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Anxiety-associated and separation distress-associated behaviours in Angelman syndrome

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Abstract

Background Anxiety is considered a 'frequent' feature in the clinical criteria for Angelman syndrome; however, the nature and severity of anxiety symptoms have not been well characterised in this population. Anxiety behaviours, especially in response to separation from a preferred caregiver, have been described clinically but have not yet been explored empirically.

Method This study used a combination of standardised and clinician-derived survey items to assess the frequency, nature and severity of behaviours associated with anxiety and separation distress in 100 individuals with Angelman syndrome. Family (e.g. income and maternal education) and individual (e.g. age, sex, genetic subtype, sleep difficulties and aggressive behaviours) variables were also gathered to assess possible predictors of higher anxiety levels. Approximately half of the sample was seen in clinic and assessed with standardised measures of development and daily functioning, allowing for an additional exploration of the association between anxiety symptoms and extent of cognitive impairment.

Results Anxiety concerns were reported in 40% of the sample, almost 70% were reported to have a preferred caregiver and over half displayed distress when separated from that caregiver. Individuals with the deletion subtype and individuals who are younger were less likely to have anxiety behaviours. Sleep difficulties and aggressive behaviour consistently significantly predicted total anxiety, the latter suggesting a need for future studies to tease apart differences between anxiety and aggression or anger in this population.

Conclusions Anxiety concerns, especially separation distress, are common in individuals with Angelman syndrome and represent an area of unmet need for this population.

Keywords Angelman syndrome, anxiety, separation distress

Introduction

Angelman syndrome (AS) is a rare neurodevelopmental disorder caused by a loss of function of the *UBE3A* gene on the maternal allele of chromosome 15q11.2–q13. Although the true prevalence of AS is not known, it is generally estimated to be approximately 1:15 000 births (Mertz *et al.* 2013). There are four molecular causes for the deficient expression of maternal *UBE3A* causing AS. The most common is a deletion of the AS critical region of chromosome 15q11.2–q13, occurring in approximately 70% of cases (Lalande & Calciano 2007). Deletions occur de novo and do not carry a

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recurrence risk. The second most common aetiology of AS is paternal uniparental disomy, occurring in approximately 5% of cases (Bird 2014). Uniparental disomy cases also occur de novo and have a very low recurrence. Imprinting defect, causing lack of expression of the maternal copy of *UBE3A*, occurs in approximately 5% of AS cases. Imprinting defect cases are familial and carry a 50% recurrence rate (Bird 2014). Point mutations of the maternally inherited copy of *UBE3A* make up approximately 10% of cases; these include insertion, deletion, nonsense, missense and splice site mutations (Bird 2014). These mutations are often inherited from the mother's paternally acquired allele, leading to a 50% recurrence rate (Van Buggenhout & Fryns 2009).

Clinical diagnostic criteria for AS were established by a consensus report in 1995 (Williams *et al.* 1995) and updated in 2005 (Williams *et al.* 2006). These criteria include a list of features seen in 100% of individuals with AS (consistent), features seen in 80% of individuals with AS (frequent) and features seen in 20% to 80% of individuals with AS (associated). Although there is some variability depending on subtype, the lack of *UBE3A* in the brain always results in a severely affected phenotype. Included in the consistent features are functionally severe developmental delays, little to no use of speech, movement disorders and a unique behavioural profile that includes frequent laughing, smiling and excitability as well as hyperactivity. Sleep difficulties, seizures and other medical and behavioural features are also frequently present (see Wheeler *et al.* 2017 for a review).

A behavioural feature less well described in this population is anxiety. Anxiety is listed as a 'frequent' feature in the consensus reports (Williams *et al.* 2006), and studies of stress and anxiety in the AS mouse model suggest that increased anxiety-like behaviour may be directly associated with loss of *UBE3A* (Godavarthi *et al.* 2012). However, to date, only three studies have explicitly described anxiety in AS (Larson *et al.* 2015; Wink *et al.* 2015; Prasad *et al.* 2018). Results of these studies suggest that, when asked in an interview, nearly half of caregivers report high anxiety in their family member with AS (Larson *et al.* 2015), with these numbers increasing significantly in individuals over 26 years of age (Prasad *et al.* 2018). However, on standardised measures of anxiety, individuals with AS were not rated as having diagnostically elevated social avoidance, generalised anxiety or obsessive–compulsive behaviour (Wink *et al.* 2015).

