The common *BDNF* polymorphism may be a modifier of disease severity in Rett syndrome

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ABSTRACT

Background: Rett syndrome (RTT) is caused by mutations in the transcriptional repressor methyl CpG-binding protein 2 (*MECP2*). Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor playing a major role in neuronal survival, neurogenesis, and plasticity, and it has been shown that BDNF expression is regulated by MeCP2 through a complex interaction. A common polymorphism of *BDNF* (Val66Met [p.V66M]) has been found to correlate with severity and course of several neuropsychiatric disorders.

Methods: We examined the association between disease severity score, assessed by the modified Percy score, and *BDNF* polymorphism, using regression methods, in 125 mutation-positive patients with RTT from the Australian Rett Syndrome Database and an Israeli cohort.

Results: Those who were heterozygous (Val/Met) had slightly more severe disease than those who were homozygous for the wild-type (Val/Val) *BDNF* polymorphism (increased severity score 2.1, $p = 0.09$). In those with p.R168X, a commonly occurring *MECP2* mutation in RTT, there was a 6-point increase in severity score for those who were heterozygous for the *BDNF* polymorphism, both unadjusted ($p = 0.02$) and adjusted for age ($p = 0.03$). Individuals with the p.R168X mutation and heterozygous for the *BDNF* polymorphism were also at an increased risk of seizure onset (hazard ratio 5.3, 95% confidence interval 1.6–17.7) compared with those homozygous for the wild-type *BDNF* allele.

Conclusions: In addition to mutation type and degree of X-chromosome skewing, the common brain-derived neurotrophic factor (*BDNF*) polymorphism appears to be another genetic modifier of Rett syndrome (RTT) severity. This suggests that BDNF function may play a significant role in the pathogenesis of RTT. ***Neurology*® 2009;72:1242–1247**

GLOSSARY

ARSD = Australian Rett Syndrome Database; **BDNF** = brain-derived neurotrophic factor; **CI** = confidence interval; **HR** = hazard ratios; ***MECP2*** = methyl CpG-binding protein 2; **Met** = methionine; **NAA** = *N*-acetylaspartate; **RTT** = Rett syndrome; **Val** = valine.

Rett syndrome (RTT) is an X-linked dominant postnatal neurodevelopmental disorder primarily caused by mutations in the methyl CpG-binding protein 2 (*MECP2*) gene.[1] MeCP2 protein may act as either a transcriptional repressor or activator depending on the target gene with which it associates.[2,3] The severity of the RTT phenotype varies considerably depending on the *MECP2* mutation type and location.[4-8] The degree of X chromosome inactivation skewing has also been shown to affect phenotypic variability in *Mecp2*-null mice,[9] and in RTT females.[10] However, these two mechanisms only partially explain this variability.

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Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that plays a major role in neuronal survival, neurogenesis, and neuronal plasticity.[11-14] It has been identified as a MeCP2 target through a candidate gene approach,[15,16] and abnormalities in BDNF homeostasis contribute to the neurologic phenotype in *Mecp2*-null mice.[3,17]

A relatively common single nucleotide polymorphism in the *BDNF* gene is a substitution of valine (Val) with methionine (Met) at codon 66 (p.V66M). This substitution is believed to disrupt folding, dimerization, and intracellular trafficking of the protein,[12,18] decreased gray matter volume,[19] and decreased dendritic arborization with neuronal loss.[20] In the US population, the frequency of the Val/Val, Val/Met, and Met/Met genotypes is 70, 25, and 5%.[12] Various studies have shown a relationship between the polymorphism type and the severity of clinical and imaging features in healthy subjects and in different neuropsychiatric and neurologic disorders.[12,21-28]

Because of the suspected role of BDNF in RTT pathogenesis, the dramatic wide-scale effect of BDNF in the CNS in general, the relative disadvantage of the p.V66M variant in culture, and clinical studies in other diseases, we decided to investigate the relationship between the presence of this *BDNF* variant and RTT clinical severity. In order to overcome the variability of severity between mutations, we also examined separately those patients with the two most common mutations, p.T158 M and p.R168X.

