Genotype and early development in Rett syndrome: The value of international data

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Abstract

Background: Rett syndrome is a neurodevelopmental disorder mostly affecting females and caused by mutations in the MECP2 gene. Originally the syndrome was characterised as having a normal prenatal and perinatal period with later regression. Previous work has speculated that the girl with Rett syndrome may not be normal at birth. Aims: to examine whether early development between birth and ten months varies by genotype in Rett syndrome. Methods: cases were sourced from two databases, the Australian Rett Syndrome Database (est. 1993) and the newly formed InterRett - IRSA Rett Phenotype Database. Data available on 320 cases included information provided by parents on perinatal problems, early developmental behaviour and mobility. Problem scores, mobility scores and a total composite score for each mutation were generated and compared. Results: overall, 58% of respondents noted unusual behaviour during the first six months and 70.6% from the period between 6 and 10 months of life. Statistically significant differences were detected between some of the common mutations. Infants with R294X (P<0.05) and R133C (P<0.03) were less likely than those with R255X to have problems in the perinatal period. The most severe profile overall for early development was associated with mutations R255X and R270X. Conclusion: This is the largest study to date examining the effects of individual mutations in Rett syndrome. With the ongoing case ascertainment and expansion of InterRett, sample size will increase rapidly and provide improved statistical power for future analyses. Results from this study will contribute to understanding the mechanism of early development in Rett syndrome and determining if and at which time(s) early intervention might be feasible.

Keywords: Rett syndrome; MECP2; Phenotype; Early development; International; Internet; Database

1. Introduction

The clinical criteria for Rett syndrome identified by Hagberg et al. [1] and then revised by Trevarthan and Moser [2] characterised Rett syndrome as a neurodevelopmental disorder with a normal prenatal and perinatal period and early development in the normal range with a later regression period. However, it has become apparent that girls with Rett syndrome may not be normal at birth [3,4] and that there are subtle differences which indicate developmental delay early in life [5,6]. Early hypotonia [7–9], motor problems [10,11] including early jerky incoordination [7], placidity and perinatal difficulties requiring admission to a special care nursery [3] have been noted in the pre-regression period.

Since the association between mutations in MECP2 and Rett syndrome was recognized [12], many studies have tried to characterise the relationship between genotype and clinical severity. In a sample of 56 girls Amir et al. [13] found respiratory dysfunction was more frequently
associated with truncating and scoliosis with missense mutations. Other studies have found a more severe clinical phenotype in truncating when compared with missense mutations [14–17]. Where investigations have taken into account mutation location as well as mutation type a more refined picture appears. Cheadle et al. [14] showed that early truncating mutations were likely to be more severe than late truncating mutations. Similarly, Hoffbuhr et al. [18] found that missense mutations in the MBD and mutations truncating the entire TRD had a more severe clinical phenotype than missense and nonsense mutations in the TRD and C-terminal. Huppke et al. [16] found that mutations in the TRD NLS were associated with a more severe phenotype than those in the MBD and in the rest of the TRD. Characterisation of phenotypic severity using a large population by Leonard et al. [19] and Colvin et al. [20] has shown differential phenotypic severity between the common mutations including confirmation that, in comparison with other MECP2 mutations, the R133C mutation has a milder clinical presentation. This was the first clear and clinically meaningful description of the association between genotype and clinical phenotype. The present study aimed to investigate the relationship between early development and individual mutations using both Australian population based data and international data.

Our previous work showed that, compared with singleton births of a similar gestation, a greater proportion of Rett syndrome cases had birthweights <3500 g and lower birth head circumference; 41% of families reported some perinatal problem and nearly half (46.5%) of families had reported their child’s behaviour to be unusual in the first 6 months of life [3]. This led us to speculate that the girl with Rett syndrome may not be normal at birth. The purpose of this current study has been to examine whether any of these early indicators of reduced optimality at birth and in the early months vary by genotype.

2. Methods

2.1. Case ascertainment

Cases were sourced from two databases, the Australian Rett Syndrome Database (ARSD) and InterRett, the IRSA Rett Phenotype Database. Established in 1993, the ARSD is a population-based database with ongoing ascertainment of Australian Rett syndrome cases born since 1976 [21]. It contains demographic, clinical, behavioural, developmental, functional and genetic information [22]. InterRett is an international phenotype database, established in 2003 with funding from the International Rett Syndrome Association and managed from the Telethon Institute for Child Health Research in Western Australia [23]. Data contributions to InterRett cases can be made individually or as multiple submissions from international collaborating centres. Both databases collect data from families and clinicians.

