The Incidence of Rett Syndrome in France

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Since the description of Rett syndrome, only a handful of epidemiologic studies based only on clinical investigation have been reported. Mutations in the MECP2 gene are associated with Rett syndrome and French laboratories have organized a clinical and molecular network to investigate the incidence of Rett syndrome in France including the results of molecular investigations. The present study, based on a large cohort of 424 patients with Rett syndrome, found that the incidence of this disease with a MECP2 mutation varied between 0.43 to 0.71 per 10,000 females. The total population of females aged 4-15 years in November 2004 in France was estimated to be 4,337,627. The data presented here indicate a prevalence of Rett syndrome of 0.558 per 10,000 females aged 4-15 years in France. The incidence of Rett syndrome is in accordance with other European epidemiologic studies based on clinical examination. Given that this is a minimum incidence because complete inventory was not possible, this study of patients with Rett syndrome reinforces the fact that the great majority of patients with Rett syndrome have a MECP2 mutation.

Introduction

Rett syndrome (OMIM 312750) is a progressive neurologic disorder which develops exclusively in young females after a period of apparently normal development. The syndrome is characterized by arrest of psychomotor development and regression after age 6-18 months, acquired microcephaly, autistic manifestations, loss of purposeful hand skills, stereotypic hand movements, and gait ataxia. The disease is frequently caused by mutations in MECP2, encoding a methyl-CpG binding protein MeCP2 [1]. Numerous studies have found various mutations (missense, nonsense, and frameshift) in the coding region of MECP2 in patients with Rett syndrome. Mutations were identified in approximately 80% of patients, suggesting that the remaining 20% may have mutations in other regions of this gene [2]. It is also possible that a second gene/locus is involved. Recent studies have identified novel large gene rearrangements that escape the common polymerase chain reaction–based mutation screening strategy [3,4] and mutations in a novel MeCP2 isoform (named MECP2B or MeCP2_e1 ) [5]. Even if Rett syndrome is not synonymous with mutations in the MECP2 gene, these new data suggest that MECP2 mutations explain the great majority of patients with Rett syndrome.

The first epidemiologic study was performed by Hagberg in 1985, who found a prevalence in southwestern Sweden of 0.65 per 10,000 females aged 6-17 years, which is approximately twice that of phenylketonuria in the same area [6]. Studies performed in other European regions and...
countries before the identification of MECP2 reported that the prevalence was 1 per 10,000 females in Sweden [7], and 0.80 per 10,000 females under 15 years old in Scotland [8]. The prevalence in Australia was similar to that in Sweden and Scotland (0.72 per 10,000 females) [9]. However, the prevalence rates reported for different regions in Japan differ considerably: 0.67 per 10,000 females aged 6-17 years (0.50 per 10,000 females if restricted to Rett syndrome of classical type) in the Tama district of Tokyo [10], 0.50 per 10,000 females aged 6-14 years (0.40 per 10,000 females if restricted to Rett syndrome not including variants) in metropolitan Tokyo [10], 0.36 per 10,000 females under 16 years old (0.37 per 10,000 females aged 7-15 years) in the Tokushima prefecture [11], and 0.22 per 10,000 females aged 6-14 years in the Fukui prefecture [12]. In a more recent study, among females aged 0 to 18 years in North Dakota, Burd et al. found the prevalence of Rett syndrome to be 0.505 per 10,000 [13]. In all of these studies, the diagnosis criteria are clinically based and dependent on the awareness concerning the disease and the follow-up of early growth and development. Diagnosis is hampered by insufficient information regarding its pathogenesis and lack of biologic markers specific to Rett syndrome. Moreover, because a description of Rett syndrome has been available in the English-language medical literature only since 1983, and that article was published in a specialty medical journal, it is highly probable that many health professionals were not fully aware of Rett syndrome when the first epidemiologic studies were implemented. Finally, some of these studies did not allow one to infer the most likely figure corresponding to the prevalence of Rett syndrome (limited size of the studied population). Differences in diagnostic practice and experience, as well as in research methodology, could cause some of the variation in prevalence figures.

Now that many of the health and education professionals are aware that mutations in the MECP2 gene have been found to be associated with Rett syndrome and that French laboratories studying this syndrome are organized into a network supported by the Groupement d’Interet Scientifique (GIS)-Institut des maladies rares and the Association Francaise du Syndrome de Rett (a parent support group), we have investigated the incidence of Rett syndrome in France using the results of molecular investigations generated by the French molecular network. This investigation is the first epidemiologic study of Rett syndrome performed in France.

**Material and Methods**

Since the implication of MECP2 in the etiology of Rett syndrome in 1999, the diagnosis is not only dependent on documentation of a child’s early growth and development and periodic evaluation of physical and neurologic status but also on MECP2 genetic analysis. Such analysis of the MECP2 gene is now systematically performed when a child neurologist, a neurologist, or a pediatrician suspected a typical, atypical, or a probable Rett syndrome in a female patient. On the contrary, the presence of one or more of the exclusion criteria previously defined by the Rett Syndrome Diagnostic Criteria Work Group excludes the genetic analysis of the MECP2 gene [14]. Moreover, the Association Francaise du Syndrome de Rett, a nonprofit organization for parents, organized 10 years ago a collection of blood samples to allow the detection of the mutation since the identification of the gene involved in the disease. All these blood samples were analyzed. As these two controlled sources are independent, we believe that almost all the cases of female patients with a MECP2 mutation were diagnosed.