There are several likely causes for this discrepancy and the lack of clear understanding about anxiety in this population. First, because individuals with AS are almost all non-verbal and have severe cognitive impairments, they are unable to provide a self-report of their emotions and experiences (Adams & Oliver 2011); therefore, reporting of anxiety becomes reliant on proxy reporting by caregivers or other observers (Flynn *et al.* 2017). Proxy reports have their own set of issues, including how the reporter interprets observable behaviours. Diagnostic overshadowing may exist, whereby raters may attribute possible symptoms of a mental health issue like anxiety to the individual's intellectual disability (ID) or other co-morbid condition and thus do not report it as anxiety (Deb *et al.* 2001). This may be an especially salient issue in AS, where behaviours such as irritability, restlessness, repetitive behaviours, crying and somatic issues could be perceived as related to anxiety or attributed to neurological issues, pain, gastrointestinal problems or frustration with lack of communication. Added to these challenges are a lack of psychometrically sound measures of psychiatric symptoms that have been validated in individuals with severe IDs (Flynn *et al.* 2017).

Separation distress is one form of anxiety that can be particularly prevalent in individuals with IDs like AS (Emerson 2003; Larson *et al.* 2011). Behaviours such as crying, becoming agitated or aggressive when a preferred caregiver is not present, following or seeking out the preferred caregiver before being able to engage in other activities and engaging in attention-seeking behaviours when the caregiver is not attending to them are all examples of behaviours reported in studies of anxiety in individuals with intellectual and developmental disabilities (IDDs) (Schuengel *et al.* 2013). Anecdotally, these behaviours have been reported in clinical settings with individuals with AS, but to date, the prevalence and severity of these behaviours of separation distress have not been empirically studied.

This study explored caregiver report of behaviours related to anxiety in 100 individuals with AS. The primary goal was to describe the frequency, severity and nature of behaviours typically associated with anxiety in this population. In addition, prevalence and

intensity of behaviours associated with separation distress in individuals with AS across age, sex and genetic aetiology are explored. The following research questions guided analyses:

1 What is the prevalence of behaviours associated with anxiety and separation distress in individuals with AS?
2 What is the severity of specific anxiety-related behaviours in individuals with AS?
3 Do anxiety behaviours differ by gender, molecular subtype or age of the individual with AS?
4 What individual and family variables are associated with anxiety-related behaviours?

Methods

All activities were approved by the institutional review board serving the authors' institution. Participants were parents or primary caregivers of 100 individuals with confirmed AS. Over half (54%) of participants were recruited from a clinical setting. For these participants, information regarding the individual with AS's developmental/cognitive age, functional skills, communication level and other behavioural concerns were collected through direct assessment. The remaining participants (46%) were recruited through distribution of a survey at the Angelman Syndrome Foundation Family conference. These participants completed questionnaires and returned them in the mail to the researchers. Duplicates among the two samples (e.g. those who attended the clinic and completed the mail survey) were removed with the questionnaires completed at the time of the clinic visit retained for analysis. Table 1 provides details about the sample as a whole and the clinic versus survey samples. Clinic participants were significantly younger than the survey sample. None of the core variables in the study were different between the clinic and survey samples; thus, we collapsed the groups for the additional analyses.

Measures

Anxiety behaviours

Anxiety behaviours were assessed using a clinician-developed questionnaire that was intended to identify behaviours associated with caregiver perceptions of anxiety in individuals with AS. Six of the items ('nervous', 'does not relax', 'tense', 'worried', 'panic attacks' and 'trembles') were selected from the General Anxiety subscale of Anxiety, Mood, and Depression Scale (Esbensen *et al.* 2003). Twenty-seven additional clinician-developed items were included to assess additional behaviours frequently seen in AS; 16 anxiety-specific behaviours included 'mood changes rapidly', 'ritualistic behaviours', 'nervous habits', 'sweats excessively', 'clingy', 'tearful', 'stomach aches', 'agitated', 'avoids confined places', 'avoids others', 'startles', 'avoids eye contact', 'fearful', 'exaggerated startle', 'paces' and 'increased eye contact'. The anxiety items were part of a questionnaire specifically addressing 'anxiety'. All items were scored on a 4-point scale from 0 (not a problem) to 3 (severe problem). An exploratory factor analysis was conducted (forcing one factor) to reduce anxiety-specific items (22 total) in the development of a total anxiety score. Twenty items with factor loadings greater than 0.40 were selected and summed (all except 'sweats excessively' and 'stomach aches'); this total anxiety score was used for comparison tests and regression models. As expected, these items demonstrated high internal consistency (standardised Cronbach's $\alpha = 0.91$).

Finally, several items assessing behaviours associated with separation distress were included in the questionnaire. These items asked whether the individual with AS shows a preference for one caregiver over others, if they display agitation if someone comes between them and their preferred caregiver, if they become agitated if the preferred caregiver attends to someone else or attempts to leave them for any amount of time, if the individual with AS displays agitation if eye contact with the preferred caregiver is broken and if the preferred caregiver experiences anxiety or fear when leaving the individual with AS. These behaviours were scored as either present or absent; they were not combined in any subscale or composite score. Nevertheless, these five items demonstrated moderate internal consistency (standardised Cronbach's $\alpha = 0.71$). The five separation distress items were also moderately correlated with the total anxiety score: *r*s ranged from 0.22 to 0.43 (*P*s ranged from <0.001 to 0.04).