The InterRett family questionnaire administered on case enrolment is similar to and has been developed from the original Australian version providing comparability between the two datasets.

As at 31st October 2003, 251 cases had been reported to ARSD and verified according to the clinical criteria for classical or atypical Rett syndrome [3]. Of the 251 verified cases, data from families have been provided in 235 and in 16, information was currently only available from the clinician. Data provision to InterRett commenced in January 2003. Cases were included in this analysis either if they had a valid pathogenic mutation or if the diagnosis of Rett syndrome was considered by the child’s doctor to be ‘definite’. As at June 2003, 88 InterRett cases meeting this study definition had been reported. Three of these cases were duplicates of ARSD cases and so were excluded from this analysis. Thus a total of 235 ARSD (including 130 cases from the original cohort [3]) and 85 InterRett cases with family information were available for this study. Ethical approval for the study was granted by the local institutional ethics committee.

2.2. Clinical data collection

This report focuses on information relating to early development provided by the family. As previously described, data were collected by paper-based questionnaires for ARSD cases [21] and by paper-based or Internet questionnaires for InterRett cases [23]. Questions were asked about problems in the first week of life, abnormal behaviours and development in the first 6 months and from after 6–10 months. Open responses were coded according to type of problem as identified by the family e.g. placid, sleeping problems, gastro-intestinal, developmental problems.

For each question, cases were generally allocated a score of 0 if there were no problems identified and a score of 1 for each problem type identified. However for the questions on admission to special nursery and for help with breathing at birth, responses were simply scored as 0 or 1. A composite perinatal score was derived from the three questions involving the perinatal period. The maximum number of problems coded for any of the three problem questions was 3. Thus each case could receive a score of between 0 and 5 for the perinatal period and a score of between 0 and 3 for the other two time periods and thus a total problem score ranging from 0 to 11. Additionally, cases were scored according to their reported mobility at 10 months with the lowest score of 0 allocated to those performing best (i.e. walking independently) and the highest score of 12 to those who could not move around. A score of 10 was allocated for rolling, 8 for commando crawling, 6 for bottom shuffling, 4 for crawling, and 2 for moving around by furniture walking or knee walking. A total composite (problem and mobility) score was computed with a possible range from 0 to 23. Cases with missing data were not allocated scores.
2.3. Mutational analyses

Mutation testing has been carried out on 197/235 (83.8%) ARSD and on 66/85 (77.6%) InterRett cases with pathogenic mutations identified in 136/197 (69.0%) and 45/66 (68.2%), respectively. This analysis concentrates particularly on those cases where a pathogenic MECP2 mutation was identified. Pathogenicity was verified as previously [19,20] by laboratory report for ARSD cases. For InterRett cases, multiple sources (clinician and family questionnaires) were used to confirm mutation status and pathogenicity was validated using the mutation database, RettBASE [24]. For the purposes of this study any ambiguous mutation data were not treated as pathogenic. Cases with any pathogenic mutation were identified and then cases with any of the seven most common pathogenic mutations (R133C, T158M, R168X, R255X, R270X, R294X and R306C) were grouped together and according to their specific mutation. Other groupings were cases with other pathogenic mutations and cases who had either not been tested or in whom a mutation had not been found. The ARSD and InterRett data were stored in Filemaker Pro 6 databases and transferred into SPSS Version 10.0 for analysis.

2.4. Data analysis

For each group the proportions reporting any or a specific problem for the three time periods, perinatal, 0–6, >6–10 months were presented. In all cases the denominators were adjusted to account for missing values on individual questions. The chi-square test was used to determine the presence of associations between categorical variables [25]. Means were calculated for individual and total problem scores, the mobility score and the composite score [including problems and mobility]. One-way analysis of variance [Kruskal Wallis for non-normally distributed variables] was used to compare differences in means according to mutation categories. P values of less than 0.1 were considered indicative of important differences and no adjustments were made for multiple testing [26].

3. Results

The distribution of cases by year, country of birth and age at diagnosis by data source is shown in Table 1. Of those 262 (81.9%) had results from molecular testing. In 181 of those tested, a valid pathogenic mutation as defined by study criteria was present. For each of the mutations R133C, T158M, R168X, R255X, R270X, R294X and R306C there were eleven or more cases. This group accounted for 122/181 (67.4%) of those cases with pathogenic mutations.

