Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome

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Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder mainly affecting females and is associated with mutations in *MECP2*, the gene encoding methyl CpG-binding protein 2. Mouse models suggest that recombinant human insulin-like growth factor 1 (IGF-1) (rhIGF1) (mecasermin) may improve many clinical features. We evaluated the safety, tolerability, and pharmacokinetic profiles of IGF-1 in 12 girls with *MECP2* mutations (9 with RTT). In addition, we performed a preliminary assessment of efficacy using automated cardiorespiratory measures, EEG, a set of RTT-oriented clinical assessments, and two standardized behavioral questionnaires. This phase 1 trial included a 4-wk multiple ascending dose (MAD) (40–120 μg/kg twice daily) period and a 20-wk open-label extension (OLE) at the maximum dose. Twelve subjects completed the MAD and 10 the entire study, without evidence of hypoglycemia or serious adverse events. Mecasermin reached the CNS compartment as evidenced by the increase in cerebrospinal fluid IGF-1 levels at the end of the MAD. The drug followed nonlinear kinetics, with greater distribution in the peripheral compartment. Cardiorespiratory measures showed that apnea improved during the OLE. Some neurobehavioral parameters, specifically measures of anxiety and mood also improved during the OLE. These improvements in mood and anxiety scores were supported by reversal of right frontal alpha band asymmetry on EEG, an index of anxiety and depression. Our data indicate that IGF-1 is safe and well tolerated in girls with RTT and, as demonstrated in preclinical studies, ameliorates certain breathing and behavioral abnormalities.

Rett syndrome (RTT), the second most common cause of severe intellectual disability in females, is associated in the majority of cases with mutations in *MECP2*, a gene on Xq28 that encodes the transcriptional regulator methyl CpG-binding protein 2 (1). The disorder is characterized by apparent normal early development followed by subsequent psychomotor regression in early childhood, affecting predominantly language and purposeful hand skills (1–3). Gait impairment and stereotypic hand movements are the other two main diagnostic criteria. Other common features, some of which are considered supportive diagnostic criteria, include growth retardation, breathing disturbances, seizures, and behavioral abnormalities (1). Current RTT treatments are focused on managing neurological symptoms (e.g., seizures, anxiety) and medical comorbidities (e.g., constipation, scoliosis), but have had limited success (4).

Initial drug trials for RTT, including two randomized placebo-controlled trials, were based on neurobiological aspects of the disorder derived from pathological and laboratory studies of affected individuals (4, 5). The identification of *MECP2* mutations, which cause a defect in synaptic maturation and maintenance (6), as the etiology of most cases of RTT, represented a major breakthrough for the development of new treatments. The creation of experimental models of the disorder led to the identification of downstream therapeutic strategies (4). Substantial reversal of mouse model neurologic phenotypes by genetic manipulations, at different developmental stages (7, 8), has supported the testing of several candidate drugs (4, 9). A particularly attractive candidate drug is recombinant human insulin-like growth factor 1 (rhIGF-1) (IGF-1). IGF-1 is one of the most potent activators of the AKT signaling pathway and may potentiate the function of brain-derived neurotrophic factor, a key target of MeCP2's transcriptional regulation (10). There is also evidence that MeCP2 regulates the expression of IGF-binding protein 3

Significance

This paper provides unique insights into mechanism-based therapeutics for Rett syndrome (RTT), a devastating neurodevelopmental disorder. This clinical trial was based on pioneer preclinical work from the laboratory of M.S. Outcome measures include clinical instruments, standardized behavioral measures, and biomarkers, the latter being not only objective but also applicable to experimental studies. We believe this work will a have major impact on the understanding and treatment of RTT, as well as other neurodevelopmental disorders.

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(IGFBP3), a major IGF-1-binding factor that is increased in brains of RTT patients and Mecp2-null mice (11). Furthermore, administration of IGF-1 restores dendritic spine dynamics in Mecp2-deficient mice (12). The most compelling data supporting IGF-1 as a treatment for RTT come from two studies demonstrating that systemic administration of either full length IGF-1 or its active peptide fragment reverses, at least partially, many RTT-relevant features in Mecp2-deficient mice (13, 14). Among the latter are locomotor function impairment, breathing abnormalities, and heart rate irregularities. These improvements seem to reflect IGF-1's effect on defective synaptic maturation and maintenance secondary to Mecp2 deficit (14).

Mecasermin, recombinant human IGF-1, is already Food and Drug Administration-approved for the long-term treatment of growth failure in children with severe primary IGF-I deficiency (Laron syndrome) (15). We carried out a multiple ascending dose (MAD) study followed by an open-label extension (OLE) period with mecasermin in a group of 12 girls with *MECP2* mutations, 9 of whom had RTT. Here, we report our findings on safety, tolerability, pharmacokinetics (PK), and preliminary assessments of efficacy. The latter include evaluations of neurobehavioral measures, exploratory biomarkers, and their corresponding pharmacodynamics (PD) data.

Results

Twelve girls with *MECP2* mutations participated in the 4-wk MAD; 10 of them continued and completed the subsequent 20-wk OLE. Fig. S1 illustrates the timeline of this phase 1 trial. Participants' demographic and baseline characteristics are shown in Table 1. Nine subjects met full diagnostic criteria for RTT and all continued in the OLE. The 4-wk MAD focused on obtaining PK data, determining cerebrospinal fluid (CSF) penetration, initial evaluations of safety and tolerability, and estimating feasibility of automated cardiorespiratory measures as biomarkers for treatment response. The OLE was designed to obtain additional information on safety, tolerability, and the aforementioned cardiorespiratory measures after chronic dosing, as well as preliminary data on neurologic and behavioral parameters of clinical relevance to RTT. These neurobehavioral evaluations were based on questionnaires and assessments used in an ongoing multisite longitudinal study [the Rett Natural History study (U54 HD061222)] and on two standardized measures of problem behaviors. Data on safety, tolerability, and PK is reported for all 12 *MECP2* mutation-positive subjects, whereas preliminary efficacy and PD data only for the 9 subjects with RTT.

During the MAD, mecasermin dosing was escalated over a 4-wk period, beginning with twice daily (BID) injections of 40 μg/kg the first week, 80 μg/kg the second week, and 120 μg/kg during the third and fourth weeks, as depicted in Fig. S2. CSF samples were obtained before drug administration and after completing the fourth week (Fig. S2). Fig. 1 illustrates levels of IGF-1 and IGFBP3, the main IGF-1-binding protein (10, 11), in serum and CSF. There was a significant increase in IGF-1 but not IGFBP3 in both compartments at the end of the MAD. At the start of the OLE, subjects went through an identical dose escalation, staying on the maximum dose of 120 μg/kg for the remaining 17 wk of treatment.

Serum IGF-1 concentrations were first analyzed by a noncompartmental analysis (16) comparing different doses. A log-linear terminal phase was observed after 4–6 h postdosing (Fig. 2*A*). The slopes of this decay allowed the estimation of terminal elimination half-lives ($t_{1/2,\lambda}$) and mean residence times in the body (MRT_b) as shown in Table S1. Maximal concentrations (C_{max}) and the times to reach them (t_{max}) were also documented. The areas under the curve (AUC_t; t, time of last observation) up to the last observation lacked dose proportionality, suggesting nonlinear kinetics (Fig. 2*B*). The starting dose of 40 μg/kg elicited a mean AUC_t = 2,050 ng·h/mL, while the area for twice that dose increased by just 75%. When the starting dose was tripled, the increment was nearly the same. Nonlinearity is also supported by the early parts of the concentrations profiles, with upward deviations after reaching maximum levels. We also carried out a compartmental analysis using a two-compartment model based on calculated Akaike and Bayesian information criteria, as well as on the residuals analysis (16). A Michaelis–Menten elimination kinetics (16) with first order absorption and a distribution clearance parameterization provided the best goodness of fit, compared with first order or mixed elimination alternatives. The volume of distribution for the central compartment (V_1/F) was estimated to be 7.71 ± 0.78 L (mean ± SE) and for the peripheral compartment it was 33.5 ± 16 L. The other parameters in the model were estimated for intercompartmental clearance as 0.38 ± 0.048 L/h, for the maximum elimination rate as 1.02 ± 0.4 $\mu g \cdot kg^{-1} \cdot h^{-1}$, and for the Michaelis–Menten constant as 4.62 ± 3.7 ng/mL. Individual subjects' noncompartmental curves are depicted in Fig. S3 and Fig. S4 demonstrates the appropriateness of the proposed models (i.e., predicted vs. observed grouped data).

