reported as a compulsory form of self-injurious forced staring (17).

As we have not been able to replicate the findings by Enoch and associates and in addition did not find any other ophthalmic abnormalities that seemed to be specific for GTS, we decided not to include these investigations in our family study for screening.

REFERENCES

1. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
2. Robertson MM. The Gilles de la Tourette syndrome: the current status. Brit J Psychiatry 1988; 154: 147-169.
3. Cohen DJ, Bruun RD, Leckman. Tourette's syndrome and Tic disorders. Wiley, New York, 1988.

4. Gilles de la Tourette GAEB. Etude sur une affection nerveuse caractérisée par l'incoördination motrice accompagnée d'écholalie et de coprolalie. Arch Neurol 1885; 9: 19-42,158-200.
5 Wetering BJM van de, Heutink P. The genetics of the Gilles de la Tourette syndrome; a review. J Lab Clin Med 1993; 121: in press.
6. Golden GS. Tics and Tourette's: a continuum of symptoms? Ann Neurol 1978; 4: 145-8.
7 Pauls DL, Towbin KE, Leckman JF, Zahner GEP, Cohen DJ. Gilles de la Tourette's syndrome and obsessive-compulsive disorder: evidence supporting a genetic relationship. Arch Gen Psychiatry 1986; 43: 1180-2.
8. Pakstis AJ, Heutink P, Pauls DL, Kurlan R, Wetering BJM van de, Weber J, Leckman JF, Sandkuijl LA, Kidd JR, Breedveld GJ, Castiglione CM, Sparkes RS, Cohen DJ, Kidd KK, Oostra BA. Progress in the search for genetic linkage with Tourette syndrome: an exclusion map covering more than 50% of the autosomal genome. Am J Hum Genet 1991; 48: 281-94.
9. Enoch JM, Itzhaki A, Lakshminarayanan V, Comerford JP, Lieberman M, Lowe T. Gilles de la Tourette syndrome: Visual effects. Neuro-ophthalmology 1988; 8: 251-8.
10. Enoch JM, Itzhaki A, Lakshminarayanan V, Comerford JP, Lieberman M, Lowe T. Visual defects detected in patients with Gilles de la Tourette syndrome: ap reliminary report. Int Ophtalmol 1989; 13: 331-44.
11. Enoch JM, Khamar BM, Lakshminarayanan V. An analysis of step-like field defects in Gilles de la Tourette syndrome. Neuro-ophthalmology 1990; 10: 299-309.
12. Enoch JM, Lakshminarayanan V, Itzhaki A, Khamar BM, Landau K, Lowe T, Surendran T, Comerford J. Anomalous kinetic visual fields found in family members of patients with a confirmed diagnosis of Gilles de la Tourette syndrome. Optomet Vis Sci 1991; 68: 807-12.
13. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). American Psychiatric Press, Washington DC, 1987.
14. Repka MX, Singer H. Gilles de la Tourette syndrome and the afferent visual system. Neuro-ophtalmology 1992; 12: 367-74.
15. Frankel M, Cummings JL. Neuro-ophthalmic abnormalities in Tourette's syndrome: functional and anatomic implications. Neurology 1984; 34: 359-61.
16. Bollen EL, Roos RAC, Cohen AP, Minderaa RB, Reulen JPH, Wetering BJM van de, Buruma OJS, Woerkom TCAM van. Oculomotor disturbances in Gilles de la Tourette's syndrome. J Neurol Neurosurg Psychiatry 1988; 51: 1081-3.
17. Kobylski TP, Licamele WL. Sun gazing by patients with Tourette's disorder. Am J Psychiatry 1991; 148: 394.

5. REFLECTIONS ON THE PHENOTYPE

5.2. Mental play in Gilles de la Tourette syndrome and obsessive-compulsive disorder.

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INTRODUCTION

Motor and vocal tics have long been considered the most characteristic symptoms of the Gilles de la Tourette syndrome (GTS). The tics usually appear during childhood and follow a waxing and waning course. Tics probably are not mere 'muscular phenomena', but also have a psychological dimension and are related to certain sensory and cognitive experiences (1,2).

Apart from the key role of tics for the diagnosis of GTS, increasing attention is currently directed at other symptoms, like phobic behavior, attentional and learning problems, conduct disorder, sleep disturbances and self injurious behavior (3,4). These symptoms are called 'associated behavioral problems' and it is currently being debated whether they are an integral part of the syndrome or not (5-7). The association of obsessive-compulsive behavior with GTS is generally accepted (7). However, it is not yet clear what the exact phenomenological relationship is between GTS and obsessive-compulsive behavior.

According to DSM-III-R, obsessive-compulsive disorder (OCD) is an anxiety disorder, characterized by recurrent obsessional anxiety provoking ideas, and/or recurrent purposeful overt behavior, designed to neutralise obsessional ideas or to prevent a dreaded event or situation (8). The obsessions and/or compulsions are considered by the patient to be excessive and/or irrational. OCD causes distress, and significantly interferes negatively with the patient's daily activities.

Obsessive-compulsive symptoms have been proposed as intrinsic to GTS (3,9,10); this is also based upon findings in family studies (7,11). Remarkably, all these studies emphasized the frequency of occurrence of various obsessive-compulsive symptoms rather than their phenomenology or psychological significance. Differentiating OCD from impulse control disorders or impulsions seems essential in understanding the phenomenology and pathophysiology (12). Most obsessive-compulsive-like symptoms in GTS patients may in fact be impulsions (6). These authors used the term 'impulsions' to mean 'a stimulus that sets the mind in action'. For a more detailed discussion see Shapiro et al (6, pp 240 and 360).

In order to clarify the relationship between GTS and OCD, we performed a pilot study of GTS and OCD patients, comparing their phenomenology. The overall results of this study will be described in detail elsewhere (13). In this paper, we present a new and presumably unique GTS-related phenomenon, referred to as mental play (for the definition, see below).

PATIENTS AND METHODS

From an out-patient population 10 GTS and 10 OCD patients were invited consecutively to participate in the study. All had been diagnosed according to DSM-III-R criteria and gave written informed consent for the study. All patients were told that the aim of the study was to gain more insight into the symptoms of their ailment.

Exclusion criteria were previous or current psychosis, affective disorder, substance abuse, and organic brain disease. On basis of these criteria, one GTS patient and one OCD patient were excluded from the study. The GTS patient had had a psychotic episode and the OCD patient had epilepsy. One OCD patient withdrew from the study without giving a reason. The GTS group therefore consisted of eight males and one female, while the OCD group contained two males and six females. The mean age in the GTS group was 26 years (s.d. 6.7) and in the OCD group 44.5 years (s.d. 16.9). In both groups ages ranged from 18-70 years. Groups were not matched for education or duration of illness.

Each patient was investigated with a semi-structured interview on videotape. The interview included specific detailed items about frequency, characteristics and subjective experiences of tics, as well as obsessions, impulsions, repeating thoughts, compulsions and repeating behavior. All videotapes were systematically scored according to a checklist by two of the authors (DCC and TCAMvW). The results were subsequently discussed until consensus was reached regarding the occurrence and psychological significance of repeating phenomena during the interview.

RESULTS

The phenomenon of 'mental play' was observed in five GTS patients and in none of the OCD patients. Mental play refers to the occurrence of repetitive, seemingly useless impulses, thoughts or images, mostly not unpleasant in their nature. It often appears to be intended as pastime, and includes visual, auditory, cognitive, word and/or number games.

In contrast to mental play, counting occurred in both groups. This offered the opportunity to compare the emotional implications of repetitive behavior in both groups. Four of the GTS patients had the habit of regular counting, compared with six OCD patients. Some of the GTS and OCD patients reported more than one counting phenomenon.

TABLE 1

*Emotional implications of mental play and counting of GTS and OCD patients.**

	Mental play		Counting	
	GTS N=5	OCD N=0	GTS N=4	OCD N=6
Pleasant	5	-	1	-
Voluntary	5	-	1	-
Easy to suppress	4	-	1	-
Egodystonic	1	-	3	5
Suffering	1	-	-	5
Accompanying fear	-	-	1	6
Accompanying tension	5	-	4	6
Resistance	-	-	1	-
Goal/purpose	1	-	-	5
neutralise/repress fear fearful thought	-	-	1	1
neutralise/repress/limit repetitive action	-	-	-	5
give effect to impulse/thought	1	-	3	-
pastime	4	-	3	-
Interwovenness symptom/other symptoms	-	-	-	6
Hours/day	0-2	-	<1	<1-4

* Some patients reported more than 1 phenomenon.

Table 1 summarizes emotional implications of mental play and counting. Four of five GTS patients could stop the symptom when desired and all experienced it as pleasant and not burdensome. It was regarded as egosyntonic by four of the five patients. There was no accompanying fear, but an increased simultaneous inner tension was reported. Apart from being a pastime in some cases, mental play was usually purposeless and not interwoven with other repetitive phenomena like evening-up, touching or arranging. It impressed to be an autonomous symptom, that consumed minutes to hours a day. Mental play included:

(a) evoking double images or projecting persons or objects in one plane by squinting
(b) thought analyzing, designing fingersetting for accordion and

playing tunes heard on radio
(c) breaking up words heard on radio into letters and making up new phrases using those letters or looking for identical letters in environment
(d) mental arithmetic tasks
(e) designing 3-item sets concerning letters and words.

Two cases are described in more detail.

Case 1. *A 30 year-old male with GTS had been a professional musician. Whenever he heard a melody, it was necessary for him to fix it in his mind so that he could analyze the tune. He then determined the fingering for the accordion in his mind and mentally played the entire tune repeatedly. He considered this behavior to be pleasant and useful in improving his playing technique. It only became unpleasant whenever it prevented him from falling asleep at night.*

Case 2. *A 25 year-old male GTS listened to the radio every night before falling asleep. He used to form phrases or sentences within a split second using all the letters of certain words that appealed to him from the songs played on the radio. A variant habit was to find letters on little flags above his bed identical to those from particular words in songs on the radio. He experienced this habit as a pleasant, exciting and voluntary pastime. It had started spontaneously and he felt neither resistance against it nor tension or anxiety when he stopped doing it.*

In the GTS group, the counting had a similar emotional impact to the mental play, regarding the items suffering, accompanying fear and tension, resistance and goal. Although the patients experienced the counting as ranging from slightly unpleasant (n=3) to pleasant (n=1), difficult to suppress (n=3), and involuntary (n=3), which was in contrast to the mental play, there was no reported actual suffering from the counting. This was in line with the experiences of mental play. Only in one case there was some concurrent fear; this GTS patient counted mile-posts, and lampposts while riding his motorcycle, while having the fearfull thought: "if I stop counting, I don't reach 100 years of age". In all cases there was some accompanying tension reported, in only 1 case any resistance to the counting. This concerned a patient who experienced attentional problems while counting letters watching TV.
The goals of counting and mental play were strikingly similar; the goal

associated with counting was a pastime in three out of the GTS counters; in four of the GTS counters the act of counting was aimless (no other goal than to give effect to a counting impulse). In one case, the aim of counting was to neutralise a fearful thought (i.e. if I stop counting I will not reach 100 years).

Only two of the GTS patients counted in fixed but seemingly arbitrarily chosen numbers. Like in mental play, the counting of the GTS group was autonomous, not interwoven with any other repetitive phenomenon. The average time per day spent on counting was less than an hour.

Various types of counting were undertaken by the patients. These included: counting letters/words while watching TV/films/posters (one GTS patient); counting tiles on walls and floors of toilet (2 GTS, 1 OCD); counting mile posts and lamp posts while driving (2 GTS); counting objects in shop-windows (1 GTS); counting burls on a wall (1 GTS); counting cars, numbers or letter combinations in fixed sets (1 GTS, 1 OCD); counting until a fixed maximum related to household tasks (3 OCD); counting until a fixed maximum related to checking routines (2 OCD); and a fixed number of repeated thoughts about daily activities (1 OCD). (Some patients reported more than one phenomenon.)