METHODS Data for this study, based on RTT subjects with a confirmed *MECP2* mutation, were ascertained from two sources. These were 1) cases in the Australian Rett Syndrome Database (ARSD) on whom DNA samples had been stored in the Westmead laboratory and available for analyses of BDNF polymorphisms and 2) cases seen at the Sheba Medical Centre, Israel. The study was approved by the Ethics Committees of the Princess Margaret Hospital, Western Australia, and the Sheba Medical Centre. Parental consent was provided for all subjects. Cases in the ARSD (n = 131) represent 42.8% of all cases known to the ARSD in December 2006. The ARSD is a population-based database of RTT subjects born since 1976,[29] so that ages ranged from 2.9 to 28.9 years at the 2004 follow-up. Israeli cases were identified from those cases seen at Sheba Medical Centre, with ages ranging from 3.5–42 years.

Clinical severity was assessed using what we have coined the Percy scale,[6,29] which takes into account early developmental characteristics as well as current clinical features and has been shown to be an appropriate measure. Severity scales provide quantitative estimates of clinical severity. Each scale is a summation of individual items related to RTT characteristics, which are graded on a discrete scale based on their specific severity or degree of abnormality, with the highest level corresponding to the most severe or abnormal presentation. We chose to use the Percy scale, which has 15 items with maximum possible score of 45, because of its reasonable balance between current functioning and developmental characteristics.[29] Data used to determine the scores were derived from information for ARSD cases that were provided to the ARSD in the 2004 follow-up questionnaire and coded for previous analyses.[30] Information for Israeli cases was extracted from case records and coded in the same way as the Australian cases. Age at onset of seizures (if present, or age at data collection if seizures were not present) was recorded for cases in both cohorts.

The *BDNF* polymorphism (p.V66M) was genotyped using TaqMan® SNP Genotyping Assays (assay ID c_11592758_10, Applied Biosystems) according to the manufacturer's protocol.

Statistical analysis was performed on the combined cohort and relevant subsets using Stata version 9. Comparison of groups was done using analysis of variance or the Kruskal-Wallis test where normality assumptions did not hold. Regression models were linear with dummy variables for categorical factors (including *BDNF* polymorphism and cohort). The effect of *BDNF* polymorphism on clinical severity using the Percy score was assessed for all cases with and without adjusting for age as a continuous variable. The relative risk of seizures was estimated using Cox regression with age as the underlying time variable. Subjects were followed up from birth to their age of first seizure, or their age at data collection, if they had experienced no seizures. Results are presented as hazard ratios (HR) and seizure-free survival curves were plotted using Kaplan-Meier estimates. The Mann-Whitney test was used to compare median ages of seizure onset (in those who had seizures) for the BDNF polymorphisms. Analyses were repeated separately for each of the two *MECP2* mutation groups, p.T158M and p.R168X.

RESULTS There were a total of 182 cases in the study with 72.0% (131) from ARSD and 28.0% (51) from Israel. The mean age at data collection for the Australian cases (15.9) was greater than for the Israeli cases (13.8) ($p = 0.02$). Eight common mutations were found in 30/46 Israeli cases and 97/131 Australian cases, with the commonest mutations being p.T158M (15.8%) and p.R168X (11.9%) (table 1). Severity scores were available for 118/131 ARSD cases and 45/51 Israeli cases. The mean severity scores for Australian cases (25.3) and Israeli cases (27.2) were similar ($p = 0.11$). The overall mean Percy score was 25.8 and ranged from 8 to 43. There was a slight increase in severity by age group from 24.6 to 26.3, although severity was greatest in those aged 16–21 years (figure 1). Of the 178 cases with known seizure status, 136 (76.4%) had commenced seizures. The median age at onset for the entire cohort (using Kaplan-Meier estimates) was 5 years (95% confidence interval [CI] 4.5–6 years). Seizures were present in 82.8% of Australian cases compared with only 60% of Israeli cases ($p = 0.04$). However,

