Elevated morning cortisol is a stratified population-level biomarker for major depression in boys only with high depressive symptoms

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Major depressive disorder (MD) is a debilitating public mental health problem with severe societal and personal costs attached. Around one in six people will suffer from this complex disorder at some point in their lives, which has shown considerable etiological and clinical heterogeneity. Overall there remain no validated biomarkers in the youth population at large that can aid the detection of at-risk groups for depression in general and for boys and young men in particular. Using repeated measurements of two well-known correlates of MD (self-reported current depressive symptoms and early-morning cortisol), we undertook a population-based investigation to ascertain subtypes of adolescents that represent separate longitudinal phenotypes. Subsequently, we tested for differential risks for MD and other mental illnesses and cognitive differences between subtypes. Through the use of latent class analysis, we revealed a high-risk subtype (17% of the sample) demarcated by both high depressive symptoms and elevated cortisol levels. Membership of this class of individuals was associated with increased levels of impaired autobiographical memory recall in both sexes and the greatest likelihood of experiencing MD in boys only. These previously unidentified findings demonstrate at the population level a class of adolescents with a common physiological biomarker specifically for MD in boys and for a mnemonic vulnerability in both sexes. We suggest that the biobehavioral combination of high depressive symptoms and elevated morning cortisol is particularly hazardous for adolescent boys.

adolescence | gender differences

Major depressive disorder (MD) is a serious mental health problem predicted to be the leading health burden worldwide by 2030 (1, 2). MD increases markedly during adolescence and young adulthood: 25% of lifetime mood disorders appear by 18 y of age and 50% by the age of 30 y (3). MD in childhood or adolescence raises the risk of future episodes in adulthood some fourfold (4), especially in adolescent boys and younger men aged 15–35 y (5, 6). The sex ratio for depression is about equal in childhood, but, by the end of adolescence, females outnumber males by a factor of around two to one (7, 8). Whether there are underlying mechanisms associated with the emergence of MD during adolescence that are themselves sex-specific is unknown. If this distinction were so, then biomarkers for MD might themselves be preferentially activated in one sex compared with another.

Current diagnostic classifications [e.g., the *Diagnostic and Statistical Manual for Mental Disorders* (DSM) and the *International Classification of Diseases* (ICD)] have proved to have low diagnostic validity for investigations on the etiology, prevention, or treatment of MD partly because they ignore heterogeneity (9). Identifying predictive biomarkers (10) has been hampered by this variation because the current taxonomic systems may conflate disorders of similar clinical phenotype that have distinctly different aetiologies (11–14). One approach to this problem is to use person-centered statistical techniques that are effective in incorporating this clinical heterogeneity by identifying distinct classes of adolescents at the population level (15, 16). In this way, it may be possible to develop longitudinal phenotypes that can enhance or improve upon current classifications systems. This principle is also consistent with the philosophy behind the National Institute of Mental Health Research Domain Criteria (RDoC) public mental health strategy, which seeks to discover new ways of classifying psychopathology based on dimensions of observable behavior and neurobiology (17, 18).

This lack of precision in identifying valid population-based subtypes at differential risk for MD is a serious impairment in targeting available interventions toward the most susceptible individuals. There is continued reliance on seeking universal interventions to prevent MD in younger populations using single-risk measures such as raised but subclinical depressive symptoms. The low validity of elevated depressive symptoms alone as a recruitment index for early interventions was demonstrated recently in the largest school-based psychological intervention reported to date. The authors showed null effects for two active treatments [cognitive behavioral therapy (*CBT*) and attentional

Significance

Clinical depression is a severe and common illness, characterized primarily by persistent low mood and lack of pleasure in usually enjoyable activities, that results in significant impairment in everyday living. It also involves alterations in cognitive and hormonal functions. There is substantial variation between depressed individuals in terms of the causes and therapeutic response, making it difficult to identify those most likely to benefit from intervention and treatment. We derived subtypes of adolescents in the population based on different levels of the hormone cortisol and subclinical depressive symptoms. A group (17%) with both high levels of cortisol and depressive symptoms of both sexes had more depressed thinking. Boys in this group were at high risk for clinical depression.

