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Atypical Presentations and Specific Genotypes are Associated with a Delay in Diagnosis in Females with Rett Syndrome

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Abstract

There is often delay between onset of Rett syndrome symptoms and its diagnosis, possibly related to symptom presentation or socio-demographic factors. We hypothesized that girls with an atypical presentation or whose family had a lower socio-economic status would receive a later diagnosis. Female subjects with a confirmed diagnosis of Rett syndrome were sourced from the Australian Rett Syndrome and InterRett Databases. Variables analyzed included timing and development of symptoms; *MECP2* mutation type; parental occupation and education; maternal age and birth-order. Residential location and socio-economic status were also analyzed for the Australian cases. Linear regression was used to determine relationships between these factors and age at diagnosis. A total of 909 cases were included. An older age of diagnosis was associated with later loss of hand function and speech, later onset of hand stereotypies and the presence of the p.R133C or p.R294X *MECP2* mutation. Socio-economic factors did not predict age of diagnosis for Australian families. For families participating in the InterRett database, a younger age of diagnosis was associated with higher levels of parental education or occupation. A clinical picture consistent with the classic presentation of Rett syndrome is associated with an earlier diagnosis. Clinicians need to be alerted to the variable presentation of Rett syndrome including the milder phenotypes of cases with the p.R133C or p.R294X mutation. Educational resources to assist this understanding including guidance on when to request genetic testing could be useful to streamline the process of diagnosis in Rett syndrome.

Keywords

Rett syndrome; age at diagnosis; socio-economic status; symptom presentation; *MECP2*

INTRODUCTION

Rett syndrome (RTT) is a severe neurodevelopment disorder mainly affecting females and associated with mutations in the *Methyl-CpG Binding Protein 2 (MECP2)* gene [Amir et al., 1999]. Core symptoms include loss of hand and speech skills, development of hand stereotypies and gait disturbances [Hagberg et al., 1983]. Although such deviations in development and behavioral changes usually occur within the first 6–18 months of life [Leonard and Bower, 1998; Naidu et al., 1986; Witt-Engerström, 1990, 1992], they are not necessarily predictive of RTT. In an Australian population-based study published in 2006,

the mean age of diagnosis was 5.3 years [Laurvick et al., 2006] suggesting that often several years may pass between the onset of Rett-related symptoms and the time at which a diagnosis of RTT is made.

More than two decades ago, Witt-Engerström [1987] investigated the early symptoms of RTT. Using medical and family reported data of the youngest ten girls from the Swedish RTT cohort, symptoms of gross motor delay prior to 15 months of age were nonspecific for RTT but by 15 months, symptoms such as loss of purposeful voluntary hand use were predictive of RTT. About the same time, she and Bengt Hagberg devised a staging system to illustrate the evolution of the disorder [Hagberg and Witt-Engerström, 1986] commencing with stagnation in development and progressing to loss of hand function and speech and the appearance of hand stereotypies. During this time Opitz and Lewin [1987] also reviewed the available literature on the genetics of Rett syndrome and cautioned against artificial truncation of the phenotypic spectrum of Rett syndrome leading to underestimation of the clinical variability. Through their observation of eight girls who all showed congenital hypotonia they questioned whether any girl with Rett syndrome was neurologically normal at birth. There have since been other studies examining the early developmental period in girls with RTT [Leonard and Bower, 1998; Burford et al., 2003; Einspieler et al., 2005a, b; Leonard et al., 2005]. The pattern of symptom presentation could relate to the timing of diagnosis, yet no study has investigated whether the timing or occurrence of particular symptoms has had any effect on the age of diagnosis of RTT.

Further, there is considerable and often genetically determined variability in the occurrence and range of severity of some of the features of RTT. For example, a recent study involving 346 cases from the International Rett Syndrome Phenotype Database (InterRett) found that subjects with a p.R133X or p.R294X mutation had delayed onset of regression, retention of more words and hand skills [Bebbington et al., 2008]. The relationships between specific mutation and age of diagnosis have not been investigated.

US studies have investigated the effect of the influence of socio-economic factors such as ethnicity, income level and residential location on the age of diagnosis of autism spectrum disorders [Mandell et al., 2002, 2005, 2007, 2009]. A later age of diagnosis was associated with African-American and Hispanic ethnicity, low income (below the poverty line) and living in a rural setting. The potential influences of family and socio-economic factors on age of diagnosis of RTT have not been investigated.

Using pooled data from the Australian Rett Syndrome Database (ARSD) and the InterRett database this study evaluated the relationship between presenting symptomatology and family and socio-economic factors and the age of diagnosis of RTT.