All the French genetic laboratories that perform MECP2 mutation analyses in public hospitals or medical schools were organized into a research network. The eight laboratories involved in this network (Paris Cochin, Paris Necker, Kremlin-Bicêtre, Nancy, Lyon, Rennes, Tours, Marseille) are geographically well distributed. All laboratories were invited to fill in a form about the genotypes they identified in Rett syndrome. Using the date of birth and the first letters of the first and last name of each patient, duplicates were excluded. Genomic deoxyribonucleic acid extracted from peripheral blood and then amplified by polymerase chain reaction was the starting material for the MECP2 mutation analysis. Five laboratories screened their panel of Rett syndrome chromosomes by indirect scanning techniques, such as denaturing gradient gel electrophoresis (Paris Cochin) [15], single-stranded conformation polymorphism (Marseille, Tours), or denaturing high-pressure liquid chromatography (Paris Necker-Enfants-Malades, Nancy, Rennes), followed by the sequencing of the polymerase chain reaction products displaying an abnormal migration. The direct sequencing of the coding region of MECP2 as the primary testing tool was used by two laboratories (Lyon, Kremlin-Bicêtre). For samples that were negative in the screening analysis and to increase sensitivity and to reduce the risk of false negatives, screening of exon 1 of MECP2B (McCP2-e1) was undertaken by direct sequencing or denaturing high-pressure liquid chromatography. Moreover, screening the gene by semiquantitative polymerase chain reaction, by Southern blot analysis, or by multiplex ligation-dependent probe amplification was systematically performed in the absence of an identified MECP2 mutation [3,5,16]. Written consent was systematically obtained from the parent/guardian.

In this study, all the Rett syndrome cases identified in France with positive MECP2 molecular analysis (i.e., 424 Rett syndrome patients) have been included. The genotype was identified at the onset of the clinical disease. However, the incidence (number of new cases of Rett syndrome by birth year) was calculated using the birth date of each patient. As the mortality is low in this genetic disease, one can assume that the mean incidence could be considered close to the prevalence. Given that the number of births of females per year in France is perfectly known (data from Institut National de la statistique et des études économiques; http://www.insee.fr), we calculated the incidence of Rett syndrome. Taking into account the fact that the rate of infant mortality is extremely low in France (4.2% [per 1,000] in 2002, data from Institut National de la statistique et des études économiques; http://www.insee.fr), we believe that when calculating the incidence this factor does not significantly modify the denominator size (population at risk).

**Results**

In this study, a total of 424 French patients with Rett syndrome have been identified. All of these clinically identified 424 patients had MECP2 mutations. The majority of patients were patients with classical sporadic Rett syndrome. However, some sporadic females with some features of Rett syndrome, but in whom the diagnosis was clinically uncertain, were also included. Molecular diagnosis was performed by one of eight specialized MeCP2 centers. In total, 121 different MECP2 mutations were identified including missense mutations, nonsense muta-
tions, small insertions or deletions, mutations that affect splicing, and large rearrangements. Large rearrangements that escape a polymerase chain reaction–based mutation searching strategy were not an exceptional event and represent 5.9% of the MECP2 mutations, suggesting that routine mutation screening in MECP2 should include quantitative analysis of the MECP2 gene. On the contrary, mutations located in the novel coding exon (exon 1 of MECP2B) represent less than 0.5% of MECP2 mutations [17].

Given that the number of births of females per year in France is perfectly known, we calculated the number of females diagnosed with Rett syndrome by birth year Table 1. The term “incidence” refers to the number of new cases of Rett syndrome by birth year. We excluded from this analysis the patients born before 1989 and after 2000 to limit a bias due to the mortality of the oldest patients and a late diagnosis of the younger patients.

Estimates of the incidence of Rett syndrome are presented in Table 1 and range from 0.431 case per 10,000 females (in 1990) to 0.707 case per 10,000 females (in 1997). The total population of females aged 4-15 years in November 2004 (born between 1989 and 2000) in France was estimated to be 4,337,627. The number of Rett syndrome females aged 4-15 years was estimated to be 251. As the mortality is low in this genetic disease [18], one can assume that the mean incidence could be considered extremely close to the prevalence. This calculation gives a prevalence of Rett syndrome of 0.578 per 10,000 females aged 4-15 years in France.