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training, respectively] delivered to groups in classroom settings against standard support given by teachers (11). They recommend a pause in the current mental-health strategy toward early detection and intervention in groups at risk for MD until more valid methods of detecting susceptible individuals can be achieved.

Nevertheless, subclinical levels of self-reported depressive symptoms alone do constitute the most robust prognostic measure for the emergence of MD in youth and young adults of both sexes (19, 20). How might the embedded valid signal for MD of this symptom profile be enhanced? Two other potential detection components have been used in prognostic studies at the community level with some modest results: elevated morning salivary cortisol and computerized tests of cognitive biases. Dysregulated cortisol rhythms and elevated morning and evening cortisol have consistently been reported as a risk factor for, or consequence of, MD (21–26). Biomarkers such as early-morning cortisol are likely to be important for some but not all young people who become ill. As yet there has been no population-based investigation of who such adolescents might be. These findings suggested to us that a population-based stratified approach may identify a subtype at highest risk for depression by combining depressive symptoms with early-morning cortisol as a risk phenotype and discriminating these from any other subtypes using cognitive measures that are associated with MD (15, 16).

The mechanisms that underpin the risk effects of depressive symptoms and/or morning cortisol are also unclear but may involve cognitive processes. For example, depressed adolescents retrieve more "overgeneral" memories of categorical autobiographical events than nondepressed psychiatric patients and community controls, rather than specific details of such events that happened at a particular time and place (27). Overgeneral memory (OGM) is often associated with MD and depressive symptoms, as well as with impaired interpersonal problem solving, difficulties in imagining specific events in the future, unproductive perseverative ruminative thought, and the avoidance of healthy exposure to negative memories (28). Enhancing the specificity of memories in adolescents has been found to reduce depressive symptoms over time (29) although further research is needed to know whether such interventions could be truly preventive.

Whether there is a relationship between OGM and cortisol is less certain. Some studies report a negative dose–response effect of administered cortisol on OGM performance in healthy volunteers (30) but not in patients with MD (31). Another study, measuring endogenous salivary cortisol in MD patients, found no correlation between basal levels of cortisol and OGM. However, the latter was negatively correlated with a decrease in cortisol in males (32). We expected more OGM in individuals classified by both higher depressive symptoms and elevated morning cortisol compared with other population subtypes.

Alterations in emotional responses expressed through impaired information processing for negative and neutral, but not positive, word classification on a Go/No-Go Task are present in well but susceptible adolescents and are associated with emotional disorders (33). We predicted that such impairments would be found most in a population subtype defined by higher depressive symptoms and morning cortisol. Etiological heterogeneity in another psychiatric disorder [attention deficit hyperactivity disorder (ADHD)] was recently revealed by recognizing subgroups nested within the normal population with distinctive cognitive profiles (34). Whether subgroups exist for clinical depressions is not known.

The extent to which distal factors, such as exposure to family adversities in childhood, have an influence on phenotypes involving both depressive symptoms and cortisol is also unclear. Our studies show that exposure to an adverse family environment in childhood is clearly associated with the emergence of both emotional and behavioral disorders by adolescence (35). Other research has shown that early adversities may, however, be associated with lowered cortisol (36). Conduct, and oppositional disorders in particular, may be associated with cortisol profiles different from those of affective disorders (37). We therefore determined whether exposure to childhood adversities before the age of 11 y discriminated between adolescent classes in the population. Subsequently, we looked to see whether there was any particular association between low cortisol and behavior disorders. If this relationship were so, it might indicate a population-based differentiation between adolescents at risk for conduct and depressive disorders, respectively.

Here, we have used a person-centered approach to analyze data from two cohorts of young people (total $n = 1,858$) recruited from schools in one locality of the United Kingdom. We found four distinct classes of adolescents defined either by elevated cortisol or the presence of depressive symptoms. Of particular interest was the fourth class, identified by both high depressive symptoms and elevated early-morning cortisol. We found that boys showed a significant increase in the odds of MD in this class, compared with the reference class (low depressive symptoms and morning cortisol). We also found that OGM was overexpressed among those in this class. However, there was no specificity between classes and behavior disorders or exposure to family adversities before 11 y of age.

Details on sample recruitment, measures used, and data analysis are found in *SI Materials and Methods* and *SI Text*.