Over a third of respondents reported that their child had experienced a problem in the first week of life, with almost the same proportion (39.3%) in the group with pathogenic mutations. Within the seven common mutations the proportions ranged from 3/11 (27.3%) in those with R133C to 9/16 (56.3%) in those with R255X (P = 0.14, Table 2). Feeding problems were the most common individual problem reported in 24 (13.5%) of those with valid mutations and in 18 (15%) of those with the seven common mutations. Parents were also asked whether the infant needed help breathing. Again there was variation with no such reports in the 11 with R133C compared with 4/15 (26.7%) in those with R255X (P = 0.06). Only 1/16 (6.3%) of R294X cases had been admitted to a special nursery compared with 5/16 (31.3%) of those with R255X (P = 0.07). Overall 81/180 (45.0%) of those with pathogenic mutations were reported to have some perinatal difficulty: either help to start breathing, admission to a special nursery or a problem in the first week of life.

Overall 184/317 (58%) of respondents reported that they had noted unusual behaviour or development during the first 6 months of their daughter’s life (Table 3). Nineteen reports included in the analysis were ambiguous in that respondents provided a response to the open but not the closed question.
### Table 2
Presence and nature of perinatal problems by mutation type

<table>
<thead>
<tr>
<th>Observation/problem</th>
<th>R133C (N=11) n (%)</th>
<th>R294X (N=17) n (%)</th>
<th>R168X (N=22) n (%)</th>
<th>T158M (N=28) n (%)</th>
<th>R255X (N=16) n (%)</th>
<th>R270X (N=16) n (%)</th>
<th>R306C (N=12) n (%)</th>
<th>Seven common mutations (N=122) n (%)</th>
<th>Other pathogenic mutations (N=59) n (%)</th>
<th>All pathogenic mutations (N=181) n (%)</th>
<th>No Mutation or Not tested (N=139) n (%)</th>
<th>All cases* (N=320) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Problems in the 1st week of life</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding</td>
<td>–</td>
<td>2(11.1)</td>
<td>2(10.0)</td>
<td>7(25.0)</td>
<td>3(18.8)</td>
<td>1(6.3)</td>
<td>3(25.0)</td>
<td>18(15.0)</td>
<td>6(10.3)</td>
<td>24(13.5)</td>
<td>27(19.8)</td>
<td>51(16.1)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>–</td>
<td>1(5.6)</td>
<td>4(20.0)</td>
<td>2(7.1)</td>
<td>–</td>
<td>1(6.3)</td>
<td>2(16.7)</td>
<td>10(8.3)</td>
<td>7(12.1)</td>
<td>17(9.6)</td>
<td>5(3.7)</td>
<td>22(7.0)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>1(9.1)</td>
<td>2(11.1)</td>
<td>3(15.6)</td>
<td>2(7.1)</td>
<td>1(6.3)</td>
<td>2(12.6)</td>
<td>1(8.3)</td>
<td>8(6.7)</td>
<td>2(3.4)</td>
<td>10(5.6)</td>
<td>6(4.4)</td>
<td>16(5.1)</td>
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<td>Breathing problems</td>
<td>–</td>
<td>–</td>
<td>1(5.0)</td>
<td>3(10.7)</td>
<td>2(12.5)</td>
<td>1(6.3)</td>
<td>2(16.7)</td>
<td>9(7.5)</td>
<td>1(1.7)</td>
<td>10(5.6)</td>
<td>4(2.9)</td>
<td>14(4.4)</td>
</tr>
<tr>
<td>Sleep</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2(7.1)</td>
<td>1(6.3)</td>
<td>3(18.8)</td>
<td>6(5.0)</td>
<td>2(3.4)</td>
<td>8(4.5)</td>
<td>4(2.9)</td>
<td>12(3.8)</td>
<td></td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1(3.6)</td>
<td>–</td>
<td>–</td>
<td>1(1.0)</td>
<td>1(1.7)</td>
<td>2(1.1)</td>
<td>–</td>
<td>2(0.6)</td>
<td></td>
</tr>
<tr>
<td>Hypotension</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1(3.6)</td>
<td>1(6.3)</td>
<td>–</td>
<td>2(1.7)</td>
<td>–</td>
<td>2(1.1)</td>
<td>–</td>
<td>2(0.6)</td>
<td></td>
</tr>
<tr>
<td>Birth defect</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1(6.3)</td>
<td>–</td>
<td>1(1.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1(0.3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2(18.2)</td>
<td>2(11.1)</td>
<td>–</td>
<td>2(7.1)</td>
<td>2(12.5)</td>
<td>–</td>
<td>2(16.7)</td>
<td>10(8.3)</td>
<td>2(3.4)</td>
<td>12(6.7)</td>
<td>11(8.1)</td>
<td>23(7.3)</td>
</tr>
<tr>
<td><strong>Special nursery help breathing</strong></td>
<td>2(8.2)</td>
<td>2(11.1)</td>
<td>2(11.1)</td>
<td>4(15.4)</td>
<td>5(31.3)</td>
<td>–</td>
<td>2(16.7)</td>
<td>19(16.0)</td>
<td>8(13.8)</td>
<td>27(15.3)</td>
<td>24(17.8)</td>
<td>51(16.8)</td>
</tr>
<tr>
<td><strong>Any perinatal difficulty</strong></td>
<td>4(36.4)</td>
<td>6(35.3)</td>
<td>10(45.5)</td>
<td>13(46.4)</td>
<td>10(62.5)</td>
<td>7(43.8)</td>
<td>7(58.3)</td>
<td>57(46.7)</td>
<td>24(41.4)</td>
<td>81(45.0)</td>
<td>64(46.4)</td>
<td>145(45.6)</td>
</tr>
</tbody>
</table>

