Predicting outcomes following cognitive behaviour therapy in child anxiety disorders: the influence of genetic, demographic and clinical information

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ABSTRACT

Background. Within a therapeutic gene by environment (GxE) framework, we recently demonstrated that variation in the Serotonin Transporter Promoter Polymorphism; *5HTTLPR* and marker rs6330 in Nerve Growth Factor gene; *NGF* is associated with poorer outcomes following cognitive behaviour therapy (CBT) for child anxiety disorders. The aim of this study was to explore one potential means of extending the translational reach of G×E data in a way that may be clinically informative. We describe a 'risk-index' approach combining genetic, demographic and clinical data and test its ability to predict diagnostic outcome following CBT in anxious children.

Method. DNA and clinical data were collected from 384 children with a primary anxiety disorder undergoing CBT. We tested our risk model in five cross-validation training sets.

Results. In predicting treatment outcome, six variables had a minimum mean beta value of 0.5: 5*HTTLPR*, *NGF* rs6330, gender, primary anxiety severity, comorbid mood disorder and comorbid externalising disorder. A risk index (range 0-8) constructed from these variables had moderate predictive ability (AUC = .62-.69) in this study. Children scoring high on this index (5-8) were approximately three times as likely to retain their primary anxiety disorder at follow-up as compared to those children scoring 2 or less.

Conclusion. Significant genetic, demographic and clinical predictors of outcome following CBT for anxiety-disordered children were identified. Combining these predictors within a risk-index could be used to identify which children are less likely to be diagnosis free following CBT alone or thus require longer or enhanced treatment. The 'risk-index' approach represents one means of harnessing the translational potential of G×E data.

INTRODUCTION

Gene-environment interaction (GxE) in the context of psychological disorders is predominantly studied within a diathesis stress framework, which proposes that individuals carrying genetic vulnerabilities are disproportionately likely to be adversely impacted by an environmental stressor. However, this model posits a truncated form of G×E, focusing exclusively on adversity and negative outcomes, neglecting positive environments and adaptive outcomes. An alternative framework, the differential susceptibility hypothesis, addresses this issue (Belsky & Pluess, 2009). Individuals considered "vulnerable" (strongly affected by adversity), may also benefit most from supportive environments. Thus individual differences in developmental plasticity may result in genetic influences that act in a "for better and for worse manner" (Belsky & Pluess, 2009). Derived from this framework is the concept of vantage sensitivity, which proposes that individuals will vary (for genetic and other reasons) in the extent to which they gain benefit from positive and enriching environmental influences (Pluess & Belsky, 2012).

One example of vantage sensitivity could be response to psychological intervention. For example, while many people experience positive outcomes of Cognitive Behaviour Therapy (CBT) for mood and anxiety disorders, a large minority (35-45%) retains significant impairments. Very few studies have investigated the source of this individual variation in treatment response despite the potential for stratified medicine and improved outcomes. Biological measures, for example genetic or physiological factors, have rarely been investigated as the source of individual differences in response to psychological interventions (Pluess & Belsky, 2012). Within the child anxiety treatment field, clinical and demographic risk factors (such as age, gender, pre-treatment severity, comorbid disorders) have proven to be modest and somewhat inconsistent predictors of treatment response. This inconsistency may in part arise from relatively small sample sizes and differences in assessment of outcome. One approach posits that the most likely source of predictors of treatment response may be the origins of the disorder; that is "cause should inform cure" (Uher, 2008). Genetic variants therefore represent plausible predictors of psychotherapy response. Testing for an interaction between a therapeutic intervention and a genetic variant represents a special case of G×E (Uher, 2011) and provides an investigation of the vantage sensitivity concept. In a therapeutic G×E study, the environment is positive and predictable, allowing for prospective analysis.

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Very few studies have investigated genetic predictors of individual differences in response to psychological therapy, a field we recently termed "therapygenetics" (Eley et al., 2012).The most widely studied marker to date is the serotonin transporter promoter polymorphism (*5HTTLPR*). Two studies have demonstrated that individuals with the low expression short/short (SS) genotype show better response to psychological therapy than those with the intermediate/high expression genotypes (Eley et al., 2012; Kohen et al., 2011), although one found the reverse (Bryant et al., 2010), and six studies showed no significant association (Bockting, Mocking, Lok, Koeter, & Schene, 2012; Furmark et al., 2010; Hedman et al., 2012; Lonsdorf et al., 2010; Sakolsky et al., 2011; Wang et al., 2009). Associations with psychological treatment response have also been investigated with other markers (Lester & Eley, 2013). These include the serotonin transporter intron 2 variable number tandem repeat (Kohen et al., 2011; Sakolsky et al., 2011), 5-hydroxytryptamine (serotonin) receptor 2A gene (Kotte, McQuaid, & Kelsoe, 2007), tryptophan hydroxylase 2 gene (Furmark et al., 2010), monoamine oxidase-A variable number tandem repeat (Reif et al., in submission), catechol-O-methyltransferase gene (Hedman et al., 2012; Lonsdorf et al., 2010), nerve growth factor gene (NGF) (Lester et al., 2012), brain-derived neurotrophic factor gene (Fullana et al., 2012; Hedman et al., 2012; Lester et al., 2012; Sakolsky et al., 2010), and glutamate receptor, ionotropic kainite 4 gene (Sakolsky et al., 2010). To date, only two of these candidate genes (*5HTTLPR, NGF)* have been associated with treatment response following CBT for child anxiety[1].