Results

Longitudinal Stability of Salivary Cortisol. Given the dearth of information available on the longitudinal stability of cortisol in young people (38), we first established that morning cortisol has a trait-like component over a 12-mo period. In cohort 1 ($n = 660$) salivary cortisol samples were collected at 0800 hours within a week of a baseline interview over four consecutive days and again 12 mo later. We used a structural equation modeling framework using a latent state-trait model (LST) to estimate the extent to which individual differences in morning cortisol are explained by a trait-like component versus occasion-specific factors (Fig. S1). The results showed that there was a substantial trait (48–60%) as well as state (40–52%) component. Full model details can be found in *SI Text*.

A Four-Class Model of Morning Cortisol and Depressive Symptoms Revealed and Replicated. We then used latent class analysis (LCA) combining longitudinal data from both cortisol and depressive symptoms to derive classes of young people in our first cohort of our participants (cohort 1), who had depressive symptoms measured at baseline and at 4, 8, and 12 mo, and subsequently replicated this class structure in the second one (cohort 2), in whom symptoms were measured at baseline and at 18 and 36 mo. Details of model-selection criteria for the LCA can be found in *SI Text*.

A four-class model provided the best fit to the data in cohort 1: the lowest Bayesian information criterion (BIC) (9,605.7), a nonsignificant likelihood ratio test (LRT) ($\chi^2 = 1,553.2$, $P = 1.0$), and a significant Lo–Mendell–Rubin (LMR) test (159.4, $P = 0.046$). Analysis of cohort 2 replicated this four-class model (lowest BIC, 12,233.6; nonsignificant LRT, $\chi^2 = 689.6$, $P = 0.323$; significant LMR, 141.9, $P = 0.001$). Mean cortisol levels and full model details can be found in Table S1 and *SI Text*. See Fig. S2 and Tables S2 and S3 for full model comparisons and Fig. 1 for an illustration. Tables 1 and 2 show the descriptive statistics for the indicators by latent class.

The average latent class probabilities for both cohorts were high, ranging from 0.86 to 0.90 (Table S2). In both cohorts, class 1 was characterized by both relatively low morning-cortisol levels and low depressive symptoms over time, class 2 by low levels of depressive symptoms but relatively high morning cortisol, class 3 by high depressive symptoms and low morning cortisol, and class 4 by high levels of both morning cortisol and depressive symptoms.

The similarity of the class structure between the two cohorts and the absence of any difference in the latent class probabilities [χ^2 (df = 3) = 0.07, $P = 0.99$] allowed us to combine cohorts 1 and

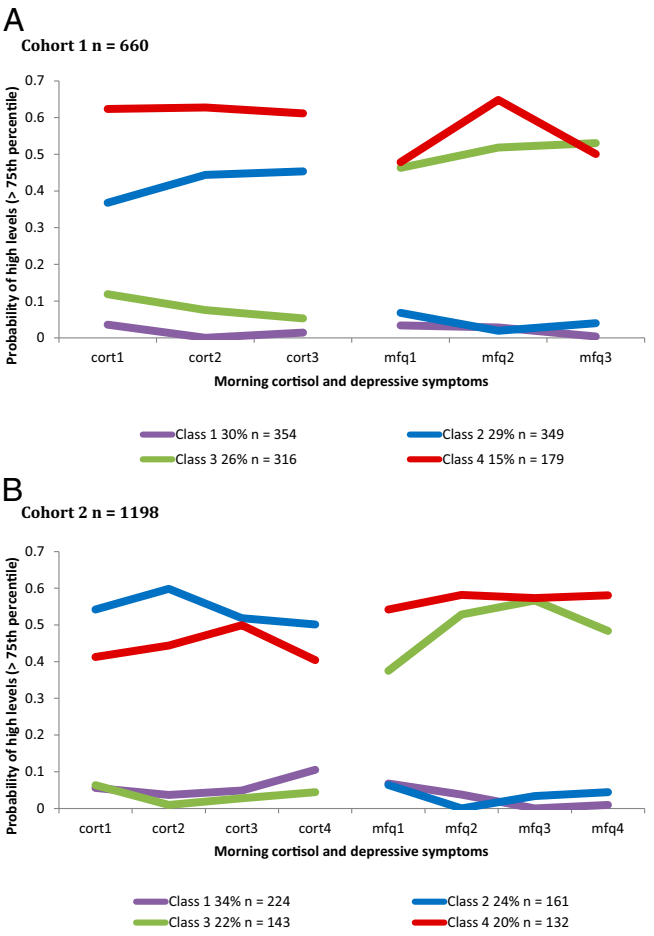

Fig. 1. The four-class model in cohort 1 (*A*) and cohort 2 (*B*). Cort1 to -4 refer to the measurements of morning cortisol whereas mfq1 to -4 refer to depressive symptoms. Lines represent actual classes, and the *y* axis refers to the probability of high levels of each variable given class membership.

