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InterRett, a model for international data collection in a rare genetic disorder

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Abstract

Rett syndrome (RTT) is a rare genetic disorder within the autistic spectrum. This study compared socio-demographic, clinical and genetic characteristics of the international database, InterRett, and the population based Australian Rett syndrome database (ARSD). It also explored the strengths and limitations of InterRett in comparison with other studies. A literature review compared InterRett with RTT population-based and case-based studies of thirty or more cases that investigated genotype and/or phenotype relationships. Questionnaire data were used to determine case status and to investigate the comparability of InterRett and ARSD. Twenty four case series, five population based studies and a *MECP2* mutation database were identified of which twenty one (70%) collected phenotype and genotype data. Only three studies were representative of their underlying case population and many had low numbers. Of one thousand one hundred and fourteen InterRett subjects, nine hundred and thirty five born after 1976 could be verified as Rett

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HL was involved in study conception and design, had overall responsibility for data collection and made a major contribution to the drafting and revision of manuscript for important intellectual content. All authors have given final approval of the version to be published.

cases and compared with the two hundred and ninety five ARSD subjects. Although more InterRett families had higher education and occupation levels and their children were marginally less severe, the distribution of *MECP2* mutation types was similar. The InterRett can be used with confidence to investigate genotype phenotype associations and clinical variation in RTT and provides an exemplary international model for other rare disorders.

Keywords

Rett syndrome; international database; rare disorder; MECP2; phenotype

Rett syndrome (RTT) is characterised by severe intellectual and physical disability and affects 1 in 8500 females by the age of 15 years (B. Hagberg, 1985; Laurvick et al., 2006). In general, the most notable characteristics of RTT are the development of stereotypic hand movements and a regression in motor and cognitive function usually after a period of normal development (Trevathan, 1988). Other features include development of motor impairments including apraxia, epilepsy, scoliosis and breathing abnormalities.

Criteria for classical Rett syndrome were originally developed in 1988 to help clinicians diagnose the disorder (Trevathan, 1988) and included the following: normal fetal and postnatal development; a normal head circumference at birth often followed by deceleration of head growth; loss of hand skills; impaired language skills; development of stereotypic hand movements; the development of an impaired gait. However in the following decade it became clear that there was considerable clinical variability in this disorder and consequently the term variant was used to describe these phenotypes which did not conform exactly to the original criteria (Hagberg & Skjeldal, 1994). Examples of these were the “preserved speech” variant (Renieri et al., 2008) where individuals retained some words or speech, the “forme fruste” variant where the clinical features were milder overall (Hagberg & Skjeldal, 1994) and the congenital variant where symptoms were present from birth (Ariani et al., 2008).

In 1999 the link between Rett syndrome and the methyl CpG binding protein 2 (*MECP2*) was identified (Amir et al., 1999). It subsequently became apparent that pathogenic *MECP2* mutations were indeed present in many individuals who did not meet the criteria for the classical form of the disorder, e.g. in those without normal early development or without demonstrated head growth deceleration. Consequently the diagnostic criteria were amended (B. Hagberg, 2002) to account for this broader phenotype.

The presence of a *MECP2* mutation often provides confirmatory evidence of RTT (B. Hagberg, 2002) and testing for *MECP2* mutations has been available in North America (Percy et al., 2007) and countries such as UK (Kerr & Prescott, 2005), Israel (Yaron et al., 2002), Australia (Colvin et al., 2004), and in European countries such as Spain (Monros et al., 2001), France (Bienvenu et al., 2000), Germany (Huppke, Held, Hanefeld, Engel, & Laccone, 2002) and Italy (Vacca et al., 2001), at least in a research capacity, since 2000. Over 200 different pathogenic *MECP2* mutations have been identified and one focus of recent research has been to investigate whether there is a relationship between specific clinical features (phenotype) and specific genetic mutations (genotype) (Bebbington et al., 2008; Chae, Hwang, Hwang, Cheong, & Kim, 2004; Charman et al., 2005; Colvin et al., 2004; Hoffbuhr et al., 2001; Huppke, Held, Laccone, & Hanefeld, 2003; Monros et al., 2001; Neul et al., 2008; Nicolao et al., 2001).

Early genotype and phenotype studies were often based on small sample sizes (Amir et al., 2000; Cheadle et al., 2000; Monros et al., 2001; Nicolao et al., 2001) and lacked the power

necessary to investigate specific *MECP2* mutations separately and differentiate between similar phenotypes. Even where data have been pooled from different sources the number of cases for specific mutations has still been relatively small. An example includes a study in which data from three countries, Japan, Australia and the United Kingdom, were combined to enable the phenotype of one specific *MECP2* mutation, p.R133C, to be compared with that of other mutations (Leonard et al., 2003).

The Australian Rett syndrome Database (ARSD), initially established in 1993, is the only ongoing RTT population based study worldwide (Laurvick et al., 2006; Leonard, Bower, & English, 1997; Moore, Leonard, Fyfe, De Klerk, & Leonard, 2005). Potential RTT cases are ascertained by the ARSD through several sources including the Australian Paediatric Surveillance unit (a national network which facilitates the reporting of rare disorders by paediatricians) and the parent support group, the Rett Syndrome Association of Australia (Leonard, Bower, & English, 1997). Ascertainment has been estimated as 93% complete (Leonard, Bower, & English, 1997) and by the end of 2007 the ARSD contained 311 diagnosed cases of RTT (Downs et al., 2008).

InterRett, an international database of RTT cases, was set up in 2002 to contribute larger case numbers for genotype and phenotype investigations than could be provided by the ARSD which is based on a national population of just over 21 million people (Bebbington et al., 2008; Fyfe, Cream, de Klerk, Christodoulou, & Leonard, 2003; Leonard et al., 2005; Moore, Leonard, Fyfe, De Klerk, & Leonard, 2005). Unlike the ARSD, InterRett participants are determined by a variety of mechanisms including bulk submission of data from a number of countries, parent listservs and advertisement in newsletters and parent support associations. InterRett data are collected worldwide from family members with a child with RTT and by clinicians who have a patient with RTT via web and paper based questionnaires. Family (FQ) questionnaires were based on those already used by the ARSD (Laurvick et al., 2006) and clinician (CQ) questionnaires were developed by an international reference panel consisting of families with a child with RTT, clinicians, therapists and researchers. These questionnaires have been translated into French, Spanish, German, Italian and Mandarin. Data have also been collected from Spain, France, China, Israel and Canada (using the mechanisms described in table 1) and cases from these sources represent over half of the 1293 non-ARSD cases currently in the database (*Anderson, personal communication, July 2008*).