At present, there is modest preliminary evidence that G×E interactions are relevant not only in the development but also the remission of psychopathology. One method by which predictive power can be potentially enhanced is through the use of analytic techniques that aggregate across multiple polymorphisms and/or genes into a single predictive parameter or risk score. In the present study, we present a first preliminary test of the combined predictive value of both genetic variants and measures of demographic and clinical factors within a therapeutic G×E design. With an end point focus and hence greater clinical utility, our outcome was non-remission from the primary anxiety diagnosis following CBT in 384 children with anxiety disorders. Our aim here was not to provide a definitive method for determining risk scores, but to use this approach to illustrate one way in which G×E data could be repurposed to

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[1] GRIK4 was associated with treatment outcome in a sample of anxiety-disordered children receiving CBT, Sertraline or CBT+Sertraline. However it is unclear whether GRIK4 interacts with treatment type (Salkosky, 2010).

potentially provide clinical utility. Our goal was to create a risk index in which scores of 0, 1, or 2 (to allow ease of use for clinicians) were allocated for each clinical and genetic variable such that those scoring high on this scale would be the individuals least likely to benefit from current CBT protocols, providing the opportunity to offer an enhanced treatment from the outset.   Although at present, it is expensive and impractical for clinicians to obtain DNA samples from clients as part of routine practice, in the future this may become a more viable option given the momentum of the field of individualized medicine.

METHOD

Participants

Three hundred and eighty-four children aged 6-13 years who met DSM IV criteria (American Psychiatric Association, 1994) for a primary diagnosis of an anxiety disorder were selected. Participants were excluded from the analyses if any of the genetic, demographic or clinical variables (see below) were missing or if they had significant physical/intellectual impairment, psychoses, and concurrent treatment (N = 186, initial sample was 570)[2]. Subjects were recruited from four trials at the Centre for Emotional Health at Macquarie University, Sydney and from two trials at the Berkshire Child Anxiety Clinic, University of Reading (see supplementary material for further information on treatment format).

Measures

Child diagnosis.

Diagnoses were made at pre-treatment and follow-up with the Anxiety Disorders Interview Schedule for DSM-IV, Parent and Child versions (Silverman & Albano, 1996). Diagnoses and Clinical Severity Ratings (CSR; 0-8) were assigned by graduate or clinical psychologists based on composite parent/child report. Where the child met diagnostic criteria and received a CSR of 4 or more, diagnosis was assigned. Children were allocated a primary diagnosis (most interfering) as well as comorbid diagnoses (see supplementary material).

Parental symptoms

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[2] Note, missingness was primarily a result of missing follow-up or parental DASS data.

Parents completed the Depression, Anxiety and Stress Scale (DASS-21) (Lovibond & Lovibond, 1995), a self-report measure of symptoms over the past week. Three 7-item scales were created for stress, anxiety and depression (range 0-21); internal consistency was .85, .76 and .90 respectively. To identify parents with significant symptoms, we used the cut-offs for the "severe" category (21, 15, and 26 for depression, anxiety and stress respectively). Parents were classified as affected if they scored above the cut-off for any of the three scales (mothers: 20.9% affected; fathers: 18.7% affected). Parental caseness scores were 0 (neither parent affected, 67.4%), 1 (1 parent, 26.6%) or 2 (both parents, 6.0%).

Ethnicity

Child ethnicity was based on reports of grandparent ancestry. As we wanted to develop a risk index of broad clinical utility, we included all individuals in our analyses regardless of ethnicity. The percentage of participants within each ethnic sub-group was: white European (62.8%); African or Caribbean (0.3%); Asian, (1.3%); Arab and Middle Eastern, (1.6%); Mixed, (7.6%) and Ancestry unknown and missing data, (26.6%).