In the six OCD cases counting was associated with a different set of experiences and motivations (Table 1). There was no playfulness, on the contrary these patients felt very uncomfortable and experienced much accompanying fear and tension in relation to their counting. There was no resistance against the counting, perhaps because it was intended either to neutralise a fearful obsessive thought, to limit a repeated action or to support anxiety-reducing checking. Also, the ideations during counting differed from GTS and implied a range of lucky or 'good' numbers to ward off danger and 'bad' numbers that should be avoided. The counting in OCD patients was not an action in itself but part of a complex obsessive-compulsive behavioral pattern, in contrast to the GTS patients. The time per day spent on counting depended on the severity of the other obsessive-compulsive phenomena, and ranged from minutes to hours.

DISCUSSION

As far as we know the repetitive phenomena described as 'mental play' have not been defined in the literature before. We define mental play as repetitive cognitive behavior, usually neutral or pleasurable in its

affective content, that is elicited by external stimuli. In this study mental play has only been found in GTS patients. In this group, it might represent a subentity of repetitive behavior like counting, evening-up and feeling forced to touch.

Mental play occurs probably in many normal individuals. A.K. Shapiro mentioned that he himself had the impulsion, to "line up a part of a person's body with an object in the room, when the conversation with that person was not too interesting" (6).

We propose to define this impulsion as a form of mental play. The difference between mental play in GTS patients and in the normal population could be the excessive time spent on it in some GTS patients. However, this remains speculative in the absence of qualitative and quantitative data on impulsions in normal populations.

Mental play in GTS might result from enhanced reactivity to provocative stimuli. In GTS patients, unexpected sensory stimuli cause an excessive urge to respond to them, for instance constructing words from observed letters. Although OCD patients are also hypersensitive to unexpected stimuli, we think that their response is entirely different. Frankel et al. (1986) have examined the obsessive-compulsive phenomenology among GTS, OCD and control subjects (9). They also found differences in the pattern of symptoms between GTS and OCD. In general, OCD subjects respond with increased anxiety, fear and behavior intended to neutralize anxiety or to prevent a dreaded event.

Mental play and counting share many subjective features within the GTS group; both are experienced as autonomous phenomena that are aimless or just a pastime. Both phenomena cause hardly any suffering or accompanying fear. The counting occurs equally often in the GTS and the OCD group. In GTS, counting is also referred to as arithmomania (4). However, there are major differences in the meaning and experience of counting between GTS and OCD patients (table 1). Counting in GTS can generally be regarded as playful, autonomous and aimless. It seems to be related merely to accidental, evocative environmental stimuli. This might explain the greater pluriformity of counting behavior seen in GTS patients. In OCD patients, counting is designed for support and is interwoven with other anxiety-reducing repetitive actions, like cleaning and checking. It is also regarded to be more unpleasant than in GTS (14). These differences in phenomenology between GTS and OCD implies that at least mental play and counting in GTS should not be diagnosed as obsessive-compulsive symptoms.

Shortcomings of this study require some remarks. The number of GTS and OCD patients was small. The groups were not matched. The

difference in sex distribution could be of major importance: recent family studies indicated sex differences in GTS patients, where more obsessive-compulsive rituals were found in female GTS patients than in males (11).

In conclusion we suggest the phenomenon of mental play should be added to the range of repetitive impulses in GTS. Mental play in GTS, as well as counting in GTS patients, should not be diagnosed as an obsessive- compulsive phenomenon. Further research is needed to elucidate the nature of the differences and resemblances in repetitive phenomena in GTS and OCD.

REFERENCES

1. Bliss J. Sensory experiences of Gilles de la Tourette syndrome. Arch Gen Psychiatry 1980; 37: 1343-7.
2. Kurlan R, Lichter D, Hewitt D. Sensory tics in Tourette's syndrome. Neurology 1989; 39: 731-4.
3. Robertson MM, Trimble MM, Lees AJ. The psychopathology of the Gilles de la Tourette syndrome: A phenomenological analysis. Brit J Psychiatry 1988; 152: 383-90.
4. Robertson MM. The Gilles de la Tourette syndrome: The current status. Brit J Psychiatry 1989; 154: 147-69.
5. Comings DE, Comings BG. A controlled study of Tourette syndrome, I-VII. Am J Hum Genet 1987; 41:701-866.
6. Shapiro AK, Shapiro ES, Young JG, Feinberg FE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
7. Pauls DL, Raymond CL, Stevenson JM, Leckman JF. A family study of Gilles de la Tourette syndrome. Am J Hum Genet 1991; 48: 154-63.
8. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). Washington, DC, 1987.
9. Frankel M, Cummings JL, Robertson MM, Trimble MR, Hill MA, Benson DF. Obsessions and compulsions in Gilles de la Tourette's syndrome. Neurology 1986; 36: 378-82.
10. Robertson MM, Gourdie A. Familial Tourette's syndrome in a large British pedigree. Brit J Psychiatry 1990; 156: 515-21.
11. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. N Eng J Med 1986; 315: 993-7.
12. Hoogduin CAL. On the diagnosis of obsessive-compulsive disorder. Am J Psychother 1986; 15: 36-51.
13. Cath DC, Hoogduin CAL, Wetering BJM van de, Woerkom TCAM van, Roos RAC, Rooymans HGM. Tourette syndrome and obsessive compulsive disorder. Adv Neurol 1992; 58: 33-41.
14. Haan E de. A man with counting rituals: a diagnostic problem. Dth 1988; 8: 127-34.

5. REFLECTIONS ON THE PHENOTYPE

5.3. The tics of Gilles de la Tourette syndrome

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INTRODUCTION

In an earlier survey of 66 Dutch patients with the Gilles de la Tourette syndrome (GTS), motor and vocal tics were described as characteristic symptoms (1). The course of the disorder is waxing and waning, with long periods from weeks to months in which the tics do not only vary in severity but also in nature and localization. The cause of these long-lasting fluctuations is unknown. Moreover, there are brief fluctuations in nature and severity of the tics, lasting minutes to days; these have been found to be associated with stress-inducing circumstances, such as frightening situations, an examination, going to another school or moving house. Especially in children with the syndrome of Gilles de la Tourette the tics are not always the most marked sign. They are presented sometimes with disturbances of attention, behavior and impulse control, sleeping problems and atypical developmental disturbances (2). Early recognition of the signs and treatment can spare patients and parents much grief, not only about the cause of any tics, but also from the lack of understanding of others.

Detailed phenomenological investigation of tics has led to a broadening of the concept of 'tic' and to a new interpretation of the obsessive-compulsive symptoms in GTS. Because this has not only consequences for research, but also for clinical practice, it seemed important to us to discuss again the tics, as they are now distinguished.

THE CLASSIC VIEW: MOTOR AND VOCAL TICS

Motor tics

Motor tics are described as abrupt, involuntary, nonrhythmic, repetitive movements of separate muscle groups (3). Motor tics are divided according to the site of occurrence, the severity and degree of complexity depending on the involvement of one or more muscle groups (4). Thus increased eye blinking is defined as a simple tic; combined hand and arm movements are defined as a multiple complex tic. Tics often seem like intentional movements and are often easy to imitate. They do, however, differ from intentional movements; tics are not preceded by a so-called 'Bereitschaft-Potential', a brain potential seen in intentional movement (5). Severe motor tics may lead to injury of the skin (for instance by repeated hitting or pinching) or of peripheral nerves (for instance by violent shaking of the head).

Vocal tics

Vocal tics are all the noises which are the result of a flow of air in throat, mouth or nose, caused by muscular activity. Thus sniffing and clearing one's throat are vocal tics, clicking one's tongue and gnashing one's teeth are not. Vocal tics may vary from simple sounds to words and sentences. A special form is coprolalia; this uttering of foul language occurs in 30% of the patients.

NEW VIEWS: SENSORY AND COGNITIVE TICS

The tic movement does not stand by itself, but seems to be part of a chain of sensory, emotional, cognitive and motor events. Unlike disturbances of other hyperkinetic movements tics can be suppressed for a shorter or longer period of time. This leads to an increasing inner tension, which, however, is not felt as fear, and is followed by a brief storm of tics, whereby the suppressed tics show up most severely and thus make the inner tension decrease. In addition, sensory and cognitive elements can often be distinguished in the developmental stage of a tic, which initially lead to a voluntary movement which then changes to an automatic one.

Meige and Feindel regard tics as coordinated, purposive movements, provoked by external circumstances or thoughts (6). Repetition of the movement would, according to them, lead to habit forming. Finally, the tic assumes autonomic and involuntary qualities, whereby form, intensity and frequency may considerably increase. The tic movement is preceded by an uncontrollable impulse, the suppression of which is accompanied by malaise (6).

The following personal observation illustrates this way of the development of tics.

Case

A 35-year-old woman, in whom the Gilles de la Tourette syndrome was diagnosed a few months earlier, was very much embarrassed by a recently developed tic. This tic was a brief shaking of her breasts. It had started a few weeks before during cycling over a bumpy road, when she felt her breasts shake. This feeling, not especially pleasant or unpleasant, could not be ignored once she had become aware of it. At first, she tried deliberately to evoke this feeling again by briefly shaking her breasts. It was pleasant when she succeeded. Eventually, it happened spontaneously and unnoticed by herself, except for the fact that her partner found it annoying and other people often gave her a strange look.

Sensory tics

Kinnier-Wilson compared the development of tics to the repeated movement of the tip of the tongue to the space between teeth following extraction of one element (7). Bliss, who suffered from GTS, brought the separate and assumed involuntary nature of the motor tics up for discussion (8). He said that compelling, sensory sensations precede the tic movement, but that the tic movement can never completely remove these sensations. Another patient could only with certain tic-like muscle movements ease an 'unpleasant' feeling in her back.

Also Lang found, on comparison of the subjectively felt 'voluntary' abnormal movements, a considerable difference between patients with tics and those with various other movement disorders. Unlike this latter group, over 90% of the patients with tics had the feeling that a certain degree of voluntariness played a role in the development and manifestation of the tic (10).

Shapiro et al. described sensory tics as 'patterns of recurring bodily sensations, described by the patients as a feeling of pressure, itching, warmth, coldness or other abnormal sensations in skin, muscles, bones or joints (4). These sensations occur each time in specific parts of the body and are often felt as definitely unpleasant. Certain movements, often in the form of tonic contractions of muscle groups, are made in order to mitigate the unpleasant sensation (this should not be confused with motor tics). This is only temporarily successful, so that the movements must be continuously repeated. To mitigate unpleasant sensations, especially in the mouth or pharynx, noises may be made. These noises then do not have the abrupt, brief nature of vocal tics, but consist, for instance, in lengthily clearing one's throat, gurgling or humming. The degree in which these sensory sensations are experienced varies from person to person. The following case illustrates the phenomenon of the sensory tic.

Case

A 26-year-old man was diagnosed to have GTS at the age of eight. He performed, in addition to his motor and vocal tics, a number of recurring ritual actions. He often had to take small sips of water from a glass, always to descend the stairs backwards, and to bite on spoons whenever he saw any. Moreover, he had recurring thoughts and images about symmetry, and he brought situations in his surroundings 'in balance' by the number of times that he glanced at them. He experienced these behaviors as only slightly disturbing to his development.

His reason for consultation was a recently increased strange sensation

in his skin. In various places of his body he felt almost continuously 'tension'. Also, there was a constant feeling that his pants did not fit properly. He was continuously aware of the material touching his skin. He plucked and fiddled with the tissue of his trousers, especially in the genital area. An itching and prickly feeling of the skin in different and variable areas of the body troubled him most. These areas had an indescribably confined and changing size. Scratching, slight rubbing, touching or taking a shower usually helped, but only temporarily. On examination there was no redness of the skin, no swelling or scratching marks. We regarded these skin sensations as a sensory tic.