2 (total n = 1,858), controlling for cohort as a cofactor. This procedure resulted in a combined group of 898 boys and 959 girls, of which 31% were in class 1, 27% class 2, 25% class 3, and 17% class 4. This four-class model (Fig. 1) showed a sex-differentiated pattern [χ^2 (3) = 145.8, $P < 0.0001$] with the ratio of boys to girls reversing between class 1 and the other three classes. Thus, class 1 contained 67% boys and 33% girls whereas class 4 was made up of 28% boys and 72% girls; the other two classes showed intermediate values (Fig. S3). As a result, sex was treated as a covariate in subsequent overall analyses. Sex-independent analyses were also carried out. Given that there were additional differences in age and puberty between cohorts (*SI Text*), these factors were also controlled for as covariates.

Classes Show Distinctive Associations with Clinical Diagnosis of Major Depression. Next, we related these four classes to the onset of subsequent MD derived from clinical interview. Data on follow-up clinical diagnostics were available for 93% (n = 1,733) of participants. Two hundred seven of 1,733 (12%) had met criteria for clinical depression at least once before 16 (cohort 1) or 17 (cohort 2) y of age. Participants in classes 2–4 were more likely to have reported MD at follow-up compared with class 1: The odds of being a depressed case increased progressively across the classes from 1.6 to 7.1 [class 2, odds ratios (OR) = 1.6, $P = 0.10$, 95% confidence interval (CI) 0.9–2.8; class 3, OR = 5.2, $P < 0.0001$, 95% CI 3.2–8.6; and class 4, OR = 7.1, $P < 0.0001$, 95% CI 4.3–11.8].

Sex Differences. Next we asked whether these sex differences applied to the association between the classes and MD. There was a significant sex × classes interaction (χ^2 = 11.8, $P < 0.01$) (Fig. 2). In boys, there was an increase in the odds of being clinically depressed for those in class 4 (high depressive symptoms, elevated morning cortisol) compared with class 1 (low symptoms, low cortisol) (OR = 14.7, $P < 0.0001$, 95% CI 6.1–35.0). Post hoc comparisons showed that class 4 boys also had a significantly increased probability of MD compared with those in the other two groups (class 2, low symptoms, high cortisol, $P < 0.001$; or class 3, high symptoms, low cortisol, $P < 0.01$). We found a similar pattern for class 4 boys when those with a current diagnosis of MD at baseline (n = 20) were removed from the analysis [OR = 10.7, $P < 0.0001$, 95% CI 4.3–26.7, significantly different from class 2 ($P < 0.001$) but not from class 3 when the Holm correction was applied ($P = 0.03$)].

Girls showed a different pattern: Class 3 individuals (high symptoms, low cortisol) were most likely to report depression compared with class 1 (OR = 3.9, $P < 0.001$, 95% CI 2.1–7.3), followed by class 4 (high symptoms, high cortisol) (OR = 3.5, $P < 0.001$, 95% CI 1.9–6.6). Post hoc comparisons showed that class 3 participants were significantly different from class 2 (low symptoms, high cortisol) ($P < 0.001$) but not class 4 ($P = 0.62$).

These sex-differentiated results imply that cortisol moderated the liability for MD with high depressive symptoms only in boys whereas, in females, higher depressive symptoms were prognostic irrespective of cortisol levels.