Table 1 Distribution of *MECP2* mutations by case source

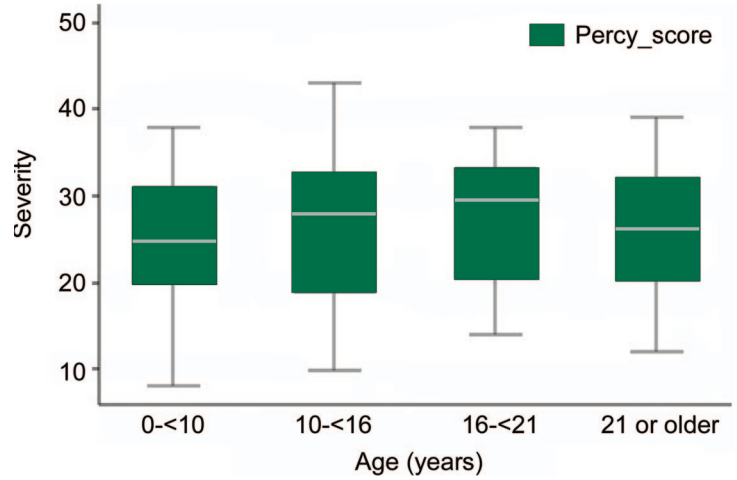
Mutation	Cases (%)	Israeli cases (%)	ARSD cases (%)
p.R106W	5 (2.8)	2 (4.3)	3 (2.3)
p.R133C	13 (7.3)	4 (8.7)	9 (6.9)
p.R168X	21 (11.9)	5 (10.9)	16 (12.2)
p.R255X	14 (7.9)	6 (13.0)	8 (6.1)
p.R270X	14 (7.9)	2 (4.3)	12 (9.2)
p.R294X	13 (7.3)	1 (2.2)	12 (9.2)
p.R306C	9 (5.1)	2 (4.3)	7 (5.3)
p.T158M	28 (15.8)	8 (17.4)	20 (15.3)
C-terminal deletions	18 (10.2)	7 (15.2)	11 (8.4)
Early truncating mutations	7 (4.0)	2 (4.3)	5 (3.8)
Large deletions	9 (5.1)	1 (2.2)	8 (6.1)
Other	26 (14.7)	6 (13.0)	20 (15.3)
Total	177 (5 cases missing)	46	131

Overall: 46 Israeli cases and 131 Australian Rett Syndrome Database (ARSD) cases.

in those cases with seizures, the median ages at diagnosis in Israeli (3 years) and Australian (4 years) cases were similar ($p = 0.63$).

For *BDNF*, 57.2% were homozygous for the wild-type (Val/Val) allele, 37.6% were heterozygous (Val/Met), and 5.2% were homozygous for the mutated (Met/Met) allele, demonstrating Hardy-Weinberg equilibrium ($p = 0.84$). Since only a small number of cases were homozygous for the Met/Met allele (4 from the Israeli cohort and 5 from the ARSD cohort), they were excluded from the severity and seizure comparisons. Overall, those heterozygous for the *BDNF* polymorphism were slightly more severe than those who were homozygous for the wild type (increased severity 2.1, $p = 0.09$). When severity was examined for those with the p.R168X mutation (n = 19 with scores), severity was associated with a 6-point increase in the severity score among heterozygotes for the *BDNF* polymorphism, both unadjusted ($p = 0.02$) and adjusted for age ($p = 0.03$). No such difference was seen with the p.T158M mutation (n = 23 with scores) (table 2, figure 2). Risk of seizure onset, after accounting for cohort effects, was not significantly affected by *BDNF* polymorphism (HR when heterozygous for the *BDNF* polymorphism 1.2, p value = 0.24, 95% CI 0.9–1.8). The median age at onset of seizures, in cases with seizures, was similar for heterozygous (4.2 years) and wild-type (3.5 years) cases ($p = 0.53$). However, for cases with the p.R168X mutation, heterozygous cases had a significantly increased risk of seizure onset (HR 5.3, p value 0.006, 95% CI 1.6–17.7), and had an earlier age at seizure onset (median = 2 years), than those who were homozygous for the wild-type *BDNF* allele (median = 7 years) (figure 3). Of the nine cases homozygous for the mutant *BDNF* allele who were not included in this analysis, six had commenced seizures (median age at onset 5 years) and three had not.

Figure 1 Severity by age group



DISCUSSION Among all these Rett patients, those who were heterozygous for the p.V66M variant were slightly more severe than those homozygous for the wild-type (Val/Val) *BDNF* allele. Patients with the p.R168X mutation who were heterozygous for the *BDNF* allele had a 6-point increase in the Percy severity score, as well as a fivefold increase in risk of seizures, after adjusting for age. Neither of these effects was seen in patients with the p.T158M mutation. The distribution of the *BDNF* polymorphism variant was found to be similar to what has been previously reported in the literature in Caucasian populations[12] including a cohort of patients with Rett syndrome.[31]