Table 1 Demographics of participants from clinic and survey samples

	All	Clinic sample	Survey sample	*P*-value*
	N = 100 Mean (SD): range	*N* = 54 Mean (SD): range	*N* = 46 Mean (SD): range	
Age (years)	12.6 (10.1): 0.8–41.6	10.2 (9.5): 0.8–37.7	15.0 (10.2): 1.7–41.6	**0.02**
Total anxiety score	12.9 (10.7): 0–44	13.8 (11.4): 0–40	12.0 (10.0): 1–44	NS
Total sleep difficulties	55.6 (15.7): 14–93	54.5 (18.2): 16–93	57.0 (12.5): 14–77	NS
Total behaviour score†	6.0 (5.7): 0–21.0	6.8 (6.0): 0–21.0	5.3 (4.4): 0–16	NS
Age at diagnosis (years)	4.1 (6.1): 0.5–36.0	4.1 (5.8): 0.5–35.0	4.1 (6.4): 0.8–36.0	NS
	N (%)	*N* (%)	*N* (%)	
Sex (female)	50 (50.0%)	28 (51.9%)	22 (47.8%)	NS
Genetic subtype				NS
Deletion	59 (59.0%)	26 (48.2%)	33 (71.7%)	
UPD	14 (14.0%)	8 (14.8%)	6 (13.0%)	
UBE3A mutation	12 (12.0%)	10 (18.5%)	2 (4.4%)	
Other	11 (11.0%)	6 (11.1%)	5 (10.9%)	
Missing	4 (4.0%)	4 (7.4%)	0 (0.0%)	
Race/ethnicity				NS
Caucasian	70 (70.0%)	34 (63.0%)	36 (78.3%)	
African American	3 (3.0%)	3 (5.6%)	0 (0.0%)	
Asian	6 (6.0%)	4 (7.4%)	2 (4.4%)	
Hispanic	7 (7.0%)	4 (7.4%)	3 (6.5%)	
Biracial	4 (4.0%)	1 (1.9%)	3 (6.5%)	
Missing	10 (10.0%)	8 (14.8%)	2 (4.4%)	
Maternal education				NS
HS or less	16 (16.0%)	9 (16.7%)	7 (15.2%)	
Some college	23 (23.0%)	13 (24.0%)	10 (21.7%)	
Assoc./Bach	24 (24.0%)	7 (13.0%)	17 (37.0%)	
Adv. degree	22 (22.0%)	12 (22.2%)	10 (21.7%)	
Missing	15 (15.0%)	13 (24.1%)	2 (4.4%)	
Family income				NS
<$25 000	8 (8.0%)	4 (7.4%)	4 (8.7%)	
$25 000–50 000	13 (13.0%)	9 (16.7%)	4 (8.7%)	
$50 000–75 000	11 (11.0%)	6 (11.1%)	5 (10.9%)	
$75 000+	56 (56.0%)	25 (46.3%)	31 (67.4%)	
Missing	12 (12.0%)	10 (18.5%)	2 (4.4%)	
Lives …				NS
With family	89 (89.0%)	46 (85.2%)	43 (93.5%)	
Residential home	3 (3.0%)	1 (1.9%)	2 (4.4%)	
Missing	8 (8.0%)	7 (13.0%)	1 (2.2%)	

*Difference tests were calculated using available data only (i.e. excluding missing data).

†Highest possible score = 21.

HS, high school; NS, not significant; SD, standard deviation; UPD, uniparental disomy.

Demographics, sleep and aggressive behaviours

Basic demographic information was collected for all participants including gender, age, AS subtype, maternal education, family income, number of siblings and whether the individual with AS lived in the parents' home. In addition, caregivers completed a 42-item survey surrounding their child's sleep behaviour. Nine items of interest were selected from the Behavioural Evaluation of Disorders of Sleep (Schreck *et al.* 2003), which asks parents to rate the frequency of common sleep challenges. The remaining items were developed by clinicians with expertise in AS. All items were scored on a scale from 0 (never) to 4 (always), as is typical for the Behavioural Evaluation of Disorders of Sleep. The 42

sleep items were summed to create a total sleep difficulties score (higher scores indicate greater difficulties); these items demonstrated moderate internal consistency (standardised Cronbach's $\alpha = 0.78$).

Caregivers also completed clinician-derived scale assessing the severity of common aggressive behaviour issues (e.g. biting, kicking, screaming, whining, throwing and hitting), rated on a scale from 0 (not a problem) to 3 (severe problem). The aggression items were summed to create a total aggressive behaviour score; these items demonstrated high internal consistency (standardised Cronbach's $\alpha = 0.85$).