Nondepressive Psychiatric Disorders and Elevated Morning Cortisol. We tested the specificity of these effects for MD by repeating the analyses using DSM IV nondepressive psychiatric disorders (NDPDs) (Fig. S4). There was also a significant association between NDPD and the classes. However, important differences from MD were revealed. Overall, there was no difference between class 1 and class 2 in the probability of reporting NDPD (OR = 1.1, $P = 0.95$, 95% CI 0.7–1.7). There were significant differences between class 1 and both class 3 (OR = 2.2, $P < 0.001$, 95% CI 1.4–3.5) and class 4 (OR = 2.9, $P < 0.0001$, 95% CI 1.8–4.9). Post hoc comparisons showed that individuals in class 3 and class 4 were significantly different from those in class 2 (P <0.001 and P <0.0001, respectively), but, unlike the association with MD, they were not significantly different from one another ($P = 0.21$). Furthermore, there was no significant sex-differentiated effect ($P = 0.40$). Overall, these results for all NDPDs support the existence of a specific effect of elevated morning cortisol as a biomarker for MD in boys with higher depressive symptoms.

Family Adversities, Behavior Disorders, and Class Membership. Because there is some evidence for low, as well as high, cortisol levels being associated with exposure to family adversities and mental illnesses such as behavior disorders, we examined the relationships between these phenomena and the classes. We found that behavior disorders (BDs) (oppositional defiant and conduct disorders combined) were differentially associated with the classes (Fig. S5). Class 3 (high symptoms, low cortisol) had a significantly higher likelihood of BD compared with class 1 (OR = 2.6, $P = 0.002$, 95% CI 1.4–4.7). Post hoc comparisons showed that this class also had a higher probability than class 2 ($P < 0.001$) and that class 4 was more likely to have BD than class 2 ($P = 0.01$). There was no significant sex × classes interaction ($P = 0.45$). This finding reveals no clear cut association between low cortisol and behavior disorders but does indicate a possible transdiagnostic effect of depressive symptoms in identifying risk for behavior as well as depressive disorders.

Using data from cohort 2 only, we found a significant overall effect of family adversity on the classes [χ^2 (1) = 30.0, $P < 0.001$]. The relative risk posed by the presence of family adversity for class 3 membership was significant [relative risk ratio (RR) = 1.9, $P < 0.001$, 95% CI 1.4–2.7] compared with the reference group (no adversity). This pattern was also observed for class 4

Table 1. Descriptive statistics by class and subsample (cohort 1, $n = 660$)

Class	1 ($n = 224$, 34%)		2 ($n = 161$, 24%)		3 ($n = 143$, 22%)		4 ($n = 132$, 20%)	
Sex M (%)	152 (67.9)		70 (43.5)		83 (58.0)		53 (40.2)	
Age	13.7 (1.1)		13.9 (1.2)		13.6 (1.2)		13.6 (1.2)	
Sex	M	F	M	F	M	F	M	F
MFQ 1	12.8 (6.8)	13.4 (7.0)	14.8 (6.7)	13.8 (5.6)	23.2 (8.0)	23.7 (8.4)	24.7 (8.0)	26.5 (8.3)
MFQ 2	7.0 (4.6)	8.7 (5.3)	7.6 (4.5)	8.6 (4.6)	20.3 (7.5)	21.4 (8.9)	22.1 (6.9)	22.0 (8.4)
MFQ 3	6.8 (4.2)	9.5 (4.1)	9.3 (4.8)	8.9 (5.8)	22.5 (7.8)	23.8 (11.3)	21.3 (9.3)	24.3 (10.6)
MFQ 4	8.7 (4.6)	10.1 (4.8)	10.9 (5.4)	10.9 (5.8)	20.5 (7.0)	22.6 (11.0)	23.4 (8.5)	23.9 (9.2)
CORT 1	2.2 (1.1)	2.3 (1.4)	3.8 (1.6)	5.0 (1.7)	2.1 (1.1)	2.4 (1.1)	4.2 (1.8)	4.0 (1.9)
CORT 2	2.0 (1.1)	2.3 (1.0)	4.1 (1.2)	4.6 (2.0)	1.9 (0.9)	2.1 (0.9)	4.0 (1.5)	4.1 (1.8)
CORT 3	2.1 (1.2)	2.3 (1.0)	3.7 (1.4)	4.7 (1.6)	2.0 (1.0)	2.1 (1.0)	4.2 (2.2)	4.5 (2.0)
CORT 4	2.1 (1.4)	2.2 (1.2)	3.9 (1.3)	4.6 (1.9)	2.1 (1.2)	2.2 (1.1)	3.5 (2.2)	4.1 (2.0)

MFQ 1–4, Mood and Feelings Questionnaire total score for time points 1–4. CORT 1–4, morning salivary cortisol for time points 1–4 in ng/mL. Values represent means with standard deviations in parentheses.

membership, which was predicted by the presence of family adversity (RR = 1.8, $P < 0.01$, 95% CI 1.2–2.6). The risk of class membership stemming from family adversity was not different between classes 3 and 4 [χ^2 (1) = 0.1, $P = 0.72$]. There was no sex × classes interaction ($P = 0.40$).