Based on direct compliance monitoring and serum levels, s.c. injections were well tolerated and no incidences of hypoglycemia or errors in dose administration were detected. During the MAD, one serious adverse event occurred (respiratory distress) and was determined to be unrelated to the study drug (10, 15). During the MAD, only two adverse events (nausea and vomiting) were considered as probably related to the study drug and preceded withdrawal from the OLE. A similar profile of safety and

Table 1. Subject demographics and characteristics at baseline

Age, y	Diagnosis	Stage	*MECP2* mutation	Concomitant medications	Breathing phenotype
3	Classic	II	R168X	None	None
7	MRD*	n/a†	C1135_1142 del	None	None
7	MRD*	n/a†	C1135_1142 del	None	None
2	Classic	II	C790_808 del	Levetiracetam	BH, HV, AE
5	Classic	III	Large del exon 3 and 4	None	BH, HV
4	Classic	III	C1159_1273 del	None	AE‡
8	Classic	III	R255X	Lamotrigine, lorazepam, melatonin	BH, AE§
4	Classic	III	R255X	None	AE
8	Classic	III	T158M	None	BH, AE§
3	MRD	n/a†	R306C	None	None
10	Classic	III	Large del exon 1 and 2	Gabapentin, diastat	BH, AE, cyanosis§
8	Classic	III	P322L	Levetiracetam	BH, AE, cyanosis§

AE, air expulsion; BH, breath holding; HV, hyperventilation.
*Subjects did not continue in OLE.
†Staging not applicable (n/a) to non-RTT.
‡Subject with mild apnea (apneic episodes >10 s and <5 apneas per hour).
§Subjects with moderate–severe apnea (apneic episodes >10 s and >5 apneas per hour).

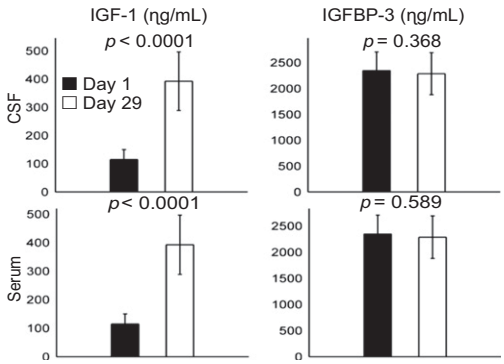

Fig. 1. IGF-1 and IGFBP-3 levels in CSF and serum pre- and post-MAD. The Mean and SE of IGF-1 and IGFBP-3 in serum and CSF are shown (*P* values based on Student's *t* test). CSF and serum samples were obtained before IGF-1 administration on day 1 and 1–2 h after dose on day 29 ($n = 12$). Levels of IGF-1 in serum and CSF more than doubled, indicating IGF-1 reaches the CNS compartment. IGFBP3, the main IGF-1-binding protein, did not significantly increase in serum or CSF.

tolerability, with no unexpected, progressive, or related serious adverse events, was observed during the OLE. Although a high proportion of the subjects had abnormal cholesterol levels at baseline, these did not worsen during the trial. For details on adverse events, see Table S2.

Using cardiorespiratory data obtained with a BioRadio device (17), we calculated the apnea (18) and hyperventilation (19) indices and compared the start and end of the MAD (pre- to post-MAD), start and end of the OLE (pre- to post-OLE), and beginning and end of the entire trial (pre-MAD to post-OLE). We applied paired *t* tests, Wilcoxon signed rank tests, and a random intercept (RI) model, illustrating time effects at each time point (post-MAD, pre-OLE, and post-OLE compared with pre-MAD). As illustrated in Table 2 (see "apnea index by time point" entries), based on the RI model which accounts for within-subjects correlation, the improvement in the apnea index was significant at the end of the OLE in comparison with start of the MAD. Improvements in the apnea index were comparable when only the five subjects with clinically significant apnea (apneic episodes >10 s), four of whom had moderate–severe

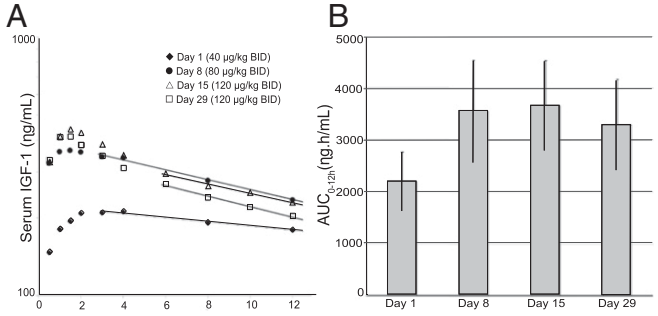

Fig. 2. (*A*) Serum IGF-1 concentrations show a log-linear terminal phase 4–6 h after dosing. Serum IGF-1 concentrations were analyzed by a noncompartmental analysis comparing escalating doses at days 1, 8, 15, and 29. A log-linear terminal phase was observed after 4–6 h postdosing. The slopes of decay allowed the estimation of $t_{1/2,\lambda}$ and MRT_b are described in Table S1. (*B*) As shown, the mean and SE of the AUC_t of IGF-1 suggests nonlinear kinetics. The AUC_t up to the last observation lacked dose proportionality, suggesting a nonlinear kinetics. The lowest dose of 40 µg/kg BID dose elicited a mean AUC_t = 2,050 ng·h/mL whereas the area for twice that dose (80 µg/kg BID) incremented just about 75%. When the lowest dose was tripled (120 µg/kg BID) at day 15, the increment was nearly the same. The Mean and SE of the AUC_t in serum and CSF are shown.

apnea, were included in the analyses (Table S3). Fig. S5 depicts the trajectories of the apnea index for all subjects. In addition, despite the small sample, we tested the effect of age as a covariate in the RI model for all nine subjects with RTT. The effect of age and its interaction with the respective time points was positive and significant, namely the improvements in the apnea index were more significant in older subjects. These patterns of improvement were not observed for the hyperventilation index (see "hyperventilation index by time point" entries in Table 2). The specificity of the apnea index improvements are underscored when other respiratory parameters (20), typically not used in the clinical context, are examined. Table S4 shows that during the OLE, for instance, the percent epoch in slow respiratory rate and the mean total respiratory cycle times (Ttot) in slow respiration also decreased significantly but not the percent epoch in rapid respiratory rate and the mean Ttot in rapid respiration. Similar results were found in the MAD. There were also changes in the cardiac parameters, namely a reduction in the percent epoch in normal heart rate with a concurrent increase in the percent epoch in rapid heart rate when the beginning and end of the OLE were compared. Variance in heart rate also decreased, although not significantly (Table S4). Similar to the breathing parameters, changes in cardiac variables demonstrated the same trend during the shorter MAD and the longer OLE. Preliminary PD analyses indicate a positive response, namely a decrease in the apnea index, over the course of treatment. Just in a few cases this decrease leveled off or, in one case, seemed to revert at the end of the MAD (Fig. S6 illustrates examples of different PD profiles).

During the OLE, preliminary efficacy data were gathered by administering two RTT-oriented clinician assessments and two standardized behavioral measures to the nine RTT subjects. We focused on established instruments already reported in the literature (21–25), and did not include parent or clinician global impression assessments, to decrease data subjectivity and allow for future comparisons with other publications. Neurologic and behavioral parameters were measured by two evaluations from the Rett Natural History study (21), as well as the Rett Syndrome Behavioral Questionnaire (RSBQ) (22, 23) and the Anxiety Depression and Mood Scale (ADAMS) (24, 25). We performed exploratory comparisons between onset and end of the OLE using *t* tests and the Wilcoxon signed rank test. Although not significantly different, total scores showed a trend toward improvement in all instruments. We then organized the subscales of these measures into neurobehavioral domains (e.g., motor, breathing/autonomic, problem behavior) and subjected them to exploratory *t* tests comparing pre- to post-OLE. We followed these hierarchical analyses by examining the items in the same subscales. These analyses revealed significant or trend-level changes in the breathing/autonomic and behavioral domains. However, the direction of change in breathing and peripheral autonomic subscales were inconsistent. For instance, breath-holding items in the RSBQ showed improvement whereas those on the clinical assessment (CA) and motor–behavioral assessment (MBA) worsened. Similar inconsistencies were present for peripheral autonomic scales/items. Subscales and items representing alertness, activity, anxious behaviors, or abnormal mood demonstrated consistent improvements, whereas those recording irritability, aggressiveness, disruptive/hyperactive behavior, communication, and motor domains did not (Table 3 and Fig. S7).

Relative right-sided resting frontal (alpha band) EEG asymmetry has been used in multiple studies as an index of anxiety and depression (26), including pediatric populations (27). Left (L) greater than right (R) alpha power is typically interpreted as more positive vs. negative (less anxious vs. more anxious) behavior, whereas R > L is viewed as the reverse. As depicted in Fig. 3, six subjects evaluated during the OLE with EEG demonstrated R > L asymmetry (i.e., more anxious). Although the degree of asymmetry was variable, five of the six showed a decrease in the asymmetry index and in three it was reversed. A paired-samples *t* test revealed that this group trend toward L > R asymmetry (i.e., reduction in anxiety) was significant. Moreover,

the group reduction in the R > L asymmetry index correlated with improvements in measures of mood abnormalities and, to lesser extent in measures of breathing abnormalities and anxiety (Table S5). Analyses of cardiorespiratory and neurobehavioral parameters excluding the two individuals in Hagberg stage II (i.e., end of regression period) did not yield significantly different results from those including all nine RTT subjects.

Discussion

Our findings indicate that IGF-1 is safe for use in girls with *MECP2* mutations, including those meeting diagnostic criteria for RTT. We found that mecasermin reaches the CNS and that its kinetics are complex, as expected from a protein that is cleaved and binds its receptor and interacting proteins (10, 11, 28, 29). Our preliminary efficacy analyses suggest that, when administered over several weeks, mecasermin improved certain aspects of the RTT phenotype, most notably, abnormal behaviors (i.e., anxiety) and breathing abnormalities (i.e., apnea). Changes in breathing abnormalities were better characterized using automated measurements of cardiorespiratory function. The evaluation of potential biomarkers also successfully delineated behavioral abnormalities with right-sided frontal alpha band EEG asymmetry, an index of anxiety and depression, showing a trend toward reversal in most RTT subjects exhibiting the phenomenon. Overall, the findings of this phase 1 trial are in agreement with preclinical data suggesting IGF-1 is a safe and beneficial treatment of RTT (13, 14).