Seventy-six percent of the 34 patients with GTS experienced sensory symptoms before 'motor or vocal tics' (11). These sensory tics were focal, generalized, or both. They could well be distinguished from feelings of diffuse, inner tension, which occurred in 24 patients (71%) before the motor or vocal tics.

Cognitive tics

At a cognitive level, patients with GTS are more sensitive to stimuli from their surroundings, are more easily distracted, and have more learning difficulties because of concentration problems (1). In them, in an increased frequency, the diagnosis of 'lack of attention disorder with hyperkinesia' has been made (14).

The patients are not only more 'open' to what happens around them, but they also react more strongly to stimuli. From that point of view, the following frequently occurring signs could be explained better: echolalia and echopraxia, the need for symmetry in their surroundings, and the impulsion to touch (délire de toucher) (7). It is compelling for many patients to count repeating elements in their immediate surroundings (the steps of a staircase, tiles, patterns in curtains or floor covering). Many patients appear to play, in their minds, with certain stimuli in their surroundings. An example is the following case history.

Case

A 28-year-old young man had besides motor and vocal tics, a range of other, sometimes ritual-like symptoms. He always counted the steps of the staircase he was walking on. When he entered a room, he had first to put a foot several times over the threshold. When talking, he accentuated in his feeling certain words by tapping continually on his throat. Occasionally, when he happened to knock his one elbow against the table, he at once had to touch the table also with his other elbow. When he saw the number plate of a car, he at once started to do sums with the figures

and to make words with the letters. He did not feel forced to do so in an annoying manner and regarded it as a playful quality. He never felt the need for suppressing it, but he felt that would be possible if necessary. We regard these phenomena as cognitive tics.

The course of sensory and cognitive tics shows much similarity to that of motor tics: the repetitive character, the changes in severity, nature and content in the course of time and the correlation with stimuli.

DISCUSSION: IMPLICATIONS FOR DIAGNOSIS AND THERAPY

The changing views on the symptomatology of GTS imply that the tics involve not only the motor system, but also the sensorium and the cognition. This view has also diagnostic and therapeutic consequences.

Sensory tics may be erroneously diagnosed, for instance as trigeminal neuralgia, or renal colic or as conversion. A personal observation (by R.A.C. Roos) concerns a woman who was referred with the diagnosis 'restless legs'. She appeared to have multiple motor and vocal tics, consistent with the diagnosis of 'the syndrome of Gilles de la Tourette'. Her 'restless legs' were caused by sensory tics in her legs.

Frequently, compulsive disorders have been described in patients with the syndrome of Gilles de la Tourette, which are regarded as an integral part of the disease (14). Compared with the signs of compulsive neurotics, the compulsive signs of the patients with GTS appear to differ essentially in a number of phenomenological aspects, because in GTS these signs are not meant to prevent fear or fright-induced impulses (12,13). They show more variation, are more cursory in nature, are briefer in duration and are not or hardly felt as embarrassing (13). We now assume that they are caused by impaired impulse control and should therefore not be called obsessive-compulsive symptoms any more.

The broadened view and interpretation of tics may possibly have consequences for treatment. With or without a combination of drug therapy, psychotherapy can improve the quality of living for many a patient with the syndrome of Gilles de la Tourette. There are three points of to address in psychotherapy, namely the direct influence of tics as such, secondly of antecedent factors, and thirdly of consequential factors, such as depressive feelings and a sociophobic development. The direct influence on the tics according to the behavioral therapeutic treatment according to Azrin and Nunn is based on self-observation and

self-registration of the tics, training in the achievement of awareness of the tics and learning of a response which is incompatible with the tic (14). Awareness of premonitory signs that precede the tic, may help to either suppress or avoid the tic. Patients with sensory tics can suppress associated motor behaviors better than patients without sensory tics (11). Sometimes the development of the tic can successfully be stopped early or be translocated to a part of the body where the manifestation is slight or inconspicuous (for instance the toe) (8). Tics influenced in this way do not lead to a rebound of tics as described above. Especially sensory tics seem to offer a good starting point for such a behavioral therapeutic treatment. In an as yet limited number of cases of the Dutch study group improvements study group were seen (15,16,17). The longitudinal study of larger groups of patients for a longer period is needed, also to differentiate between 'therapeutic' and 'spontaneous' improvements in severity during the course of this naturally waxing and waning disorder.

Future phenomenological studies may possibly show whether the actual differentiation of tics reflects a more general pathogenetic process. Moreover, study of sensory and cognitive tics may considerably contribute towards the differential understanding of obsessive-compulsive disorders as well as of disorders of impulse control.

REFERENCES

1. Wetering BJM van de, Cohen AP, Minderaa RB, Roos RAC, Woerkom TCAM van. Het syndroom van Gilles de la Tourette. Ned T Geneesk 1988; 132: 21-5.
2. Minderaa RB, Gemert TM van, Wetering BJM van de. Onverwachte presentatie-wijzen van het syndroom van Gilles de la Tourette. Ned T Psychiatrie 1988; 30: 246-54.
3. Lees AJ. Tics and related disorders. Churchill Livingstone, London-New York, 1985.
4. Shapiro AK, Shapiro ES, Young YG, Feinberg TE. Gilles de la Tourette syndrome (2nd edition). Raven Press, New York, 1988.
5. Obeso JA, Rothwell JC, Marsden CD. Simple tics in Gilles de la Tourette syndrome are not prefaced by a normal premovement EEG potential. J Neurol Neurosurg Psychiatry 1981; 44: 735-8.
6. Meige H, Feindel E. Der Tic, sein Wesen und seine Behandlung. (Deutsche autorisierte Ausgabe von O. Giese). Deuticke, Leipzig-Wien, 1903.
7. Kinnier-Wilson SA. The tics and allied conditions. J Neurol Psychopathol 1927; 8: 93-109.
8. Bliss J. Sensory experiences of Gilles de la Tourette syndrome. Arch Gen

Psychiatry 1980; 37: 1343-7.
9. Bullen JG, Hemsley R. Sensory experience as a trigger in Gilles de la Tourette's syndrome. J Behav Ther Exp Psychiatry 1983; 14: 197-201.
10. Lang A. Patient perception of tics and other movement disorders. Neurology 1991; 41: 223-8.
11. Kurlan R, Lichter D, Hewitt D. Sensory tics in Tourette's syndrome. Neurology 1989; 39: 731-4.
12. Haan E de. De man die telt; een diagnostisch probleem. Directieve Therapie 1988; 2: 127-35.
13. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooijmans HGM. Mental play in Gilles de la Tourette syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-5.
14. Azrin NH, Nunn RG. Habit Reversal: a method of eliminating nervous habits and tics. Behav Res Ther 1973; 11: 619-28.
15. Evers RAF, van de Wetering BJM. Behandeling van motorische tics op basis van een spanningsreductiemodel. Gedragstherapie 1990; 23: 167-78.
16. Hoogduin CAL, Reinders M. Overwegingen bij de behandeling van tics en het syndroom van Gilles de la Tourette. Dth 1987; 7: 245-53.
17. Evers RAF, Wetering BJM van de. Een patiënte met het syndroom van Gilles de la Tourette en een klokmaag. Dth 1990; 10: 168-75.

6. GENERAL DISCUSSION AND CONCLUSIONS

INTRODUCTION

The recent developments of DNA techniques and advanced statistical methods for the analysis of genetic data have opened new vistas for biological psychiatric research. New investigative strategies were aimed at the identification of genetic vulnerability markers, stimulated by the first successful localization of the gene for Huntington's disease on chromosome 4 (1). This finding initiated searches for genes involved in other neuro-psychiatric disorders, like schizophrenia, bipolar affective disorder, autism, anxiety disorders, alcoholism, neuroticism, Alzheimer's disease, sleep disorders and Gilles de la Tourette syndrome (2-4).

These linkage studies have as the goal to understand the genetic mechanisms of psychiatric illnesses. The localization of the defective gene(s) responsible for the illness by positional cloning is an important initial step in this approach (5). The subsequent characterization of the DNA sequence of identified candidate genes and the defective gene product will become essential cornerstones. The resolution of diagnostic controversies might come into view, but also insight into the pathogenesis, the interaction between genotype and environment, improved options for precise genetic counselling and the development of new therapeutic strategies.

In spite of the fact that the first report of linkage between blood group and colour vision markers on the X-chromosome and bipolar affective disorder was reported already in 1969, the exact mode of genetic transmission for the majority of neuropsychiatric conditions studied remained obscure as they do not seem to follow clearly Mendelian patterns of inheritance (6). Positional cloning has resulted though in the mapping of the gene responsible for Huntington's chorea on chromosome 4 and Alzheimer's disease on chromosome 14 and 21 (1,7-9). Linkage has been reported to chromosome 5 for schizophrenia in a number of Icelandic and British families and both to the X-chromosome and chromosome 11 for bipolar affective disorder (10-13). However, most of these findings in the major psychiatric syndromes have not been replicated and many questions concerning the methodology of a number of these studies have been raised (14-20). Decrease of evidence for linkage in these studies was the result of updating diagnostic information, the use of more informative markers and identification of the effects of applying erroneous or simplified genetic models in the linkage analysis (20-23).

The assumption of genetic homogeneity might be another reason for the lack of success in linkage studies (24).

The current status in the search for the GTS susceptibility gene(s), as described in this thesis, reflects the general situation in psychiatric genetic research. With the actually available evidence, favoring an autosomal dominant pattern of transmission, GTS seemed to provide a good starting point for a linkage study (25). Since then about 80% of the genome has been excluded after testing more than 600 DNA markers on the autosomes in the 26 families from the international consortium (26). The consequence is that all steps from the method of ascertainment of the families to the interpretation of polymorphic marker readings, the genetic models of transmission and the parameters used in the statistical analyses have to be carefully scrutinized again with the knowledge and experience that has been acquired since the start of the study.

ASCERTAINMENT AND ASSESSMENT OF THE FAMILIES

The effects of selection mechanisms of the families in the current study have to be considered. Firstly, the probands came from a 'clinical' sample. If this implies a selection in severity and/or complexity of symptoms as the most severely affected cases will be more readily referred for diagnosis and treatment, there may be a bias in ascertainment. This was illustrated by Caine who found that of the 41 Gilles de la Tourette cases identified in about 140,000 school-age children in Monroe County (Rochester, NY, USA), from the 24 previously diagnosed cases 19 (79%) had associated behavioral problems versus 1 (6%) of the 17 new cases (27).

Secondly, a family was included in the Dutch GTS family study if there was a positive family history for tics and three generations were available for examination. This could cause a bias of selecting families with an (autosomal) dominant inheritance, as multiply affected, multigenerational families had the highest chance to become included. Nine of the 10 families described in chapters 2.1 and 2.2 were ascertained because of a positive family history in a first degree relative and one in a second degree relative.

As most of the families were caucasian and came from the South-Western part of the Netherlands, racial and geographical restrictions with respect to the generalization of our results might also be considered.

Diagnosis

Familial loading of a psychiatric illness can be studied by investigating all available (non-)biological relatives and/or by taking histories about the relatives from a limited number of family members. Medical records can provide further information about individuals. To confirm a clinical diagnosis, (biological) markers (when available) for the illness are invaluable in the definite assignment of 'cases'. As in other neuropsychiatric diseases, there are no biological markers available in GTS. The finding of characteristic ophthalmological abnormalities in GTS patients and their relatives by Enoch and associates could not be reproduced in Dutch GTS patients as described in chapter 5.1. (28,29). Accordingly, the assignment of cases in our family studies was based on observation and historical information provided by the subject and relatives on current and past symptoms, and supported, whenever possible, by the data from the medical history.