*Denominator used taking into account missing values for individual questions.

### Table 3
Presence and nature of abnormal development or behaviour in first 6 months of life by mutation type

<table>
<thead>
<tr>
<th>Observation/problem</th>
<th>R133C (N=11) n (%)</th>
<th>R294X (N=17) n (%)</th>
<th>R168X (N=22) n (%)</th>
<th>T158M (N=28) n (%)</th>
<th>R255X (N=16) n (%)</th>
<th>R270X (N=16) n (%)</th>
<th>R306C (N=12) n (%)</th>
<th>Seven common mutations (N=122) n (%)</th>
<th>Other pathogenic mutations (N=59) n (%)</th>
<th>All pathogenic mutations (N=181) n (%)</th>
<th>No Mutation or Not tested (N=139) n (%)</th>
<th>All cases* (N=320) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unusual behaviour in the 1st 6 months</strong></td>
<td>4(36.4)</td>
<td>9(52.9)</td>
<td>15(68.2)</td>
<td>19(67.9)</td>
<td>11(68.8)</td>
<td>9(56.3)</td>
<td>6(50.0)</td>
<td>73(59.8)</td>
<td>33(56.9)</td>
<td>106(58.9)</td>
<td>78(56.9)</td>
<td>184(58.0)</td>
</tr>
<tr>
<td>Placid</td>
<td>–</td>
<td>3(17.6)</td>
<td>3(13.6)</td>
<td>4(14.3)</td>
<td>4(25.0)</td>
<td>2(12.5)</td>
<td>4(33.3)</td>
<td>20(16.4)</td>
<td>12(20.7)</td>
<td>32(17.8)</td>
<td>26(19.0)</td>
<td>58(18.3)</td>
</tr>
<tr>
<td>Feeding</td>
<td>–</td>
<td>–</td>
<td>3(13.6)</td>
<td>5(17.9)</td>
<td>1(6.3)</td>
<td>4(25.0)</td>
<td>1(8.3)</td>
<td>14(11.5)</td>
<td>8(13.8)</td>
<td>22(12.2)</td>
<td>17(12.4)</td>
<td>39(12.3)</td>
</tr>
<tr>
<td>Developmental</td>
<td>–</td>
<td>1(5.9)</td>
<td>1(4.5)</td>
<td>5(17.9)</td>
<td>3(18.8)</td>
<td>3(18.8)</td>
<td>–</td>
<td>12(9.8)</td>
<td>5(8.6)</td>
<td>17(9.4)</td>
<td>18(13.1)</td>
<td>35(11.0)</td>
</tr>
<tr>
<td>Floppy</td>
<td>–</td>
<td>–</td>
<td>5(22.7)</td>
<td>5(17.9)</td>
<td>1(6.3)</td>
<td>2(12.5)</td>
<td>–</td>
<td>13(10.7)</td>
<td>5(8.6)</td>
<td>18(10.0)</td>
<td>14(10.2)</td>
<td>32(10.1)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>1(9.1)</td>
<td>3(17.6)</td>
<td>1(4.5)</td>
<td>2(7.1)</td>
<td>2(12.5)</td>
<td>2(12.5)</td>
<td>–</td>
<td>11(9.0)</td>
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<td>12(6.7)</td>
<td>9(6.6)</td>
<td>21(6.6)</td>
</tr>
<tr>
<td>Eyes</td>
<td>–</td>
<td>–</td>
<td>1(4.5)</td>
<td>1(3.6)</td>
<td>1(6.3)</td>
<td>–</td>
<td>1(8.3)</td>
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<td>4(2.2)</td>
<td>11(8.0)</td>
<td>14(4.7)</td>
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<tr>
<td>Sleep</td>
<td>1(9.1)</td>
<td>1(5.9)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2(1.6)</td>
<td>4(6.9)</td>
<td>6(3.3)</td>
<td>3(2.2)</td>
<td>9(2.8)</td>
</tr>
<tr>
<td>Strange behaviour</td>
<td>–</td>
<td>1(5.9)</td>
<td>1(5.9)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3(2.5)</td>
<td>1(1.7)</td>
<td>4(2.2)</td>
<td>4(2.9)</td>
<td>8(2.5)</td>
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<td>Hand behaviour</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>5(3.6)</td>
<td>5(1.6)</td>
</tr>
<tr>
<td>Other</td>
<td>3(27.3)</td>
<td>2(11.8)</td>
<td>1(4.5)</td>
<td>1(3.6)</td>
<td>1(6.3)</td>
<td>–</td>
<td>1(8.3)</td>
<td>9(7.4)</td>
<td>2(3.4)</td>
<td>11(6.1)</td>
<td>1(0.7)</td>
<td>12(3.8)</td>
</tr>
</tbody>
</table>