Classes and Cognition. Using data available from cohort 1 only, we showed that there were significant differences between the classes on the test of overgeneral memory (OGM). Class 4 participants showed more OGM responses compared with class 1 (OR = 2.3, $P < 0.01$, 95% CI 1.3–3.9). Follow-up comparisons showed that class 4 was also significantly different from both class 2 (low symptoms, high cortisol; $P < 0.001$) and class 3 (high symptoms, low cortisol; $P = 0.01$) (Fig. S6). There was, however, no significant sex × classes interaction ($P = 0.83$).

There were no significant differences between the classes on our measure of information-processing bias (*SI Text*).

Discussion

Consistent with recent mental health strategy recommendations (16, 39), the present study explored ways of identifying a potential biomarker to detect subtypes of adolescents at the population level with higher risk for one of the most debilitating and costly mental health problems, major depressive disorder (MD) (1, 2). The results suggest the presence of longitudinally derived and population-based phenotypes characterized by varying combinations of morning cortisol and depressive symptoms. We demonstrate that a combination of higher self-reported depressive symptoms and elevated morning cortisol identifies a substantial subtype of adolescent boys in the community at large with the highest risk for MD and that this subtype could be a valid stratified target for etiological, preventative, and therapeutic studies (9).

This clinical finding emphasizes the need for new lines of sex-differentiated inquiry into mechanisms that lead to MD. In terms of public mental health strategy, early detection and intervention of adolescent boys in class 4 in the population at large could be valuable for reducing the longer-term burden of mental illness. Because we found an associated relation between this class membership and OGM, a targeted strategy on adolescent boys with higher depressive symptoms and elevated morning cortisol, focusing on methods to improve the specificity of recalling autobiographical memories through a training intervention (29), may be more clinically effective and cost-effective than current universal programs. However, this is only a testable hypothesis at this stage.

Consistent with the only other report (38), we provide clear-cut confirmatory evidence for longitudinal stability of morning-cortisol levels. This finding demonstrates a trait-like property, a key pre requisite for acting as a physiological biomarker. Subsequently, we derived and then replicated four classes of population-based adolescents using repeated measures of depressive symptoms and early-morning levels of cortisol. This study represents previously unidentified use of longitudinal data in an adolescent population for these purposes and is valuable for improving the validity of both measures and the formation of latent classes. These classes represent subtypes of adolescents and provided a platform for testing new hypotheses regarding the liability for clinical disorders and the mechanistic role of cognitive processes associated with MD (27, 29, 33, 40). The subtype approach used here is also consistent with the RDoC research strategy, which heralds a move away from an over-reliance on psychiatric diagnosis. Another critical test of the usefulness of such subtypes will be in assessing how well these markers predict interventions, such as mental health education programs, as well as treatment response (18).

Table 2. Descriptive statistics by class and subsample (cohort 2, $n = 1,198$)