The family history method is often the easiest and fastest way to obtain information about the family, including the deceased relatives. In fact, for the study of dementia, a late onset condition that shortens life span, that is often the only option. By applying standardized methods including confronting informants with discrepant reports, Silverman et al (1986) obtained sufficiently reliable information in a study on familial dementia (30). However, that method was shown to be less adequate to identify the affected family members for a number of other psychiatric diseases (31-33).

All the available biological and non-biological relatives from the families described in section 2 have been interviewed using the structured interview by Pauls and Hurst (34). In addition, family and medical history data were obtained to complete as much as possible information on the family and to identify possibly affected relatives. For the non-biological relatives this information was used to get insight whether they might be a gene-carrier.

The criteria of the DSM-III-R seem unequivocal in classifying the occurrence of tics as GTS or CMT (35). When applied to the written precoded abstracts from the interviews the Dutch panel of experts reached almost complete consensus. However, the critical issue in the diagnostic and classification process is to recognize the tics during observation and from the history obtained from the subject (and his relatives). Tics, especially their mild forms, remain often unnoticed by the subjects or their relatives and are therefore not reported. Moreover, tics vary in frequency and severity and are frequently suppressed during examination. When this is confirmed by the subject, it is very

suggestive for GTS, but on the other hand it hampers the direct observation of the tics. All subjects in the Dutch study have been examined by the same investigator; regularly accompanied by a second investigator. There have never been disagreement about the observed or presumed tics. However this approach obviously has serious limitations. At the time the resources did not allow for (videotaped) interviews, systematicly rated by multiple observators.

As long as it is unknown which particular symptoms are directly related to the underlying genetic defect the interviewer should be as possibly objective and a priori unbiased by being aware which subset of symptoms has to be looked for and how each subject is related to the proband. A positive family history for a psychiatric illness is generally considered to be an additional confirmation of that specific diagnosis in a given patient. It raises the a priori chance for that diagnosis to be assigned to a given mentally ill family member. Subsequently, the report of the interview should ideally be classified on the basis of a complete consensus by a panel of independent experts to determine the degree of 'caseness'.

However, as all the relatives were interviewed by the principal investigator, the relationship with the families became less distant, leading to subsequent unrequested but essential additional information about children being born, newly identified cases and changes of addresses. This greatly increased the precision of the data and reflects the importance of a family study in quite another way; families with a certain genetic condition suddenly feel recognized by the medical and research profession.

Broadening the phenotype

The accurate definition of the phenotype is essential to understand the patterns of inheritance of an illness in a family and analyze the results of the DNA linkage studies. It has been argued that only the most strictly defined phenotypes should be appointed 'cases' in the genetic studies as these are most likely related directly to the genotype (36,37). Others favor multiple analyses using systematically broadened phenotypes to avoid loss of information by applying too strict diagnostic classifications (38). As pointed out in chapter 3.1. a higher lod-score is then needed to correct for multiple testing.

There is general agreement that milder tic syndromes in families of GTS probands are part of the phenotype. From clinical experience the hypothesis has evolved that the spectrum of tics is not restricted to motor and vocal domains, but also includes sensory phenomena and

cognitive behaviors. Further study is needed to confirm our current views that are described in chapters 5.2 and 5.3. Increased attention for these subtle features in the phenomenology of GTS symptoms has contributed to a refined distinction of GTS from the group of hyperkinetic disorders, to which it has been classified for a long time. The diagnostic resolution will thereby become increased, which could lead to a more detailed definition of the GTS-spectrum phenotype.

As isolated tics, not related to a genetic GTS susceptibility, are relatively frequent in the general population, non-genetic cases could be over-represented in our families. Recently, Pauls (personal communication) suggested that only subjects with tics should be included when they also showed OCD. However, the question about the relation between OCD and GTS is not resolved. In the first place OCD is estimated as frequent in the general population as tics. Secondly, phenomenological analysis of the 'OCD-like' behaviors in GTS patients indicates that they are essentially different from OCD as described in chapter 5.2.

It becomes even more complicated when tics might go unnoticed as associated behavioral symptoms like conduct disorder, attention deficit disorder with hyperkinesia, self injurious behaviors and stuttering (amongst other) predominate the clinical picture (36). Comings et al have suggested that these associated behaviors and a variety of other psychiatric conditions like phobia, autism and alcoholism are related to the same underlying genetic vulnerability with a relatively high frequency in the population. On basis of his family material he has suggested that a semi-dominant, semi-recessive model provides the best genetic model of transmission. In none of the families in the Dutch family study we have found an excess of psychopathology in either the biological or non-biological relatives of GTS probands. Associated behavioral symptoms are found in cases with tics suggesting that they either are intrinsically related to the disorder (as a form of disturbed impulse control) or are secondary to having this disorder with its peculiar symptoms affecting psychological and social development.

THE GENETIC MODELS

The distribution of tics in the first degree, second degree and more distant biological relatives in our kindreds does not directly reflect a 'simple' autosomal dominant model of transmission. The recurrence risks do not show the 50% drop for every succeeding degree of

relationship. That might result from the method of ascertainment, as the presence of another affected relative was required according to the inclusion criteria. As all but one of these additional cases were first degree relatives, it does not seem likely that originally families with a high loading of affected cases in the second degree and more distant relatives have been included. Moreover, the number of females with CMT in our study exceeds that observed in the USA by Pauls et al. (37). One explanation could be that the inheritance of GTS does not follow either classic multifactorial or monogenic patterns. As with other psychiatric disorders, more complex models of transmission need to be invoked to explain the results of the Dutch family study.

This unexpected complexity could also explain the results of the segregation analyses. By applying the parameters from the earlier studies, the segregation analyses showed autosomal dominant transmission to be the second best solution. A non-Mendelian way of transmission, reflected by the better results of the tau2 model, appeared to be more likely. More extensive analyses applying other values for the ascertainment probability and additional estimates for population prevalence for males and females are planned in the near future.

These findings shed new light on the fact that the linkage approach did not yet result in conclusive evidence for the chromosomal localization of the GTS susceptibility gene(s).

In this thesis the state of the art in GTS genetic research has been described. The current results, including our observations indicate new ways for future research. The GTS phenotype needs more detailed phenomenological analysis to refine the diagnostic classification of affected cases in the pedigrees. Though the different collaborating groups have applied similar methods of investigating the families, a much more strict and uniform system of classification needs to be used. In addition the current families will be re-investigated in order to study the stability of diagnosis over time and examine the younger family members that have been growing up. The number of (nuclear) GTS families will be extended to perform segregation analysis on a larger sample.

With regard to the DNA analyses an association study is performed on a large sample of GTS cases to screen for candidate genes. New statistical approaches, like the sib-pair analysis and the affected pedigree member method, which are less sensitive to genetic heterogeneity, will be applied to the DNA marker data.

It has been 40 years ago that Watson and Crick felt that they had

found 'the secret of life' by discovering the double helix structure of DNA. From then, the mystery of how traits were passed through generations seemed to be revealed. Many genes responsible for a variety of disorders with Mendelian patterns of inheritance have been localized, but fewer identified, since then. It took 10 years to identify the gene for Huntington's disease after its had been localization on chromosome 4 (7).

The era of the unravelling of complex disorders is proceeding in the conviction that the follow through along complex pathways has still many promises for new horizons.

REFERENCES

1. Gusella JF, Wexler NS, Conneally MP, Naylor SL, Anderson M, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young AB, Shoulson I, Bonilla E, Martin JB. A polymorphic DNA marker genetically linked to Huntington's disease. Nature 1983; 306: 234-8.
2. Vandenberg SG, Singer SM, Pauls DL. The heredity of behavior disorders in adults and childeren. Plenum, New York, 1986.
3. Parkes JD Lock CB. Genetic factors in sleep disorders. J Neurol Neurosurg Psychiatry 1989; 4: 101-8.
4. Wetering BJM van de, Heutink P. The genetics of the Gilles de la Tourette syndrome: a review. J Lab Clin Med, 1993; 121, in press.
5. Collins F. Positional cloning: let's not call it reverse anymore. Nature Genet 1992; 1: 3-6.
6. Reich T, Clayton P, Winokur G. Family histroy studies; V. the genetics of mania. Am J Psychiatry 1969; 125: 1358-69.
7. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72: 1-20.
8. Schellenberg GD, Pericak-Vance MA, Wijsman EM, Moore DK, Gaskell PC, Yamaoka LA, Bebout JL, Anderson L, Welsh KA, Clark CM, Martin GM, Roses AD, Birds TD. Linkage analysis of familial Alzheimer disease, using chromosome 21 markers. Am J Human Genet 1988; 48: 563-83.
9. Broeckhoven C van, Backhovens H, Cruts M, Winter G de, Bruyland M, Cras P, Martin JJ. Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. Nature Genet 1992; 2: 335-39.
10. Sherrington R, Brynjolfson J, Petursson H. Localization of a susceptibility locus for schizophrenia on chromosome 5. Nature 1988; 336: 164-7.
11. Mendlewicz J, Rainer JD. Evidence for X-linkage in the transmission in manic-depressive illness. JAMA 1972; 222: 1624-7.
12. Baron M, Risch N, Hamburger R, Mandel B, Kushner S, Newman M, Drumer D, Belmaker RH. Genetic Linkage between X-chromosome markers and bipolar affective illness. Nature 1987; 326: 289-92.

13. Egeland JA, Gerhard DS, Pauls DL, Sussex JN, Kidd KK, Allen CR, Hostetter AM, Housman DE. Bipolar affective disorders linked to DNA markers on chromosome 11. Nature 1987; 325: 783-7.
14. Kennedy JL, Giuffra LA, Moises HW. Evidence against linkage of schizophrenia to marker on chromosome 5 in a Northern Swedish pedigree. Nature 1988; 336; 167-70.
15. St. Clair D, Blackwood D, Muir W. No linkage of Chromosome 5q11-q13 markers to schizophrenia in Scottish families. Nature 1989; 339: 305-7.
16. Hallmayer J, Maier W, Ackenheil M, Ertl, Schmidt S, Minges J, Lichterman D, Wildenauer D. Evidence against linkage of schizophrenia to chromosome 5q11-q13 markers in systematically ascertained families. Biol Psychiatry 1992; 31: 83-94.
17. Hebebrand J. A critical appraisal of X-linked bipolar illness. Br J Psychiatry 1992; 160; 7-11.
18. Baron M, Freimer NF, Risch N, Lerer B, Alexander JR, Straub RE, Asokan S, Das K, Peterson A, Amps J, Endicott J, Ott J, Gilliam TC. Diminished support for linkage between manic depressive illness and X-chromosome markers in three Israeli pedigrees. Nature Genet 1993; 3: 49-55.
19. Detera-Wadleigh SD, Berrettini, Goldin LR, Boorman D, Anderson, Gershon ES. Close linkage of c-Harvey-ras-1 and the insulin gene to affective diorder is ruled out in three North American pedigrees. Nature 1987; 325: 806-8.
20. Kelsoe JR, Ginns EI, Egeland JA, Gerhard DS, Goldstein AM, Bale SJ, Pauls DL, Long RT, Kidd KK, Conte G, Housman DE, Paul SM. Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old order Amish. Nature 1989; 342: 238-43.
21. Clerget-Darpoux, Bonaiti-Pelli C, Hochez J. Effects of misspecifying genetic parameters in lod score analyses. Biometrics 1986; 42: 393-9.
22. Greenberg DA, Hodge SE. Linkage analyses under 'random' and 'genetic' reduced penetrance. Genet Epidemiol 1989; 6: 259-64.
23. Cox NJ, Hodge SE, Marazita ML, Spence MA, Kidd KK. Some effects of selection strategies on linkage analyses. Genet Epidemiol 1988; 5: 289-97.
24. Pauls DL. Behavioural disorders: lessons in linkage. Nature Genet 1993; 3: 4-5.
25. Pauls DL, Leckman JF. The inheritance of Gilles de la Tourette's syndrome and associated behaviors. New Eng J Med 1986; 315: 993-7.
26. Heutink P, Breedveld GJ, Niermijer MF, Wetering BJM van de, Oostra BA. Progress in gene localization. In: Kurlan R (ed). The handbook of Tourette's syndrome and associated tic and behavioral disorders. Marcel Dekker, New York, 1993: 317-35.
27. Caine ED, McBride MC, Chiverton P, Bamford KA, Rediess S, Shiao J. Tourette's syndrome in Monroe County school children. Neurology 1988; 38: 472-4.
28. Enoch JM, Itzhaki A, Lakshminarayanan V, Comerford JP, Lieberman M, Lowe T. Gilles de la Tourette syndrome: Genetic marker? Neuro-ophthalmology 1988; 8: 259-65.
29. Enoch JM, Khamar BM, Lakshminarayanan V: Static and kinetic perimetric field defects in Gilles de la Tourette syndrome: quantifying step-like visual