In 2006, an investigation of genotype and phenotype relationships was carried out using the InterRett database(Bebbington et al., 2008). The study demonstrated that p.R270X and p.R255X *MECP2* mutations were associated with a more severe phenotype and p.R133C and p.R294X with a milder phenotype. At the time of publication there was no other known RTT study which had used an international dataset as large to examine specific genotype and phenotype relationships. While InterRett is a large dataset, it does contain data from diverse sources and is not population-based.

This study aimed to:

1. examine the strengths and limitations of InterRett in relation to other registers, databases, or studies involving approximately 30 or more cases.
2. to determine whether the socio-demographic, clinical and genetic characteristics of InterRett subjects are congruent with those of a population-based study (the Australian Rett syndrome database – ARSD).

Methods

a) Literature Review

A review of the literature identified studies published in English speaking journals investigating Rett syndrome genotypes and/or phenotypes. Articles published between 2000 and 2008 (inclusive) were selected from a Medline search using the search terms “Rett syndrome” and “phenotype” or “genotype”. A broad search of RTT population based studies and case series was conducted for studies before 2000.

Parameters such as sample size, data source and case validation method were then compared. Estimated RTT cases per year were determined for studies that appeared to collect cases from a specific catchment area by dividing the catchment area population by 1000, multiplying by the birth rate and then multiplying the reciprocal of the sex birth-ratio plus one. Population data were extracted from the CIA world fact book (The Central Intelligence Agency, 2008). The catchment specific RTT birth estimate was then divided by the RTT incidence in Australia as the ARSD is the only current RTT population based study.

b) Comparison of socio-demographic, clinical and genetic characteristics of InterRett and ARSD subjects

Data source

Participants: Data on a total of 1114 cases (12 of whom were deceased) on whom information had been submitted to InterRett from 2003 to June 2007, were available for analysis. Data from 312 validated ARSD [3] cases, born after 1976, including 39 deceased, were available for comparison. InterRett and ARSD cases were mutually exclusive.

Data management: Case status was defined using a uniform verification system applicable to both databases (Fig 1). This was devised to account for the differing data content provided by the various sources. Data could be available for both InterRett and ARSD participants from a questionnaire completed by a family member (FQ only), clinician (CQ only) or both (FQ & CQ). Included amongst the InterRett participants were the Spanish (n=314) and French (n=232) cases (Table 2). Participants with a clinician's diagnosis were coded as clinically definite, those without a clinician's diagnosis but who met Hagberg's revised criteria (B. Hagberg, Hanefeld, Percy, & Skjeldal, 2002) were classified as phenotypically definite and those with insufficient information to meet either of these criteria but with a known *MECP2* mutation were classified as genotypically definite. Subjects who met any of these three classifications were regarded as verified RTT cases for this study (Fig 1). Socio-demographic, clinical (level of severity) and genetic (genetic testing and results) variables were selected for analysis (Table 2).

Variables relating to the socio-demographic characteristics of a subject and/or their family could only be assessed from family questionnaires. Follow-up questionnaires provided additional information on parents' occupation, employment and/or birth order (2000, 2002 and 2004) that were not provided in original ARSD family questionnaires (Table 2). Variables relating to clinical characteristics could be assessed from both family and/or clinician questionnaires. Genetic testing and results were assessed from both family and clinician questionnaires (table 2).

Data coding

Socio-demographic variables: Education was categorised using mothers' and fathers' highest educational qualifications [20]. An additional category, “Some Schooling” was created to account for parents who had completed some high school. The Australian and

New Zealand Standard for coding of Occupation (ANZSCO) (Australian Bureau of Statistics, 2006) was used to code most recent occupation type.

Clinical severity: For clinical severity a modified version of the Pineda scale, first defined by the Spanish group (Monros et al., 2001) and later used to assess Australian (Colvin et al., 2004) and international (Bebbington et al., 2008) data, was used. Items within the Pineda scale assess phenotype severity using mainly developmental characteristics including onset of hand stereotypies and age milestones for crawling, sitting and walking. A maximum score of 31 is possible from the assessment of 10 items within the Pineda scale with higher score indicating greater severity.

Mutation type: *MECP2* mutation type was coded as follows; the eight most common missense and nonsense *MECP2* mutations (p.R106W, p.R133C, p.T158M, p.R168X, p.R255C, p.R270X, p.R294X and p.R306C), large deletions and other (e.g. frameshift).

Statistical Analysis: STATA version 9.2 (StataCorp, 2005) was used for analysis. Continuous data were compared between the ARSD and InterRett using an independent sample t-test and categorical information was assessed by Chi squared tests. Multinomial logistic regression reporting relative risk ratios (RRR) was used to adjust for confounders, specifically mother's age at questionnaire completion. Analysis was restricted to subjects born within and after 1976.

Results

Twenty four studies using case series, 5 studies using population based data and 1 *MECP2* mutation database met the inclusion criteria for the literature search (Table 3). Of the studies 1 (3.3 %) provided only phenotypic information, 7 only genotypic information (23.3%) and 21 (70.0%) both phenotypic and genotypic information. Only for the population-based studies was there sufficient information to complete all the parameters with information generally poorly provided on case source and country of origin of participants. Only 2 parameters (18.2%) could be completed for the *MECP2* mutation database (“RettBASE: IRSA MECP2 Variation Database”). This was largely attributed to the difficulty of determining the exact number of RTT cases as there appeared to be no mechanism in place to identify duplicated cases and the variable nomenclature used to describe the same mutation.