**Discussion**

This large epidemiologic study of Rett syndrome based on 424 female cases (251 individuals born between 1989 and 2000) produced a prevalence estimate of 0.578 case per 10,000 females. In comparison with other studies of Rett syndrome, this study included almost two times the number of cases than were included in one of the previous largest studies of Rett syndrome prevalence (the Texas Rett syndrome registry) [19]. In contrast to previous epidemiologic studies based only on clinical investigation, one of the strengths of including molecular confirmation of the diagnosis is that the atypical cases not meeting the rigorous clinical criteria will not be excluded [19]. However, the incidence reported in the present study is in accordance with other European epidemiologic studies based on clinical examination, implying that these are fairly accurate. Hagberg found a prevalence in southwestern Sweden of 0.65 per 10,000 females, Kerr and Stephenson reported a prevalence of 0.67 per 10,000 females for the western part of Scotland, and Talvik et al. a prevalence of 0.67 per 10,000 females in Estonia [6,8,20]. In the large epidemiologic study from Texas, the prevalence was estimated to 0.438 case per 10,000 females from 2 to 18 years of age. This low figure of Rett syndrome prevalence could be explained by the difficulty of standard ascertainment and diagnosis of Rett syndrome in groups of older individuals [20]. The results presented herein suggest that this latter hypothesis is probable.

In France, the public health system covers similarly all the regions including economically deprived regions geographically separated from major medical centers. As this study included eight laboratories geographically well distributed, there was good coverage of the population base. Moreover, for example, in the MeCP2 center at Cochin Hospital in Paris, a total of 357 patients suspected of having Rett syndrome had been screened for MECP2 mutations during the last 4 years. The current detection rate was 31.6%, probably owing to the high proportion of patients with atypical clinical presentations or other diagnoses (mental retardation with or without behavior problems). All these results suggest that only few Rett syndrome patients are missing from the study and that clinicians suspect Rett syndrome probably more often than necessary.

However, we cannot exclude that few Rett syndrome patients were diagnosed and evaluated in countries bordering France (such as Belgium, Switzerland, Germany). Because of this latter potential bias and because the probability of undiagnosed cases with a MECP2 mutation is minimal but not null, figures provided in this study represent a minimum incidence. However, it is worth mentioning that this incidence is in accordance with previous European studies based only on clinical documentation. This study of a large cohort of Rett syndrome patients reinforces the fact that in the great majority of patients with Rett syndrome the disease is probably the result of a mutation in the MECP2 gene. The recent data presented at international meetings indicate that up to 95% of Rett cases have MECP2 mutations [21]. A small proportion of sporadic cases and familial cases, especially among atypical Rett syndrome cases, could be caused by mutations in other genes (such as CDKL5) [22]. However, in the present study, we cannot estimate the probable low

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**Table 1. Comparison of the number of cases of Rett syndrome by birth year during the years 1989–2001 in France**

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>Number of Cases</th>
<th>Number of Live Female Births</th>
<th>Incidence and Standard Deviation (x10,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>24</td>
<td>373,824</td>
<td>0.642 (0.131)</td>
</tr>
<tr>
<td>1990</td>
<td>16</td>
<td>371,095</td>
<td>0.431 (0.108)</td>
</tr>
<tr>
<td>1991</td>
<td>19</td>
<td>369,817</td>
<td>0.514 (0.118)</td>
</tr>
<tr>
<td>1992</td>
<td>16</td>
<td>361,914</td>
<td>0.442 (0.110)</td>
</tr>
<tr>
<td>1993</td>
<td>19</td>
<td>347,021</td>
<td>0.548 (0.126)</td>
</tr>
<tr>
<td>1994</td>
<td>20</td>
<td>346,716</td>
<td>0.577 (0.129)</td>
</tr>
<tr>
<td>1995</td>
<td>24</td>
<td>356,200</td>
<td>0.674 (0.138)</td>
</tr>
<tr>
<td>1996</td>
<td>23</td>
<td>357,335</td>
<td>0.644 (0.134)</td>
</tr>
<tr>
<td>1997</td>
<td>25</td>
<td>353,611</td>
<td>0.707 (0.141)</td>
</tr>
<tr>
<td>1998</td>
<td>23</td>
<td>360,005</td>
<td>0.639 (0.133)</td>
</tr>
<tr>
<td>1999</td>
<td>23</td>
<td>362,659</td>
<td>0.634 (0.132)</td>
</tr>
<tr>
<td>2000</td>
<td>19</td>
<td>377,430</td>
<td>0.503 (1.115)</td>
</tr>
<tr>
<td>Period 1989–2000</td>
<td>251</td>
<td>4,337,627</td>
<td>0.578 (0.038)</td>
</tr>
</tbody>
</table>
proportion of patients suspected of having Rett syndrome without MECP2 mutations; this is mainly because the presence of cases that were never referred to any of the centers involved in the study cannot be excluded.

This study, based on current molecular diagnostic strategies, suggests that Rett syndrome occurred more commonly than phenylketonuria (1/16,394), as previously demonstrated by epidemiologic studies [23]. Mutations in the MECP2 gene represent one of the most frequent causes of mental retardation in females, and the availability in the future of efficient therapeutic strategies would warrant a systematic neonatal screening in the general population.

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References