Genotyping

Genomic DNA was extracted from buccal swabs using established procedures (Freeman et al., 2003). The genotying procedure is reported in the online supplementary materials. Genotyping of *5HTTLPR* was performed by polymerase chain reaction with the amplified products (S-469 bp, L-512 bp) separated by electrophoresis on a 3.5% agarose gel and stained with ethidium bromide. For *NGF* rs6330 genotyping was performed using the Sequenom MassARRAY ® iPLEX Gold technology (Sequenom, San Diego, CA, USA). Genotype distribution conformed to the Hardy-Weinberg Equilibrium for *5HTTLPR* (SS: 21.1%; LS: 47.4%; LL: 31.5%; $\chi^2_1 = 0.67$, *p* = .414 and *NGF* rs6330 TT: 21.6%; CT: 48.4%; CC: 30.0%; $\chi^2_1 = 0.23$, *p* = .632).

Procedure

Ethical approval was received from Human Ethics and Biosafety Committees at both sites. Parents provided informed consent, children provided assent. Buccal swabs were collected either at the clinic or through the post. Families

provided data at pre, post (N = 373; 97.1%) and one follow-up point (N = 384; 100%) which differed across trials: 52

were assessed at 3 months, 307 at 6 months, and 25 at 12 months.

Statistical analysis

We classified treatment outcome on absence/presence of the primary anxiety disorder. We focused on outcome

at follow-up as this was where we saw the strongest effect in our previous studies (Eley et al., 2012; Lester et al., 2012).

To aid in computing a cumulative risk score for non-remission (primary anxiety disorders still present at follow-up), all

predictor variables were di- or trichotimised. We focused on the alleles associated with risk for poor treatment outcome,

the L allele for 5*HTTLPR* and the C allele for *NGF* rs6330. A recessive model was used for 5*HTTLPR* (i.e., SS coded as 0;

SL/LL coded as 1), and an additive model for *NGF* rs6330 (TT coded as -1; CT coded as 0 and CC coded as 1) in line with

previous analyses (Eley et al., 2012). The remaining variables were coded as follows: age (0 = lower 50%, 1 = upper 50%

of distribution); gender (0 = male, 1 = female); pre-treatment anxiety severity (0 = CSR of 4-6, 1 = CSR of 7-8); comorbid

mood disorders (0 = absence, 1 = presence); comorbid externalising disorders (0 = absence, 1 = presence) and parental

psychopathology (0 = neither parent affected, 1 = 1 parent affected, 2 = both parents affected).

We tested the predictive performance of the risk index using a cross-validation technique (Hastie, Tibshirani, &

Friedman, 2009)[3]. We performed five rounds of cross-validation using a repeated random sub-sampling validation

method. On each round the sample was partitioned into a training set (80% of the sample) and a validation set (20% of

the sample). For each round of cross-validation, multiple linear[4] regression analyses with robust standard errors were

modelled in the training set to provide parameter estimates (unstandardised beta coefficients) for each predictor of

treatment outcome (coded 0 = absence of primary anxiety diagnosis, i.e. remission, and 10= presence of primary

anxiety diagnosis, non-remission)[5]. Risk scores were then calculated for each individual in the validation set using two

methods: i.) by assigning a score for each predictor variable from the unstandardised beta coefficient estimated in the

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[3] Cross-validation involves partitioning a dataset into training and validation sets. For each partition, a model is fitted within the training set and then the predictive performance of the model is assessed in the validation set. Multiple rounds of cross-validation are undertaken using different partitions to reduce variability and the validation results are averaged.

[4] Linear rather than logistic regression was used to obtain additive rather than multiplicative parameter estimates that could easily be combined into a risk score.

[5] The scale was modified here so the beta values rounded to 1 rather than .1. The scale is reverted to 0 and 1 for analyses using the validation datasets.

training set and ii.) by assigning a score for each predictor using a mean unstandardised beta coefficient computed across the five training sets and then rounded to the nearest integer. This later approach is appealing as by averaging parameter estimates across training sets, variability is reduced. Furthermore, while rounding parameter estimates may somewhat reduce precision, it may increase clinical utility through its simplicity. In both approaches, the risk score took the following form:

risk score = b_1*5HTTLPR + b_2*NGF + b_3*Age + b_4*Gender + b_5*Severity + b_6*ComorbidMood + b_7*ComorbidExternalising + b_8*ParentalPsychopathology

Predictive accuracy was assessed in each validation set by testing the extent to which the risk score[6] correctly classified remission and non-remission. Area under the curve (AUC) values were computed using non-parametric receiver operating characteristic analyses. Finally, we report exploratory analyses that investigate the extent to which the rounded risk score was associated with treatment outcome in the entire sample (N = 384).

RESULTS

Descriptive statistics

Table 1 provides descriptive data and test statistics for the genetic, demographic and clinical predictors comparing training and validation sets for each of the five cross-validation partitions. Within each cross-validation partition, the training and validation sets did not differ significantly on any variables reported. More broadly, rates of remission did not differ significantly between the Reading and Sydney sites, χ^2 (1) = 0.81, p = .37. The rates of remission for the entire sample were 60.9 % (n = 234).