Using the number of potential RTT cases within a defined catchment area as an estimate of RTT cases in the study, only the Texan (Kozinetz et al., 1993), Australian (Colvin et al., 2004) and French (Bienvenu et al., 2006) studies could be considered representative of their underlying population of cases. Of the remaining studies where sufficient information was provided, case numbers were all substantially less than would be expected based on the estimated RTT cases born per year. For example Huppke et al (Huppke, Held, Hanefeld, Engel, & Laccone, 2002) reported on 123 participants, approximately only the number that would be expected to be born in Germany over three years, although it was clear that the age range would have been much greater. Moreover age distribution of cases wasn't available for many of the studies.

Overall, the North American study by Percy et al. (2007) contained the most participants (n=1928), InterRett the most culturally diverse subjects (with greater than six countries represented within the sample), and the most common data source was from family and clinicians combined (33.3%). Of the severity scales used the four main types of scales were Percy (also known as the Clinical Severity Score(Neul et al., 2008); used by 20.3 %), Kerr (25 %), Pineda (20.5%), British Isles Rett Syndrome questionnaire (8.3 %) while Leonard et

al (Leonard et al., 2005), Smeets et al (Smeets et al., 2003), Hoffbuhr et al (Hoffbuhr et al., 2001), Huppke et al (Huppke, Held, Hanefeld, Engel, & Laccone, 2002), Cheadle et al (Cheadle et al., 2000) and Chae et al (Chae, Hwang, Hwang, Cheong, & Kim, 2004) also used their own specific scales.

Only 21 (70.0 %) of the 30 identified studies investigated phenotype and genotype associations. Overall there was general agreement that pRI33C and C-terminal deletions were associated with milder RTT phenotypes (Bebbington et al., 2008; Charman et al., 2005; Colvin et al., 2004; Hoffbuhr et al., 2001; Huppke, Held, Hanefeld, Engel, & Laccone, 2002; Neul et al., 2008)and that p.R270X, p.R168X and p.R255X mutations were associated with more severe RTT phenotypes (Bebbington et al., 2008; Colvin et al., 2004; Kerr & Prescott, 2005; Neul et al., 2008; Smeets et al., 2003). Inconsistent findings were reported on the influence of late truncating mutations, missense and nonsense mutations. An early study (Cheadle et al., 2000) reported that late truncating mutations were associated with a more severe RTT phenotype, but this was refuted in two later studies (Charman et al., 2005; Nectoux et al., 2008) which found that late truncating mutations were associated with milder RTT phenotypes. The same early study (Cheadle et al., 2000) reported that missense mutations were associated with a more severe phenotype while four subsequent studies (Chae, Hwang, Hwang, Cheong, & Kim, 2004; Monros et al., 2001; Schanen et al., 2004; Smeets et al., 2003) reported the association of missense mutations with a less severe phenotype. In addition, the sample sizes reported generally increased over time.

Participants

Of the 1114 individuals contacted by InterRett, 1004 (90.1%) could be verified as RTT cases by the study protocol (Figure 1). Of the 1004 InterRett participants, 935 were born within and after 1976. Five hundred and forty nine cases (58.7%) were validated by a clinician's diagnosis (clinically definite), a further 369 cases (39.5%) were categorised by the revised criteria (phenotypically definite) and 17 (1.8%) were verified only by the presence of a pathogenic *MECP2* mutation (genotypically definite). Geographically the majority of InterRett cases originated from Southern Europe (29.3%), Western Europe (22.4%) and Northern America (20.9%).

Of the 312 cases in the ARSD, 295 (94.6%) could be verified by the study protocol. One hundred and ninety cases (64.4%) were categorised as clinically definite by a clinician's diagnosis, a further 92 cases (31.2%) by the revised criteria only (phenotypically definite) and 13 (4.4%) only by the presence of a pathogenic *MECP2* mutation (genotypically definite).

Genetic and Clinical Characteristics

As seen in Table 4 InterRett participants (born 1976 and subsequently) were older at time of questionnaire completion ($p<0.001$) but similar at age of diagnosis ($p=0.697$), compared with ARSD participants.

Despite being older InterRett participants had marginally lower severity than ARSD participants on the modified Pineda scale ($p=0.043$) (Table 5 and Fig 2). The distribution of mutations in the 2 groups was similar ($p=0.385$) with the most common 8 mutations accounting for 64.1% of the InterRett and 64.4% of the ARSD mutations. Of the 8 most common mutations p.T158M (12.4%) was the most frequently occurring mutation in InterRett and p.R168X (11.2%) in the ARSD. In both InterRett (3.5%) and the ARSD (3.9%) p.R106W was the least common of this group (Table 5).

Socio-demographic Characteristics of Subjects and Families

There were differences in the socio-demographic characteristics of InterRett and ARSD subjects and families available for analysis (Tables 4 and 6). InterRett mothers were on average, about 2 years older at subject's birth than ARSD mothers ($p<0.001$) (Table 4). InterRett mothers were more likely to have fewer children after having a child with RTT than those in the ARSD ($p=0.027$). InterRett participants were more likely to be an only (16.5%) or youngest child (44.0%) compared with ARSD participants (10.7% and 34.9% respectively).

Both mothers ($p<0.001$) and fathers ($p<0.001$) of participants in InterRett were more highly educated than those of the ARSD. After adjustment for maternal age, mothers of children in InterRett were over 7 times as likely (RRR=7.49 [CI: 3.83, 14.65]) to have school accreditation; more than 3 times as likely (RRR=3.29 [CI: 1.69, 6.43]) to have a vocational education and 12 times as likely (RRR=12.34 [CI: 6.49, 23.44) to have a higher education, compared to mothers of cases in the ARSD (Table 6). Of the 229 InterRett fathers for whom this information was available, 115 (50.2%) had a higher educational qualification compared with 16.7 % (41/245) of ARSD fathers (Table 6). InterRett mothers ($p<0.001$) and fathers ($p<0.001$) were also more likely to have occupations requiring a higher skill level (Table 6).