Cognitive and functional skills

For clinic participants only, the cognitive scale of the Bayley Scales of Infant and Toddler Development, third edition (Bayley-3) (Bayley 2005) and the Vineland Adaptive Behaviour Scales, second edition (Vineland-II) (Sparrow *et al.* 2005) were administered to obtain an estimate of developmental and functional skills, respectively. Although the Bayley-3 is designed for children aged 0 to 42 months, it has been used commonly with older children with AS, given the nature of their developmental delays, including as the primary outcome measure in a multicentre AS Natural History Study (ClinicalTrials.gov: NCT00296764) and in all previous clinical trials in AS (Peters *et al.* 2010; Bird *et al.* 2011; Grieco *et al.* 2014; Ovid Therapeutics Inc. 2016; Tan *et al.* 2018). The Vineland-II, which assesses the extent to which an individual can participate in activities of daily living independently, was completed by a parent or guardian to assess functional skills.

Data analysis

Descriptive analysis including frequencies, means, standard deviations and ranges was calculated for all items and domains assessed. As described, 20 anxiety-related items were summed to create a total anxiety score. All items from the sleep survey and all items related to aggressive behaviour were summed to create sleep difficulties and aggressive behaviour scores, respectively. Comparison tests (*t*-tests, χ^2-tests and analyses of variance) were performed to assess differences in anxiety, sleep difficulties, aggressive behaviour, developmental skills, functional skills and separation distress by child's genetic aetiology, age and gender. When we found significant differences in composite scores, we probed at the item level.

Ordinal least squares regression models were used to identify predictors of the total anxiety score; predictors included child age and gender, maternal education, family income, sleep behaviours, developmental skills (Bayley-3), functional skills (Vineland-II) and total aggressive behaviours. Because only clinic participants completed the cognitive scale of the Bayley-3 and the Vineland-II, 61% and 53% of participants had missing data for these measures, respectively. Missing data for other variables entered into the regression model ranged from 0% (child gender) to 13% (maternal education). Multiple imputations (50 imputations) were used to generate missing data prior to the regression, and continuous variables (i.e. all measures except for child gender) were standardised (mean = 0; standard deviation = 1). First, due to the high proportion of missing data in survey participants, the regression model was limited to the clinic sample (Model 1). Next, the regression model was expanded to include the full sample, with developmental and functional skills removed as predictors, as the survey participants did not have these data (Model 2). Finally, the regression model included all participants and included developmental and functional predictors (Model 3). Estimates in each of the 50 datasets were pooled across regression models. While Model 3 included a high proportion of imputed data for the Bayley-3 and Vineland-II measures, the accompanying pooled estimates and standard errors reflect the uncertainty of the missing data (Rubin 1987).

Results

Prevalence and severity of anxiety and separation distress

Forty per cent of caregivers reported they, or someone else, had concerns regarding anxiety in their loved one with AS. More than half of the sample (61.5%) reported that the individual with AS prefers one caregiver over others. Almost half (48.4%) reported that the individual with AS displays agitation if someone comes between them and their preferred

caregiver or if the preferred caregiver attends to someone else or attempts to leave for any amount of time. Fewer (8.7%) reported agitation when the preferred caregiver breaks eye contact. Thirty per cent of caregivers reported experiencing fear or anxiety about separating from their loved one with AS.

The most severely rated behaviour associated with anxiety was *clingy*, with 40% of the sample being described as having a moderate to severe problem. Other highly rated behaviours included *does not relax* (38%), *trembles* (36%), *agitated* (28%), *nervous habits* (25%) and *fearful* (25%). See Figs 1–5 for an illustration of prevalence of behaviour concerns associated with anxiety that were rated as being a *moderate* to *severe* problem and separation distress behaviours for the full sample as well as by the per cent of those in each age and Angelman subtype group whose caregiver endorsed the behaviours.

Differences by molecular subtype, age and gender

Individuals with the deletion subtype had significantly fewer anxiety-related behaviours ($F = 2.95$, $P = 0.04$). Specifically, they were less likely to be reported as *tense* ($F = 2.74$, $P = 0.05$) and as having *nervous habits* ($F = 8.40$, $P < 0.001$). Those with the deletion were also less likely to have *outbursts of anger* ($F = 3.88$, $P = 0.01$). There were no significant differences between molecular subtypes on any of the separation distress items, nor were there differences in sleep difficulties or functional skills. Individuals with *UBE3A* mutations received significantly higher developmental scores (Bayley-3; Table 2; $F = 9.09$, $P < 0.001$) but were also rated as having significantly more aggressive behaviours ($F = 4.99$, $P = 0.006$), including *whining* ($F = 3.78$, $P = 0.014$), *throwing objects* ($F = 10.35$, $P < 0.0001$) and *hitting* ($F = 8.27$, $P < 0.0001$).