[Insert Table 1 about here]

Predicting treatment outcome in the training set

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[6] The constant was not included in our risk score computations as it is irrelevant for discrimination between those who do and do not remit following treatment.

Table 2 reports unstandardised beta coefficients for each predictor variable, and *p* values. We also report mean unstandardised beta coefficients calculated across the five cross-validation partitions, and rounded beta values based on these mean beta coefficients.

[Insert Table 2 about here]

Genetic, demographic, and clinical predictors in combination significantly predicted treatment outcome, with all model *p* values < .05. The proportion of the variance in treatment outcome accounted for by the model (R^2) approximated 8%. The most consistent predictor of treatment outcome was *NGF* rs6330 genotype, which significantly predicted treatment outcome in all five data sets (*p values* from .008 to .03). With each extra C allele, participants were at a significantly greater risk of retaining their primary anxiety diagnosis at follow-up. Gender, primary anxiety severity, comorbid mood disorders and comorbid externalizing disorders also significantly predicted treatment outcome, albeit not consistently across the five training sets. Where these variables were significant predictors, poor outcome was associated with being female, greater anxiety severity pre-treatment, and the presence of comorbid mood and externalizing disorders. Parental psychopathology, *5HTTLPR* genotype and age did not make a significant contribution to the prediction of treatment outcome in these analyses. However, the purpose of this step was not to determine statistical significance per se but, instead, to estimate effect sizes for each predictor, irrespective of significance level, that could then be carried forward to calculate a risk score in the validation sets.

Calculating and testing the predictive fit of a risk score in the validation set

As described above, we calculated two risk score variants: *sample specific* and *rounded* risk scores. To test how well the risk scores predicted outcome, we conducted logistic regression analyses with robust standard errors with risk score entered as a continuous predictor of presence (1) or absence (0) of primary anxiety disorder at follow-up. Table 3 (top panel) reports descriptive statistics for the sample specific risk score, odds ratios, *p* values and area under the curve (AUC) values with associated confidence intervals.

[Insert Table 3 about here]

There was some variability in the predictive performance of the sample specific risk score across the five validation sets, with two of the five models providing statistically significant prediction ($p<.05$) of treatment outcome in the validation data set. However, as anticipated, all models reported odds ratios exceeding 1 (range 1.32 – 1.69), indicating that as risk score increased, the odds of non-remission (i.e. still having primary anxiety diagnosis) increased. For example, an odds ratio of 1.69 was identified in validation set 2: this indicates that the odds of non-remission increased by 1.69 times for every single point increment on the risk score. For the sample specific risk score, AUC[7] ranged from 0.62 to 0.68 with a mean value of 0.65.

Table 3 (bottom panel) reports summary statistics for the cross-validation *rounded* risk score. Four of the five models attained statistical significance. All models reported odds ratios exceeding 1 (range 1.38 – 1.70), again indicating that as the rounded risk score increased, the odds of non-remission also increased. For example, an OR of 1.70 was reported in validation set 2 indicating that the odds of non-remission increased by 1.7 times for every point increment on the risk score. AUC ranged from 0.65 to 0.69, with a mean AUC of 0.66. The rounded risk score and sample specific risk score therefore discriminated between remission and non-remission to a similar extent. Age and parental caseness had rounded scores of zero indicating that they did not contribute to prediction of outcome over and above the other variables in the model. As a result of the zero scores, age and parental caseness do not contribute to the risk index.

Characterising treatment outcome as a function of risk score in the entire sample

As a final step, we performed exploratory analyses to investigate the extent to which the rounded risk score was associated with treatment outcome in the entire sample. From a statistical perspective, this has the obvious limitation that it is not an independent sample from that in which the risk score was developed. However these exploratory analyses were undertaken solely to demonstrate the potential clinical utility of the risk score approach for predicting treatment outcome. Figure 1 depicts the distribution of risk scores in remitters and non-remitters. The distribution of

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[7] AUC measures the discriminative ability of the risk score to correctly classify those with and without their primary anxiety diagnosis. The AUC represents the proportion of randomly drawn pairs, consisting of one individual with their primary anxiety diagnosis and one individual without their primary anxiety diagnosis who would be correctly classified as such on the basis of their risk score. The participant with the higher risk score should be the one from the group with their primary anxiety diagnosis remaining.

risk scores was shifted to the right for non-remission compared to remission with a mean risk score of 3.64 (*SD* 1.45) and

2.87 (*SD 1.30*) respectively, $t(382) = -5.44$, $p < .001$, $d = 0.57$.

[Insert Figure 1 about here]

Figure 2 reports the percentage of participants with each risk score who retained their primary anxiety at follow-up.