As recently reported by Pini et al. (30), mecasermin administration is relatively safe and well tolerated. In our own phase 1 study, several expected adverse events, such as increased tonsil size and related snoring, were observed but were relatively mild and nonprogressive and did not lead to withdrawal from the trial. Most subjects had elevated cholesterol; however, this preceded IGF-1 administration and did not worsen with the drug. Therefore, concerns about metabolic syndrome raised by a recent animal study (31) were not supported by our trial. The most common adverse event, early signs of puberty, may be significant as some reports have shown accelerated puberty (i.e., early adrenarche) in RTT (32, 33). Nonetheless, because hormonal levels were within normal ranges throughout the study, this issue deserves further investigation. In summary, at the doses used in this (240 $\mu g \cdot kg^{-1} \cdot d^{-1}$) and the previously published (200 $\mu g \cdot kg^{-1} \cdot d^{-1}$) trial (30), mecasermin is a safe treatment.

Our data indicate that mecasermin administration increases IGF-1 levels in the CNS (10); therefore, our data on efficacy and some of the adverse events could be attributed to the presence of IGF-1 in the brain. The IGF-1 increase in CSF depicted in Fig. 1 is comparable to the one in positive responders to fluoxetine (34) or adrenocorticotropic hormone (35). The levels of IGFBP3, the main IGF-1-binding protein (10), were unaffected by the increase in IGF-1. This suggests that increased IGFBP3 as a mechanism underlying RTT pathophysiology (11) is not corrected by mecasermin, at least at the dosages used in this trial.

Despite their normal serum levels, our subjects exhibited a nonlinear PK profile of IGF-1 (36). This is not unexpected for a protein with complex regulation, mechanism of action, and pleiotropic effects (10, 37, 38). Recent data demonstrating activation of different signaling pathway by full length IGF-1 and its active breakdown product (29) highlights this issue. The dose used in this study, 240 $\mu g \cdot kg^{-1} \cdot d^{-1}$, was selected based on the investigational medicinal product's current approved labeling and its efficacy in preclinical studies (13, 14). Several PK parameters in our study (e.g., C_{max}, $t_{1/2}$, t_{max}) and their changes with increasing doses of mecasermin (C_{max}) are comparable to those found in healthy volunteers and children with primary IGF-1 deficiency (39). As we observed, chronic treatment PK studies have suggested a plateau effect for doses between 160–240 $\mu g \cdot kg^{-1} \cdot d^{-1}$, probably reflecting saturation of IGFBP3 (38). The nonlinear PK kinetics, greater volume distribution in the peripheral than the central compartment, and lack of change in IGFBP3 in serum and CSF suggest that serum levels of IGF-1 for may not be the best basis for dosing and that higher or chronic dosing in RTT may not necessarily result in higher exposures or a sustained exposure–response relationship. This leads to careful consideration of dosing for future studies where acute intermittent pulses of mecasermin may be more effective than chronic dosing. The mouse model may be useful in exploring optimum dosing regimens.

Our study confirmed the feasibility of automated cardiorespiratory measurements as biomarkers of treatment response (40). It also indicates that these breathing evaluations may be more reliable and valid than clinical instruments because parent questionnaire data were in disagreement with clinicians' observations. Whether these discrepancies reflect different lengths of observations (i.e., days to weeks for parents vs. minutes for clinicians) is unclear. Regardless, the measurements obtained during both the MAD and OLE demonstrate a consistent trend toward improved breathing. Other parameters obtained during the automated assessments further emphasize IGF-1's selective effect on slow breathing, initially shown in the RTT mouse model (14). Although the lack of improvement in hyperventilation may have been influenced by technical issues (e.g., movement artifact), the selective effect on apnea is still desirable as it is perceived as more concerning clinically (41). Our preliminary dose–response analyses suggest that reduction in the apnea index is the result of IGF-1 administration; nonetheless, the reverting trend observed in a few subjects toward the end of the MAD (Fig. S6) may reflect the aforementioned saturation kinetics of IGF-1. Additional PD analyses, focusing on exposure–response relationships, need to be conducted to clarify this issue. The effects of IGF-1 on cardiac function were challenging to interpret. Although decreased heart rate variability may be seen as positive, its association with a trend toward higher heart rate may be considered a potential side effect. However, heart rate values remained within the wide normal range (42). Although the possible effect of mecasermin on heart rate warrants further investigation, our findings are in line with the partial correction of bradycardia in the Mecp2-null mouse (14).

Our preliminary efficacy evaluations on neurobehavioral parameters provided a mixed picture. Whereas some measures indicated improvements, others worsened. This was particularly the case for abnormalities in breathing and peripheral autonomic function. A similar inconsistent pattern was found for externalizing problem behaviors, such as disruptive and irritable behaviors. Two other important domains—communication and motor function, including abnormal movements—did not show a change.

Table 2. Summary of breathing indices for all RTT subjects by time point (n = 9)

Breathing indices	Pre-MAD	Post-MAD	Pre-OLE	Post-OLE	Pre-MAD to Post-OLE
Apnea index (mean ± SE)	10.11 ± 19.34	5.11 ± 9.68	4.67 ± 6.81	3.00 ± 5.72	−7.12 ± 4.58
Student's *t P*	–	–	–	–	0.159
Wilcoxon signed rank *P*	–	–	–	–	0.094
RI model *P*	–	–	–	–	0.018
Hyperventilation index (mean ± SE)	3.55 ± 6.71	3.00 ± 6.59	6.44 ± 16.86	3.66 ± 8.97	0.12 ± 0.93
Student's *t P*	–	–	–	–	0.908
Wilcoxon signed rank *P*	–	–	–	–	0.875
RI model *P*	–	–	–	–	0.963

Table 3. Neurobehavioral measures between V1 and V5

Measure	V1 mean	V5 mean	Mean difference	Mean difference SE	Student's *t P*	Wilcoxon signed rank *P*
Behavioral subtotal (MBA)	24.00	19.88	−4.11	1.11	0.006	0.016
Passive/unengaged (CA)	0.33	0.00	−0.33	0.17	0.081	0.250
Intermittent laughter (CA)	0.33	0.00	−0.33	0.17	0.081	0.250
Fear/anxiety subtotal (RSBQ)	3.55	2.77	−0.79	0.66	0.274	0.281
Spells of laughter at night (RSBQ)	0.77	0.44	−0.33	0.17	0.081	0.250
Social avoidance subtotal (ADAMS)	4.55	3.11	−1.44	0.84	0.122	0.109

V1, visit 1 of OLE; V5, visit 5 of OLE.

However, behaviors under the categories of anxiety (i.e., including fear and avoidance) and mood abnormalities (e.g., inappropriate laughter) showed modest although consistent improvements among measures that included two standardized behavioral scales (i.e., RSBQ, ADAMS). These findings were supported by the partial or complete reversal of right-sided alpha band frontal EEG asymmetry in five of the six subjects presenting with this phenomenon, which correlated with improved scores on mood abnormalities and anxiety. Because EEG frontal asymmetry has been linked to depression and particularly to anxiety in children (26, 27), its use in RTT and other neurodevelopmental disorders may serve as an effective tool for assessing drug efficacy. Our findings of IGF-1's effect on anxiety are in agreement with data from studies in the animal model (14).

The data presented here suggest that administration of IGF-1 is a promising treatment for RTT. Its safety and tolerability profiles are acceptable considering the severity of the targeted symptoms. However, the potential long-term use of mecasermin should be weighed against its potential effects on puberty, which is already accelerated in RTT (32, 33). The complex pharmacology of IGF-1 makes the determination of an optimal dosage difficult; the positive effects reported here indicate that long-term treatment may be necessary, which is not surprising considering IGF-1's likely effects on synaptic maturation and maintenance (6, 13, 14). The effect of IGF-1 was mild and selective, influencing certain cardiorespiratory and neurobehavioral features of RTT. Although this may seem unexpected given the context of IGF-1's extensive efficacy in the mouse model (14), it is not surprising compared with trial results in other neurodevelopmental disorders. In fragile X syndrome, mGluR5 antagonists (43) and GABA-B agonists (44) had similarly selective effects in human trials, but were preceded by a more generalized reversal of the phenotype in preclinical studies (45, 46). Interaction between the primary genetic defect and the individual's own genetic background is one of several mechanisms that may contribute to these discrepancies.

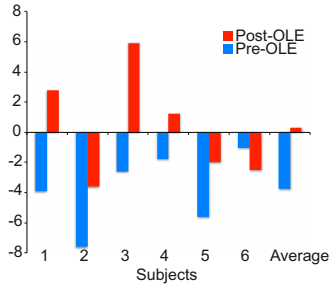

Fig. 3. Right-sided frontal alpha band EEG asymmetry shows a trend toward reversal. Greater relative L vs. R alpha activity has been interpreted as greater positive effect/less anxiety and greater R vs. L the opposite. Six subjects evaluated before the OLE demonstrated R > L asymmetry. Although the degree of asymmetry was variable after OLE, five of the six showed a decrease in the asymmetry index and in three there was a reversal. A paired-samples *t* test revealed significant group differences pre- and post-OLE.