Anxiety scores were highest for adolescents and lowest for children 5 years or younger (Table 3; $F = 4.43$, $P = 0.006$). The youngest children (0 to 5 years) were the least likely to be rated as being *tense* ($F = 5.53$, $P = 0.002$), having *nervous habits* ($F = 7.38$, $P < 0.001$), being *worried* ($F = 4.10$, $P = 0.009$) and *trembling* ($F = 2.99$, $P = 0.04$). Adolescents (13 to 17 years) were most likely to be rated as *tense* and *worried*. There were no age-related differences in any of the separation distress items, nor were there differences in sleep difficulties or aggressive behaviours. Differences in developmental skills

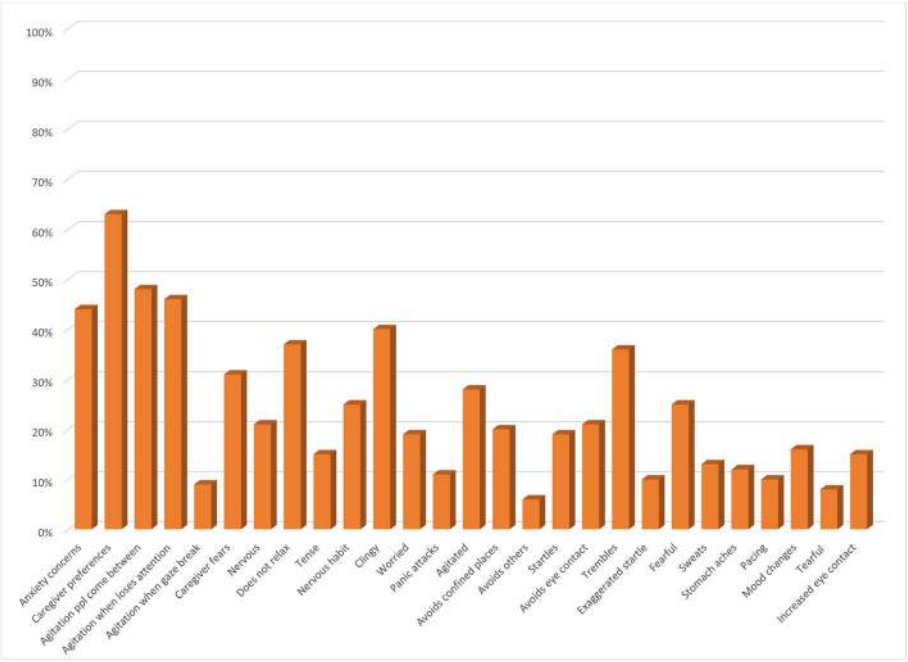

Figure 1. Per cent of full sample with endorsed behaviours. [Colour figure can be viewed at wileyonlinelibrary.com]

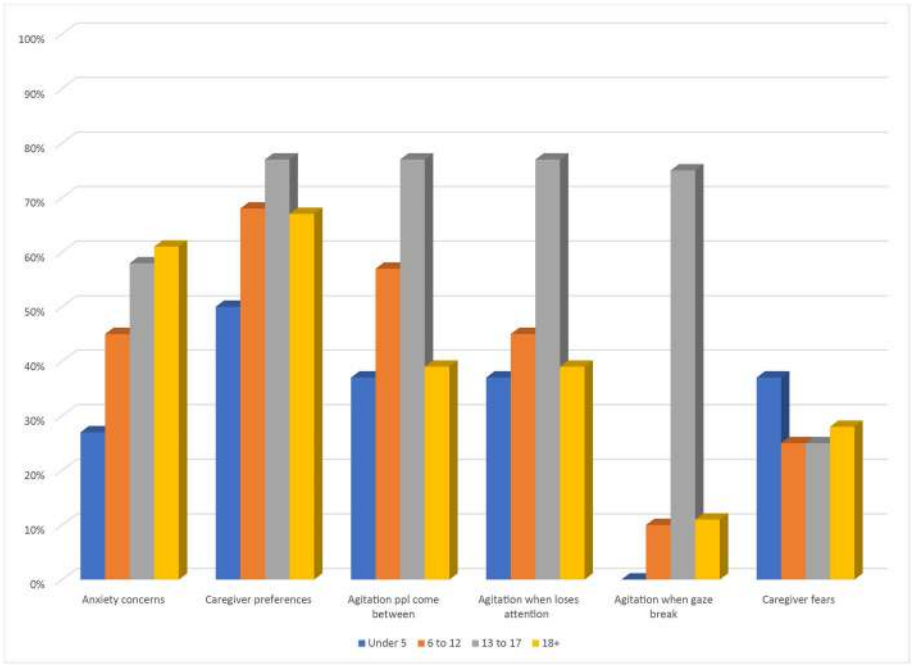

Figure 2. Per cent of individuals within each age group with separation distress behaviours. [Colour figure can be viewed at wileyonlinelibrary.com]