Both the type of mutation and degree of skewing of X-chromosome inactivation have an effect on RTT severity, including pattern of seizure onset[32] and frequency.[33] There were some differences in the distribution of mutations between the two case sources, e.g., the p.R270X mutation was more common in the Australian cohort, and C-terminal deletions were more common in the Israeli cohort. The Australian cohort had also experienced more seizures. The Australian cohort was population-based, including older patients and those who had died, in whom the prevalence of seizures was likely to have been higher, so it is perhaps not surprising that the seizure prevalence was higher in the Australian than Israeli cohort. However, paroxysmal nonepileptic events, which mimic epilepsy, also occur in RTT and differentiating between epileptiform and nonepileptiform events can be difficult without video-electroencephalographic monitoring.[34] This was not feasible in the Australian cohort. Therefore we, like others,[35] re-

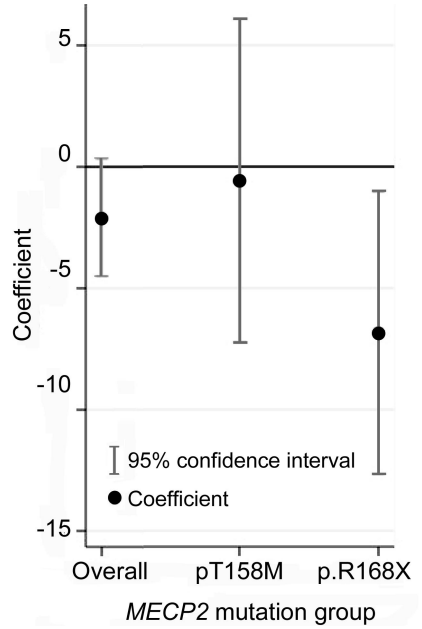
Table 2 Severity and brain-derived neurotrophic factor polymorphism

Model	All cases, heterozygous (Val/Met)	p.R168X cases, heterozygous (Val/Met)	p.T158M cases, heterozygous (Val/Met)
No adjustment			
Coefficient*	2.1	6.8	0.6
p Value	0.09	0.02	0.86
95% CI	−0.3, 4.5	1.0, 12.6	−6.1, 7.2
Age adjusted			
Coefficient*	2.3	6.8	−0.632
p Value	0.07	0.03	0.86
95% CI	−0.1, 4.8	0.8, 12.8	−8.0, 6.8
Cohort adjusted†			
Coefficient*	1.8	6.5	0.8
p Value	0.15	0.04	0.81
95% CI	−0.6, 4.2	0.4, 12.5	−5.9, 7.5
Age and cohort adjusted			
Coefficient*	2.0	6.4	0.3
p Value	0.11	0.05	0.94
95% CI	−0.5, 4.4	0.1, 12.7	−7.2, 7.8

*Change in Percy score for heterozygotes compared with wild type (Val/Val).
†Israel = 0, Australian Rett Syndrome Database = 1.
Val = valine; Met = methionine; CI = confidence interval.

lied on parental report of seizures for this cohort and accepted their assessment that events were epileptic. This could be further contributing to the difference in epilepsy diagnosis between the two countries. However, adjusting for the cohort effect in our analysis should have ensured that this difference did not affect the relationship with the BDNF polymorphism.

Figure 2 Change in Percy score for the brain-derived neurotrophic factor polymorphism compared with wild-type (valine/valine) brain-derived neurotrophic factor