It is important to recognize the limitations of the present study. The first limitation is the relatively small sample and age range considering the dynamics of RTT. Nine of the subjects met RTT diagnostic criteria and only seven were at a stable period (Hagberg stage III) (2). Nevertheless, analyses excluding the two individuals in stage II did not yield different results. Although the inclusion of twins with *MECP2*-related disorder (MRD) allowed for the examination of safety and PK in individuals with other MRDs, it also decreased the variability of the sample. This study was designed to assess CNS penetration and PK profile of IGF-1, and to test the feasibility of automated cardiorespiratory measures; as such, RTT subjects were not selected on the basis of breathing abnormalities or specific profiles of neurobehavioral impairment. This increased the heterogeneity of the already small sample, leading to diminished statistical power. Analyses of the clinically oriented measures used discovery type statistics without correcting for multiple comparisons and emphasizing the consistency of the body of data rather than specific parameters. On the other hand, comparisons between onset and end of the OLE, without considering intermediate time points may have overlooked transient positive effects of IGF-1. Although measures from the Rett Natural History study (21) were selected because of their relevance, these instruments have not been validated as outcome measures, and discrepancies between the parent questionnaire and clinician assessment need to be further examined. Also, the ADAMS (24, 25), has not been validated in RTT. Increased care and placebo effect could have also influenced our neurobehavioral findings. Nonetheless, the use of automated measures such as the BioRadio for cardiorespiratory function (17) or EEG asymmetry profiles for anxiety and mood (26, 27) strengthened clinician- and parent-reported data and support future exploration of biomarkers. Additional biomarker data—namely the Q sensor (47) for recording motion and hand stereotypies and visual evoked potentials for examining cortical function (48)—was collected as part of this trial and needs to be analyzed and reported in future publications.

Methods

Sample. Characteristics of our cohort are shown in Table 1 and *SI Methods*. The study was approved by the Institutional Review Board of Boston Children's Hospital and informed consent was obtained from the parent of each participant. Further information is provided in *SI Methods*.

Study Design and Safety Measures. Unblinded phase 1 study designed to establish PK profile (4-wk MAD) and long-term safety and tolerability (20-wk OLE) of IGF-1 in girls with RTT (Fig. S1). Subjects received twice daily (BID) s.c. injections at 40 μg/kg (week 1), 80 μg/kg (week 2), and 120 μg/kg (weeks 3, 4, OLE) (Fig. S2). Safety was assessed by evaluations listed in Table S6. Detailed information is provided in *SI Methods*.

PK and PD Analyses. Sera were obtained at different daily time points during the MAD, and at each visit during the OLE, while CSF only at the beginning and end of the MAD (Fig. S2). Methodologies for IGF-1 and IGFBP3 measurements, and PK and pharmacodynamics analyses, are detailed in *SI Methods*.

Automated Cardiorespiratory Measures. Time synchronized chest respiratory inductive plethysmography, three lead electrocardiography, and video recordings are detailed in *SI Methods*.

Neurobehavioral Assessments. Table S4 lists the multiple measures of neurologic and other functions obtained during the OLE. Additional information is presented *SI Methods*.

EEG Recordings. EEG recording, spectral power analysis, and frontal asymmetry scores were performed as reported (49–51) and detailed in *SI Methods*.

Statistical Analyses. Standard descriptive and comparative statistics were employed. Specific tests are specified in *Results* and *SI Methods*.

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1. Neul JL, et al.; RettSearch Consortium (2010) Rett syndrome: Revised diagnostic criteria and nomenclature. *Ann Neurol* 68(6):944–950.
2. Hagberg B (2002) Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev* 8(2):61–65.
3. Marschik PB, et al. (2013) Changing the perspective on early development of Rett syndrome. *Res Dev Disabil* 34(4):1236–1239.
4. Tarquinio DC, Kaufmann WE (2014) Targeted treatments in Rett syndrome. *New Developments in Treatment for Neurodevelopmental Disorders: Targeting Neurobiological Mechanisms*, eds Hagerman R, Hendren R (Oxford Univ Press, New York).
5. Percy AK (2002) Clinical trials and treatment prospects. *Ment Retard Dev Disabil Res Rev* 8(2):106–111.
6. Kaufmann WE, Johnston MV, Blue ME (2005) MeCP2 expression and function during brain development: Implications for Rett syndrome's pathogenesis and clinical evolution. *Brain Dev* 27(Suppl 1):S77–S87.
7. Guy J, Gan J, Selfridge J, Cobb S, Bird A (2007) Reversal of neurological defects in a mouse model of Rett syndrome. *Science* 315(5815):1143–1147.
8. Robinson L, et al. (2012) Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome. *Brain* 135(Pt 9):2699–2710.
9. Khwaja OS, Sahin M (2011) Translational research: Rett syndrome and tuberous sclerosis complex. *Curr Opin Pediatr* 23(6):633–639.
10. Guan J, Mathai S, Liang HP, Gunn AJ (2013) Insulin-like growth factor-1 and its derivatives: Potential pharmaceutical application for treating neurological conditions. *Recent Patents CNS Drug Discov* 8(2):142–160.
11. Itoh M, et al. (2007) Methyl CpG-binding protein 2 (a mutation of which causes Rett syndrome) directly regulates insulin-like growth factor binding protein 3 in mouse and human brains. *J Neuropathol Exp Neurol* 66(2):117–123.
12. Landi S, et al. (2011) The short-time structural plasticity of dendritic spines is altered in a model of Rett syndrome. *Sci Rep* 1:45.
13. Tropea D, et al. (2009) Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. *Proc Natl Acad Sci USA* 106(6):2029–2034.
14. Castro J, et al. (2012) Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett Syndrome. *Soc Neurosci*, 2012 (abstr).
15. Backeljauw PF, Chernausek SD (2012) The insulin-like growth factors and growth disorders of childhood. *Endocrinol Metab Clin North Am* 41(2):265–282, v.
16. Gabrielsson J, Weiner D (2007) *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications* (Swedish Pharmaceutical Press, Stockholm), 4th Ed.
17. Leino K, Nunes S, Valta P, Takala J (2001) Validation of a new respiratory inductive plethysmograph. *Acta Anaesthesiol Scand* 45(1):104–111.
18. Iber C (2007) *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications* (American Academy of Sleep Medicine, Westchester, IL).
19. Kerr AM, Julu POO (1999) Recent insights into hyperventilation from the study of Rett syndrome. *Arch Dis Child* 80(4):384–387.
20. Hsu YW, et al. (2004) Dexmedetomidine pharmacodynamics: Part I: Crossover comparison of the respiratory effects of dexmedetomidine and remifentanil in healthy volunteers. *Anesthesiology* 101(5):1066–1076.
21. Percy AK, et al. (2010) Rett syndrome diagnostic criteria: Lessons from the Natural History Study. *Ann Neurol* 68(6):951–955.
22. Mount RH, Charman T, Hastings RP, Reilly S, Cass H (2002) The Rett Syndrome Behaviour Questionnaire (RSBQ): Refining the behavioural phenotype of Rett syndrome. *J Child Psychol Psychiatry* 43(8):1099–1110.
23. Kaufmann WE, et al. (2012) Social impairments in Rett syndrome: Characteristics and relationship with clinical severity. *J Intellect Disabil Res* 56(3):233–247.
24. Esbensen AJ, Rojahn J, Aman MG, Ruedrich S (2003) Reliability and validity of an assessment instrument for anxiety, depression, and mood among individuals with mental retardation. *J Autism Dev Disord* 33(6):617–629.
25. Cordeiro L, Ballinger E, Hagerman R, Hessl D (2011) Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: Prevalence and characterization. *J Neurodev Disord* 3(1):57–67.
26. Thibodeau R, Jorgensen RS, Kim S (2006) Depression, anxiety, and resting frontal EEG asymmetry: A meta-analytic review. *J Abnorm Psychol* 115(4):715–729.
27. Kagan J, Snidman N (1999) Early childhood predictors of adult anxiety disorders. *Biol Psychiatry* 46(11):1536–1541.
28. O'Kusky J, Ye P (2012) Neurodevelopmental effects of insulin-like growth factor signaling. *Front Neuroendocrinol* 33(3):230–251.
29. Corvin AP, et al. (2012) Insulin-like growth factor 1 (IGF1) and its active peptide (1-3) IGF1 enhance the expression of synaptic markers in neuronal circuits through different cellular mechanisms. *Neurosci Lett* 520(1):51–56.
30. Pini G, et al. (2012) IGF1 as a potential treatment for Rett syndrome: Safety assessment in six Rett patients. *Autism Res Treat* 2012:679801.
31. Pitcher MR, et al. (2013) Insulinotropic treatments exacerbate metabolic syndrome in mice lacking MeCP2 function. *Hum Mol Genet* 22(13):2626–2633.
32. Knight O, et al. (2013) Pubertal trajectory in females with Rett syndrome: A population-based study. *Brain Dev* 35(10):912–920.
33. Baş VN, et al. (2013) Report of the first case of precocious puberty in Rett syndrome. *J Pediatr Endocrinol Metab* 26(9-10):937–939.
34. Makkonen I, Kokki H, Kuikka J, Turpeinen U, Riikonen R (2011) Effects of fluoxetine treatment on striatal dopamine transporter binding and cerebrospinal fluid insulin-like growth factor-1 in children with autism. *Neuropediatrics* 42(5):207–209.
35. Riikonen RS, Jääskeläinen J, Turpeinen U (2010) Insulin-like growth factor-1 is associated with cognitive outcome in infantile spasms. *Epilepsia* 51(7):1283–1289.
36. Tercica, Inc. (2007) *Investigator's Brochure: Recombinant Human Insulin-Like Growth Factor-1* (Tercica, Inc., a subsidiary of the Ipsen Group, Brisbane, CA).
37. Skaper SD (2011) Peptide mimetics of neurotrophins and their receptors. *Curr Pharm Des* 17(25):2704–2718.
38. Rosenbloom AL (2009) Mecasermin (recombinant human insulin-like growth factor I). *Adv Ther* 26(1):40–54.
39. Grahnén A, et al. (1993) Pharmacokinetics of recombinant human insulin-like growth factor I given subcutaneously to healthy volunteers and to patients with growth hormone receptor deficiency. *Acta Paediatr Suppl* 82(Suppl 391):9–13, discussion 14.
40. Peña F, García O (2006) Breathing generation and potential pharmacotherapeutic approaches to central respiratory disorders. *Curr Med Chem* 13(22):2681–2693.
41. Rohdin M, et al. (2007) Disturbances in cardiorespiratory function during day and night in Rett syndrome. *Pediatr Neurol* 37(5):338–344.
42. Fleming S, et al. (2011) Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: A systematic review of observational studies. *Lancet* 377(9770):1011–1018.
43. Dölen G, et al. (2007) Correction of fragile X syndrome in mice. *Neuron* 56(6):955–962.
44. Henderson C, et al. (2012) Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. *Sci Transl Med* 4(152):ra128.
45. Jacquemont S, et al. (2011) Epigenetic modification of the FMR1 gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci Transl Med* 3(64):ra1.
46. Berry-Kravis EM, et al. (2012) Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: A randomized, controlled, phase 2 trial. *Sci Transl Med* 4(152):ra127.
47. Poh MZ, Swenson NC, Picard RW (2010) A wearable sensor for unobtrusive, long-term assessment of electrodermal activity. *IEEE Trans Biomed Eng* 57(5):1243–1252.
48. Stauder JE, Smeets EE, van Mil SG, Curfs LG (2006) The development of visual- and auditory processing in Rett syndrome: An ERP study. *Brain Dev* 28(8):487–494.
49. Jasper H (1958) Report on the committee on methods of clinical examination in electroencephalography. *Electroencephalogr Clin Neurophysiol* 10:370–375.
50. McManis MH, Kagan J, Snidman NC, Woodward SA (2002) EEG asymmetry, power, and temperament in children. *Dev Psychobiol* 41(2):169–177.
51. Marshall PJ, Fox NA, Bucharest Early Intervention Project Core Group (2004) A comparison of the electroencephalogram between institutionalized and community children in Romania. *J Cogn Neurosci* 16(8):1327–1338.