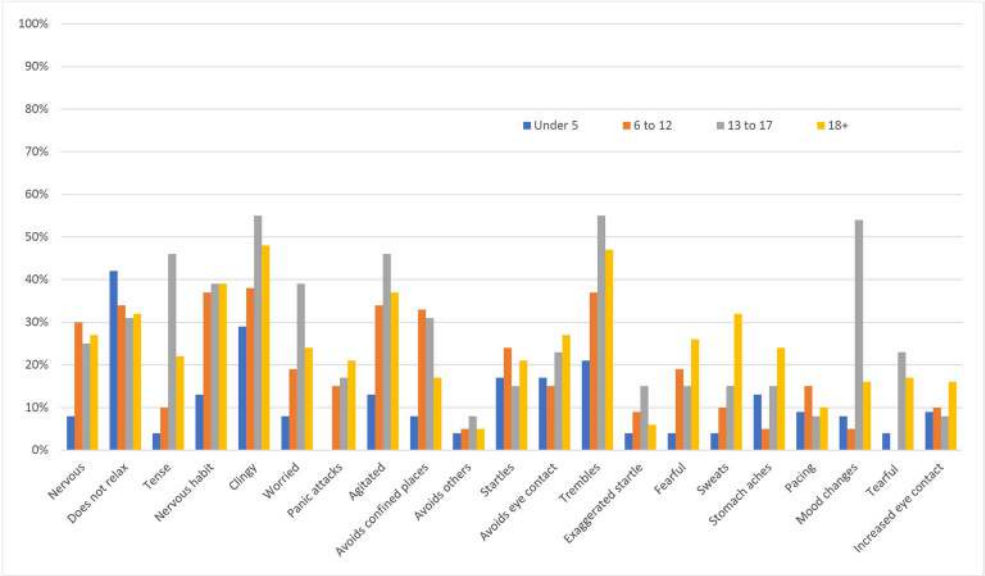

Figure 3. Per cent of individuals within each age group with moderate–severe anxiety-related behaviours. [Colour figure can be viewed at wileyonlinelibrary.com]

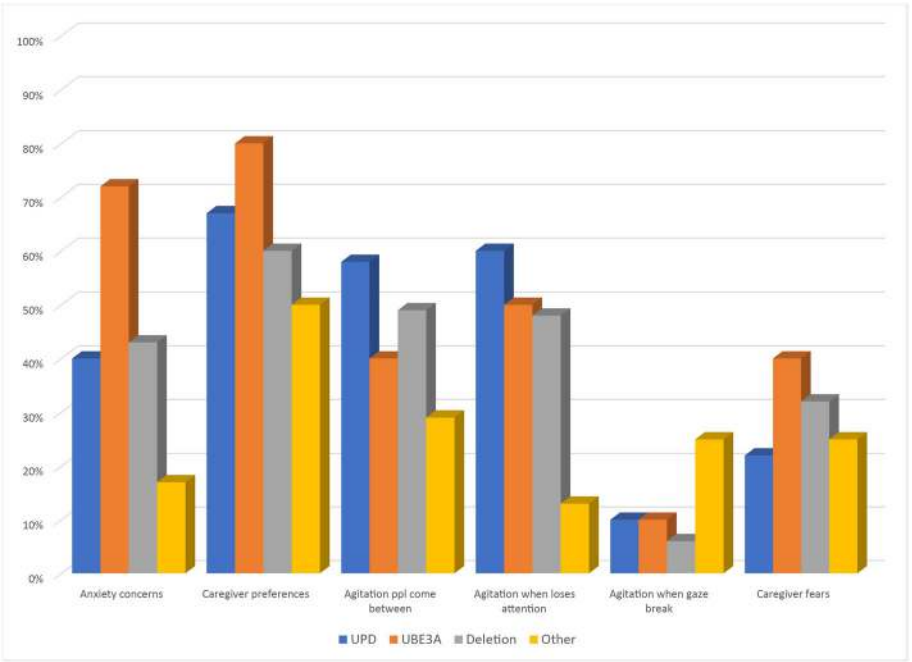

Figure 4. Per cent of individuals within each Angelman syndrome subtype with separation distress behaviours. UPD, uniparental disomy. [Colour figure can be viewed at wileyonlinelibrary.com]