*Denominator used taking into account missing values for individual questions.
A very similar proportion of unusual behaviour was reported in those cases with pathogenic mutations. For the seven specific mutations examined, the proportion varied from 4/11 (36.4%) in cases with R133C to 11/16 (68.8%) in cases with R255X mutations ($P = 0.10$, Table 3). When the open responses were coded into specific categories the most commonly reported category was *placid* reported in 18.3% of all cases and in 16.4% of those with the common mutations. Some examples of how the families expressed this are shown in Box 1. Other high rankings were reports of a *floppy* baby in 10.1% of all cases and 10.7% of those with common mutations and of *developmental* problems in 11.0% of all cases and 9.8% of those with common mutations.

Participants were then asked whether they had noticed anything unusual about their child’s development or behaviour in the period from after six to ten months of age. Overall 221 of the 313 (70.6%) reported in the affirmative (7 cases with ambiguous responses in that they provided a response to the open but not the closed question were also included) and 122/180 (67.8%) of those cases with a valid mutation (Table 4). Thus the proportion reporting in the affirmative among those who did not have a mutation was marginally higher than among those who did (74.4 vs 67.8%, $P = 0.20$). For the seven specific mutations the proportions varied from 9/17 (52.9%) in those with R294X to 13/16 (81.3%) in those with R270X. The comparison between these two approached significance at $P = 0.08$. Respondents’ most predominant recollection within this time period, was concern specifically about their child’s *development* (Box 2). This was mentioned in nearly half (45.7%) of all cases and in 42.6% of those with common mutations. Other categories were much less frequently reported and generally in less than 10% of all cases (Table 4).

For 311 cases a description of the child’s mobility at 10 months was available. In 62/311 (19.9%) of all cases and in 15.0% of those with the common mutations the child was reported not to be moving around at 10 months. In 88/311 (28.3%) of all cases and a quarter (25.8%) of those with the common mutations the child was reported to be moving around by rolling. The child was commando crawling in 29/311 (9.3%) of all cases and in 7.5% of those with the common mutations and bottom shuffling in 40/311 (12.9%) and 15.8%, respectively. Sixty one (19.6%) of all cases and nearly a quarter (24.2%) of those with the common mutations were crawling at this time. Whilst 2.3 and 3.3%, were said to be able to walk independently, a further 8.0% of all cases and 8.3% of those with the common mutations were using other forms of mobility such as furniture or knee walking.

The mobility and other scores for each of the seven common mutations are shown in Fig. 1. For mobility the highest score indicating the least mobility at 10 months was for R270X (mean = 8.25) and the lowest mean scores indicating the best mobility were for R306C and R294X (5.17, 5.76). After R294X and R306C the next most mobile were the R133C and T158M cases, followed by R168X, and R255X. However the only statistically significant differences were between R306C and both R255X and R270X.