MATERIALS AND METHODS

Cases were ascertained from both the ARSD and InterRett. The ARSD was established in 1993 [Leonard, 1994; Leonard et al., 1997] and is a population-based register of Australian RTT cases born since 1976. Cases are ascertained through the Australian Paediatric Surveillance Unit [Elliott and Chant, 1994] and the parent support group, the Rett Syndrome Association of Australia. Data are collected through the submission of family and clinician questionnaires on recruitment [Laurvick et al., 2006]. Additional questionnaire data are collected every two to three years from 2000 (2000, 2002, 2004, 2006 and 2009). InterRett was established in 2003 by the Australian RTT study group [Fyfe et al., 2003]. Cases are ascertained through a variety of sources including parent support groups, the listserv Rettnet [Leonard, 2004] and also bulk submission of de-identified data by clinicians outside

Australia [Bebbington et al., 2008; Louise et al., 2009]. InterRett questionnaires are completed online by families and clinicians at one time point.

Female cases in both databases were verified as RTT by either having a pathogenic mutation or fulfilling the 2002 diagnostic criteria for RTT [Hagberg et al., 2002]. Only InterRett cases in which a family questionnaire had been completed were included and only any Australian cases born before 1976 to avoid any overlap with the ARSD cohort. For cases in the ARSD cohort, age of diagnosis was determined using the ages specified in the family and clinician questionnaires. If no age was available a surrogate age was estimated from the date of ascertainment, date of known genetic testing or through contact with the families. For InterRett cases, age of diagnosis was available from the family questionnaire only.

The age at hand function loss and speed of loss, age at appearance of stereotypies and age at loss of speech (either babble or words) were reported by parents. For those in whom a common pathogenic *MECP2* mutation had been identified, the mutation type was classified as either p.R106W, p.R133C, p.T158M, p.R168X, p.R255X, p.R270X, p.R294X, p.R306C, p.R306H, large deletion, C-terminal deletion or early truncating mutation.

Family and socio-demographic factors, which were examined, included: birth order, maternal age at the time of birth, level of paternal and maternal education (classified as some high school, school accreditation, vocational and training, or tertiary education), and paternal and maternal occupation (categorized using the Australian and New Zealand Standard Classification of Occupation 1st Edition (ANZSCO)) [The Australian Bureau of Statistics, 2006a]. For InterRett cases birth order was not included in an earlier version of the questionnaire. Where possible, there was follow-up for families with available contact information with missing data.

Residential location and socio-economic status were also determined for Australian cases according to the earliest available postcode for the family. Residential location was coded using the Accessibility/Remoteness Index for Australia version *plus* (ARIA+) [The Australian Bureau of Statistics, 2003; The National Key Center for Social Applications of Geographic Information Systems, 2010] and scores were then categorized into: major cities of Australia, regional Australia (includes inner and outer regional) and remote Australia (includes remote and very remote locations) [Glover and Tennant, 2003]. The families' socio-economic status was investigated using the 2006 Socio-Economic Indexes for Area (SEIFA) [The Australian Bureau of Statistics, 2006b, c] specifically the Index of Relative Socio-economic Disadvantage (IRD) and the Index of Economic Resources (IER).

Analysis

Cases from the two data sources were pooled to examine relationships between age of diagnosis and symptom presentation, birth order and maternal age. Continuous variables such as age at development of symptoms and maternal age at the time of birth were categorized into quartiles due to their non-linear relationship with age of diagnosis. Linear regression analysis adjusting for birth year and data source was conducted to analyze the relationship between age at diagnosis and each variable and to provide adjusted mean ages at diagnosis for each factor. The adjusted means were at set levels of the co-variants where appropriate. Socio-economic status variables were analyzed separately for the ARSD and InterRett cases. Separate analyses of child factors were conducted for all subjects and for those with a *MECP2* mutation.

RESULTS

Information was available on a total of 909 verified cases, of which 570 were sourced from InterRett and 339 from the ARSD. Only clinician information was available for 16 ARSD cases. Genetic testing had been conducted in 87.7% of individuals ($n=797/909$), and of those with a known mutation result, a pathogenic *MECP2* mutation was identified in 85.3% ($n=654/795$) (Table I). More than half (56.14%) of cases with RTT had at least one older sibling and the mean maternal age at the child's birth was 28.6 years (CI 28.5–29.2; range 14.1–44.0 years).

Most (756/849, 89.1%) reported that their child had developed and subsequently lost functional hand use. By the age of 22 months half of these had lost hand skills, with mean age at loss being 27.5 months (95% CI 25.5–29.5). A pathogenic mutation was present in 71.3% (539/756) of cases that lost hand function and 80.6% ($n=75/93$) of those who did not. Earlier loss of hand function (<16 months) was associated with a younger age at diagnosis (adjusted mean 58.8 months) compared to loss after the age of 32 months (adjusted mean 74.1 months). The same pattern existed for mutation positive only individuals. The speed at which hand function was lost was described by families as being gradual by 76.8% and sudden by 23.2%.