Supporting Information

Khwaja et al. 10.1073/pnas.1311141111

SI Methods

Sample. All subjects completed the 4-wk multiple ascending dose (MAD) period of phase 1 and 10 subjects (83%) went on to complete the 20-wk open-label extension (OLE). All subjects were female; 10 subjects were Caucasian (83%) and 2 were Asian (17%). Based on the 2010 revised diagnostic criteria (1), 9 subjects (75%) had classic Rett syndrome (RTT) and 3 (25%) had a *MECP2*-related disorder. Based on their detailed developmental and medical history, two subjects were determined to be in Hagberg stage II (undergoing regression) and 7 subjects were in the more stable (pseudostationary) stage III (2). Four subjects (25%) were taking antiepileptic drugs; 1 of the 4 subjects also took melatonin as a sleep aid (Table 1).

Study Design and Safety Measures. Subjects were admitted for baseline, each dose escalation, and study termination, and evaluated in an outpatient research facility 2 d after discharge. Cerebrospinal fluid (CSF) was obtained by performing sterile lumbar punctures under light conscious sedation (midazolam and fentanyl) with the subject in the left lateral position and appropriate sampling of 10–20 mL CSF at each procedure. All subjects tolerated lumbar punctures well with one subject developing a post-procedure headache that resolved spontaneously. The 20-wk OLE was offered to all participants. The time-off treatment, between the MAD period and OLE, varied by patient and ranged from 12 to 30 wk. During the OLE, subjects underwent outpatient evaluations every 5 wk to monitor safety and pilot outcome measures for a subsequent phase 2 trial, including automated cardiorespiratory measures, EEG, visual evoked potentials (VEPs), and neurobehavioral assessments. Fig. S1 illustrates the trial design. During the screening and subsequent visits, many laboratory parameters were assessed. Regarding metabolic parameters, at-baseline serum levels of glucose were normal in all 12 subjects. However, serum lipid levels were abnormal in several subjects: total cholesterol (4 borderline abnormal and 3 markedly abnormal), low-density lipoprotein (4 borderline abnormal and 3 markedly abnormal), high-density lipoprotein (4 markedly abnormal), and triglycerides (2 borderline abnormal and 2 markedly abnormal). Safety measures are listed in Table S6.

Pharmacokinetic and Pharmacodynamics Analyses. Levels of insulin-like growth factor 1 (IGF-1), IGF-binding protein 3, and brain-derived neurotrophic factor (BDNF) were measured by ELISA as described by us for BDNF (3). BDNF levels in CSF were undetectable and, therefore, not reported. For the noncompartmental analysis, the number of data points used to characterize the log-linear terminal phase was chosen based on the log-linear regression of the concentrations successfully added from the latest time toward earlier ones (4). The areas under the concentrations vs. time curves were estimated numerically by the trapezoidal rule for increasing concentrations followed by the log-trapezoidal rule for those decaying. The compartmental analysis (4) was carried out by nonlinear Bayesian regression using the Monolix4.2 software package (Lixoft Co.). The maximum likelihood objective function was minimized using a Markov chain Monte Carlo algorithm, and upon convergence, the Fisher information matrix was calculated to better estimate the parameters variability. Pharmacodynamic (PD) analyses examined the relationship between IGF-1 doses and apnea indices over time. Observed trends were simply compared with the observed and modeled IGF-1 concentrations.

Automated Cardiorespiratory Measures. Respiratory inductive plethysmography (RIP) and electrocardiography (EKG) were recorded using BioRadio (Great Lakes Neurotech), a wireless data-acquisition device (5). The device was programmed to record signals at a sampling rate of 960 Hz and 16-bit resolution. We developed software in MATLAB (MathWorks) to annotate the peaks and troughs of respiratory effort in the RIP chest signal and R peaks in the QRS complex of the EKG signal. Instantaneous total respiratory cycle times (Ttot) were calculated from the respiratory annotations. Inspiratory peaks were defined as breaths with a minimum amplitude >20% of the typical amplitude. Ttot values were categorized as normal, apneic, and tachypneic based on age-defined norms (6). Clinically significant apneas were measured as a pause in detected inspiration >10 s. An apnea index was calculated as the number of events per hour (7). Hyperventilation was defined as five or more consecutive tachypneic breaths with an amplitude greater than twice the typical amplitude. A hyperventilation index was calculated as the number of hyperventilation periods per hour (8). R–R intervals were calculated from the EKG annotations and normal-to-normal (NN) intervals were analyzed using standard heart rate variability measures (9).

Neurobehavioral Assessments. The neurobehavioral assessments included clinician-based global impression measures (e.g., Clinical Global Impression scales) as well as parental ratings of symptoms [e.g., Parent-Targeted Symptom Visual Analog Scale (PTSVAS)] and other instruments that are being adapted to RTT [e.g., Mullen Scales of Early Learning (MSEL)] (Table S4). However, we focused on instruments already reported in the literature for our preliminary assessments of efficacy. These allowed for future comparisons with previous publications, added some validation to the present evaluations, and facilitated data interpretation. We used the RTT-oriented instruments from the Rett Natural History study (U54 HD061222) (10), a major project delineating longitudinally the RTT phenotype, which included two clinician-administered assessment forms [i.e., the clinical assessment (CA) and motor–behavioral assessment (MBA)]. The MBA combines historical items with items from direct clinician evaluations. These instruments cover major neurobehavioral domains: alertness, communication and socialization, motor function and movement disorders, and problem behaviors (both externalizing such as irritability and aggression, and internalizing, such as anxiety and mood abnormalities). Breathing and peripheral autonomic features, which reflect CNS function to a large extent, are major components of the RTT phenotype (1, 2) and are also included in these instruments. In addition, we used two standardized measures of problem behavior. The Rett Syndrome Behavioral Questionnaire (RSBQ) (11, 12) is a validated rating scale covering a wide range of behaviors of relevance to RTT, as well as salient breathing and motor features of the disorder. The Anxiety Depression and Mood Scale (ADAMS) (13, 14), an instrument focused on internalizing problem behaviors, was developed for typically developing individuals but has recently been applied to fragile X syndrome.