**Box 1.** Examples of family expression of unusual behaviour from the first 6 months of life

**Code: Placid**

‘Unusually passive—happy always! Never crying not even when hungry—or when had chicken pox at 6 months’
‘Was very placid and slept a lot compared to friends babies’
‘She was always ‘too good’ a baby. Very placid, fed well (breast fed)’
‘Over placid. Did not cry much’
‘She was ‘too good’. Extremely placid with no acknowledgement of the world around her’
‘Extremely placid, very good sleeper’
‘Very placid, easily satisfied’
‘Low tone, slow to smile, very placid, sleepy’
‘She was a placid baby with a voracious appetite and a good feeder’

**Code: Floppy**

‘Poor feeder. Very floppy. Cried a lot’
‘She was floppy compared to other children’
‘Until 6 months was unable to support her own head—hypotonic—floppy’
‘She always seemed to have a very floppy tone compared with my first child and did not seem to be making her milestones’
‘Her doctor as the time classed her as a ‘floppy baby’’
‘**** was a very ‘floppy’ baby—however in the first 6 months we just saw this as her being a very cuddly baby’
‘Kind of ‘floppy’ tone’
‘Lots of reflux, otherwise a very content ‘floppy’ baby’
‘She was a little floppy/soft’
For the combined perinatal score, which included data from three questions, R255X had the highest mean score of 1.27 followed by R306C (1.17) and T158M (1.08) and the lowest scores were for R294X (0.43) and R133C (0.45). The differences between R255X and R133C and between R255X and R294X were significant ($P < 0.05, 0.03$). Additionally the mean of the total problem scores for each mutation are also shown (Fig. 1). R255X, T158M and R306C had the highest mean scores (3.00, 2.84 and 2.75) and R294X and R133C had the lowest (1.50, 1.64). The differences between the lowest scoring mutation R294X and the highest two R255X and T158M were just significant ($P < 0.05$). However when the mobility and problem scores were combined the most severely affected was R255X (mean = 10.73) closely followed by R270X (mean = 10.60) with R294X and R306C the least severe (mean = 7.50, 7.91). The differences between R294X and R255X ($P < 0.06$) and R270X ($P < 0.07$) approached significance.

4. Discussion

This study has found that, in general, cases with R255X and R306C mutations were slightly more likely to have problems at birth with appreciable differences between R255X and both R294X and R133C. For the period after birth up to 10 months cases with R255X and R270X mutations were more likely to have problems. Cases with R270X were likely to be least and cases with R306C most mobile at 10 months. When all the scores were combined R255X and R270X were the most severe and R294X the least severe. R306C had the least consistent pattern overall, with relatively high combined perinatal problems but particularly good mobility and thus scoring overall on the mild side.

This is the largest study to date examining the effects of individual mutations in numbers of this magnitude. This has only been possible because we have been able to combine cases from our Australian population-based registry of juvenile Rett syndrome ascertained over a 10 year period [21,22] with cases from the international phenotype database InterRett ascertained so far over only a 6 month period [23]. Rett syndrome is fortunately a rare disorder with an incidence of 1:10,000 females such that in Australia we only expect 12 cases per birth year [21]. Given that T158M, the commonest mutation, only represents less than 10% of Australian Rett Syndrome cases we would only expect to ascertain approximately one case per birth year with the frequency for the other common mutations being even less. With the introduction of online-based questionnaires [23,27] we are able to reach a wider international audience with minimal costs. Therefore, this form of data collection utilised by InterRett provides an efficient mechanism to maximise case numbers for genotyp-phenotype studies in a rare condition, which has multiple

<table>
<thead>
<tr>
<th>Observation/problem</th>
<th>R133C</th>
<th>R294X</th>
<th>R270X</th>
<th>R255X</th>
<th>R270X</th>
<th>T158M</th>
<th>R306C</th>
<th>All cases*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unusual behaviour  &gt; 6-10 months</td>
<td>6(45.4)</td>
<td>9(63.7)</td>
<td>10(62.5)</td>
<td>5(32.8)</td>
<td>16(61.5)</td>
<td>13(65.0)</td>
<td>9(75.0)</td>
<td>81(66.4)</td>
</tr>
<tr>
<td>Development</td>
<td>3(27.3)</td>
<td>5(29.4)</td>
<td>10(55.3)</td>
<td>5(32.8)</td>
<td>14(53.6)</td>
<td>13(65.0)</td>
<td>9(75.0)</td>
<td>74(61.4)</td>
</tr>
<tr>
<td>Floppy</td>
<td>0</td>
<td>1(9.1)</td>
<td>2(11.8)</td>
<td>2(12.5)</td>
<td>1(6.3)</td>
<td>2(10.5)</td>
<td>1(8.3)</td>
<td>6(5.0)</td>
</tr>
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</tr>
</tbody>
</table>

*Denominator used taking into account missing values for individual questions.
different mutations. An additional strength of this and further studies that will follow is that it provides the opportunity for collaboration with other centres such as has already occurred with the Chinese group (XW, XB, HP).