Class	1 ($n = 354$, 30%)		2 ($n = 341$, 29%)		3 ($n = 316$, 26%)		4 ($n = 179$, 15%)	
Sex M (%)	236 (66.7)		134 (38.5)		135 (42.7)		35 (19.6)	
Age	14.5 (0.3)		14.5 (0.3)		14.5 (0.3)		14.5 (0.3)	
Sex	M	F	M	F	M	F	M	F
MFQ 1	9.4 (5.2)	10.0 (5.3)	9.6 (6.9)	10.9 (7.0)	21.1 (8.6)	23.8 (10.2)	21.7 (5.5)	24.6 (10.1)
MFQ 2	7.4 (5.7)	8.4 (5.8)	8.0 (4.8)	9.2 (5.4)	20.5 (11.1)	25.3 (11.8)	21.7 (15.4)	28.3 (14.6)
MFQ 3	6.9 (4.5)	7.9 (4.7)	8.7 (5.9)	10.0 (6.3)	22.4 (9.7)	24.1 (13.3)	23.7 (14.5)	23.1 (11.5)
CORT 1	2.5 (1.1)	2.6 (1.2)	4.4 (1.8)	4.8 (2.1)	2.6 (1.3)	3.3 (2.0)	5.1 (1.5)	5.9 (2.1)
CORT 2	2.3 (1.0)	2.4 (1.1)	4.6 (1.8)	5.0 (1.7)	2.5 (1.2)	2.7 (1.5)	5.1 (1.6)	5.7 (1.9)
CORT 3	2.3 (1.1)	2.5 (1.2)	4.4 (1.6)	4.9 (1.9)	2.4 (1.1)	2.6 (1.4)	4.8 (1.4)	5.6 (2.1)

MFQ 1–3, Mood and Feelings Questionnaire total score for time points 1–3. CORT 1–3, morning salivary cortisol for time points 1–3 in ng/mL. Values represent means with standard deviations in parentheses.

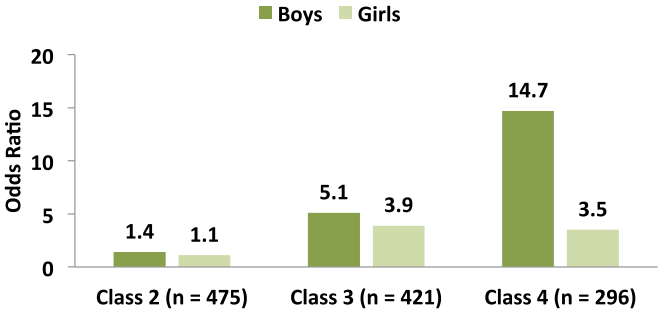

Fig. 2. The odds ratios for MD in each class by sex. The reference group is class 1 (n = 539). The associations are adjusted for cohort, age, and pubertal status.

Focusing on the class 4 population subtype, we show sex-differentiated results. Boys in this class on average show higher depressive symptoms and are at highest risk for MD arising through a corticoid-moderated process associated with cognitive deficits in autobiographical memory. This finding resonates with the results of a naturalistic follow-up of severely depressed adolescents, which demonstrated that boys were at significantly higher risk than girls for persistent and severe mental illness into young adult life (6).

Importantly, neither boys nor girls with elevated cortisol levels but low depressive symptoms (class 2) show such a set of associations. This result clearly implicates the importance of both higher depressive symptoms and elevated morning-cortisol levels in combination, identifying a vulnerable class of adolescent boys. There is a growing literature on the sex-differentiated impact of stress on cortisol. Boys appear more likely than girls to show elevated levels following stress under real-life and laboratory controlled conditions (41), a mechanism that may be in place before birth (42). Might boys be more vulnerable than girls to the neurotoxicity effects of persistently elevated cortisol within the normal range? Because adolescent girls have higher free (salivary) cortisol levels than boys (25), there may be other pathways involving cortisol operating to increase mental illness risk in females not revealed in this study. For example, the differential effects of stress on girls and boys may depend on the nature, timing, and patterning of early life stresses and/or acute proximal adolescent stresses not included in this report, as well as neural responses to stress (43, 44).

The clinical specificity of these findings for MD was enhanced by the fact that there was no specific association between being a member of class 4 for either sex and the presence of nondepressive psychiatric disorder or, more specifically, behavior disorder. Interestingly, these analyses also demonstrated the transdiagnostic nature of self-reported depressive symptoms, as both classes with elevated depressive symptoms (classes 3 and 4) showed increased risk for nondepressive disorders as well as MD compared with class 1 (low depressive symptoms and cortisol). The latter finding supports the notion that, in adolescents, there is a lack of precision within clinical measures for MD (45).