Stereotypies occurred in 95.1% (839/882) of cases at a mean age of 27.4 months (CI 26.2–28.5), with appearance before four years of age in 90% of cases. Development of stereotypies was not reported in 43 children (4.9%), 74.4% ($n=32$) of whom had a pathogenic mutation. Diagnosis was earlier in those who developed stereotypies (adjusted mean 63.7 months) compared to those who did not (adjusted mean 75.4 months) and earlier if they developed before 18 months (adjusted mean 49.5 months) compared to after 36 months (adjusted mean 80.1 months). This pattern was similar as when restricted to cases with a pathogenic mutation. Hand stereotypies developed prior to loss of hand function in just over half (58.4%) of cases and 69.9% of these had a pathogenic mutation. If stereotypies developed more than 15 months after the loss of hand function, the age of diagnosis was older (adjusted mean 85.1 months) compared with loss of hand function prior to the development of stereotypies (adjusted mean 56.8 months). In those with a pathogenic mutation, the relationships were similar.

For those in whom it was achieved, most (89.3%, 691/774) lost speech ability at a mean age of 24.4 months (CI 22.5–26.2). A pathogenic mutation was present in 70.6% of those with lost speech ability and 89.2% which did not. Individuals who lost speech skills before 15 months (adjusted mean 56.3 months) had a younger age of diagnosis than those who lost speech skills after 24 months of age (adjusted mean 79.7 months). This pattern was similar in those with a pathogenic mutation only.

A slightly younger age of diagnosis was seen in those with a pathogenic mutation (adjusted mean 60.9 months) compared with those without (adjusted mean 70.7 months). Cases with a p.R133C mutation (adjusted mean 76.5 months) and those with a p.R294X mutation (adjusted mean 69.6 months) had a later age of diagnosis compared to those with a p.T158M mutation (adjusted mean 50.5 months). The youngest ages at diagnosis were in those with the mutations p.R255X (adjusted mean 43.7 months) and p.R168X (adjusted mean 43.5 months).

Most of parents from the InterRett cohort had achieved higher levels of education than those from the ARSD (Table II). In the InterRett cohort, children whose fathers had completed a 'tertiary education' were diagnosed younger (adjusted mean 54.7 months) than those whose fathers had only achieved 'vocational and training' qualifications (adjusted mean 68.7

months). There was no relationship between level of parental education and age at diagnosis for the ARSD cohort. The most frequently described maternal occupation for those participating in the ARSD were 'home duties' (52.1%), whilst the highest proportion of InterRett mothers was 'professionals' (35.2%). Fathers from the ARSD reported 'technical and trades' category professions most frequently (28.2%) while fathers participating in InterRett most frequently reported occupations in the category 'professionals' (35.87%). Children of fathers from InterRett whose occupation was categorized as 'technical and trades' had an older mean age of diagnosis (adjusted mean 75.4 months) than whose occupation was in the 'professionals' category (adjusted mean 59.8 months). However for ARSD families there was no relationship between occupation and the age at diagnosis.

Over half (59.45%) of Australian cases lived within the major cities with less than 5% in remote Australia (Table III). There was no apparent relationship between residential location and age of diagnosis. Families were most frequently categorized into the 2nd and 3rd highest categories of SEIFA, and there was no apparent relationship between their corresponding SEIFA score and age at diagnosis.

DISCUSSION

The loss of acquired hand function, speech abilities and the development of stereotypies at an earlier age, together with a shorter time period between the development of hand stereotypies and hand function loss were associated with an earlier age of diagnosis in RTT. Conversely, an older diagnosis was associated with late onset and atypical presentations of symptoms, often characteristic of subjects with a p.R133C or p.R294X mutation. To a lesser extent, lower levels of parental education or type of work in InterRett families were associated with a later age of diagnosis. Birth order and maternal age were not related to the age of diagnosis.

We have found that a clinical picture consistent with the classic presentation of RTT including earlier loss of speech, hand function loss, and the development of hand stereotypies within a close time frame was associated with an earlier diagnosis of RTT. In Fragile-X syndrome [Bailey et al., 2009] higher numbers of co-occurring symptoms were found to be associated with an earlier diagnosis, while in ASD [Mandell et al., 2005] children exhibiting symptoms such as sustained unusual play received an earlier diagnosis. However, the spectrum of phenotypes in RTT includes classical and also milder, less typical presentations [Zappella, 1992; Leonard et al., 2003; Colvin et al., 2004; Bebbington et al., 2008] and our study found that these atypical presentations sometimes involving a delayed onset of regression were often diagnosed at a later age. Clinicians rely on an overall clinical picture including the development of stereotypies and if symptoms are not typical, may observe the evolving clinical picture for a longer period of time prior to considering the diagnosis. It is important that, in considering the diagnosis, clinicians are alerted to the possible later development of hand stereotypies or even occasionally the total absence of hand stereotypies. Clinicians may be less aware of the range of phenotypes in RTT and earlier genetic testing especially in cases with unexplained developmental "slurring" [Julu et al., 2008] could be warranted. When the clinical presentation is atypical, families may receive an alternate diagnosis in the interim or no diagnosis at all as a wait-and-see approach is adopted. In particular girls may often be diagnosed with autism prior to receiving a diagnosis of RTT [Witt-Engerström and Gillberg, 1987; Young et al., 2008], with the likelihood increasing with a later age at regression or a better level of mobility [Young et al., 2008]. A general fall in the age of diagnosis has accompanied the development of genetic testing techniques over the last decade [Fehr, 2010]. To facilitate earlier diagnosis, improved educational resources about the clinical variability of RTT as a supplement and guide for

clinical assessment and genetic testing could be distributed. There may even be a case for broadening the current diagnostic criteria.