Given the low number of participants for some scores within the scale, we divided the sample into the following four risk

categories: 0-2, 3, 4, or 5-8.

[Insert Figure 2 about here]

Non-remission increased in a linear manner with each risk category with just 23% of those scoring 0-2 retaining their

primary anxiety disorder at follow-up compared to 62.3% of those scoring 5-8. Risk score significantly predicted

treatment outcome at follow-up (OR = 1.51 [95% CI: 1.28 – 1.77], $p < .001$). AUC was also commensurate to that seen in

the validation sets (0.66). [8]

DISCUSSION

   This paper presents a novel risk score approach to combining genetic, demographic and clinical data to predict

non-remission to psychological therapy for child anxiety disorders. In doing so, we illustrate one clinically informative

method of repurposing G×E data that may assist in the identification of individuals who may require an enhanced

treatment package. Our combined clinical trial dataset also permitted greater clarification regarding the importance of

clinical factors in predicting remission of anxiety diagnoses. We found that greater pre-treatment severity, comorbid

mood disorders, comorbid externalizing disorders and female gender were all significantly associated with poorer

remission rates in at least one of our 5 validation sets when controlling for other variables (including genetic factors). In

line with our hypothesis, the risk score combining genetic and demographic/clinical factors had an odds ratio

significantly greater than 1 in all analyses. This indicates that the odds of non-remission increased for every one-point

increment on the risk score. The risk index showed moderate predictive ability, with a mean AUC of .65 and .66 using

the sample specific and rounded risk scores respectively. Finally, in the entire sample, the risk score was significantly

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[8] We tested this in the white only subset and the index performed in a similar manner (OR = 1.73 [95% CI: 1.40 –
2.15], $p < .001$; AUC = 0.70).

higher in non-remitters than remitters and those with scores in the highest risk band (5-8) were almost 3 times as likely not to remit following treatment as those in the lowest band (0-2).

Implications: Predictors of treatment outcome

Previous research examining non-genetic predictors of treatment outcome for child anxiety has been limited by low power, resulting in inconsistent findings. In our larger sample, we were able to examine the role of demographic and clinical factors whilst also taking into account preliminary genetic findings. As previously demonstrated (see Rapee, et al., 2009), higher pre-treatment severity of child anxiety predicted poorer outcome, even after accounting for both *5HTTLPR* and *NGF* rs6330 genotypes. In line with some (Liber et al., 2010) but not all previous research, comorbid mood and externalizing disorders also significantly predicted poorer outcome over and above all other variables. Previous studies have produced inconsistent findings with regards to the impact of non-anxiety comorbidity on treatment outcome, possibly due to their low frequency in children. The discrepant findings may also be due to different definitions of treatment response (e.g. diagnostic status at outcome versus change in symptom severity). The findings in this study are unique in showing that, even after accounting for pre-treatment severity, children with comorbid non-anxiety diagnoses have significantly worse end-points following treatment than children without comorbid diagnoses.

Finally, we also demonstrated that female gender was associated with poor treatment outcome. The majority of studies comparing treatment outcome for girls and boys have shown that gender does not moderate treatment outcome (Rapee, Schniering, & Hudson, 2009). The current findings cannot be explained by increased severity or comorbidity as the effect was evident after controlling for both. It is possible that factors associated with increased anxiety in girls also increase the likelihood of non-remission. This finding requires replication and further investigation.

Of note, neither age nor parental psychopathology were significant predictors of outcome, although our sample was restricted to children thirteen years and under. Previous evidence regarding the role of parental psychopathology in treatment outcome following CBT for child anxiety is mixed (Rapee, et al., 2009). In our analyses parental psychopathology was not significant in any of the five validation sets, and the rounded mean beta coefficient was zero. If entered alone parental psychopathology did predict outcome in this sample, but this effect was no longer present once other variables were included. However, we also note that our measure of parental psychopathology relied solely on a

self-report questionnaire which may have led to underreporting of parent symptoms, thus reducing the strength of this variable as a unique predictor of child outcome (Bogels & Brechman-Toussaint, 2006). Measurement of parental psychopathology using structured diagnostic interviews may result in a more accurate picture of symptoms.

Implications: Risk index for poor treatment outcome

Our risk index created from genetic, demographic and clinical factors was moderately good at predicting remission at follow-up. These results provide early promise that combining genetic information with clinical and demographic risk factors may be an informative approach to predicting individual differences in response to psychological treatment. Our data also support for the idea that genetic factors (over and above demographic and clinical variables) moderate response to a positive environmental influence, namely psychological therapy (Pluess & Belsky, 2012). Our risk index had similar predictive ability regardless of whether we used the sample specific or rounded beta coefficients. The next step is clearly to replicate these results, and to extend them, taking into account other potential predictors of non-remission, including additional genetic polymorphisms. What is more encouraging is that those in the highest band of our risk index were almost three times as likely not to remit following treatment as those in the lowest band, suggesting that the approach is worth pursuing further.