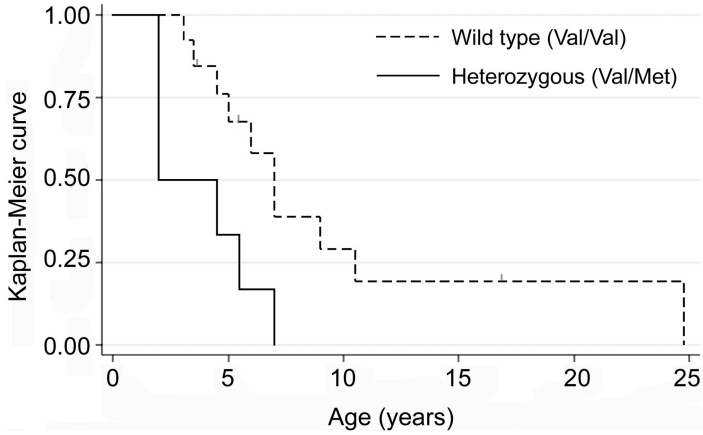
Information on the X-inactivation skewing was not available for the majority of our cohort, so that it could not be used as a variable in this analysis. This missing information could affect our results in both directions, especially as we have previously demonstrated the contribution of X-inactivation to the variability of phenotype in these specific mutations.[10] Despite this shortcoming, the fact that we did find a relationship between the p.V66M polymorphism and severity, in keeping with the direction we hypothesized, is consistent with previous studies in normal individuals, and other neuropsychiatric and neurologic diseases. This would suggest a role for BDNF activity in the pathogenesis of RTT. This role may be nonspecific, as suggested in the other diseases, and may relate to the general role of BDNF in neuronal survival, and plasticity. It could also be explained by an as yet untested possibility that the two proteins (MeCP2 and BDNF) have certain overlapping effects. In addition, it could be that MeCP2 dysfunction reduces overall neuronal activity, thereby indirectly resulting in decreased BDNF,[36] further accentuating possible adverse effects of a less efficient BDNF protein variant. On the other hand, it could also suggest a direct role of MeCP2 protein in *BDNF* gene expression, and as a consequence a significant role of Bdnf/BDNF protein activity in the pathogenesis of RTT in both the mouse model and the human.

In mice, BDNF has distinct effects if depleted during early development, including extreme impairment in learning and memory compared with depletion only in the adult brain, which results in diminished long-term potentiation in the hippocampus, a process believed to be the cellular mechanism of learning and memory. There is also a gender-specific effect. BDNF-deficient males are hyperactive but show no depression-related behaviors, whereas BDNF-deficient females show no motor changes but express depression-related behaviors.[37]

The existence of two distinct possible mechanisms by which BDNF may affect function in the developing and mature brain may suggest a different role and impact in infants and young children with evolving RTT and in adolescent and adult RTT patients. Investigating the effect of the common *BDNF* polymorphism on different aspects of the RTT phenotype which are age related would be worthwhile but would require a much larger dataset.

In a recent publication,[31] the effect of the common *BDNF* functional polymorphism was investigated in a cohort of 81 girls with various mutations looking at both a general score (using the Kerr scor-

Figure 3 Kaplan-Meier curve for age at onset of seizures for cases with p.R168X (with censor points marked as small gray lines)



ing system[38]) and also on 14 different clinical features separately (combining all mutations together). Although the authors did not find a significant relationship with the overall severity score, they did find that the *BDNF* genotype distribution tended to be different in the areas of hand skills and age at seizure onset (defined simply as a categorical variable, with early onset described as before 2 years of age). They suggested that the polymorphism might even be protective against seizures. In contrast to their findings, and consistent with our hypothesis regarding the relative dysfunction of the p.V66M polymorphism, we found that age at onset of seizures was earlier in those heterozygous for the *BDNF* polymorphism, particularly for people with the p.R168X mutation. We did not categorize age at seizure onset but instead we retained the actual ages at onset or ages at data collection in people without seizures and used survival analysis to take account of censoring. We also found that overall severity was greater in those who were heterozygous for the p.V66M allele compared with those with the wild-type *BDNF* sequence, particularly in those individuals with the p.R168X *MECP2* mutation. It may be that both the earlier age at seizure onset and the additional severity we find with the Val/Met variant in subjects with the p.R168X mutation are manifestations relating to the general role of BDNF expression in RTT pathogenesis. Further research could investigate the effect of the common *BDNF* polymorphism on specific phenotypic characteristics, such as the degree and type of breathing abnormality and other abnormalities of autonomic function, based on the specific role on these functions of BDNF found in brainstem of the *Mecp2*-null mice.[39]

The exact role of BDNF in brain development and maturation and its contribution to the pathogenesis of RTT remain unclear, even in face of new data demonstrating that BDNF expression is specifically reduced in the *Mecp2*-null mouse model.[3] However, we have been able to show in a large Australian/Israeli combined RTT dataset that the presence of the common *BDNF* functional polymorphism appears to confer some mutation-specific additional severity and increase the risk of seizure onset, particularly in those with the p.R168X mutation. Further international collaborative studies combining national data from individual countries (as already done through the infrastructure of an international Rett syndrome database[7]) and examining specific phenotypic and age-related features could be beneficial.

AUTHOR CONTRIBUTIONS

Statistical analysis was performed by Ami Bebbington, assisted by Nick de Klerk.

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