The advantage of studies using the ARSD over other studies has always been the fact that it is population-based and has shown to be effective in representing juvenile Rett syndrome in Australia (population approximately 20 million) [21]. InterRett has the capacity to provide much larger numbers and although we hope that in the future it will contain representative data for many countries it will never fully represent its target population—the world. Similarly, although for our Australian study [3] we were able to use state and national population norms for comparing birthweight and head circumference, this becomes much more difficult in an international multi-ethnic sample and for this reason we did not include such an analysis here.

We acknowledge that our research relies on retrospective parental recall. Nevertheless with a rare condition such as this a prospective epidemiological study involving the period prior to regression is not logistically feasible and hence for information on early development one is dependent on a study design, which uses retrospective report. In studies of this nature there is always concern about whether the information is recalled accurately but more importantly about whether differential recall between groups is biasing the results [28]. Our experience of collecting data from both clinicians and families is that the information provided by families is often more detailed and comprehensive than that provided by clinicians. Also when information is requested of them we note that clinicians will often return to the families to supplement or validate their own data. With respect to differential recall we do not have any evidence to suggest that this is likely to be systematically different between families of children with Rett syndrome whose subsequent outcome is more or less severe because of their mutation type. Whilst the primary purpose of the present study was to compare early development in different categories of children with Rett syndrome, Majnemer and Rosenblatt [29] found little evidence of bias in a case control study of parental recall of developmental milestones in high risk and healthy newborns. In our study we encouraged parents to use the child’s baby book to help answer some of the questions. As this record was made at the time there should be little chance that later outcome would affect their recording of this event and thus their reporting.

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**Box 2. Examples of family expression of unusual behaviour from 6 to 10 months of life**

**Code: Development**

‘Her development was slower than my other two children’
‘Did not sit up and very slow to roll over’
‘No crawling or weight bearing/standing’
‘No attempts to crawl despite being able to sit well from 5.5 months’
‘In between those months rolling around on the floor became less frequent, and moving around in a baby walker began to stop. Also, no crawling or sitting independently eventuated’

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Fig. 1. Mean score for problem and mobility and problem/mobility composite scores for seven common mutations.
The data collection methods have not been entirely uniform for the whole dataset in that all the Australian data were provided by paper-based questionnaires while for the InterRett cohort, the emphasis was on online data collection using the Internet. In future research we plan to investigate differences in data produced by the two approaches but this is beyond the scope of this paper. A proportion (40\%) of the records used were previously presented [3] but the early analysis did not examine the effect of genotype. In other reports [22] we have used four scales – Kerr, Percy, Pineda and WeeFIM to help characterise phenotype. One of the twenty items contributing to the Kerr scale did relate to early development, whilst the Percy scale did not seek information about crawling and the Pineda scale included an item on age at sitting. Age at sitting was not used in this analysis. The nature of our data makes our information on crawling incomplete and so in this previous analysis we had to use surrogate measures [22]. However, in both cases (for the Kerr and Percy scores) these developmental items only contributed ~5–6\% of the total score.

In our previous reports [19,20] we have included X inactivation data and have found no protective effect on the phenotype by the presence of skewed X inactivation when specific mutations have been examined. However more recently, we demonstrated that skewed X inactivation was associated with an overall protective effect (including in cases where no mutation was found) in particular reference to health service utilisation and hospital admissions [30]. In this present study X inactivation data were only available for the Australian cohort and therefore were not included.

Apparently normal pre- and perinatal period and apparently normal psychomotor development through the first six months of life are two of the diagnostic criteria for Rett syndrome set out by Trevathan et al. in 1988 [2]. Despite this the issue of early normality in Rett syndrome has been questioned for many years in case reports [5,7,8,11,31]. These clinical observations were corroborated by our epidemiological studies where we compared birth weight and head circumference in Rett syndrome with population norms and reported on parental perceptions of the perinatal period and early months of life [3]. Much more recently and independently Huppke et al. [4] also reported that birth weight, length and head circumference in a group of 120 mutation positive Rett syndrome cases were significantly lower than in the general population. In 2002 it was suggested that the clinical criteria be revised such that early normality was no longer mandatory [32].