Mutation type was associated with different ages of diagnosis, with a later diagnosis more likely in subjects with a p.R133C or a p.R294X mutation. Investigations of relationships between phenotype severity and genotype have found that individuals with p.R133C, p.R294X and p.R306C have been reported to have delayed onset of regression, later onset of stereotypies, more retention of words and hand function, and to have learnt to walk [Leonard et al., 2003; Colvin et al., 2004; Charman et al., 2005; Bebbington et al., 2008; Neul, 2008; de Lima et al., 2009]. The more severe phenotypes are found in individuals with p.R255X, p.R270X and p.R168X, who were more likely to have a younger age of regression, loss of social interaction and onset of stereotypies. Certain mutation types such as p.R133C have also been shown to manifest fewer diagnostic criteria than p.R270X and p.R255X [Charman et al., 2005]. Thus far, the findings of the genotype/phenotype studies are consistent with our findings of an older age of diagnosis for girls with the p.R133C or p.R294X mutation compared with those with the p.T158M mutation. The earliest diagnoses were in those with p.R255X or p.R168X mutation.

Our results would suggest that equality in the access to medical services in the ARSD cohort is better than in the InterRett cohort for which the US represents the highest proportion (55.5%) of families. We used multiple measures to capture the many facets of the socio-economic construct [Braveman, 2005; McCracken, 2001] in the population-based ARSD and did not find any relationship between socio-economic status and the age of diagnosis. In contrast, higher levels of paternal education were associated with earlier age at diagnosis in InterRett families and this finding was within a group already comprising a higher percentage of advantaged families [Louise et al., 2009]. Consistent with our findings, Mandell *et al* [2005] found that children living in households with lower incomes received a diagnosis of autism at a later age. It would appear that health care systems are variable in offering equity of access to services necessary to diagnosing rare disorders such as RTT. It is important that data collection at a population level is undertaken so that the availability of medical services can be better determined within countries.

Birth order and maternal age were not related to the age of diagnosis. We had hypothesized that mothers who had had previous children or who were older might either be more alert to developmental deviations or better able to advocate for diagnosis but this was not the case. Rather, specific features of the symptoms and some aspects of socio-economic status predicted the age of diagnosis.

Two sizeable data sources were pooled to increase the power of the study and capture available variability. The Australian sample is population-based and this strengthens the study. Our analysis included adjustments for cohort and period effects. Limitations of this study included the use of parent-reported data which could have been subject to recall error and InterRett not being population-based. Due to data collection methods that rely on families having access to the internet to fill out the questionnaires [Louise et al., 2009] the InterRett cohort is most likely representative of families with higher socio-economic status.

Nevertheless, factors relating to age at diagnosis of RTT have not previously been investigated. Relationships between symptom presentation and age of diagnosis could provide greater clarity for understanding clinical practice during which clinicians typically see small series of cases. Our findings were mixed in relation to socio-economic factors and further study of the complexities of this construct is recommended. In conclusion, clinicians need to be alert to the variable presentation of RTT including the milder phenotypes of cases with the p.R133C or p.R294X mutation. Educational resources to assist this understanding

including guidance on when to request genetic testing could be useful to streamline the process of diagnosis in RTT.

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REFERENCES

- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl- CpG-binding protein 2. *Nat Genet.* 1999; 23:185–188. [PubMed: 10508514]
- Bailey DB, Raspa M, Bishop E, Holiday D. No change in the age of diagnosis for fragile X syndrome: findings from a national parent survey. *Pediatrics.* 2009; 124:527–533. [PubMed: 19581269]
- Bebbington A, Anderson A, Ravine D, Fyfe S, Pineda M, de Klerk N, Ben-Zeev B, Yatawara N, Percy AK, Kaufmann WE, Leonard H. Investigating genotype-phenotype relationships in Rett syndrome using an international dataset. *Neurology.* 2008; 70:868–875. [PubMed: 18332345]
- Braveman PA. Socioeconomic status in health research: one size does not fit all. *JAMA.* 2005; 294:2879–2888. [PubMed: 16352796]
- Burford B, Kerr AM, Macleod HA. Nurse recognition of early deviation in development in home videos of infants with Rett disorder. *J Intellect Disabil Res.* 2003; 47(Pt 8):588–596. [PubMed: 14641806]
- Charman T, Neilson TC, Mash V, Archer HL, Gardiner MT, Knudsen GP, McDonnell A, Perry J, Whatley SD, Bunyan DJ, Ravn K, Mount RH, Hastings RP, Hulten M, Orstavik KH, Reilly S, Cass H, Clarke AJ, Kerr AM, Bailey ME. Dimensional phenotypic analysis and functional categorisation of mutations reveal novel genotype-phenotype associations in Rett syndrome. *Eur J Hum Genet.* 2005; 13:1121–1130. [PubMed: 16077736]
- Colvin L, Leonard H, De Klerk N, Davis M, Weaving LS, Williamson SL, Christodoulou J. Refining the phenotype of common mutations in Rett syndrome. *J Med Genet.* 2004; 41:25–30. [PubMed: 14729826]
- de Lima FT, Brunoni D, Schwartzman JS, Pozzi MC, Kok F, Juliano Y, Pereira LV. Genotype-phenotype correlation in Brazilian Rett syndrome patients. *Arq Neuropsiquiatr.* 2009; 67:577–584. [PubMed: 19722030]
- Einspieler C, Kerr AM, Prechtl HF. Abnormal general movements in girls with Rett disorder: the first four months of life. *Brain Dev.* 2005a; 27(Suppl 1):S8–S13. [PubMed: 16182501]
- Einspieler C, Kerr AM, Prechtl HF. Is the early development of girls with Rett disorder really normal? *Pediatr Res.* 2005b; 57(5 Pt 1):696–700. [PubMed: 15718369]
- Elliott E, Chant KG. Rare disease surveillance. *J Paediatr Child Health.* 1994; 30(6):463–465. [PubMed: 7865253]
- Fehr, S. Diagnosing Rett syndrome: processes, influencing factors and family response [Honours]. Nedlands: University of Western Australia; 2010. 151 p.