EEG Recordings. EEG was recorded over the frontal and parietal areas of the brain based on the International 10/20 System (15) and spectral power analysis was computed and analyzed as reported (16, 17). Alpha power was defined as the sum of the spectral power between 7 and 13 Hz. Frontal asymmetry scores were calculated by subtracting the sum of the natural log alpha power on the left side from the sum of the natural log alpha power on the right (17). A

positive score reflects greater activation on the left frontal lobe. Although all 10 subjects in the OLE period underwent EEG analyses, due to either quality of the recordings or seizures that transpired during the recording, only 6 data sets were considered appropriate for analysis.

Statistical Analyses. Preliminary evaluations of efficacy used discovery approaches, mainly two-sided paired *t* tests and the Wilcoxon signed rank test without correction for multiple comparisons. Considering the relatively small subject sample, which could lead to nonnormal, nonlinear data distribution, most of the analyses included parametric and nonparametric tests as alternative approaches. Furthermore, the cardiorespiratory measures were also analyzed used mixed-effects models to account for the correlations among the subjects.

1. Neul JL, et al.; RettSearch Consortium (2010) Rett syndrome: Revised diagnostic criteria and nomenclature. *Ann Neurol* 68(6):944–950.
2. Hagberg B (2002) Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev* 8(2):61–65.
3. Makkonen I, et al. (2011) Brain derived neurotrophic factor and serotonin transporter binding as markers of clinical response to fluoxetine therapy in children with autism. *J Pediatr Neurol* 9(1):1–8.
4. Gabrielsson J, Weiner D (2007) *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications* (Swedish Pharmaceutical Press, Stockholm), 4th Ed.
5. Leino K, Nunes S, Valta P, Takala J (2001) Validation of a new respiratory inductive plethysmograph. *Acta Anaesthesiol Scand* 45(1):104–111.
6. Fleming S, et al. (2011) Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: A systematic review of observational studies. *Lancet* 377(9770):1011–1018.
7. Iber C (2007) *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications* (American Academy of Sleep Medicine, Westchester, IL).
8. Kerr AM, Julu POO (1999) Recent insights into hyperventilation from the study of Rett syndrome. *Arch Dis Child* 80(4):384–387.
9. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 17(3):354–381.
10. Percy AK, et al. (2010) Rett syndrome diagnostic criteria: Lessons from the Natural History Study. *Ann Neurol* 68(6):951–955.
11. Mount RH, Charman T, Hastings RP, Reilly S, Cass H (2002) The Rett Syndrome Behaviour Questionnaire (RSBQ): Refining the behavioural phenotype of Rett syndrome. *J Child Psychol Psychiatry* 43(8):1099–1110.
12. Kaufmann WE, et al. (2012) Social impairments in Rett syndrome: Characteristics and relationship with clinical severity. *J Intellect Disabil Res* 56(3):233–247.
13. Esbensen AJ, Rojahn J, Aman MG, Ruedrich S (2003) Reliability and validity of an assessment instrument for anxiety, depression, and mood among individuals with mental retardation. *J Autism Dev Disord* 33(6):617–629.
14. Cordeiro L, Ballinger E, Hagerman R, Hessl D (2011) Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: Prevalence and characterization. *J Neurodev Disord* 3(1):57–67.
15. Jasper H (1958) Report on the committee on methods of clinical examination in electroencephalography. *Electroencephalogr Clin Neurophysiol* 10:370–375.
16. McManis MH, Kagan J, Snidman NC, Woodward SA (2002) EEG asymmetry, power, and temperament in children. *Dev Psychobiol* 41(2):169–177.
17. Marshall PJ, Fox NA; Bucharest Early Intervention Project Core Group (2004) A comparison of the electroencephalogram between institutionalized and community children in Romania. *J Cogn Neurosci* 16(8):1327–1338.

Fig. S1. The phase 1 study design included a 4-wk escalating dose (MAD) period and a 20-wk OLE period. Subjects were admitted at days 1, 8, 15, and 29 for serial pharmacokinetic (PK) sampling and seen in an outpatient research facility for safety monitoring 2 d after discharge. Time between the MAD period and start of the OLE varied by patient and ranged from 12 to 30 wk. In the OLE, subjects were evaluated every 5 wk in an outpatient research facility for safety monitoring and preliminary efficacy assessments.

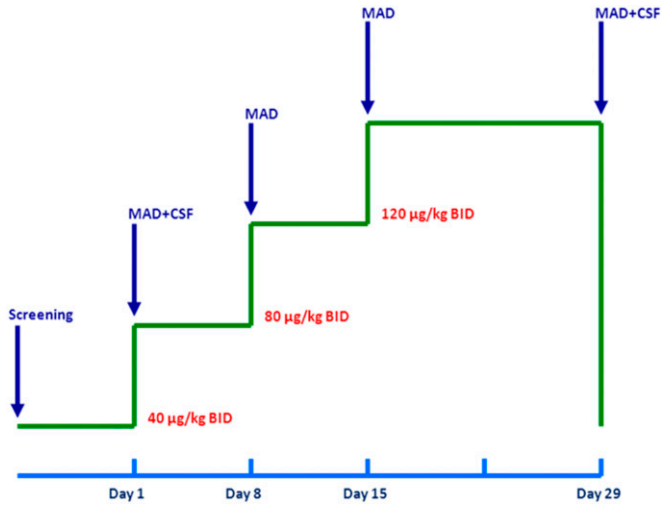

Fig. S2. The MAD period schedule of dose titration and CSF and PK collection time points. Subjects' dosing started at 40 µg/kg twice daily (BID) on day 1, escalated to 80 µg/kg BID on day 8, and reached the target dose of 120 µg/kg at day 15 and continued through day 29. Serial serum PK sampling occurred on days 1, 8, 15, and 29. CSF was collected before IGF-1 administration on day 1 and 1–2 h after dose administration on day 29.

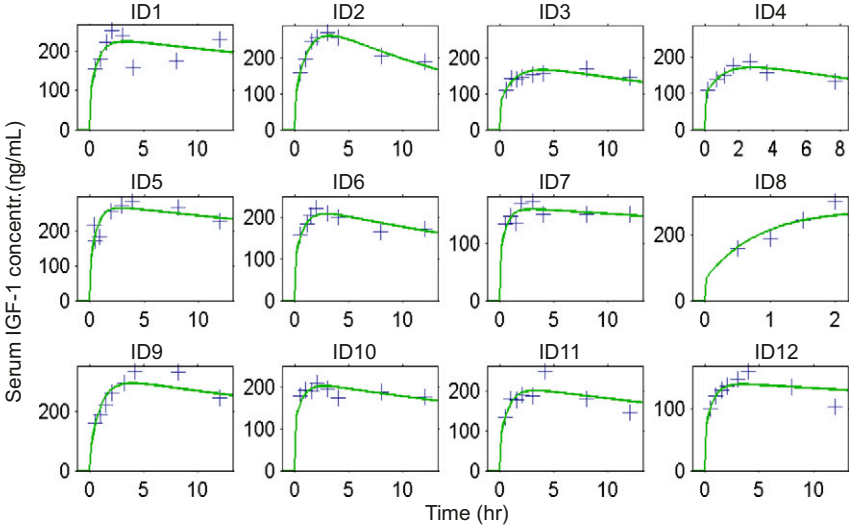

Fig. S3. Individual subject profiles of IGF-1 concentration. Typical individual fits for IGF-1 serum concentrations resulting from the day 1 dose of 40 µg/kg for each subject in the cohort (n = 12).

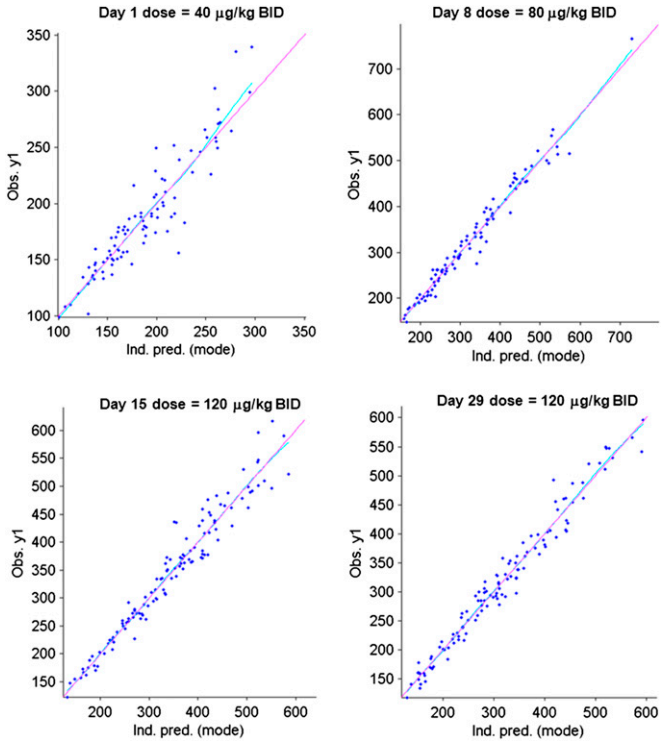

Fig. S4. Fitness of PK compartmental model. Observed vs. predicted IGF-1 serum concentrations at days 1, 8, 15, and 29 according to the final compartmental model.