Limitations

We note a number of limitations. First, our sample remains small for a G×E study, even though it is the largest of its kind to date. Second, we recruited from six trials across two sites. However, not only does this make the data a closer representation of reality than utilising data from a single trial, it also means that our findings rose above the noise our sampling strategy inevitably incurred. Third, we focus here on the follow-up data, as our previous papers indicate that the effects of genotype occurred at this time-point (Eley et al., 2012). We note, however, that the follow-up time-point was not consistent across participants, which may have influenced our findings. We have previously discussed that we believe the late emergence of a significant genetic effect may be explained by continued practising of techniques learned during CBT. It is possible that what our risk index measures is the likelihood of a child continuing to benefit from treatment once it has ended. Fourth, this study focuses exclusively on two candidate genes. We anticipate in the future

that multiple genes (of very small effect) will be identified to predict treatment outcome. The field of therapygenetics is in its infancy and future work will be better placed to use polygenic risk scores. Finally, although our sample size allowed a greater number of children with mood disorders to be identified compared to smaller studies, there remained a low prevalence of mood disorders (8.9%), resulting in large standard errors around their odds ratios. Replication of these findings will be important.

In conclusion, we have illustrated that combining genetic, demographic and clinical data into a risk score is an informative means of repurposing G×E data. Our data provide preliminary evidence that children with a high number of genetic, demographic and clinical risks are least likely to benefit from standard CBT. If replicated, a risk index using both genetic and clinical information could be applied in a clinical context to help decide whether a child is likely to benefit from standard CBT alone or whether enhanced treatment is required.

Acknowledgements

Table 1: Sample characteristics for the entire sample and test statistics comparing training and validation sets for each cross-validation sample

	Entire sample (N = 384)	Cross Validation 1		Cross Validation 2		Cross Validation 3		Cross Validation 4		Cross Validation 5	
Pre-treatment (N = 384)		X^2/t	p	X^2/t	p	X^2/t	p	X^2/t	p	X^2/t	p
Child ancestry[1] (white; other; missing)	62.8; 10.7; 26.6	4.09	.13	0.38	.83	3.58	.17	1.92	.38	1.01	.61
Child age[2]	9.34 (1.87)	0.43[i]	.51	0.15	.70	0.43	.51	0.44	.51	0.15	.70
Child gender (M:F)[3]	195: 189	0.55	.46	0.05	.82	0.29	.59	1.09	.30	0.29	.59
Primary anxiety severity[4]	6.30 (.86); 43.2	0.01[i]	.94	1.85	.17	0.01	.94	0.91	.34	0.19	.66
5HTTLPR genotype[1] (LL; LS; SS)	31.5; 47.4; 21.1	1.99	.37	0.74	.69	0.97	.61	2.28	.24	0.89	.64
NGF rs6330 genotype[1] (CC; CT; TT)	30.0; 48.4; 21.6	1.50	.47	1.83	.40	0.19	.91	1.00	.61	1.29	.53
Comorbid mood disorder[1]	8.9	1.60	.21	0.01	.94	0.01	.94	0.13	.71	0.96	.33
Comorbid externalising disorder[1]	20.0	0.25	.62	0.02	.89	2.11	.15	0.21	.65	0.02	.89
Maternal DASS affected[1] (N = 382)	20.9	1.67	.20	0.84	.36	0.35	.56	0.08	.77	0.43	.51
Paternal DASS affected[1] (N = 363)	18.7	0.00	.96	0.61	.44	1.40	.24	0.20	.66	0.10	.75
Parental DASS affected[1] (0 parent; 1 parent; 2 parents)	67.4; 26.6; 6.0	2.02	.36	0.20	.91	1.89	.39	0.05	.97	1.70	.43
Post-treatment (N = 373)											
Primary anxiety severity[2]	3.42 (2.06)	0.59	.56	0.12	.91	0.00	.99	0.34	.73	0.54	.59
Primary anxiety remission[1]	50.7	0.28	.60	0.42	.52	0.15	.70	0.60	.44	0.00	.99
Follow-up (N = 384)											
Primary anxiety severity[2]	2.85 (2.03)	0.26	.79	0.93	.35	0.68	.50	0.95	.34	1.52	.13
Primary anxiety remission[1]	60.9	0.29	.59	1.05	.31	0.58	.45	1.14	.29	0.29	.59

Data reported: [1]percentage; [2]Mean (SD); [3]N; [4]Mean (SD); % in severe primary anxiety severity category (CSR 7-8). Analyses: [i]Test statistics compare frequencies of a binary variable (e.g. young vs. old age group; severe vs. moderate primary anxiety severity)