Discussion

Much research has attempted to investigate genotype and phenotype relationships with small studies, which consequently have been insufficiently powered to detect the real differences. Therefore findings have at times been conflicting (Charman et al., 2005; Cheadle et al., 2000; Nectoux et al., 2008). Often investigators, particularly in early studies, have attempted to group mutations to increase power. However, in the grouping, mutations with directly opposing characteristics (such as p.R294X and p.R270X, both nonsense mutations) may have been combined, thus nullifying effects. Our results clearly demonstrate that with time and larger case numbers, which include international collaborations, the true relationship between genotype and phenotype is gradually defined better. Furthermore international collaboration is also needed to investigate and draw conclusions about less frequently occurring clinical characteristics of RTT such as gall bladder disease where national studies may also be inadequately powered.

X inactivation has been demonstrated to influence the variability of RTT phenotypes (Archer et al., 2007) but it is not known whether this effect might vary by mutation. Such an analysis would require very large sample sizes. Only a minority of projects included X inactivation data on all (Archer et al., 2007) or some (Hoffbuhr et al., 2001; Leonard et al., 2003; Nectoux et al., 2008; Neul et al., 2008) of the data. In most countries these studies, which also require parental samples, are not included as part of routine testing. Therefore their inclusion would require special sample collection and specific funding and is not appropriate for InterRett which generally does not instigate genetic investigations but instead takes advantage of the power of combining together existing data from different sources.

Early studies investigating genotype phenotype relationships in RTT were generally based on small numbers (Hoffbuhr et al., 2001; Monros et al., 2001; Nicolao et al., 2001) and the appropriateness of generalising results were not considered. However for a study to be valid it must be possible to use its findings to make inferences beyond the study sample. The representativeness of the study sample and the nature of the population, from which it is drawn are two important characteristics that need to be taken into account when considering study validity. To provide such a population the ARSD has been maintained and nurtured over the past 15 years and in any study using these data we have always strived to relate the

study participants back to the whole population in terms of their representativeness. However this is the first time the characteristics of study subjects in another Rett syndrome research sample, in this case InterRett, have been compared with those of the ARSD population-based cohort.

We found that the greatest differences between the InterRett sample and ARSD related to socio-demographic characteristics, with InterRett participants more likely to be an only or youngest child with older, more highly educated and more highly skilled parents. The question is whether the preferential inclusion of a more educated group of parents who directly report data to InterRett will result in data which are biased in some way. It is possible that higher parental socio-economic status may influence children's access to treatment and thus the InterRett group could be more advantaged with respect to some aspects of their health status (Halldorsson, Kunst, Kohler, & Mackenbach, 2002; Leonard, Fyfe, Leonard, & Msall, 2001). This may account for their severity being slightly less than expected and may be a factor to take into consideration when planning future clinical trials in Rett syndrome.

In terms of mutation distribution however, the InterRett dataset was comparable to the ARSD. Although InterRett participants were marginally less severe than ARSD individuals the distribution of severity scores was similar in both groups. This is an important finding as mutation type is an increasing focus for differentiating between subjects and the link to phenotype provides important information to parents and clinicians about the likely outcomes of this disorder for the child. Knowing that mutation distribution and phenotypic severity can be generalised gives confidence in the genotype phenotype association found in the InterRett dataset and validates the findings from a phenotype-genotype study based on InterRett data (Bebbington et al., 2008).

Strong international collaborations particularly with Spain, Israel (Bebbington et al., 2008) and France have helped build the links which have led to the submission of bulk data and made a major contribution to the total number of subjects within the InterRett sample. This is the most resource efficient mechanism for data inclusion but is dependent on the strength, trust and good will of the collaborative relationship and the capacity to ensure that data submitters are offered the opportunity to be involved in subsequent research output. Data that are submitted in bulk are also much more likely to be representative of the underlying population than individually-provided data as they are more likely to be systematically collected from this population. Therefore, they impact positively on the total representativeness of the InterRett dataset especially when, as they do, they comprise a large proportion (66.3%) of the total sample.

The ability to use different sources of data generally expands, the quality of data available for research and the number of RTT cases (Kozinetz et al., 1995). The submission of data from two independent sources (family and clinician) ensures that data within InterRett are reflective of a child's optimal functioning and not limited by their performance in a clinical assessment at a specific point in time. In addition by collecting data from two sources InterRett is able to ensure that families of RTT cases, where clinician data are not available, can still participate and provide valuable information to the study.

InterRett collects data from different sources around the world and in some cases may be dependent on receiving the data in the format which it has been originally collected which, as with the Spanish data, differs from the formats InterRett uses for data collection. This might be considered a weakness but by building a specific database compatible with the format of the Spanish data we have been able to overcome this as a problem. In other instances, as with the French data, certain questions were omitted during the translation

process. Therefore, data sources vary with respect to the amount of data available for analysis. The issue of missing data is however a common but often unrecognised problem.

Recruitment will continue to increase as the Internet becomes more accessible. Some InterRett families and clinicians chose to submit questionnaires online, a unique feature which has contributed to the large number of countries, 32, represented within the InterRett sample. Whilst the ability to submit questionnaires online is limited by the availability and access to Internet services, internet usage worldwide has increased to nearly 1.5 billion users as of March 2008 (“Internet World Stats: Usage and Population Statistics”) and is increasing daily. It is anticipated that with increasing sample size and greater accessibility to the Internet that the InterRett sample will become more representative in terms of the socio-demographic characteristics investigated in this study.

We have shown that with few exceptions most studies investigating genotype phenotype relationships in Rett syndrome have not been able to demonstrate how their data relate to the underlying population and could have poor external validity. In comparison to our work with the population-based ARSD we had previously been concerned that, although InterRett is a large and statistically powerful dataset, the data might not allow us to generalise our findings, especially in making genotype-phenotype comparisons. However our review of literature in this area has reassured us that very few other studies have been able to take this factor into account. Moreover the results from the comparison with the ARSD give us confidence that conclusions drawn from these international data are comparable to those from a population cohort, especially as the distribution of common mutations is much the same as in the Australian RTT population.

Therefore we would conclude that data analyses using InterRett are no more likely to be biased than any other non-population-based studies.