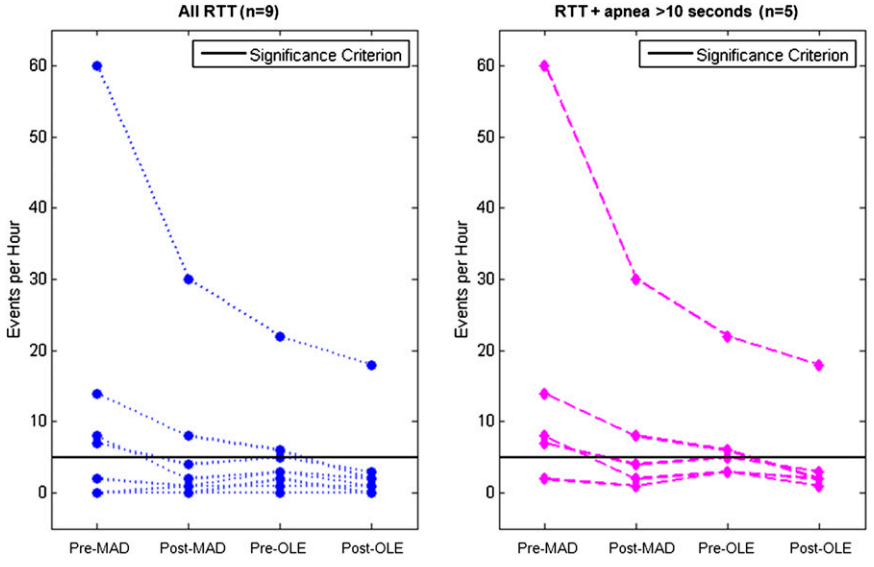

Fig. S5. Time course of apnea indices during the trial. The graphs illustrate changes in the apnea index during the different periods of the study. Clinically significant apnea was defined as apneic episodes >10 s in length, and moderate–severe apnea when these apneic episodes were more than five per hour (four subjects met this criterion). The apnea index for all RTT subjects ($n = 9$) (*Left*) and subjects with clinically significant apnea ($n = 5$) (*Right*) are shown. The four subjects with moderate–severe apnea decreased their severity to mild (less than five episodes per hour) by the end of the OLE.

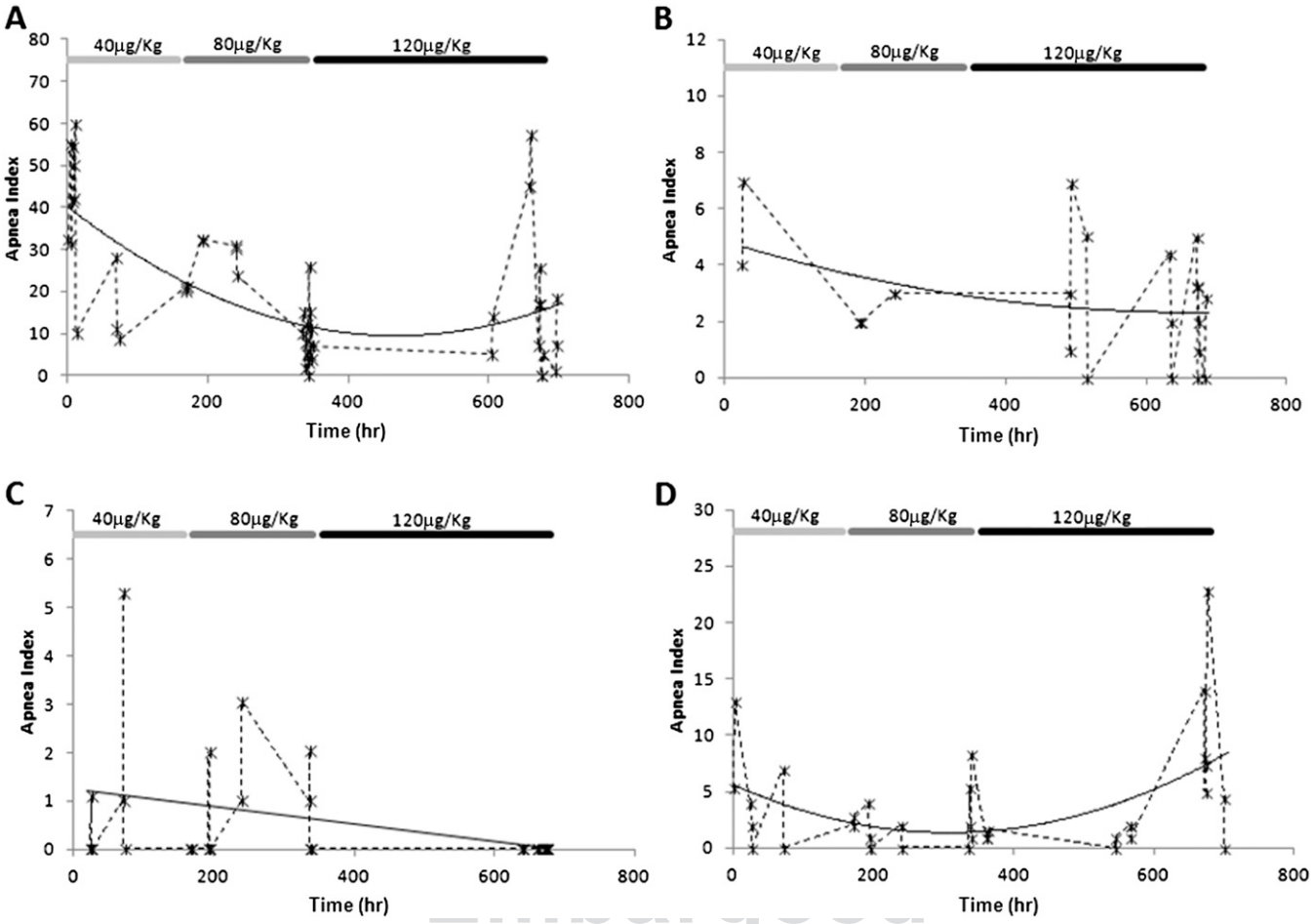

Fig. S6. Examples of PD profiles from the MAD period. Trending curves (continuous solid line) depicting the relationship between IGF-1 doses and the apnea index (discrete dashed line) for four representative subjects. *B* and *C* illustrate a sustained decrease of the apnea index across the entire 4-wk MAD period, whereas *A* and *D* illustrate a more gradual decrease with an upward trend at the end of the fourth week.

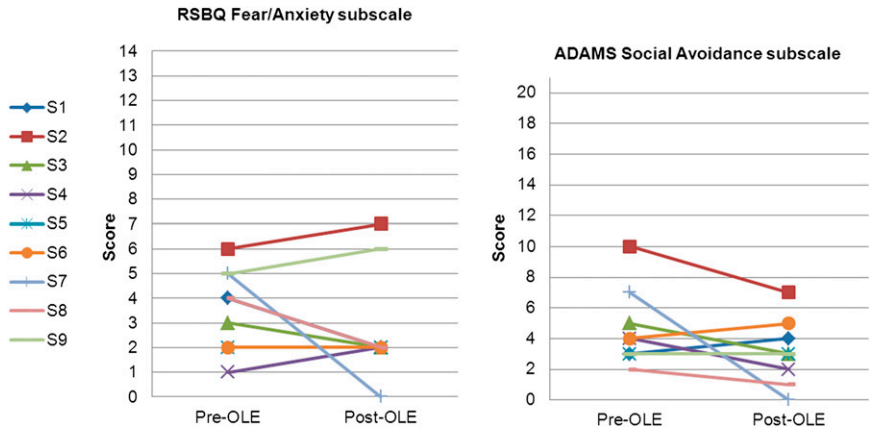

Fig. S7. IGF-1's selective effect on anxiety and social avoidance. Score profiles of individual subjects demonstrating mild but consistent improvements in the fear/anxiety subscale of the RSBQ (*Left*) and the social avoidance subscale of the ADAMS (*Right*) after the administration of mecasermin. S1–S9 represent the 9 RTT subjects.

Table S1. Basic noncompartmental PK parameters (mean ± SE)

	Dose, μg/kg			
	40	80	120	
PK parameters	Week 1	Week 2	Week 3	Week 4
t_{max}, h	3.4 ± 0.5	2.0 ± 0.3	1.9 ± 0.2	1.5 ± 0.2
C_{max}, ng/mL	234.9 ± 16.6	407.9 ± 42.0	458.5 ± 29.5	434.1 ± 29.3
AUC_t, ng·h/mL	2,050.0 ± 235.2	3,599.6 ± 319.3	3,745.6 ± 268.5	3,348.9 ± 261.9
MRT_b, h	5.5 ± 0.4	5.7 ± 0.1	5.4 ± 0.1	5.3 ± 0.1
$t_{1/2,\lambda}$; h	18.2 ± 11.3	14.6 ± 1.5	14.0 ± 2.0	12.9 ± 1.7