Testing for depressogenic cognitions provided further validity for the distinction between the class 4 subtype and the rest of the population. Class 4 individuals showed more overgeneral autobiographical memory (OGM) than both the reference class (class 1) and class 3 (high depressive symptoms, low cortisol). Deficits in information processing bias compared with class 1 were not significant after correcting for multiple comparisons. These cognitive findings build on previous research (mostly case-control studies) that have highlighted overgeneral memory being associated with MD (28, 40, 46) and are consistent with psychobiological models of depression (47). Here, we present a previously unidentified demonstration that these deficits are present in both sexes in a subsample of adolescents defined by a psychoendocrine index in the population at large.

These findings do not reveal precise cognitive mechanisms that lead to the formation of depressive symptoms (including depressogenic thinking) in boys in class 4 who are most at risk. Other processes may be at work, including impairments in reward sensitivity and learning under stress and nonstress conditions (48).

It is important to note that not all MD cases are associated with dysregulated early-morning cortisol (21) or elevated depressive symptoms so other cognitive mechanisms are also likely to be operating. Differences in such mechanisms may be particularly true in girls with elevated depressive symptoms. Furthermore, the latent classes derived in the present study represent longitudinal phenotypes, and so conclusions concerning causality should be made with caution. The interactions between factors at prior, unmeasured points in time are unknown to us and could reflect more complex developmental trajectories. It is interesting to note that, although we found a more distal effect of family adversity on class membership, this effect was largely driven by high depressive symptoms. Similarly, all models are limited by the variables that constitute them, and it is possible that further subtypes may emerge in future studies that include additional biological or cognitive measures. Therefore, we cannot rule out corticoid-moderated mechanisms via other processes for girls simply on the basis of the current findings. Our findings could be considered as epiphenomena of MD; however, the available evidence suggests that, in community-ascertained adolescent participants, higher depressive symptoms (19), morning cortisol (24), and OGM (29) all precede the emergence of MD. In addition, our results suggest that a combination of high depressive symptoms and elevated morning cortisol in boys may have a causal role in predicting future MD.

It may also be the case that current classifications, as used in this study, such as DSM and ICD are simply not optimally specified. That is, they are not yet able to take into account cortisol-moderated pathophysiologies or sex differences as well as quantitative implications of depressive symptoms. These results add to the growing evidence for pathogenic heterogeneity within psychiatric disorders pointing to the presence of subtypes, which has been used to argue that diagnostic categories may need updating (49).

In conclusion, this study indicates that combining two easily measurable factors in an epidemiologically principled way can be used to identify population-based classes of adolescents, representing longitudinal phenotypes. There is greater direct clinical vulnerability for boys in the highest risk subtype characterized by higher depressive symptoms and elevated morning cortisol. In contrast, higher depressive symptoms alone are a risk factor for girls. Further research should focus on the specific cognitive mechanisms involved for boys and girls. New models of public mental health education and intervention in the youth population are suggested for both sexes.

Materials and Methods

Participants. The present study incorporated 1,858 young people (12–19 y of age) from two cohorts (cohort 1, n = 660; cohort 2, n = 1,198) (see *SI Text* for further information). Both studies received approval from the local regional ethics committees and were conducted according to Helsinki good practice guidelines (50).

Measures Used to Derive Latent Classes. Self-reported depressive symptoms were obtained in both cohorts using the 33-item version of the Moods and Feelings Questionnaire (MFQ) (51), and cortisol assay was measured by ELISA on 20-μl samples of saliva without extraction (antibody; Cambio). Results are reported in ng/mL.

Validation Measures. ***DSM IV psychiatric diagnoses.*** Diagnoses were assessed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) semistructured interview (52).

Depressogenic cognition. In cohort 1, an experimental test of overgeneral autobiographical memory was taken (53). In cohort 2, a computerized task was used from the Cambridge Neuropsychological Test Automated Battery (54) measuring information processing bias [Affective Go/No-Go Task (AGN)].

Family Adversity. The quality and personal impact of the family environment in the childhood years was assessed retrospectively from parental interview using the Cambridge Early Experience Interview (35).

Further details of measures are given in *SI Text*.

Control Measures. ***General cognitive functioning.*** Participants completed a short form of the Wechsler Intelligence Scale for Children including block design and vocabulary (version II in cohort 1 and version III in cohort 2).

Puberty. Pubertal status was assessed using a self-reported Tanner scale. Participants were categorized into pre/early pubertal stage (Tanner stages 1 and 2) and mid/late puberty (Tanner stages 3–5).

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