Furthermore, InterRett with its ongoing recruiting strategies targeting individual families and its linkage with a number of clinical centres throughout the world has now accrued one of the largest collections of phenotype and genotype data on Rett syndrome. Providing a dataset with the power to identify small but real phenotypic differences based on genotype is an important evolution in the epidemiology of Rett syndrome. Using InterRett data we have already replicated genotype and phenotype associations evident within a national population based cohort (ARSD) (Bebbington et al., 2008). This therefore ratifies the value of collecting large amounts of data from disparate sources as a means of increasing understanding of clinical expression in rare disorders. The development of a mechanism for both families and clinicians from across the globe to submit data both on individual cases and in bulk has strengthened research capacity for collaborative worldwide studies and provides a tested model for the investigation of this and other rare disorders.

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Abbreviations

RTT	Rett syndrome
MECP2	Methyl CpG binding protein 2
ARSD	Australian Rett syndrome database

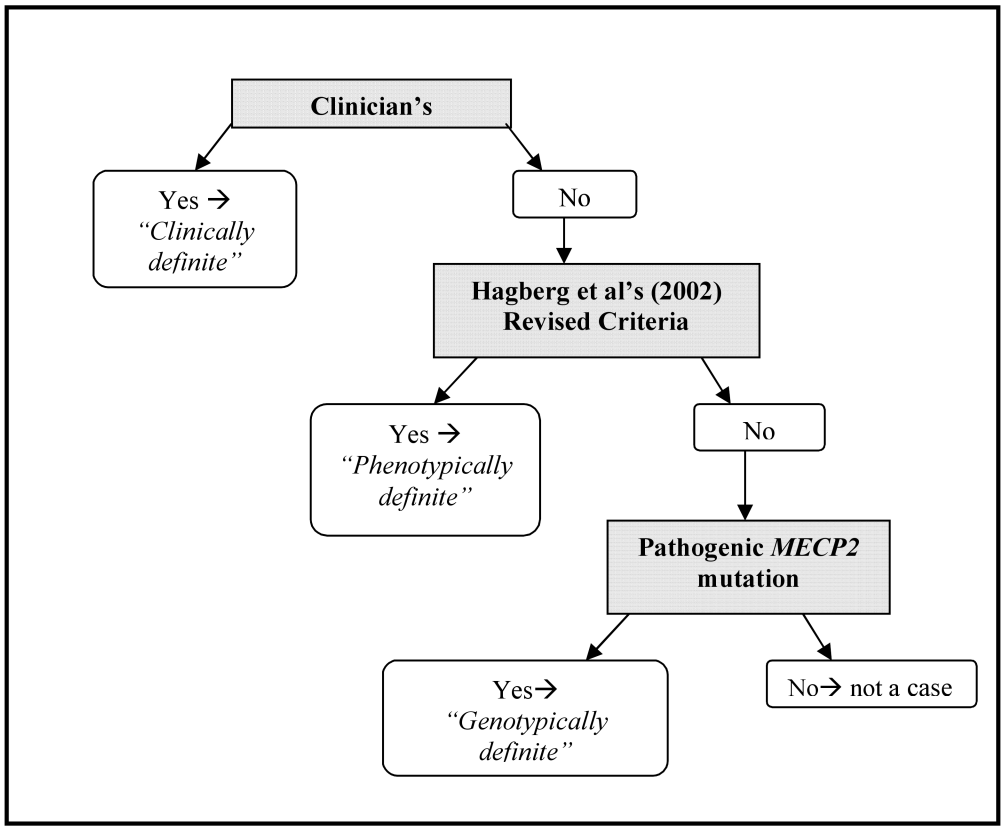

Figure 1. Flow diagram of methodology used to verify a subject's cases status

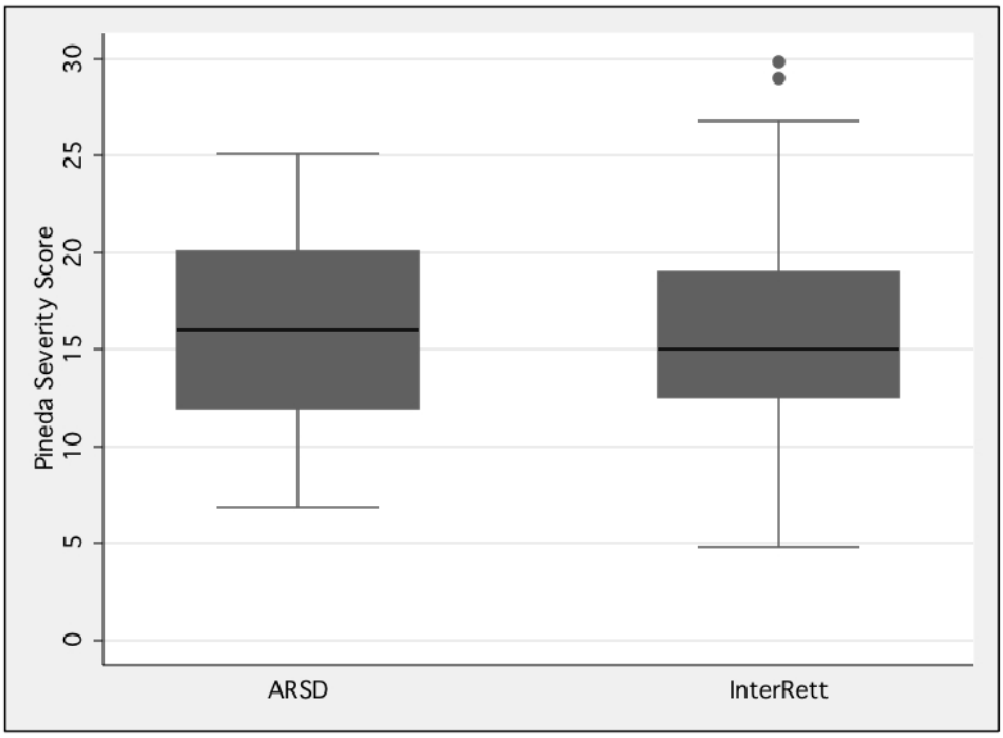

Figure 2. The distribution of ARSD and InterRett severity scores using a modified version of the Pineda severity score among *MECP2* mutation positive subjects