Table S2. Summary of adverse events during MAD and OLE

Reported adverse events	Not related*	Possibly related†	Probably related‡	Total
Adverse event subtotals by relationship	8 (21)	19 (50)	11 (29)	38 (100)
Blood and lymphatic system disorders	1 (3)	0 (0)	0 (0)	1 (3)
Anemia	1 (3)	0 (0)	0 (0)	1 (3)
Endocrine disorders	0 (0)	6 (16)	0 (0)	6 (16)
Polydipsia	0 (0)	6 (16)	0 (0)	6 (16)
Metabolism and nutrition disorders	1 (3)	0 (0)	0 (0)	1 (3)
Anorexia	1 (3)	0 (0)	0 (0)	1 (3)
Nervous system disorders	1 (3)	0 (0)	0 (0)	1 (3)
Complex partial seizure	1 (3)	0 (0)	0 (0)	1 (3)
Respiratory, thoracic and mediastinal disorders	3 (8)	1 (3)	6 (16)	10 (26)
Respiratory distress	1 (3)	0 (0)	0 (0)	1 (3)
Upper respiratory tract infection	2 (5)	0 (0)	0 (0)	2 (5)
Tonsillar hypertrophy	0 (0)	0 (0)	2 (5)	2 (5)
Snoring	0 (0)	1 (3)	4 (11)	5 (13)
Gastrointestinal disorders	2 (5)	3 (8)	1 (3)	6 (16)
Gastroenteritis viral	1 (3)	0 (0)	0 (0)	1 (3)
Vomiting	0 (0)	1 (3)	1 (3)	2 (5)
Gingival hyperplasia	0 (0)	1 (3)	0 (0)	1 (3)
Salivary hypersecretion	1 (3)	1 (3)	0 (0)	2 (5)
Tooth loss	0 (0)	1 (3)	0 (0)	1 (3)
Skin and s.c. tissue disorders	0 (0)	2 (5)	0 (0)	2 (5)
Nonscarring hair loss	0 (0)	2 (5)	0 (0)	2 (5)
Renal and urinary disorders	0 (0)	2 (5)	0 (0)	2 (5)
Polyuria	0 (0)	1 (3)	0 (0)	1 (3)
Malodorous urine	0 (0)	1 (3)	0 (0)	1 (3)
Reproductive system and breast disorders	0 (0)	4 (11)	2 (5)	6 (16)
Premature thelarche	0 (0)	1 (3)	0 (0)	1 (3)
Precocious puberty	0 (0)	3 (8)	1 (3)	4 (11)
Prematrue adrenarche	0 (0)	0 (0)	1 (3)	1 (3)
General disorders and administration site conditions	0 (0)	1 (3)	2 (5)	3 (8)
Injection site hematoma	0 (0)	0 (0)	1 (3)	1 (3)
Injection site irritation	0 (0)	0 (0)	1 (3)	1 (3)
Hyperhidrosis	0 (0)	1 (3)	0 (0)	1 (3)

At each level of summation (overall, system organ class, preferred term), subjects that had the same adverse event more than once are counted only one time using the closest relationship to study medication. Numbers in parentheses represent percent.
*Includes all events reported as "not related" or "remote" relationship to study medication.
†Includes all events reported as "possibly related" relationship to study medication.
‡Includes all events reported as "probably related" or "highly probable" relationship to study medication.

Table S3. Summary of breathing indices for RTT subjects with apnea >10 s by time point ($n = 5$)

Breathing indices	Pre-MAD	Post-MAD	Pre- to post-MAD	Pre-OLE	Post-OLE	Pre- to post-OLE	Pre-MAD to post-OLE
Apnea index by time point							
Mean	18.20	9.00	−9.20	7.80	5.20	−2.60	−13.00
SE	23.75	12.04	5.60	8.04	7.19	0.62	7.47
Student's *t P*	—	—	0.157	—	—	0.012	0.157
Wilcoxon signed rank *P*	—	—	0.062	—	—	0.062	0.062
RI model *P*	—	—	0.057	—	—	0.787	0.012
Hyperventilation index by time point							
Mean	6.20	4.00	−2.20	11.60	6.60	−5.00	0.40
SE	8.38	8.94	1.56	22.23	11.69	4.75	1.75
Student's *t P*	—	—	0.232	—	—	0.352	0.830
Wilcoxon signed rank *P*	—	—	0.500	—	—	0.500	1.000
RI Model *P*	—	—	0.579	—	—	0.069	0.918

RI, random intercept.

Table S4. Changes in breathing and heart rate characteristics pre-OLE to post-OLE

	All RTT subjects ($n = 9$)				RTT subjects with apnea index >5 ($n = 4$)			
Characteristics	Mean	SD	Difference	*P* value	Mean	SD	Difference	*P* value
Breathing								
Epoch apnea, %	36.059	21.701	−5.029	0.167	39.730	20.765	−8.640	0.043
Epoch normal respiratory rate, %	45.663	27.488	−1.259	0.791	42.368	28.070	6.206	0.306
Epoch tachypnea, %	18.278	21.854	6.289	0.107	17.902	16.714	2.433	0.490
Mean Ttot	3.330	0.815	−0.381	0.006	3.649	0.677	−0.464	0.001
Mean Ttot apnea	7.596	3.616	−1.073	0.041	8.952	3.791	−1.701	0.008
Mean Ttot normal resp. rate	2.899	0.370	0.024	0.718	3.105	0.265	−0.008	0.880
Mean Ttot tachypnea	1.744	0.199	−0.054	0.215	1.825	0.150	−0.041	0.354
SD Ttot	2.585	1.996	−0.728	0.012	3.350	2.050	−1.217	0.001
SD Ttot apnea	5.075	6.329	−2.135	0.019	7.002	7.073	−3.251	0.008
SD Ttot normal resp. rate	0.417	0.070	0.027	0.022	0.440	0.064	0.011	0.383
SD Ttot tachypnea	0.277	0.069	0.034	0.030	0.312	0.046	0.032	0.023
Mean irregularity Ttot	0.448	0.224	0.014	0.719	0.511	0.230	−0.059	0.244
SD irregularity Ttot	0.927	0.898	−0.210	0.091	1.200	0.998	−0.423	0.012
Heart rate								
Epoch bradycardia, %	0.086	0.184	−0.054	0.048	0.077	0.188	−0.061	0.043
Epoch normal heart rate, %	90.157	14.062	−38.357	0.000	85.444	15.346	−37.358	0.000
Epoch tachycardia, %	9.758	14.063	38.411	0.000	14.478	15.337	37.419	0.000
Mean NN interval	572.709	33.328	−65.599	0.000	585.626	29.440	−58.795	0.000
Mean NN interval bradycardia	346.502	491.728	−218.206	0.007	272.765	470.230	−158.855	0.085
Mean NN interval normal heart rate	587.418	42.685	−54.010	0.000	607.724	35.031	−51.324	0.005
Mean NN interval tachycardia	412.946	162.013	53.127	0.011	454.909	123.753	25.560	0.173
SDNN	62.764	36.894	−20.865	0.003	71.250	42.772	−25.052	0.011
SDNN bradycardia	5.046	13.035	1.765	0.682	2.836	6.562	−1.382	0.349
SDNN normal heart rate	56.990	31.252	−21.501	0.000	63.132	36.686	−24.166	0.004
SDNN tachycardia	11.473	9.080	4.052	0.004	15.229	8.713	1.592	0.309
Mean irregularity NN	0.030	0.015	−0.010	0.002	0.030	0.017	−0.009	0.043
SD irregularity NN	0.036	0.019	−0.010	0.014	0.038	0.023	−0.014	0.015

NN, NN heartbeat interval length measured in milliseconds; SDNN, SD of the NN interval. A normal heart rate is defined as being between the 1st and 99th percentile for age, bradycardia is defined as a heart rate below the 1st percentile of rate for age, and tachycardia is defined as a heart rate greater than the 99th percentile of rate for age. Statistical test = paired Student *t* test. The epoch length was 30 min.

Table S5. Correlation between change in neurobehavioral subscales and EEG alpha symmetry index between V1 and V5

Subscales	Pearson's *r*	*P* value	Spearman's rho	*P* value
Behavioral (MBA)	0.369	0.471	0.147	0.781
Motor (MBA)	0.338	0.512	0.152	0.774
Orofacial/respiratory (MBA)	0.485	0.329	0.334	0.518
Fear/anxiety (RSBQ)	0.653	0.159	0.508	0.305
Breathing (RSBQ)	0.625	0.184	0.698	0.123
Social avoidance (ADAMS)	0.609	0.199	0.319	0.538
Depressed mood (ADAMS)	0.841	0.036	0.617	0.192

Table S6. Safety and outcome measures categorized by subject matter

Safety
Vital signs
Scoliosis X-ray
Clinical assessment (physical examination)
Clinical laboratory tests
MOSES
Growth measurements
EKG
Problem behavior
MBA
CA
RSBQ
ADAMS
ABC
Motor
MBA
CA
Global/quality of life
CSS (Kerr)†
CSS (Percy)†
Clinical Global Impression Scale–Clinician
Clinical Global Impression Scale–Parent
PTSVAS (1)
CHQ-PF50
Cognition and adaptive behavior*
MBA
CA
MSEL (2)
VABS-II
Quantitative biomarkers
VEPs
Quantitative Measures of Respiration (BioRadio)
Accelerometry (Q sensor)

ABC, Aberrant Behavior Checklist; CHQ-PF50, Child Health Questionnaire–Parent Form 50; CSS, Clinical Severity Scale; MOSES, Monitoring of Side-Effect System; VABS-II, Vineland Adaptive Behavior Scales II.

*Includes clinical assessments of alertness, communication, and socialization.

†Two distinct Clinical Severity Scales were used: one by Alan Percy, MD and the other by Alison Kerr, FRCP.

1. Berry-Kravis E, et al. (2013) Outcome measures for clinical trials in fragile X syndrome. *J Dev Behav Pediatr* 34(7):508–522.
2. Mullen EM (1995) *Mullen Scales of Early Learning*, ed AGS (American Guidance Service Inc., Circle Pines, MN).