The results of the present analysis with respect to parental recall of the perinatal period and first 6 months of life confirm our previous findings [3]. The proportion reporting any kind of perinatal abnormality (46.4\%) in this study was very similar to the 41\% reported in the previous study. However a higher proportion, nearly 60\% of respondents indicated that they had concerns about their daughter’s behaviour or development in the first 6 months compared with just under half in the original study. Considering that the 108 Australian cases ascertained since 1995 had a younger average age at ascertainment than the original cohort, this could be due to better recall when parents did not have access to baby books. This age differentiation was a phenomenon noted by Charman et al. [6] who also found that normal pre-regression development was the exception and occurred in only 30\% cases. Our findings are also corroborated by the recent work of Burford et al. [33] and Einspieler et al. [34]. In Burford’s study health visitors were asked to review home videos taken during the first year of life in girls who were later diagnosed with Rett syndrome and in a comparison group without developmental problems at 5 years. The nurses were significantly more likely to be alerted to signs of developmental deviation in the Rett syndrome cases again suggesting that the neurodevelopmental aberration in Rett syndrome is present at a much earlier age than has typically been recognised. Einspeler et al. on the other hand conducted a detailed video analysis of movements, posture and behaviour of 22 cases of Rett syndrome during the first 6 months of life. They found that there was an abnormal quality of general movements in all cases and in over half the quality of tongue protrusion, postural stiffness, eye and finger movements were abnormal. Thus our research adds to the accumulating and complementary body of literature which is scrutinising early development in Rett syndrome through a range of methodologies.

One of the four case reports cited by Huppke et al. [4] to illustrate the phenotypic spectrum in Rett syndrome (a child with a C terminal deletion who achieved few milestones) exemplifies well how abnormal early development may be. However, none of these or any studies, of which we are aware, has attempted to examine early development in the context of specific mutations. In fact other than our own [19,20], there has been little analysis of the effects of specific mutations, although Huppke et al. also demonstrated the severity of truncating mutations involving the TRD-NLS [16]. We acknowledge that even with a sample size of 320 cases the statistical power to discriminate between individual mutations, when even the commonest of these will not be responsible for more than one sixth of mutation positive cases (and <9\% of all cases in this study), needs to be improved. Yet the present analysis does show that two of the three mutations with the lowest problem and mobility scores, namely R133C and R294X, were those we had previously identified as having a mild phenotype [19,20]. Conversely the mutations with the highest scores were the R255X and R270X. Thus the pattern we [20] and Huppke et al. [16] had seen in regard to general functioning of individuals with mutations truncating the TRD-NLS was also seen in terms of early development. Also particularly striking was the better mobility with the R294X mutation which was the aspect of the associated phenotype which most contributed to its low composite score. As mentioned the pattern with R306C, a missense mutation located in the TRD after the NLS was more variable with apparently more
problems at birth but good mobility at 10 months. Previously we would have graded R306C as being neither particularly severe or mild although we did find that the onset of regression tended to be a little later on average [20].

The results of this study have important implications from a variety of perspectives. Our findings support the increasing body of literature showing that in general the onset of this disorder is much earlier than originally appreciated. It now becomes evident that those children previously coined congenital Rett syndrome [35], and those subsequently described by Huppke et al.[4] and by ourselves [36] and those diagnosed as newborn encephalopathy [37–39] are a clearly defined part of the phenotypic spectrum. We have now been able to show that the manifestation of this early developmental aberration does seem to be associated with the underlying genotype. These findings also have major implications for the diagnosis and recognition of this disorder. It is clear we have to reverse the entrenched clinical dogma that a diagnosis of Rett syndrome should not be considered unless the child’s early development is normal. Previously and especially before the identification of the association with the MECP2 gene, such children may have remained underdiagnosed. Moreover, the information is particularly relevant to understanding the mechanism of early development in Rett syndrome and determining if and at which time(s) early intervention might be feasible.

This is the first report to have made use of this new source of international Rett syndrome data. However, InterRett is still in its infancy with data collection only commencing in 2003. Despite having comparatively large numbers of subjects, statistical power will be further improved as InterRett expands and population data from countries such as Israel, Spain, Italy, Netherlands, Belgium, Turkey and France are included. Current negotiations with these countries for data contributions will yield in excess of 1000 cases.

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References