Table 1
Mechanism used to collect and submit bulk data to InterRett

Source	Mechanism of data collection &/or data submission	n
Spain	A FileMaker database was provided to clinician A Clinician A entered cases based on own protocol.	318
Israel	Clinician B extracted data from clinical records and entered in online questionnaire	74
	OR Physiotherapist X translated questionnaires into Hebrew and administered to families. Questionnaires then entered online in English.	13
Canada	Data from clinical records of clinician C patients extracted and entered data online.	71
China	Clinician D translated family questionnaires into English. Questionnaires administered families attending D's clinic. Responses translated back into English and then returned to InterRett in paper form.	31
France	Clinician E adapted family questionnaire and added new questions to E's area of interest. Questionnaires administered through a family association. Responses returned to InterRett in form of de identified excel spreadsheet.	232
Total		739

Table 2

Percentage of variables for which data where provided from different data sources

		ARSD (n=295)	InterRett (n=1004)				
			FQ (n=160)	CQ (n=132)	FQ & CQ (n=188)	French (n=213)	Spanish (n=311)
SOCIO-DEMOGRAPHIC	**Age at Diagnosis**	89.8	95.0	NA	98.4	89.2	99.0
	Subject's age at questionnair e completion	95.3	100.0	91.7	100.0	98.6	98.1
	Mother's Age at Subject's Birth	93.6	96.3	NA	89.9	92.0	NA
	Mother's Occupation	99.7	97.5	NA	100.0	97.2	NA
	Father's Occupation	99.7	97.5	NA	100.0	98.1	NA
	Mother's Education	90.8	97.5	NA	93.6	NA	NA
	Father's Education	83.1	96.3	NA	91.5	NA	NA
	Birth Order	72.9	53.8	NA	60.1	98.1	NA
GENETIC	***MECP2* test performed**	98.3	94.4	97.0	100.0	99.1	96.8
	Results Of *MECP2* test	99.2+	73.4+	98.4+	77.3+	59.9+	99.0+
	***MECP2* mutation identified**	100.0*	100.0*	100.0*	100.0*	100.0*	100.0*
CLINICAL	**Severity**	80.0^	62.4^	31.3^	96.0^	NA	94.2^

+ Results known given a family and clinician indicated MeCP2 test was performed

* Percentage calculated given that family/ clinician indicated yes results form MeCP2 test was performed

^ Percentage based on MeCP2 positive subjects only

NA -Data not collected

Table 3

Summary of articles, concerning RTT phenotypes and/or genotypes identified from literature search

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
Population-based studies											
2008	Downs et al	240 (2-29 years) in 2004	Australia (83% of cases in ARSD)	14	ARSD (Australian Paediatric Surveillance unit and Rett Syndrome Association of Australia)	Hagberg 2002	Information on fractures (234, 98.3%)	215 (91.9%), 76.3%	Family and/or clinicians	Fractures	Fracture risk was increased specifically in cases with p.R270X mutations and in cases with p.R168X mutations.
2007	Jian et al.	162 (2-25 years) in 2000	Australia (81% of live cases in ARSD)	14	ARSD (Australian Paediatric Surveillance unit and Rett Syndrome Association of Australia)	Hagberg 2002	Information on seizure frequency in 2000 (162,100%)	154 (95.1%), 72.6%	Family and/or clinicians	Seizure rate	Seizure rates lower in those with p.R294X, p.R255X mutations and C terminal mutations.
2006	Jian et al	288 (2-29 years) in 2004	Australia	14	ARSD (Australian Paediatric Surveillance unit and Rett Syndrome Association of Australia)	Hagberg 2002	Information on onset of seizures (275, 95.5%)	254 (88.2%), 73.2%	Family and/or clinicians	Onset of seizures	p.R168X mutation conferred a protective effect for seizure onset within the first 4 years, as did p.R294X and C-terminal mutations
2004	Colvin et al	247 (2-25 years)	Australia	14	ARSD (Australian Paediatric Surveillance unit and Rett Syndrome Association of Australia)	Trevathan 1988	295, 100%	196 (79.3 %), 66%	Family and/or clinicians	Pineda, Percy and Kerr severity scales	Mutations in NLS of TRS associated with more severe phenotype p.R133C associated with milder

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
											phenotypes while p.R270X associated with most severe phenotypes
1993	Kozinetz et al	103 (2-18 years)	Texas, USA	17	Health and education agencies, services and facilities	Trevathan 1988	103,100%	Not available, study prior to *MECP2* testing	Physician or non-physician based	NA	NA
Case series											
2008	Neul et al	245	USA		Blue Bird Circle Rett Centre at Texas Children's Hospital or UAB Rett Centre between 1990-2004	Hagberg 2002	245, 100%	245 (100%), 96.3%	Clinical evaluation and medical history	Clinical Severity Score	p.R168X associated with a more severe phenotype than p.R294X and p.R133C mutations p.R168X less likely to walk, use hands or use words
2008	Nectoux et al	81 (5-50 years)	France	47	Not specified	Trevathan 1988	81,100%	81 (100%), 93.8%	Clinical examination (n=70) and questionnaires (n=11)	Kerr	Missense mutations associated with more severe epileptic phenotypes and Nonsense and late truncating mutation associated with less severe phenotype regarding walking

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
2008	Bebbington et al	346 (2-45years)	Spain, France, Israel, USA, UK and other		Parent listserv Rettnet, advertisemt in newsletters of parent support associations and presentation s at meetings as well as bulk data (see Table 1)	Pathogenic MECP2 mutation	346, 100%	346 (100%), 100%	Family and/or clinician	Modified Pineda Percy and Kerr severity scale.	Overall p.R270X and p.R255X associated with most severe phenotype. p.R133C and p.R294X mutations were associated with the least severe phenotypes.
2007	Zahorkova et al	87	Czech & Slovak Republics & Ukraine	Czech:5 Slovakia3 Ukraine 25	Neurologica l, genetic and paediatric departments	Trevathan 1988	Not specified	87 (100%), 78.2%	Not specified	Not assessed	NA
2007	Archer et al[+]	83	Australia UK		ARSD, Cardiff Rett & University of Glasgow Rett studies	Hagberg 2002	83,100%	83 (100%), 100%	Family and/or clinicians	Kerr and Pineda scales	Statistically significant reduction in clinical severity for the two mutations (p.T158M and p.R168X) associated with skewed X inactivation
2007	Percy et al	1928	USA and Canada	USA:251 Canada:2 0	IRSA members USA and Canada	Clinical diagnosis	Not collected	1165 (60.4%), 78.5%	Data provided by family indicating, dob, diagnosis, mutation testing and testing results	Not assessed	N/A
2007	Scala et al [44]	77 (~2-33years)	Italy	28	Italian RTT database and biobank	Hagberg 2002	64, 83.1%	77 (100%), 23.4%	Not specified	Modified version of Kerr score	Large deletions associate with classic RTT phenotype