- Fyfe S, Cream A, de Klerk N, Christodoulou J, Leonard H. InterRett and RettBASE: International Rett Syndrome Association databases for Rett syndrome. *J Child Neurol.* 2003; 18:709–713. [PubMed: 14649554]
- Glover, J.; Tennant, S. Remote areas statistical geography in Australia: notes on the Accessibility/remoteness index for Australia (ARIA+ version). Adelaide, SA, Australia: Public Health Information Development Unit, Commonwealth Department of Health and Ageing; 2003.
- Hagberg B, Aicardi J, Dias K, Ramos O. A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Ann Neurol.* 1983; 14:471–479. [PubMed: 6638958]
- Hagberg B, Hanefeld F, Percy AK, Skjeldal OH. An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. *Eur J Paediatr Neuro.* 2002; 6:293–297.
- Hagberg B, Witt-Engerström I. Rett syndrome: a suggested staging system for describing impairment profile with increasing age towards adolescence. *Am J Med Genet Suppl.* 1986; 1:47–59. [PubMed: 3087203]
- Julu POO, Engerström IW, Hansen S, Apartopoulos F, Engerström B, Pini G, Delamont RS, Smeets EEJ. Cardiorespiratory challenges in Rett's syndrome. *The Lancet.* 2008; 371:1981–1983.
- Laurvick CL, Klerk ND, Bower C, Christodoulou J, Ravine D, Ellaway CJ, Williamson S, Leonard H. Rett syndrome in Australia: a review of the epidemiology. *J Pediatr.* 2006; 148:347–351. [PubMed: 16615965]
- Leonard H. The Australian Rett Syndrome Study. *J Paediatr Child Health.* 1994; 30(3):A22.
- Leonard H. How can the Internet help parents of children with rare neurologic disorders? *J Child Neurol.* 2004; 19:902. [PubMed: 15658796]
- Leonard H, Bower C. Is the girl with Rett syndrome normal at birth? *Dev Med Child Neurol.* 1998; 40:115–121. [PubMed: 9489500]
- Leonard H, Bower C, English D. The prevalence and incidence of Rett syndrome in Australia. *Eur Child Adolesc Psychiatry.* 1997; 6(Supplement 1):8–10. [PubMed: 9452912]
- Leonard H, Colvin L, Christodoulou J, Schiavello T, Williamson S, Davis M, Ravine D, Fyfe S, de K, Matsuishi T, Kondo I, Clarke A, Hackwell S, Yamashita Y. Patients with the R133C mutation: is their phenotype different from patients with Rett syndrome with other mutations? *J Med Genet.* 2003; 40:e52. [PubMed: 12746406]
- Leonard H, Moore H, Carey M, Fyfe S, Hall S, Robertson L, Wu XR, Bao X, Pan H, Christodoulou J, Williamson S, de Klerk N. Genotype and early development in Rett syndrome: The value of international data. *Brain Dev.* 2005; 27(Supplement 1):S59–S68. [PubMed: 16182492]
- Louise S, Fyfe S, Bebbington A, Bahi-Buisson N, Anderson A, Pineda M, Percy A, Ben-Zeev B, Wu XR, Bao X, MacLeod P, Armstrong J, Leonard H. InterRett, a model for international data collection in a rare genetic disorder. *Res Autism Spectr Disord.* 2009; 3:639–659.
- Mandell DS, Ittenbach RF, Levy SE, Pinto-Martin JA. Disparities in diagnoses received prior to a diagnosis of autism spectrum disorder. *J Autism Dev Disord.* 2007; 37(9):1795–1802. [PubMed: 17160456]
- Mandell DS, Listerud J, Levy SE, Pinto-Martin JA. Race differences in the age at diagnosis among medicaid-eligible children with autism. *J Am Acad Child Adolesc Psychiatry.* 2002; 41:1447–1453. [PubMed: 12447031]
- Mandell DS, Novak MM, Zubritsky CD. Factors associated with age of diagnosis among children with autism spectrum disorders. *Pediatrics.* 2005; 116:1480–1486. [PubMed: 16322174]
- Mandell DS, Wiggins LD, Carpenter LA, Daniels J, DiGuseppi C, Durkin MS, Giarelli E, Morrier MJ, Nicholas JS, Pinto-Martin JA, Shattuck PT, Thomas KC, Yeargin-Allsopp M, Kirby RS. Racial/ethnic disparities in the identification of children with autism spectrum disorders. *Am J Public Health.* 2009; 99:493–498. [PubMed: 19106426]
- McCracken K. Into a SEIFA SES cul-de-sac? *Aust N Z J Public Health.* 2001; 25(4):305. [PubMed: 11529609]
- Naidu S, Murphy M, Moser H, Rett A. Rett Syndrome- Natural History in 70 Cases. *Am J Med Genet.* 1986; 24(Supplement 1):61–72.