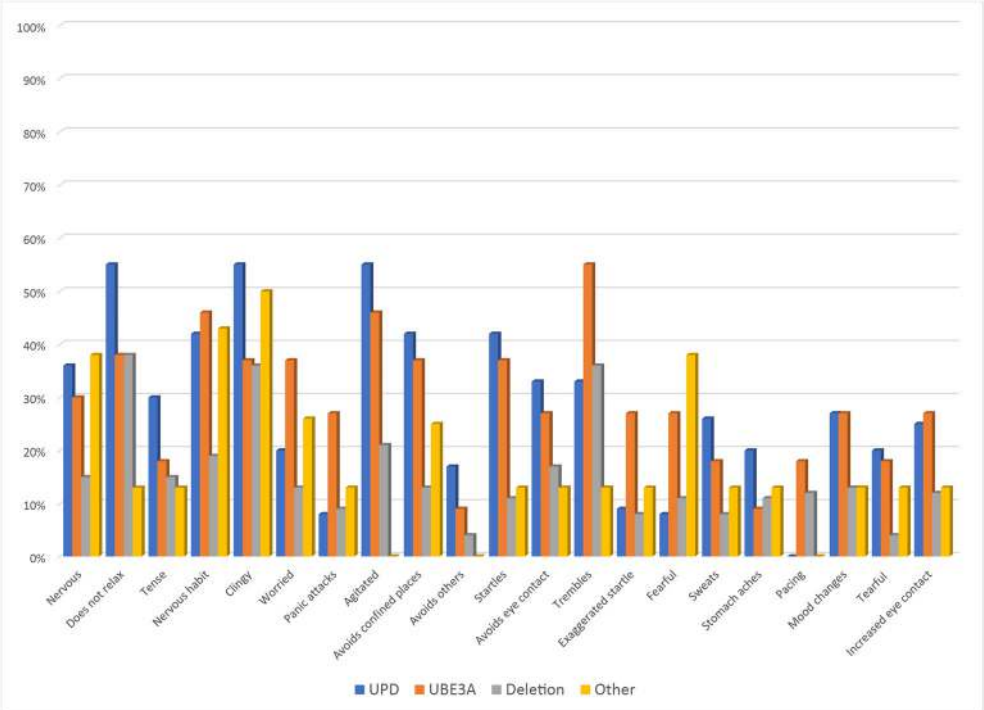

Figure 5. Per cent of individuals within each Angelman syndrome subtype with moderate–severe anxiety-related behaviours. UPD, uniparental disomy. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Key variables by Angelman subtype

	UPD	*UBE3A*	Deletion	Other*	*P*-value
	N = 14	*N* = 12	*N* = 59	*N* = 11	
Age	Mean (SD): range 13.54 (7.18): 2.09–23.08	Mean (SD): range 14.85 (8.22): 6.06–31.07	Mean (SD): range 10.79 (10.14): 1.00–41.07	Mean (SD): range 15.48 (11.97): 1.06–37.08	NS
Total anxiety score	14.7 (10.6): 2–35	19.1 (13.3): 1–40	10.7 (9.7): 0–44	16.9 (10.2): 1–31	**0.04**
Total sleep difficulties	59.6 (12.9): 42–80	61.7 (20.2): 28–93	52.1 (15.0): 14–81	61.8 (13.3): 47–81	NS
Total behaviour score	7.8 (5.7): 0–16	10.6 (6.3): 0–21	4.9 (4.3): 0–17	5.7 (5.6): 0–15	**0.003**
	N = 6	*N* = 9	*N* = 21	*N* = 1	
Bayley-3 cognitive age equivalent (months)	20.7 (8.3): 11–33	22.7 (7.4): 5–30	11.0 (5.2): 5–22	9.0	**<0.001**
	N = 6	*N* = 10	*N* = 23	*N* = 5	
Functional skills (Vineland-II standard score)	49.8 (16.9): 20–68	40.3 (17.4): 20–64	47.6 (15.4): 20–73	40.6 (26.6): 20–80	NS

*Includes imprinting centre defects and abnormal methylation.
NS, not significant; SD, standard deviation; UPD, uniparental disomy.

Table 3 Key variables by participant age

	5 and under	6–12	13–17	18+	*P*-value
	N = 31	*N* = 23	*N* = 14	*N* = 22	
Total anxiety score	Mean (SD): range 8.3 (7.4): 0–34	Mean (SD): range 12.8 (9.8): 1–33	Mean (SD): range 18.9 (13.0): 1–40	Mean (SD): range 16.1 (11.5): 1–44	**0.006**
Total sleep difficulties	53.3 (16): 16–81	57.5 (16.4): 21–82	55.6 (19.3): 14–93	56.6 (12.7): 28–78	NS
Total behaviour score	4.5 (4.9): 0–15	6.7 (5.7): 0–16	8.2 (6.3): 0–21	5.30 (3.9): 0–13	NS
	N = 19	*N* = 8	*N* = 5	*N* = 8	
Bayley-3 cognitive age equivalent (months)	11.1 (5.8): 3–22	16.6 (9.1): 6–27	25.8 (7.6): 15–33	22.3 (6.6): 15–30	**<0.001**
Functional skills (Vineland-II standard score)	56.7 (12.0): 38–80	52.5 (9.1): 33–64	34.8 (8.1): 27–47	21.4 (2.9): 20–28	**<0.001**

NS, not signifcant; SD, standard deviation.