field defects. Clin Vis Sci 1991; 6: 361-69.
30. Silverman JM, Breitner JCS, Mohs RC, Davis KL. Reliability of the family history method in genetic studies of Alzheimer's disease and related dementia's. Am J Psychiatry 1986; 143: 1279-82.
31. Andreasen NC, Endicott J, Spitzer RL, et al. The family history method using diagnotic criteria: reliability and validity. Arch Gen Psychiatry 1977; 34: 1229-35.
32. Gershon ES, Guroff JJ. Information of relatives: diagnosis of affective disorders. Arch Gen Psychiatry 1984; 41: 173-80.
33. Pauls DL, Kruger SD, Leckman JF, Cohen DJ, Kidd KK. The risk of Tourette's syndrome and chronic multiple tics among relatives of Tourette's syndrome patients obtained by direct interview. J Am Acad Child Psychiatry 1984; 23: 134-7.
34. Pauls DL, Hurst CR. Schedule for Tourette's syndrome and other behavioral syndromes. Yale University Child Study Center, 1987.
35. American Psychiatric Association. Diagnostic and Statistical Manual of mental Disorders (3rd edition, revised). American Psychiatric Press, Washington, 1987.
36. Minderaa RB, Gemert TM van, Wetering BJM van de. Onverwachte presentatie wijzen van het syndroom van Gilles de la Tourette. T Psychiatrie 1988; 30: 246-54.
37. Pauls DL, Raymond CL, Stevenson JM, Leckman JF. A family study of Gilles de la Tourette syndrome. Am J Hum Genet 1991; 48: 154-63.

SUMMARY

Introduction

The Gilles de la Tourette syndrome (GTS) is a neuropsychiatric disorder with childhood onset and characterized by motor, vocal, sensory and cognitive tics that may vary from relatively mild to very severe. The tics are often accompanied by echo- and coprophenomena, all in a characteristic waxing and waning course. In clinicial populations obsessive-compulsive symptoms, attention deficit disorder with hyperkinesia, conduct disorder and self-injurious behaviors are frequently observed in GTS patients. The severity of the GTS symptoms differs from person to person and may vary within an individual during his life. The results of treatment, that consists of (supportive) counseling, pharmacotherapy and/or (behavioral) therapy, are often not satisfactory, especially in the more severe cases. The exact etiology of GTS is unknown. Disturbances of the central dopaminergic, noradrenergic, serotonergic, GABA-ergic and endorphin systems in the brain are suggested to be involved in the pathogenesis of GTS.

In view of the unknown etiology of this condition and the unsatisfactory treatment outcome in the majority of cases, the American Tourette Syndrome Association has launched research initiatives into the etiology of the disorder. In this program a number of American and European research groups, including a Dutch group, collaborate in trying to unravel the genetic background of GTS. Systematic family studies are carried out to establish the mode of inheritance and ultimately to map and clone the susceptibility gene(s). The study comprised the following steps:

1. The ascertainment of families of GTS probands and the diagnostic classification of the family members (described in section 2).
2. The statistical analysis of the family data to test the most likely mode of inheritance by segregation analysis and the power for linkage analysis by simulation studies (described in section 3).
3. The analysis of DNA marker data by linkage analysis (described in section 4).

The Dutch Family Study

The Dutch GTS family study is a joint project of the Departments of Psychiatry and Clinical Genetics of the University Hospital Rotterdam - Dijkzigt and the Erasmus University Rotterdam. All biological and non-biological (married-in) relatives of GTS probands were interviewed using a structured interview developed at the Child Study Center of Yale University. This interview contains a detailed tic section, a general

biographical section, a section on academic performance, speech difficulties, a general psychiatric interview and the Yale-Brown Obsessive-Compulsive scale.

In **Chapter 2.1** of this thesis the frequency distribution of GTS and chronic multiple tics (CMT) in the first ten families of the Dutch study, comprising 9 families of Dutch and 1 of Norwegian origin, are reported. GTS, other tic syndromes and obsessive-compulsive symptoms were diagnosed using the information from observation, structured interview and a Dutch version of the Leyton Obsessional Inventory.
Tic disorders were found in 30% of the 233 biological relatives. Of the subjects with tics, 42% could be classified as having GTS. The male:female ratio is for the complete sample 1.4:1 for GTS and 1:1.6 for CMT.

The percentages for first degree biological relatives, both sexes combined, were 19.5 for GTS and 17.1 for CMT. The male:female ratio for GTS is 1.6:1 and for CMT 1:6. In second degree biological relatives, both sexes combined, the rates were 13.7 for GTS and 19.6 for CMT. The male:female ratio's are for GTS 1:1.2 and for CMT 1:1. The percentages for more distant biological relatives of both sexes combined were 9.9 for GTS and 16.3 CMT. The male:female ratio's are for GTS 1.4:1 and for CMT 1:1.6.

In **Chapter 2.2** the frequency distribution of OCS is reported. Fifty-one biological relatives (22%) were affected by one or more obsessive-compulsive symptoms, which was significantly different from the non-biological relatives.

Tic syndromes and OCS occurred relatively more frequently in biological relatives than in non-biological relatives. The only non-biological relative with OCD had never shown any tics. Separating the group of biological relatives into gender and affected status with regard to any of the tic categories ('All Tics', GTS, CMT) did not result in any significant differences with regard to the occurrence of OCS. There was no difference in occurrence of OCD and OCS in the group differentiated by a high IDB-score

STATISTICAL ANALYSES

Segregation analysis (chapter 3.1)

Segregation analysis is generally used to distinguish between monogenic and polygenic contributions in the etiology of inherited traits and diseases. Two earlier segregation studies of GTS families which made

use of direct interviews consistently favored a single, dominantly acting GTS gene with incomplete penetrance and variable expression.

In this study we used pedigree information and diagnostic classifications on 24 GTS families ascertained by four different research groups: University Hospital Rotterdam-Dijkzigt and Erasmus University, Rotterdam, The Netherlands (10 families), University of Iowa, Iowa City, Iowa, USA (16 families), Marshfield Medical Research Foundation, Marshfield, Wisconsin, USA (1 family) and Hospital for sick Children, Toronto, Canada (1 family). Segregation analysis was performed using the computer program POINTER. The analyses were performed separately using four diagnostic schemes 1) GTS, 2) GTS and CMT, 3) GTS, CMT and OCS and 4) GTS and OCS, respectively. The diagnostic criteria for GTS and CMT were based on the DSM-III-R (21). OCS included both obsessive-compulsive disorder according to DSM-III-R and marked repetitive behaviors that did not necessarily cause distress.

A range of hypotheses about models of transmission were tested. All tests were carried out in a hierarchical fashion. First, the null hypothesis of no transmission (NTR) was compared to an alternative hypothesis that transmission was due to a single major genetic locus with polygenic background (MIX: the so-called mixed model hypothesis). If there was significant evidence for transmission (i.e. the null hypothesis could be rejected), the maximum likelihood of the mixed model was compared to the maximum likelihoods of the hypotheses of i) polygenic inheritance (POLY: no single major locus), and ii) single gene inheritance (MEN: no polygenic background).

From the parameter estimates obtained in the segregation analysis, gender specific penetrance values could be calculated for the different diagnostic schemes.

The results of our segregation analysis support the hypothesis that the clinical GTS spectrum is caused by a single, dominantly acting gene. Penetrance of this gene was highly gender-specific in the more restricted diagnostic schemes. The observation that penetrances leveled when OCS was included in the phenotype suggests that OCS in females is a variant expression of the GTS susceptibility gene.

A remarkable finding was the good performance of the tau_2 model, which is regarded as a general test for (non-)Mendelian inheritance. Several explanations can be given. Firstly, the difference in prevalence of GTS and CMT between males and females that has been reported in the literature is not entirely supported by our data. Secondly, it should be realized that our family sample was selected specifically for the purpose of a linkage study, which can be expected to introduce a bias towards

large, multiply affected families. Thirdly, it has been suggested that the implementation of non-Mendelian transmission probabilities in the POINTER program might be incorrect. Finally, it has been argued that rejection of a simple Mendelian model in favor of a non-Mendelian model should not be regarded as conclusive evidence against a prominent role for a single gene. It is not clear whether the results obtained with the tau_2 model reflect a true biologic phenomenon in GTS, or rather are an artifact of ascertainment or of a too stringent statistical approach. The possibility that the inheritance of GTS is not consistent with a Mendelian pattern should not be discarded, but other explanations need to be investigated before a definite conclusion can be made.

Simulation Studies (chapter 3.2)

For a linkage study it is important to ascertain family material informative enough to detect linkage. The statistical power of a linkage sample can be determined with simulation studies which are relatively straightforward for simple Mendelian diseases. For complex traits additional genetic parameters such as reduced penetrance, frequency of phenocopies and variable expression of the phenotype have to be taken into account.

Until now the power of the 24 families, used by the collaborative group, to detect linkage was not systematically investigated. The results of an extensive simulation study on 24 families is reported in this chapter. This family material is shown as sufficiently informative to detect linkage even in the case of extensive genetic heterogeneity. Instability analysis on the most informative family revealed the importance of clinical diagnosis and definition of the phenotype. It has been proposed to use only a narrowly defined phenotype to circumvent diagnostic uncertainties. Alternatively, multiple testing procedures could be used. Decisions to use one of these strategies have been made arbitrarily. In this simulation study we systematically investigated which of these strategies will give the best chances of detecting linkage for GTS. We conclude that for GTS the testing of multiple diagnostic models instead of using a narrowly defined phenotype gives the best chances of detecting linkage.

The simulation studies presented here are generally applicable to other complex disorders and can facilitate the planning of a linkage study and the interpretation of the results obtained from such a study.

LINKAGE STUDIES

The objective of linkage analysis is to correlate the inheritance of a distinct segment of DNA - a 'marker' with known chromosomal localization - with the inheritance of a disease. Several strategies can be used to determine the position of the GTS gene on the human genome:

1. Search for chromosomal abnormalities associated with the disease.
2. Co-segregation of other inherited disease within GTS families.
3. Candidate gene approach.
4. Systematic screening for polymorphic markers linked to the disease locus.

The linkage study on GTS is more extensively described in the companion thesis 'Gene Mapping of Complex Diseases' by Peter Heutink. In this thesis examples of approaches 1 and 3 are given.

Search for chromosomal abnormalities associated with the disease (chapter 4.1)
Comings and collaborators (1986) reported a 46,t(7;18)(q22;q22.1) balanced reciprocal translocation in six relatives suffering from GTS. That observation suggested a localization of the GTS gene near the 18q22.1 breakpoint. In our present study no evidence for genetic linkage on chromosome 18 and chromosome 7 was obtained. Data from the markers tested made it possible to exclude the complete chromosome 18 and the chromosome 7q21.3-qter region as a locus for the GTS gene.