- Neul JL. Specific mutations in Methyl-CpG-Binding Protein 2 confer different severity in Rett syndrome. *Neurology*. 2008; 70:1313–1321. [PubMed: 18337588]
- Opitz JM, Lewin SO. Rett syndrome--a review and discussion of syndrome delineation and syndrome definition. *Brain Dev*. 1987; 9:445–450. [PubMed: 3324794]
- The Australian Bureau of Statistics. Census Geography Paper 03/02-ASGC Remoteness Classification- Purpose and Use. Canberra, ACT, Australia: 2003.
- The Australian Bureau of Statistics. 1220.0 Australian and New Zealand Standard Classification of Occupations. Canberra, ACT, Australia: 2006a.
- The Australian Bureau of Statistics. SEIFA, Census collection districts, Data cube only 2006. Canberra, ACT, Australia: 2006b. 2033.0.55.001. Census of population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia- Data only, 2006.
- The Australian Bureau of Statistics. 2039.0 Information Paper: An Introduction to Socio-Economic Indexes for Areas (SEIFA), 2006. Canberra, ACT, Australia: 2006c.
- Accessibility/Remoteness indexes for Australia version plus. 2010. The National Key Center for Social Applications of Geographic Information Systems.
- Witt-Engerström I. A retrospective pilot study on potential early predictive symptomatology. *Brain Dev*. 1987; 9:481–486. [PubMed: 3434723]
- Witt-Engerström I. Rett syndrome in Sweden. Neurodevelopment--disability--pathophysiology. *Acta Paediatr Scand Suppl*. 1990; 369:1–60. [PubMed: 1701067]
- Witt-Engerström I. Rett syndrome: the late infantile regression period--a retrospective analysis of 91 cases. *Acta Paediatr*. 1992; 81:167–172. [PubMed: 1515763]
- Witt-Engerström I, Gillberg C. Rett syndrome in Sweden. *J Autism Dev Disord*. 1987; 17(1):149–150. [PubMed: 3571139]
- Young DJ, Bebbington A, Anderson A, Ravine D, Ellaway CJ, Kulkarni A, de Klerk N, Kaufmann WE, Leonard H. The diagnosis of autism in a female: could it be Rett syndrome? *Eur J Pediatr*. 2008; 167:661–669. [PubMed: 17684768]
- Zappella M. The Rett girls with preserved speech. *Brain Dev*. 1992; 14:98–101. [PubMed: 1621933]

TABLE I
Frequencies and Adjusted Mean Ages at Diagnosis for Pooled Analysis Variables