Table 2: Unstandardised beta coefficients for demographic, clinical and genetic predictors in each training set and mean beta values calculated across the five training sets

	Training 1		Training 2		Training 3		Training 4		Training 5		Mean *b*	
Model *p* value	.0001		.009		.002		.001		.002			
R^2	.08		.07		.08		.08		.08			
	b	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*Mean*	*Rounded*
5HTTLPR	1.13	.09	1.15	.09	.44	.51	.91	.19	1.09	.10	0.94	1
NGF	**.86**	**.02**	**.83**	**.03**	**.89**	**.02**	**.98**	**.008**	**.99**	**.009**	0.91	1
Age	.01	.98	.12	.83	-.51	.38	.20	.74	-.29	.62	-0.09	0
Gender	.63	.26	.86	.13	.52	.35	**1.12**	**.05**	.71	.20	.77	1
Primary anxiety severity	1.10	.06	**1.22**	**.03**	.87	.13	1.09	.06	**1.12**	**.05**	1.08	1
Comorbid mood	1.76	.07	1.12	.28	**2.47**	**.02**	1.29	.20	1.51	.17	1.63	2
Comorbid externalising	**1.45**	**.05**	1.16	.11	1.19	.11	.91	.20	1.05	.15	1.15	1
Parental caseness	.26	.58	-.27	.57	.13	.79	.25	.61	.16	.76	.11	0
Constant	1.64	.03	1.50	.06	2.59	.001	1.70	.04	2.02	.01		

Table 3: Model fit statistics and logistic regression analyses using the sample specific and rounded risk score as a predictor of treatment outcome in the five validation sets

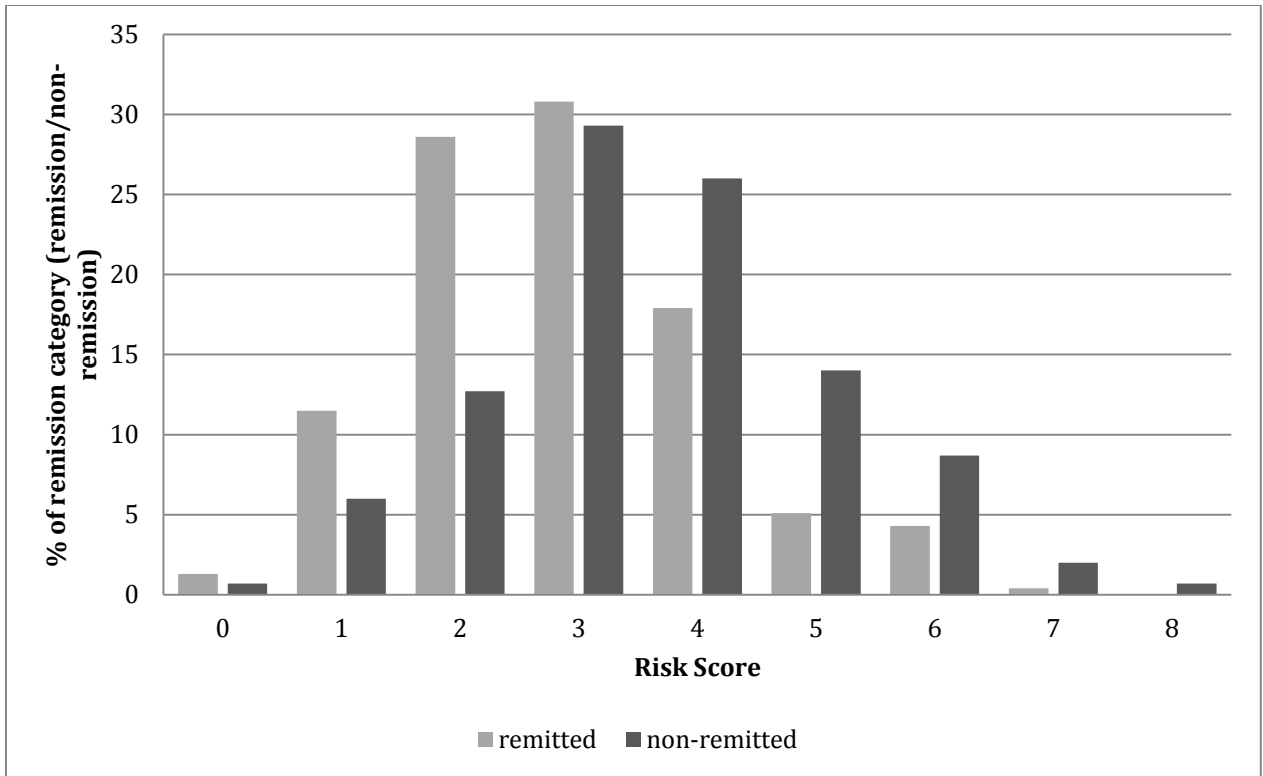|	Sample specific risk score				
	Validation 1	**Validation 2**	**Validation 3**	**Validation 4**	**Validation 5**
Mean	3.07 (1.39)	2.86 (1.40)	2.19 (1.29)	3.32 (1.38)	3.16 (1.41)
Range	.01 – 7.46	0 – 7.29	-.51 – 5.39	0 – 6.19	0 – 7.17
OR	1.32	1.69	1.34	1.41	1.50
(95% CI)	(.90 – 1.94)	(1.17 – 2.43)	(.94 – 1.93)	(.98 – 2.03)	(1.05 – 2.16)
p	.15	.005	.10	.06	.03
AUC	.65	.68	.62	.63	.65
	(.52 – .78)	(.56 - .81)	(.49 - .75)	(.50 - .76)	(.52 -.77)
	Rounded risk score				
Mean	3.06 (1.35)	2.97 (1.55)	3.25 (1.37)	3.16 (1.41)	3.36 (1.49)
Range	0 – 7	0 -8	0 – 6	0 – 6.0	0 – 8
OR	1.38	1.70	1.58	1.49	1.49
	(.92 – 2.06)	(1.22 – 2.38)	(1.09 – 2.29)	(1.03 – 2.14)	(1.06 – 2.08)
p	.12	.002	.02	.03	.02
AUC	.65	.69	.67	.65	.65
	(.53 - .78)	(.57 - .81)	(.55 - .79)	(.53 - .78)	(.52 - .77)