Candidate gene approach (chapter 4.2)
A genetic linkage study of fifteen families (n = 166) ascertained through probands diagnosed with GTS was carried out for the D_2-dopamine receptor and flanking loci on chromosome 11q22-q23. Tight linkage was excluded for all probes and regions of exclusion up to ± 20% recombination were obtained. Overlapping regions of exclusion based upon primary map data permit exclusion of the entire region of the DRD2 locus in GTS.

THE PHENOTYPE (section 5)

The initial optimism that the phenotype was well defined and could be diagnosed objectively during a single observation of the patient has

since long been refuted. It is now well understood that tics may vary from severe to mild, even to the extent that the ticcing person is unaware of them. Tics may be suppressed consciously or unconsciously during the interviews, making it more difficult to document them. They may also be masked by their conscious or unconscious integration into apparently purposeful movements (for example, a hand-to-face tic may become smoothing the hair). Furthermore, compared to the rapid, sudden tic movements, the slow dystonic tics are often more difficult to detect. Moreover, a diagnosis of GTS of a currently symptom-free individual during examination based solely on a history of a small number of tics during early childhood is not as reliable as a diagnosis based on a variety of observed tics in addition to a positive history.

In addition to a clinical diagnosis, the existence of any biological markers may be invaluable for the identification of the affected individuals in the family. For GTS no neuro-anatomical, -chemical or -physiological marker has been found. Enoch and associates reported a series of visual field defects, considered as specific for GTS and also found in biologic relatives of GTS patients, but not in a normal control group (15). If indeed, these field defects might be linked to tic disorders in biological relatives of GTS probandi, they could become promising as a biological marker for GTS.

We tried to confirm these neurophthalmologic abnormalities in GTS in a pilot-study (**chapter 5.1**). Ten consecutive GTS patients had a routine ophthalmologic examination. Afterwards they were investigated by technicians unaware of the expected findings. Kinetic Goldmann perimetry and electrophysiologic ophthalmologic examinations like electroretinography, electro-oculography and visually evoked cortical potential registration did not show any indication of abnormalities specific for GTS. In nine of the patients perimetry was repeated after about one year. This time the investigator was instructed to look for step defects and fatigue effects. Again no specific abnormalities could be demonstrated.

Essential for gene mapping studies is a clear and rigorous definition of the phenotype. Such studies are vastly more difficult if the disease phenotype is ambiguous, proper subject assignement being clouded by incomplete penetrance, cases mild enough to escape detection, or genetically unrelated but highly associated traits. Indeed, because of just these features the initial optimism about the 'well-defined and easy-to-objectivate' phenotype of GTS has gradually been tempered.

The tics appeared to vary in frequency and severity, are often sup-

pressed during examination or might go unnoticed as associated behavioral symptoms like disturbed impulse control and, moreover, as obsessive-compulsive disorder (OCD) can predominate the clinical picture. Phenomenological analysis of the symptoms has lead to an extended view on tics and the 'obsessive-compulsive behaviors' of GTS patients as described in **chapters 5.2** and **5.3**. In **chapter 5.2** a new phenomenon, found only in Gilles de la Tourette (GTS) patients, which we have called 'mental play' is described. It was compared with the phenomenon of counting, which occurred in both GTS and obsessive-compulsive patients. In the GTS patients both mental play and counting were best characterized as playful impulsions. In contrast to the GTS patients, counting of the obsessive-compulsive patients was in line with their obsessive-compulsive behaviour. These findings suggest that repetitive symptoms in GTS patients, even when they share superficial similarities with obsessive-compulsive symptoms, should not be diagnosed automatically as obsessive-compulsive. In **chapter 5.3** we propose to distinguish cognitive tics in addition to the motor, vocal and sensory tics that have been described in the literature before. The phenomenon of mental play is a clear example of cognitive tics instead of obsessive-compulsive behavior as seen in obsessive-compulsive disorder according to DSM-III-R criteria. This extension of the tic concept implies that GTS is more likely a condition characterized by a disturbed impulse control than just one of the hyperkinetic disorders.

CONCLUSION

The distribution of tics in the first degree, second degree and more distant biological relatives in our kindreds does not reflect a 'simple' autosomal dominant model of transmission. The recurrence risks do not show the 50% drop for every succeeding degree of relationship. This could be a result of the method of ascertainment, as the presence of another affected relative belonged to the inclusion criteria. As all but one of these additional cases were first degree relatives, it does not seem likely that primarily families with a high loading of affected cases in the second degree and more distant relatives have been included. Moreover, the number of females with CMT is much higher than expected from the studies by Pauls et al (37). One explanation could be that the inheritance of GTS does not follow either classic multifactorial or monogenic patterns. As with other psychiatric disorders, more complex models of transmission need to be developed to explain the results of

the Dutch family study.

This could also explain the results of the segregation analyses and the simulation studies, also of the combined sample. By applying the parameters from the earlier studies, the segregation analyses, showed autosomal dominant transmission to be the second best solution. A non-Mendelian way of transmission, reflected by the better results of the tau2 model, appeared to be more likely. More extensive analyses applying other values for the ascertainment probabilities and additional estimates for population prevalence for males and females will be carried out in the future to refine the current models.

Considering these facts, it is not surprising that the linkage approach did not as yet result in conclusive evidence for the chromosomal localization of the GTS susceptibility gene(s). Assuming that there is a single genetic vulnerability factor identical in all families, about 80% of the human genome could be excluded as possible site for the GTS gene since over 600 DNA markers have been tested in an international collaborative effort. This result necessitates the application of new statistical methods and the consideration of other genetic models with respect to the accumulated data. The difficulties faced in unravelling the genetic basis of phenotypically variable inherited disorders more generally are exemplary of the current status in the relatively young field of genetic psychiatric research.

SAMENVATTING

Inleiding

Het syndroom van Gilles de la Tourette (GTS) wordt gekenmerkt door motorische, vocale, sensorische en cognitieve tics. De eerste verschijnselen ontstaan meestal op de kinderleeftijd. Naast tics komen echo- en coprofenomenen voor evenals drang- en in mindere mate dwangsymptomen, aandachttekort-stoornissen met hyperkinesie, gedragsstoornissen en automutilatie bij GTS patiënten. De ernst van de symptomen kan niet alleen van persoon tot persoon verschillen, maar ook per persoon wisselen in verschillende perioden in het beloop. Met name bij de ernstige vormen zijn de resultaten van behandeling, die bestaat uit (een combinatie van) steun en voorlichting, medicamenteuze therapie en/of gedragstherapie, vaak onbevredigend. De oorzaak van het syndroom is onbekend. Verondersteld wordt dat stoornissen in centrale dopaminerge, noradrenerge, serotonerge, GABA-erge en endorfinesystemen invloed hebben op het ontstaan en de ontwikkeling van de symptomen.

De Amerikaanse Tourette Syndrome Association heeft initiatieven ontplooid om onderzoek te stimuleren teneinde meer inzicht te krijgen in de oorzaken en verbeterde mogelijkheden tot behandeling van GTS. Als onderdeel van dit onderzoeksprogramma werkt een aantal Amerikaanse en Europese onderzoeksgroepen samen om de genetische achtergrond van GTS op te helderen. Met systematisch familieonderzoek wordt de wijze van overerving te onderzocht om door onderzoek van het erfelijk materiaal het verantwoordelijke gen te localiseren en identificeren. Het gehele onderzoek omvat de volgende stappen:

1. Het verzamelen van families van GTS patiënten en de diagnostische classificatie van de familieleden (zie sectie 2).
2. De statistische analyse van de familiegegevens om de meest waarschijnlijke wijze van overerving vast te stellen met behulp van segregatieanalyse en om uit te zoeken wat de potentiële waarde van de families is voor koppelingsstudie door middel van simulatie onderzoek (zie sectie 3).
3. De analyse van DNA-merker gegevens met behulp van koppelingsonderzoek (zie sectie 4).

Het Nederlandse familieonderzoek

Het Nederlandse familieonderzoek is een samenwerkingsproject van de afdelingen Psychiatrie en Klinische Genetica van het Academisch Ziekenhuis Rotterdam-Dijkzigt en de Erasmus Universiteit Rotterdam.

Met behulp van een gestructureerd interview, dat ontwikkeld is in het Child Study Center van de Yale University in New Haven (V.S.), werden alle biologische en alle aangetrouwde familieleden van GTS patiënten onderzocht. Dit gestructureerde interview omvat gedétailleerde vragen over tics, de biografie, de schoolprestaties, over spraak- en taalstoornissen en een algemeen psychiatrisch onderzoek en een dwangvragenlijst (de Yale-Brown Obsessive-Compulsive Scale).

In **Hoofdstuk 2.1** van dit proefschrift wordt de frequentie van vóórkomen van GTS en chronische multipele tics (CMT) in de eerste 10 families, negen Nederlandse en één Noorse, uit het Nederlandse onderzoek weergegeven. De diagnoses werden gesteld op basis van eigen waarnemingen, de resultaten van het gestructureerde interview en een Nederlandse vertaling van een Amerikaanse dwangvragenlijst. Dertig procent van de 233 geïnterviewde biologische verwanten bleek een ticstoornis te hebben. Van de personen met tics voldeed 42% aan de criteria voor GTS. De verhouding tussen mannen en vrouwen was voor GTS 1,4:1 en voor CMT 1:1,6.

19,5% van de eerstegraads bloedverwanten had GTS en 17,1% CMT. De man:vrouw verhouding in de eerstegraads bloedverwanten voor Tourette was 1,6:1 en voor CMT 1:6. GTS kwam voor in 13,7% van de tweedegraads bloedverwanten en CMT in 19,6%. Hier waren de verhoudingen tussen mannen en vrouwen voor GTS 1:1,2 en voor CMT 1:1. Voor de overige bloedverwanten (derdegraads en verder) waren de percentages voor GTS 9,9 en voor CMT 16,3. De man:vrouw verhouding was 1,4:1 voor GTS en 1:1,6 voor CMT.

In **Hoofdstuk 2.2** wordt de frequentieverdeling voor obsessief-compulsieve symptomen (OCS) weergegeven. Bij 51 biologische verwanten (22%) werden één of meer duidelijk obsessief-compulsieve symptomen gevonden, wat een significant verschil was met de niet-biologische verwanten. De combinatie van tic-syndromen en obsessief-compulsieve symptomen kwam, zoals te verwachten, vaker voor bij biologische verwanten dan bij niet-biologische verwanten. Er werd één aangetrouwde persoon met de diagnose obsessief-compulsieve stoornis gevonden, echter deze had nooit tics gehad. Obsessief-compulsieve stoornissen kwamen niet vaker voor bij vrouwen vergeleken bij mannen en ook niet vaker bij personen met GTS ten opzichte van CMT. Er werd geen verschil gevonden tussen de IDB-sores van personen met of zonder tics.

STATISTISCHE ANALYSES

Segregatieanalyse (hoofdstuk 3.1)
Met behulp van segregatieanalyse kan een onderscheid gemaakt worden tussen monogene en polygene invloeden op de etiologie van erfelijke kenmerken of aandoeningen. Op basis van twee vroegere segregatie analyses van GTS-families, waarbij gebruik gemaakt werd van directe interviews, werd verondersteld dat het meest waarschijnlijke model van overerving berust op een autosomaal dominant gen met incomplete penetrantie en een variabele expressie.

Bij de segregatie analyse maakten wij gebruik van de familiegegevens van 24 GTS-families, die door verschillende onderzoeksgroepen verzameld zijn: Erasmus Universiteit Rotterdam en Academisch Ziekenhuis Rotterdam-Dijkzigt (10 families), de Universiteit van Iowa, Iowa City, Iowa, V.S. (16 families), Marshfield Medical Research Foundation, Marshfield, Wisconsin, V.S. (1 familie), en het Hospital for Sick Children, Toronto, Canada (1 familie). Segregatieanalyse werd uitgevoerd met behulp van het computerprogramma POINTER. Vier diagnostische modellen, te weten: 1) GTS, 2) GTS en CMT, 3) GTS, CMT en OCS, en 4) GTS en OCS, werden gebruikt ten behoeve van de analyses. Diagnostische criteria voor GTS en CMT waren gebaseerd op de DSM-III-R-criteria. De classificatie OCS omvatte zowel obsessief-compulsieve stoornissen volgens de DSM-III-R alsmede opvallende herhalende gedragingen die niet noodzakelijk verbonden waren aan het reduceren van angst en niet als beperkend werden ervaren.