Variable	All individuals			Mutation positive individuals only		
	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	P-value	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	P-value
Birth Order	(n=725) ^a					
First born	318 (43.86)	62.5 (57.9–67.2)	<i>a</i>			
Second born	262 (36.14)	63.3 (58.2–68.5)	0.08			
Third born or later	145 (20.00)	72.0 (65.2–78.9)	0.28			
Maternal Age	(n=858)					
25 years	222 (25.87)	79.9 (74.2–85.6)	<i>a</i>			
25 to 29 years	227 (26.46)	63.0 (57.4–68.6)	0.42			
29 to 33 years	221 (25.76)	59.0 (53.3–64.6)	0.90			
> 33 years	188 (21.91)	51.7 (45.5–57.8)	0.64			
Hand skills lost	(n=849) [*]			(n=614)		
Yes	756 (89.05)	64.4 (61.4–67.4)	<i>a</i>	539 (87.79)	60.1 (56.6–63.6)	<i>a</i>
No	93 (10.95)	60.7 (52.2–69.3)	0.10	75 (12.21)	57.4 (48.1–66.7)	0.3
Speed of Hand Skill Loss	(n=732) ⁺			(n=523)		
Gradual	562 (76.78)	59.6 (51.1–68.2) ^b	<i>a</i>	404 (77.25)	60.3 (56.4–64.2)	<i>a</i>
Sudden	170 (23.22)	52.4 (42.4–62.4)	0.05	119 (22.75)	57.5 (50.3–64.7)	0.18
Age of Loss of hand skills	(n=654) ⁺			(n=461)		
16 months	174 (26.61)	58.8 (52.9–64.7)	<i>a</i>	123 (26.68)	52.7 (45.7–59.8)	<i>a</i>
16 to 22 months	166 (25.38)	53.8 (47.7–59.8)	0.36	125 (27.11)	51.8 (44.8–58.8)	0.75
22 to 32 months	155 (23.70)	62.9 (56.6–69.1)	0.32	117 (25.38)	59.8 (52.6–67.1)	0.17
>32 months	159 (24.31)	74.1 (68.0–80.3)	0.01	96 (20.82)	73.9 (65.8–81.9)	0.002
Hand Stereotypies	(n=882)			(n=642)		
Developed	839 (95.12)	63.7 (60.8–66.6)	<i>a</i>	610 (95.02)	59.9 (56.5–63.2)	<i>a</i>
Not Developed	43 (4.88)	75.4 (62.7–88.2)	0.003	32 (4.98)	66.6 (52.1–81.1)	0.16
Age of Development	(n=764) ⁺			(n=553)		
18 months	258 (33.77)	49.5 (44.7–54.3)	<i>a</i>	180 (32.55)	43.0 (37.5–48.6)	<i>a</i>
18 – 24 months	191 (25.00)	59.2 (53.6–64.7)	0.26	142 (25.68)	52.6 (46.4–58.9)	0.20

Variable	All individuals			Mutation positive individuals only		
	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	P-value	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	P-value
24–36 months	187 (24.48)	63.2 (57.6–68.8)	0.015	141 (25.50)	61.3 (55.0–67.6)	0.006
>36 months	128 (16.75)	79.9 (73.1–86.7)	<0.001	90 (16.27)	76.6 (68.8–84.5)	<0.001
Duration between hand function loss and stereotypy development						
(n=580)^a						
Stereotypies developed prior loss of hand function	339 (58.45)	56.9 (52.9–60.8)	<i>a</i>	237 (58.09)	50.5 (45.7–55.3)	<i>a</i>
0 to 6 months	117 (20.17)	56.2 (49.4–63.0)	0.63	83 (20.34)	55.3 (47.2–63.4)	0.40
6 to 15 months	67 (11.55)	61.0 (52.1–70.0)	0.93	47 (11.52)	55.8 (45.0–66.6)	0.59
Greater than 15 months	57 (9.83)	84.4 (74.6–94.1)	0.003	41 (10.05)	85.5 (73.9–97.0)	0.01
Speech lost						
(n=774)[*]						
Yes	691 (89.28)	65.43 (62.3–68.6)	<i>a</i>	488 (86.83)	61.2 (57.6–64.8)	<i>a</i>
No	83 (10.72)	50.8 (41.8–59.9)	0.80	74 (13.17)	49.2 (39.9–58.5)	0.76
Age at speech loss						
(n=621)						
15 months	182 (29.31)	56.3 (50.2–62.5)	<i>a</i>	130 (29.75)	49.3 (42.1–56.6)	<i>a</i>
15–18 months	157 (25.28)	61.7 (55.1–68.4)	0.67	113 (25.86)	57.6 (49.8–65.3)	0.65
18–24 months	134 (21.58)	67.0 (59.8–74.2)	0.13	99 (22.65)	62.7 (54.4–71.0)	0.15
>24 months	148 (23.83)	79.7 (72.8–86.5)	<0.001	95 (21.74)	82.4 (73.9–90.9)	<0.001
MECP2 Mutation Status						
(n=749)^c						
Positive	654 (87.32)	60.0 (56.7–63.3)	<i>a</i>			
Negative	95 (12.68)	70.4 (61.7–79.0)	0.11			
MECP2 Mutation Outcome						
(n=528)^d						
C-Terminal	61 (11.55)	61.4 (51.–1.3)	0.47			
Deletions	36 (6.82)	50.1 (37.–3.1)	0.26			
Early Truncating	29 (5.49)	71.2 (56.–5.7)	0.83			
p.R106W	27 (5.11)	54.6 (39.–9.6)	0.87			
p.R133C	44 (8.33)	75.1 (63.–6.9)	0.004			
p.R168X	63 (11.93)	43.1 (33.–2.9)	0.56			
p.R255X	61 (11.55)	43.5 (33.–3.5)	0.51			
p.R270X	48 (9.09)	59.0 (47.–0.3)	0.75			
p.R294X	48 (9.09)	70.1 (58.–1.3)	0.04			
p.R306C	35 (6.63)	73.8 (60.–7.0)	0.15			

Variable	All individuals		Mutation positive individuals only	
	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)
p.R306H	8 (1.52)	48.8 (21.–6.4)		
p.T158M	68 (12.88)	50.5 (41.–9.9)		

a- variable used as baseline,

a only n=755 were able to answer this question (for InterRett cases only 308 completed the questionnaire and an additional 153 were followed up, while for those from the ARSD only 294 answered the question)

* individuals would have first had to develop these skills to lose them and thus those which did not (Hand skills n=28 and speech n=90) have not been included.