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
2007	Li et al	121 (oldest: 24 years)	Chinese	1017	Not specified	Hagberg 2002	Not Specified	121 (100%), 84.3%.	Not specified	Not assessed	N/A
2007	Sampieri et al	219	Italy	28	Medical Genetics Unit of the University Hospital of Siena	Hagberg 2002	Not collected	187 (100%), 74.9%	Genetic test results	Not assessed	N/A
2006	Bienvenu et al	424 (4-15 years)	France	47	Association Francaise du Syndrome Rett	*MECP2* mutation testing	Not collected	424 (100%), 100%	Eight French genetic laboratories	Clinical severity not assessed	NA
2005	Jian et al [+]	524 n=174 (2-29) n=350 (1-54)	Australia UK		ARSD, BIRS and Cardiff study	Trevathan 1988 *MECP2* mutation testing	Not applicable to this study-mortality study	524 (100%), 100%	Family and/or clinicians	Clinical severity not assessed	Mortality was significantly increased in those with p.R270X compared with other mutations
2005	Leonard et al.[+]	235 (2-27 years) 85 (3-41 years)	Australia China, UK & US		ARSD InterRett	Trevathan 1988 Reported clinical diagnosis or *MECP2* mutation testing	320,100%	263 (82%), 68.8%	Family and/or clinicians	Scoring system developed to assess characteri stics in perinatal period and early developm ent	Infants with p.R294X and p.R133C (PZ0.03) were less likely than those with p.R255X to have problems in the perinatal period. The most severe profile overall for early development was associated with mutations p.R255X and p.R270X.

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
2005	Kerr & Prescott	886	UK	UK: 37	British Paediatric Surveillance and pre existing cohort data from 1982	Clinical examination	834, 94.1%	440 (49.7%), 100%	Clinical examination s, reports and postal questionnair es	British Isles scoring system	p.T158M, p.R255X and p.R168X associated with more severe phenotype and p.R306C and p.R133C associated with less severe phenotype.
2004	Schanen et al	85	Not Specified		By referral to study or through contact in clinical setting	52/85 clinical examination	85, 100%	85 (100%), 100%	Medical records, parent report and physical examination	Modified scale by Monros and Amir	Missense mutations associated with less severe phenotypes. No difference in the severity of subjects with mutation located in MBD or TRD.
2003	Smeets et al	30 (women born between 1941 to 1987)	Not specified		Patients seen by first author	Clinically diagnosed	30, 100%	30 (100%) , 80%	Medical examination	2 scoring systems Kerr 2001 & a simplified scoring system devised for study	Generally missense mutations associated with milder phenotypes when compared to early truncating mutations Most severe mutations p.R255X and p.R298X.
2003	Chae et al	42	Korea	27	Not specified	Trevathan 1988	42,100%	42 (100%), 71.4%	Clinical examination	Based on hand and motor performan ce an	Nonsense mutations

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
										language and skill at 5 years	associated with more severe language retardation and earlier regression than missense mutations
2002	Huppke et al	123	Germany	38	Not specified	Clinical diagnosis	123,100%,	123 (100%), 95.1%	Clinical data	Own protocol	C deletion group associated with lower severity. Mutations within the truncating NLS associated with higher severity when compared to other truncating mutations
2001	Hoffbuhr et al	116 (2-34 years)	USA		Rett syndrome clinic at Kennedy Krieger Institute	Clinical examination	104, 89.7%	116 (100%), 62.9%	Clinical examination and telephone interviews OR review of medical history	Severity based on 5 clinical features	Overall c-terminal and nonsense mutations in TRD associated with less sever phenotype.
2001	Giunti et al	75	Italy	28	Rett syndrome research project	Clinical Evaluation	Not specified	75 (100%), 85.3%	DNA bank of Genetics and Molecular Medicine Unit, Dept of Paediatrics, Uni of Florence	NA	N/A
2001	Vacca et al	62	UK and Italy		Not specified	Trevathan 1988	Not specified	62 (100%), 69.4%	Not specified	Severity not measured	N/A
2001	Monros et al	46 +1 male	Spain	23	Not specified	Trevathan 1988	47,100%	46 (100%), 100%	Not specified	Pineda severity scale	Truncating mutations associated with more severe phenotype

Publication year	Author	N (age distribution in years if available)	Origin of Subjects	Estimate d cases per birth year	Source of cases	Case validation method	Phenotype data (n, % complete data)	Genotype data (n (%), % *MECP2* positive)	Data source	Disease severity method	Results of genotype and phenotype associations
											and missense mutations with less severe phenotypes. Large deletions identified in subjects with both.
2000	Cheadle et al	60	Not specified	N/A	UK Rett syndrome Association Genetic bank and samples taken from combined metabolic and genetic studies	Adaptations of Trevathan 1988 and Hagberg 1993	Not specified	60 (100%), 75%	Not specified	Derived from assessing hand use, speech and walking	Missense mutations and late truncating mutations associated with more severe phenotypes compared to truncating and early mutations respectively.
2000	Amir et al	78	Texas		Blue Bird Circle Rett Centre	Hagberg 1995	48,61.5%	78 (100%), 71.8%	Most recent Clinical profile	Clinical Severity Score	Scoliosis associated with missense mutations
***MECP2* mutation database**											
2008 (last updated)	http://mecp2.chw.edu.au/	3393[1]	Not Specified		Merging published and unpublished mutation and Polymorphi sm data	Not specified	Not specified	Not specified	Published and unpublished data from international collaborators	Not assessed	NA

Exact number of cases could not be determined as RTT cases from published papers were not identified.