De resultaten van de segregatie analyse steunen de hypothese dat het spectrum van GTS-symptomen berust op een enkel, dominant gen. De penetrantie van dit gen lijkt in sterke mate geslachtsafhankelijk in de beperkte diagnostische modellen. De bevinding dat het verschil in penetrantie afnam wanneer OCS in het fenotype betrokken werd, is een aanwijzing dat OCS bij vrouwen beschouwd zou kunnen worden als een andere vorm van expressie van het GTS-gen.

De resultaten van de segregatie analyses, zoals in hoofdstuk 3.1 beschreven, houden voorts in dat rekening gehouden moet worden met de mogelijkheid van een wijze van overerving die niet geheel volgens de wetten van Mendel verloopt. Daarmee is het overigens niet uitgesloten dat het om een 'single gene' gaat. Echter er dient verder onderzoek te gebeuren om deze mogelijkheden definitief uit te sluiten dan wel te bevestigen.

Simulatie onderzoek (hoofdstuk 3.2)
Het is voor koppelingsonderzoek van groot belang dat de families die onderzocht worden voldoende informatie bevatten om koppeling te kunnen vinden. Dit kan getest worden met behulp van simulatie onderzoek. In dit hoofdstuk worden de resultaten van simulatie onderzoek bij 24 families vermeld. De conclusie is dat het familiemateriaal dat thans beschikbaar is voldoende moet zijn om linkage te kunnen vinden, zelfs wanneer er sprake is van genetische heterogeniteit. Het simulatie onderzoek onderstreept nog eens het belang van de diagnostiek en classificatie van aangedane personen. Op basis van de beschikbare gegevens is de conclusie gerechtvaardigd, dat het testen van verschillende diagnostische modellen meer kansen oplevert uiteindelijk koppeling te vinden dan het gebruik van het meest strikte diagnostische model.

KOPPELINGSONDERZOEK

Het doel van koppelingsonderzoek is om een koppeling te vinden tussen overerving van een bekend stukje DNA - een 'merker' met een bekende chromosomale lokalisatie - en de overerving van een erfelijk kenmerk of erfelijke ziekte. Daarbij kunnen verschillende benaderingswijzen worden gevolgd, uitgaande van:

1. bestaande chromosomale afwijkingen bij personen die de ziekteverschijnselen hebben.
2. het gezamenlijk vóórkomen van de onderzochte ziekte met een andere erfelijke ziekte bij dezelfde personen.
3. bekende chromosomale localisatie van enzymen die betrokken zijn in de pathogenese.
4. het systematische onderzoek met behulp van polymorfe merkers.

Het koppelingsonderzoek bij GTS wordt uitvoerig beschreven in het proefschrift van Peter Heutink met als titel 'Gene Mapping of Complex Diseases'. In mijn proefschrift worden voorbeelden gegeven van 1 en 3.

Chromosomale afwijkingen geassociëerd met de ziekte (hoofdstuk 4.1)
De onderzoeksgroep van Comings vond in één familie een gebalanceerde translocatie tussen chromosoom 18 en 7 (46,t(7;18)(q22;q22.1)) in zes bloedverwanten die allen aan GTS leden. Die observatie suggereerde dat het Tourette-gen in de buurt van het 18q22.1 breekpunt zou kunnen liggen. Wij hebben echter geen koppeling kunnen vinden tussen

merkers op chromosoom 18 en 7 en GTS en CMT. Op basis van onze bevindingen was het mogelijk om het gehele chromosoom 18 en het gebied chromosoom 7q11.3-qter uit te sluiten als locus voor het GTS-gen.

Candidaat-genen (hoofdstuk 4.2)
Vanwege de gunstige uitwerking van dopamine receptor blokkerende middelen op de tics, werd gedacht dat (veranderingen in) dopamine receptoren mogelijk betrokken zouden kunnen zijn bij GTS. Een koppelingsonderzoek bij 15 families bestaande uit in totaal 166 personen werd uitgevoerd op chromosoom 11 in het gebied van de D_2-dopaminereceptor en direct naastliggende gebieden. Koppeling kon worden uitgesloten voor dit gebied.

HET FENOTYPE (sectie 5)

Het aanvankelijke optimisme dat het fenotype van GTS goed omschreven was en relatief gemakkelijk vastgesteld kon worden gedurende een eenmalige observatie is gaandeweg verdwenen. Het is nu duidelijk dat tics kunnen variëren in ernst, zozeer dat zelfs de persoon met tics zich er niet van bewust is. Bovendien kunnen tics bewust of onbewust onderdrukt worden gedurende het onderzoek, waardoor de aanwezigheid ervan moeilijk is vast te stellen. Tics kunnen ook gemaskeerd worden als ze opgenomen worden in doelgerichte bewegingen. Langzame tics met een dystoon karakter zijn moeilijker op te merken dan snelle, plotselinge tics. Tenslotte, de diagnose GTS is bij een persoon die op het moment van het onderzoek geen symptomen meer heeft, maar bij wie er duidelijk tics in de voorgeschiedenis zijn, minder betrouwbaar dan een diagnose die gebaseerd is op tics die zowel waargenomen zijn als in de anamnese vermeld.

In aanvulling op een klinische diagnose zouden biologische merkers van grote waarde kunnen zijn voor het opsporen van aangedane individuen binnen een familie. Voor GTS zijn er echter geen neuroanatomische, neurochemische en/of neurofysiologische merkers bekend. De onderzoeksgroep van Enoch heeft afwijkingen bij gezichtsveldonderzoek, die zij als specifiek voor GTS beschouwen, beschreven in GTS patiënten en hun familieleden. In een vooronderzoek hebben wij getracht bij 10 patiënten de bevindingen van Enoch te reproduceren. Routine oogheelkundig onderzoek en gezichtsveldonderzoek met behulp van Goldmann perimetrie werd voorafgaand aan

electrofysiologisch onderzoek uitgevoerd. Bij geen van de genoemde onderzoeken werden specifieke afwijkingen, die gerelateerd zouden kunnen zijn aan GTS, gevonden.

In de **hoofdstukken 5.2 en 5.3** worden de resultaten van nadere fenomenologische beschouwingen van de tics, zoals die voorkomen bij het syndroom van Gilles de la Tourette, in vergelijking met obsessief-compulsieve symptomen beschreven. In **hoofdstuk 5.2** wordt een symptoom beschreven, aangeduid als 'mental play', dat wij regelmatig bij GTS patiënten aantroffen. Mental play werd vergeleken met het tellen of de teldrang van GTS patiënten en patiënten met een dwangstoornis. Bij de GTS patiënten konden zowel mental play als het tellen het best omschreven worden als drangverschijnselen, met een min of meer speels karakter, vaak uitgevoerd als tijdverdrijf. Bij de dwangpatiënten daarentegen maakte het tellen deel uit van het dwanggedrag, vaak om dwanghandelingen te begrenzen. Deze bevinding maakt het waarschijnlijk dat de herhalingsverschijnselen bij Tourette-patiënten, zelfs wanneer ze oppervlakkig gezien gelijkenis vertonen met dwangsymptomen, niet als dwangverschijnsel gediagnostiseerd dienen te worden. In **hoofdstuk 5.3** stellen wij voor om in analogie van de motorische, vocale en de sensorische tics, cognitieve tics te onderscheiden. Het verschijnsel mental play hoort tot de categorie van cognitieve tics. Een nadere fenomenologische beschouwing van de tics zoals die bij het syndroom van Gilles de la Tourette voorkomen doet eerder denken dat het hierbij gaat om een stoornis in de impulscontrole dan om enige vorm van dwang.

CONCLUSIES

Het vóórkomen van tics in de eerste-, tweede- en verdere graads bloedverwanten in de door ons onderzochte families wijst niet zonder meer op een eenduidig autosomaal dominant model van overerving. Het herhalingsrisico vertoont niet de kenmerkende 50%-vermindering in iedere verdere graad van bloedverwantschap. Dit zou het gevolg kunnen zijn van de selectie van de families, waarbij de aanwezigheid van een tweede persoon met tics vereist was om de familie tot het onderzoek toe te laten. Echter, aangezien deze tweede aangedane personen tot de eerstegraads bloedverwanten bleken te horen, is het niet waarschijnlijk dat families met juist aangedane personen in de tweede- en derdegraad, ongewild geselecteerd werden voor dit onderzoek. Daarnaast is het zo dat het aantal vrouwen met tics hoger is dan in

eerdere studies, o.a. van de groep van Pauls, is gevonden. Een verklaring daarvoor zou kunnen zijn dat de overerving van Tourette geen duidelijke klassieke multifactoriële of monogene manier van overerving vertoont. Het is aannemelijk dat, zoals bij andere psychiatrische stoornissen, ingewikkelder modellen van overerving ontwikkeld moeten worden om de resultaten te verklaren.

Gezien deze aspecten, is het verklaarbaar waarom het koppelingonderzoek nog niet heeft geleid tot het vinden van de chromosomale lokalisatie van het gen verantwoordelijk voor GTS. Nu ongeveer 80% van het genoom uitgesloten lijkt na het testen van meer dan 600 DNA merkers binnen de gangbare genetische modellen, dienen nieuwe wegen te worden gezocht door middel van verder onderzoek gebruik makend van de ervaring en de kennis die inmiddels is opgedaan. Dit houdt in dat verfijnde diagnostische methoden, statistische modellen en methode van analyse van de DNA-gegevens tot ontwikkeling gebracht zullen worden. De recente identificering van het gen voor de chorea van Huntington, tien jaar nadat de chromosomale lokalisatie werd vastgesteld, is een voorbeeld hoe wetenschappelijke kreativiteit en vasthoudendheid in dit onderzoeksveld tot goede resultaten kan leiden.

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I would never have started this study if Theo van Woerkom had not introduced me to the Gilles de la Tourette syndrome (GTS) and Lodewijk Sandkuijl had not discussed the subject with the staff at the Departments of Clinical Genetics and Celbiology of the Erasmus University. His involvement as collegue and friend in the design and description of the statistical analyses of the genetic data has significantly contributed to this thesis.

Once at the doorstep of the project, Joost Schudel kept opening the doors in front of me. As promotor he learned me to deal with the obstacles inherent to research and gave me the confidence to proceed.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 26 juni 1953 te 's-Hertogenbosch geboren. Hij bezocht het Gymnasium Paulinum te Driehuis-Westerveld en het Bonaventura College te Leiden, waar hij in 1971 het diploma Gymnasium-ß behaalde. Van 1971 tot 1972 studeerde hij aan de Katholieke Sociale Academie te 's-Gravenhage om in 1972 aan te vangen met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. In 1979 werd hij tot arts bevorderd. Na vervulling van de militaire dienstplicht op de afdeling Psychiatrie van het Militair Hospitaal Dr. A. Matthijsen te Utrecht, begon hij zijn specialisatie psychiatrie in het psychiatrisch centrum Rosenburg te 's-Gravenhage (opleider: P.J. Stolk). De keuzestage bestond uit een stage neuropsychiatrie op de afdeling Neuro-psychiatrie van dat ziekenhuis (opleider: Dr. T.C.A.M. van Woerkom). De stage Sociale Psychiatrie werd gevolgd bij de afdeling sociale psychiatrie van de GG&GD te 's-Gravenhage, in de periode dat deze overging in de RIAGG (opleider: Prof.Dr. W.J. Schudel). Op 1 januari 1985 werd hij als psychiater ingeschreven in het specialistenregister. Van 1 januari tot 1 november 1985 was hij als psychiater werkzaam in het psychiatrisch centrum Rosenburg deels op de mediumstay afdeling Midsland, deels op de geriatrische observatie afdeling.
Op 1 november 1985 werd hij als staflid aangesteld in de kliniek Psychiatrie van het Academisch Ziekenhuis Rotterdam-Dijkzigt op Unit 5-Zuid. Vanaf 15 december 1992 bekleedt hij de functie van chef de policlinique.
Hij is thans voorzitter van de adviesraad van de Stichting Gilles de la Tourette en international Associate van de Medical Advisory Board van de Tourette Syndrome Association.