+ only those which had lost this skill or developed stereotypes and had specified an age which it had occurred were included,

b the mean was adjusted for the appropriate set of co-variants for this variable

^ these needed to have specified both the age which hand skills were lost and the age stereotypes occurred,

c there were an additional 29 cases who had been tested yet their result was unknown and 19 cases where the mutation which was detected had an unknown pathogenicity

d only cases with these mutations were included in this section, there were an additional 63 cases which were classified as other (less common point mutations, frame-shift), and 63 who were positive but the result was unknown.

TABLE II
Frequencies and Adjusted Mean Age at Diagnosis of ARIA+ and SEIFA Scores (ARSD Subjects Only)

Variable	ARSD			InterRett		
	Mother Frequency (%)	Mother Adjusted mean age at diagnosis in months (95% CI)	P-value	Father Frequency (%)	Father Adjusted mean age at diagnosis in months (95% CI)	P-value
Education	(n=316)			(n=510)		
Tertiary qualifications	75 (23.73)	59.7 (50.-3.8)	<i>a</i>	247 (48.43)	57.2 (51.-2.5)	<i>a</i>
Vocational and training	86 (27.22)	60.3 (51.-7.5)	0.73	110 (21.57)	66.8 (57.-4.7)	0.14
School accreditation	50 (15.82)	68.2 (56.-7.4)	0.41	132 (25.88)	72.9 (63.-0.1)	0.13
Some high school	105 (33.23)	64.6 (56.-2.4)	0.60	21 (4.12)	79.0 (60.-7.1)	0.71
Occupation	(n=305)			(n=512)		
Professionals	41 (13.44)	64.5 (51.-7.5)	<i>a</i>	180 (35.16)	64.9 (56.-9.3)	<i>a</i>
Managers	18 (5.90)	81.8 (62.-01.3)	0.60	34 (6.64)	57.9 (49.-8.7)	0.57
Technical and trades workers	9 (2.95)	55.9 (28.-3.5)	0.40	10 (1.95)	88.5 (62.-15.7)	0.20
Community and personal service	28 (9.18)	71.1 (55.-6.7)	0.91	42 (8.20)	62.5 (52.-8.3)	0.345
Clerical and administrative	29 (9.51)	55.1 (39.-0.5)	0.31	47 (9.18)	68.6 (54.-8.5)	0.40
Sales workers	8 (2.62)	50.1 (20.-9.4)	0.24	15 (2.93)	62.7 (34.-8.0)	0.32
Machinery operators and drivers	1 (0.33)	19.3 (-63.-02.2)	0.25	3 (0.59)	108.7 (41.-38.0)	0.52
Laborers	8 (2.62)	58.4 (329.-7.7)	0.64	6 (1.17)	78.5 (40.-09.2)	0.05
Unemployed	4 (1.31)	90.4 (48.-31.8)	0.11	5 (0.98)	39.4 (9.-4.6)	0.79
Home duties	159 (52.13)	60.3 (53.-6.8)	0.18	170 (33.20)	66.6 (60.-3.2)	0.21

α - variable used as baseline

only cases which were able to be classified into the above variables were included, the numbers of those which were excluded are as follows: ARSD father occupation: 10 could not be defined, ARSD mother occupation: 5 could not be defined, InterRett father's occupation: 45 could not be defined, InterRett mothers occupations: 29 could not be defined. Also only families which the families knew of the occupation/education of the mother and father could have answered the question (excluding adoptive families, unless knew biological mother/father, and also separated families).

TABLE III

Frequencies and Adjusted Mean Ages of Diagnosis for Parental Occupation and Education (ARSD and InterRett Cohorts)

Variable	Frequency (%)	Adjusted mean age at diagnosis in months (95% CI)	P-value
Residential Location	(n=331)*		
Major Cities of Australia	195 (59.45)	59.7 (53.–5.8)	<i>a</i>
Remote Australia	118 (35.98)	69.4 (61.–7.2)	0.23
Very Remote Australia	15 (4.57)	58.0 (36.–9.8)	0.67
Socio-economic Index for Area	(n=308)*+		
Index relative socio-economic disadvantage			
1	55 (17.86)	57.8 (46.–9.3)	<i>a</i>
2	52 (16.88)	70.5 (58.–2.3)	0.29
3	71 (23.05)	59.5 (49.–9.6)	0.55
4	75 (24.35)	63.6 (53.–3.4)	0.68
5	55 (17.86)	57.7 (46.–9.1)	0.92
Index economic resources			
1	48 (15.58)	61.4 (49.–3.7)	<i>a</i>
2	58 (18.83)	59.1 (47.–0.3)	0.75
3	68 (22.08)	63.2 (52.–3.5)	0.72
4	69 (22.40)	65.3 (55.–5.6)	0.85
5	65 (20.10)	59.0 (48.–9.5)	0.77

a- variable used as baseline,

* cases needed to have had a known postcode available,

+ cases needed to have a SEIFA score allocated to their collection district