[+]Studies include population data and are also supplemented by cases series of another country of origin

Table 4

Continuous Socio-demographic Characteristics of InterRett Subjects and Families compared with the ARSD

		ARSD	InterRett (complete)	InterRett (≥1976)
Mother's age at subject's birth	n	276	519	479
	Mean ± Std dev	28.04±5.49	29.76±5.26	30.03±5.23
	95%CI	(27.39,28.69)	(29.30,30.21)	(29.56,30.50)
	p value		<0.001[a]	<0.001[a]
Age of subject at questionnaire completion	n	281	987	922
	Mean ± Std dev	7.92±5.01	12.17±8.77	10.62±6.64
	95%CI	(7.33,8.51)	(11.62,12.71)	(10.19,11.05)
	p value		<0.001[a]	<0.001[a]
Age of subject at diagnosis	n	265	838	790
	Mean ± Std dev	4.88±3.47	5.90±5.70	4.97±3.88
	95%CI	(4.46,5.30)	(5.51,6.29)	(4.70,5.24)
	Median	3.83	4	3.5
	p value		<0.001[a]	0.697[a]

[a] when compared to the ARSD

Table 5
Clinical and Genetic characteristics of InterRett Subjects compared with the ARSD

		ARSD	InterRett (complete)	InterRett (≥1976)
Clinical severity	n	164	372	354
	Median	16	15	15
	p value		0.039[a]	0.043[a]
***MECP2* Genetic test performed**	n	290	982	915
	Yes (%)	90.3	91.2	91.4
	No (%)	9.7	8.8	8.6
	Overall χ^2 p value		0.638[a]	0.594[a]
Results of *MECP2* test	n	260	922	723
	Negative (%)	21.2	22.9	23.6
	Positive (%)	78.8	77.1	76.4
	Overall χ^2 p value		0.560[a]	0.412[a]
Types of *MECP2* mutations present	n	205	596	552
	R106W (%)	3.9	3.5	3.4
	R133C (%)	7.8	6.4	6.3
	R168X (%)	11.2	10.1	10.5
	R255X (%)	7.3	11.1	11.8
	T158M (%)	10.7	12.4	12.3
	R306C (%)	6.8	7.1	6.2
	R294X (%)	8.8	5.7	5.6
	R270X (%)	8.8	7.7	8.0
	Large Del (%)	6.3	3.4	3.6
	Other (%)	29.3	32.7	32.3
	Overall χ^2 p value		0.358[a]	0.385[a]

[a] when compared to the ARSD

Table 6

Categorical Socio-demographic Characteristics of InterRett Subjects and Families compared with the ARSD

		ARSD	InterRett (complete)	InterRett (≥1976)	RRR	95%CI
Birth Order	n	215	408	370		
	Only (%)	10.7	16.5	16.4	1.89*	(1.02, 3.48)
	1st (%)	33.0	25.8	24.6	0.93	(0.59, 1.47)
	2nd (%)	15.4	10.1	10.2	0.74	(0.41, 1.35)
	3rd (%)	4.6	2.8	2.8	0.52	(0.18, 1.45)
	≥4th (%)	1.4	1.0	1.0	0.42	(0.07, 2.68)
	Youngest (%)	34.9	43.8	45.0		
	Overall χ^2 p value		0.041[a]	0.027 [a]		
Mother's Education	n	268	332	299		
	Some High School (%)	36.5	6.8	6.5		
	School Accreditation (%)	17.2	26.3	24.4	7.49*	(3.83, 14.65)
	Vocational Education and Training (%)	28.0	18.3	18.7	3.29*	(1.69, 6.43)
	Higher Education (%)	18.3	48.6	50.4	12.34*	(6.49, 23.44)
	Overall χ^2 p value		<0.001 [a]	<0.001[a]		
Father's Education+	n	245	326	295		
	Some High School (%)	21.6	9.6	9.6		
	School Accreditation (%)	18.4	21.8	21.4		
	Vocational Education and Training (%)	43.3	19.4	18.8		
	Higher Education (%)	16.7	49.2	50.2		
	Overall χ^2 p value		<0.001 [a]	<0.001[a]		
Mother's Occupation	n	294	551	505		
	Managers (%)	5.8	18.2	18.2	15.65*	(3.87, 63.36)
	Professional (%)	14.6	25.4	25.4	9.69*	(2.55, 36.85)

		ARSD	InterRett (complete)	InterRett (≥1976)	RRR	95%CI
	Trade & Technician (%)	5.4	8.9	8.7	8.54*	(2.07, 35.30)
	Community & Personal Service (%)	12.3	10.5	10.9	4.89*	(1.25, 19.07)
	Clerical & Admin (%)	19.4	7.8	8.1	2.79	(0.72, 10.80)
	Sales (%)	7.8	3.4	3.2	3.97	(0.94, 16.71)
	Machine Operator & Driver (%)	0.0	0.4	0.2	0	-
	Labourers (%)	5.8	0.5	0.6		
	Miscellaneous (%)	28.9	24.9	24.7		
	Overall χ^2 p value		<0.001 [a]	<0.001[a]	N/A	
Father's Occupation[+]	n	295	553	506		
	Managers (%)	18.0	16.8	16.8		
	Professional (%)	13.6	34.2	34.2		
	Trade & Technician (%)	23.1	14.5	14.0		
	Community & Personal Service (%)	4.4	6.2	6.7		
	Clerical & Admin (%)	1.4	6.5	6.9		
	Sales (%)	3.4	3.3	3.2		
	Machine Operator & Driver (%)	8.2	4.3	4.4		
	Labourers (%)	10.2	1.4	1.4		
	Miscellaneous (%)	17.7	12.8	12.4		
	Overall χ^2 p value		<0.001 [a]	<0.001[a]		

[+] Multinomial logistic regression was not performed

[a] when compared to the ARSD

* RRR: p<0.05

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