LIST OF PUBLICATIONS

1. Balkom AJLM van, Wetering BJM van de, Tavy DJL, Hekster RAM, Woerkom TCAM van. Cerebellar atrophy due to chronic bromisovalum abuse demonstrated by computer tomography. J Neurol Neurosurg Psychiatry 1985; 48: 342-7.
2. Wetering BJM van de, Martens CMC, Fortgens C, Slaets JPJ, Woerkom TCAM van. Late components of the auditory evoked potential in Gilles de la Tourette syndrome. Clin Neurol Neurosurg 1985; 87: 181-6.
3. Woerkom TCAM van, Fortgens C, Martens CMC, Wetering BJM van de. Long-latency evoked potentials in an auditory decision task in Gilles de la Tourette syndrome. Electroenceph Clin Neurophysiol 1986; 63: 37P.
4. Woerkom TCAM van, Fortgens C, Rompel-Martens CMC, Wetering BJM van de. Auditory event-related potentials in adult patients with Gilles de la Tourette syndrome in the oddball paradigm. Electroenceph Clin Neurophysiol 1988; 71: 443-9.
5. Bollen EL, Roos RAC, Cohen AP, Minderaa RB, Reulen JPH, Wetering BJM van de, Buruma OJS, Woerkom TCAM van. Oculomotor disturbances in Gilles de la Tourette's syndrome. J Neurol Neurosurg Psychiatry 1988; 51: 1081-3.
6. Wetering BJM van de, Cohen AP, Minderaa RB, Roos RAC, Woerkom TCAM van. Het syndroom van Gilles de la Tourette. Ned Tijdschr Geneesk 1988; 132: 21-5.
7. Woerkom TCAM van, Fortgens C, Wetering BJM van de, Martens CMC. Contingent Negative Variation in adult patients with Tourette Syndrome. J Neurol Neurosurg Psychiatry 1988; 51: 630-4.
8. Minderaa RB, Gemert TM van, Wetering BJM van de. Onverwachte presentatiewijzen van het syndroom van Gilles de la Tourette. Tijdschr Psychiatrie 1988; 30: 246-54.
9. Kooyman CG, Wetering BJM van de, Mast RC van der. Patiënten aan de spoedopname van een algemeen ziekenhuis. Medisch Contact 1988; 41: 11-4.
10. Bouvy P, Wetering BJM van de, Bruijn JA, Meerwaldt JD. A case of organic brain syndrome following head injury succesfully treated with carbamazepine. Act Psychiat Scand 1988; 77: 361-3.
11. Kooiman CG, Wetering BJM van de, Mast RC van der. Clinical and demographic characteristics of emergency department patients in the Netherlands. A review of the literature and a preliminary study. Am J Emergency Med 1989; 7: 632-7.
12. Wetering BJM van de, Tan MHG, Oostra BA. Een personal computer als ondersteuning van familieonderzoek. COBO Bull 1989; 22: 27-33.
13. Devor EJ, Grandy DK, Civelli O, Litt M, Burgess AK, Isenberg KE, Wetering BJM van de, Oostra BA. Genetic linkage is excluded for the D_2-dopamine receptor lambda HD2G1 and flanking loci on chromosome 11q22-q23 in Tourette syndrome. Hum Hered 1990; 40: 105-8.
14. Dieleman BP, Broek WW van den, Wetering BJM van de. Misbruik van anticholinergica. Ned Tijdschr Geneesk 1990; 134: 625-7.
15. Heutink P, Wetering BJM van de, Breedveld GJ, Weber J, Sandkuijl LA, Devor EJ, Heiberg A, Niermeijer MF, Oostra BA. No evidence for genetic linkage of Gilles de la Tourette syndrome on chromosomes 7 and 18. J Med Genet 1990; 27: 433-6.

16. Evers RAF, Wetering BJM van de. Een patiënte met het syndroom van Gilles de la Tourette en een klokmaag. Dth 1990; 2: 168-75.
17. Evers RAF, Wetering BJM van de. Behandeling van motorische tics op basis van een spanningsreductiemodel. T Gedragstherapie 1990; 3: 167-78.
18. Woerkom TCAM van, Fortgens C, Cohen AP, Minderaa RB, Wetering BJM van de. Auditory event-related potentials in children with Gilles de la Tourette's syndrome in the oddball paradigm. In: Brunia CHM, Gaillard AWK, Kok A (eds). Psychophysiological Brain Research. Tilburg University Press, 1990: 243-7.
19. Heutink P, Wetering BJM van de, Breedveld GJ, Weber JL, Pakstis AJ, Sandkuijl LA, Pauls DL, Niermeijer MF, Kidd KK, Oostra BA. Study on the heredity of Gilles de la Tourette Syndrome. Am J Hum Genet 1990;47: A183.
20. Filloux F, McMahon WM, Wetering BJM van de, Leppert M. Arithmomania: a common manifestation of Tourette syndrome? Ann Neurol 1990; 28: 425.
21. Heutink P, Sandkuijl LA, Wetering BJM van de, Oostra BA, Weber J, Wilkie P, Devor EJ, Pakstis AJ, Pauls D, Kidd KK. Unjustified claim for linkage of Gilles de la Tourette syndrome. Lancet 1991; 337: 122-3.
22. Tulen JHM, Moleman P, Wetering BJM van de, Kruijk MPCW, Saher RA von, Boomsma F, Steenis HG van, Man in't Veld AJ. Responses to cumulative doses of clonidine in healthy volunteers: cardiovascular, noradrenergic and subjective parameters. Eur Neuropsychopharm 1991; 1: 466.
23. Tulen JHM, Wetering BJM van de, Luteijn LB, Boomsma F, Smeets F, Steenis HG van. Stability of baselines in a repeated measurements design: Details of cardiovascular variability, plasma catecholamines and subjective mood. In: Boelhouwer AJW, Brunia CHM (eds). Proceedings of the First European Psychophysiology Conference. Tilburg, 1991: 150.
24. Tulen JHM, Wetering BJM van de, Kruijk MPCW, Saher RA von, Moleman P, Boomsma F, Steenis HG van, Man in't Veld AJ. Dose-related effects of clonidine i.v. on subjective mood, cardiovascular activity and noradrenergic function. In: Boelhouwer AJW, Brunia CHM (eds). Proceedings of the First European Psychophysiology Conference. Tilburg, 1991: 151.
25. Wetering BJM van de. Het syndroom van Gilles de la Tourette. Lundbeck, Amsterdam, 1991.
26. Pakstis AJ, Heutink P, Pauls DL, Kurlan R, Wetering BJM van de, Weber J, Leckman JF, Sandkuijl LA, Kidd JR, Breedveld GJ, Castiglione CM, Sparkes RS, Cohen DJ, Kidd KK, Oostra BA. Progress in the search for genetic linkage with Tourette syndrome: an exclusion map covering more than 50% of the autosomal genome. Am J Hum Genet 1991; 48: 281-94.
27. Wetering BJM van de, Pauls DL, Heutink P, Sandkuijl LA, Oostra. The Gilles de la Tourette syndrome: segregation analysis and simulation studies. Am J Hum Gen 1991; 49: A485.
28. Wilkie P, Ahmann P, Weber J, Pakstis A, Kidd K, Wetering BJM van de, Heutink P, Oostra BA. Tourette syndrome linkage map. Am J Hum Gen 1991; 49: A363.
29. Wetering BJM van de, Cath DC, Roos RAC, Woerkom TCAM van, Hoogduin CAL, Minderaa RB. De tics bij het syndroom van Gilles de la Tourette. Ned Tijdschr Geneesk 1992; 136: 1644-7.
30. Cath DC, Wetering BJM van de, Woerkom TCAM van, Hoogduin CAL, Roos RAC, Rooymans HGM. Mental play in Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Brit J Psychiatry 1992; 161: 542-5.

31. Cath DC, Hoogduin CAL, Wetering BJM van de, Woerkom TCAM van, Roos RAC, Rooymans HGM. Tourette syndrome and obsessive-compulsive disorder. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 33-41.
32. McMahon WM, Leppert M, Filloux F, Wetering BJM van de, Hasstedt S. Tourette syndrome in 161 related family members. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 159-65.
33. Heutink P, Wetering BJM van de, Breedveld GJ, Oostra BA. Genetic study on Tourette syndrome in the Netherlands. In: Chase TN, Friedhoff AJ, Cohen DJ (eds). Tourette syndrome: Genetics, neurobiology, and treatment. Raven Press, New York, 1992: 167-72.
34. Cath DC, Wetering BJM van de, Hoogduin CAL, Roos RAC, Woerkom TCAM van. Het syndroom van Gilles de la Tourette en obsessief-compulsieve stoornissen: drang en dwang vergeleken. Dth 1992; 4: 368-76.
35. Kidd K, Pakstis AJ, Heutink P, Pauls DL, Kurlan R, Weber J, Wilkie P, Wetering BJM van de, Oostra BA. Genetics of Gilles de la Tourette syndrome. Movement Dis 1992; 7: 3.
36. Tulen JHM, Wetering BJM van de, Kruijk MPCW, Saher RA von, Moleman P, Boomsma F, Steenis HG van, Man in't Veld AJ. Cardiovascular, neuroendocrine, and sedative responses to four graded doses of clonidine in a placebo-controlled study. Biol Psychiatry 1992; 32: 485-500.
37. Foeken K, Wetering BJM van de. Manie, lithium en de schildklier; een model. Acta Neuropsychiatrica 1992; 4: 52-7.
38. Heutink P, Breedveld GJ, Niermeijer MF, Wetering BJM van de, Breedveld GJ, Oostra BA. Progress in gene localisation. In: Kurlan R (ed). The handbook of Tourette's syndrome and associated tic and behavioral disorders. Marcel Dekker, New York, 1993: 317-35.
39. Wetering BJM van de, Heutink P. The genetics of the Gilles de la Tourette syndrome: a review. Am J Clin Lab Med, 1993: 121, in press.
40. Evers RAF, Wetering BJM van de. Lucide dromen. Submitted.
41. Wetering BJM van de, Tan MH, Bonapart IE, Duivenvoorden HJ, Pauls DL, Niermeijer MF, Schudel WJ. The Dutch family study on the Gilles de la Tourette syndrome: I. Tic syndromes. In preparation.
42. Wetering BJM van de, Tan MH, Duivenvoorden HJ, Pauls DL, Niermeijer MF, Schudel WJ. The Dutch family study on the Gilles de la Tourette syndrome: II. Obsessive-compulsive symptoms. In preparation.
43. Wetering BJM van de, Heutink P, Pauls DL, Sandkuijl LA, Oostra BA, Niermeijer MF. Segregation analysis of familial Gilles de la Tourette syndrome. In preparation.
44. Heutink P, Wetering BJM van de, Pakstis AJ, Weber JL, Barr C, Devor EJ, Oostra BA, Sandkuijl LA. Simulation studies of statistical power on families with Gilles de la Tourette syndrome. Submitted.
45. Eapen V, Trimble MR, Wetering BJM van de, Robertson MM. Suicide in Gilles de la Tourette syndrome. Submitted.

ADDENDUM

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