Figure 1: Distribution of risk scores for participants with and without their primary anxiety disorder diagnosis remaining at follow-up
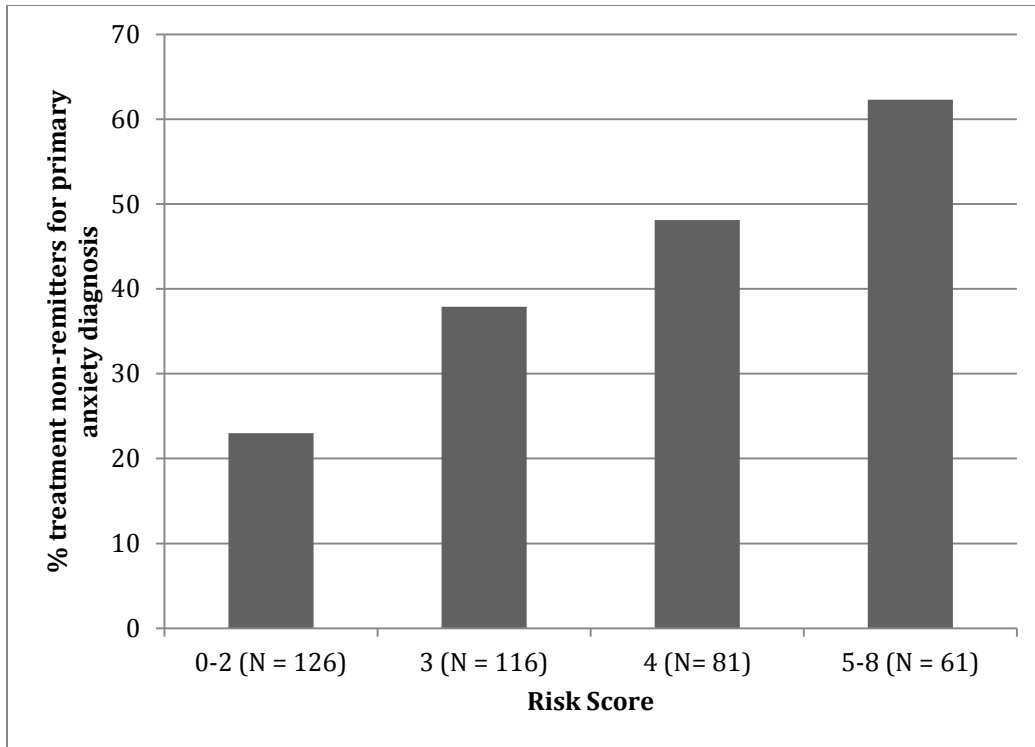
Figure 2: Percentage of non-remission (participants with their primary anxiety disorder diagnosis remaining at follow-up) by risk index score category

Key Points
1. We created a risk index from genetic, demographic and clinical factors to predict remission following CBT for child anxiety
2. The odds of non-remission increased for every one-point increment on the risk score.
3. 5*HTTLPR* and *NGF* rs6330 genotypes, pre-treatment severity of child anxiety, comorbid mood and externalizing disorders and gender predicted treatment outcome
4. Combining these predictors within a risk-index could be used to identify which children are less likely to be diagnosis free following CBT alone or thus require longer or enhanced treatment

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