(Bayley-3: $F = 7.34$, $P < 0.001$) and functional skills (Vineland-II: $F = 27.49$, $P < 0.001$) were as expected.

There were no significant differences in total anxiety, sleep difficulties, aggressive behaviours, functional skills, developmental skills or separation distress items by child gender.

Predictors of anxiety-related behaviours

Table 4 provides a summary of the regression results. Aggressive behaviours, sleep difficulties, functional skills, developmental skills and covariates explained 51–70% of the variance across models. Consistently, the total anxiety score was significantly predicted by the total sleep difficulties (Model 1: $\beta = 0.24$, $P = 0.01$; Model 2: $\beta = 0.17$, $P = 0.04$; and Model 3: $\beta = 0.19$, $P = 0.03$) and total aggressive behaviours (Model 1: $\beta = 0.41$, $P < 0.001$; Model 2: $\beta = 0.55$, $P < 0.001$; and Model 3: $\beta = 0.47$, $P < 0.001$). Child's gender, family income, maternal education, and developmental skills (Bayley-3) were not significantly related to total anxiety in any models, while higher functional skills (Vineland-II) was related to lower anxiety when assessing the clinic sample only (Model 1: $\beta = -3.47$, $P = 0.005$). Older age was related to higher total anxiety in Model 2 ($\beta = 0.27$, $P < 0.001$), the model in which we did not control for developmental (Bayley-3) or functional skills (Vineland-II). In a sensitivity analysis, agitation was removed from the total anxiety score, and the models were rerun; a similar pattern of results was shown, with total aggression continuing to be related to total anxiety across all models (Model 1: $\beta = 0.40$, $P < 0.001$; Model 2: $\beta = 0.54$, $P < 0.001$; and Model 3: $\beta = 0.52$, $P < 0.001$).

Discussion

The primary goal of this study was to explore the frequency and severity of behaviours associated with perceived anxiety and separation distress in individuals with AS. Although anxiety and separation distress have been reported among other groups of individuals with intellectual or developmental disabilities, to the authors' knowledge, this is the first to describe these behaviours in any comprehensive way for AS.

As expected, a high rate of anxiety concerns was present in this sample, with 40% of all caregivers indicating concerns. However, the prevalence of anxiety symptoms was lower than is suggested by its designation as a 'frequent' feature of AS (which would suggest upwards of 80% experience anxiety). This finding suggests that, although clearly elevated relative to the typical population, anxiety symptoms in AS may not be as common as is currently thought. However, it could also be a factor of the current rather than lifetime nature of the way the question was

Table 4 Predictors of the anxiety in Angelman syndrome

	Model 1 Clinic only sample β (95% CI)	**Model 2 Full sample β (95% CI)**	**Model 3 Full sample β (95% CI)**
Gender (female)	−0.18 (−0.58, 0.21)	−0.10 (−0.40, 0.19)	−0.15 (−0.49, 0.19)
Age	−0.41 (−1.02, 0.21)	0.27 (0.11, 0.42)***	0.00 (−0.66, 0.66)
Family income	−0.02 (−0.31, 0.26)	0.00 (−0.17, 0.17)	−0.04 (−0.25, 0.17)
Maternal education	−0.12 (−0.32, 0.07)	−0.03 (−0.20, 0.14)	−0.02 (−0.20, 0.16)
Total sleep difficulties	0.24 (0.05, 0.43)*	0.17 (0.01, 0.34)*	0.19 (0.02, 0.67)*
Developmental skills (Bayley-3)	0.96 (−0.67, 2.61)	–	1.04 (−1.08, 3.16)
Functional skills (Vineland-II)	−3.48 (−5.92, −1.03)**	–	−0.96 (−3.11, 1.19)
Total aggressive behaviour	0.41 (0.18, 0.64)***	0.55 (0.38, 0.72)***	0.47 (0.23, 0.71)***
R^2	0.70	0.51	0.56
F (NDF, DDF)	8.55 (8, 4143.6)***	14.54 (6, 33 677)***	5.62 (8, 1117.5)***

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
CI, confidence interval, DDF, denominator degrees of freedom, NDF, number of degrees of freedom.

asked. In other words, although 40% of those asked reported anxiety concerns at any given point in time, upwards of 80% will experience anxiety concerns in their lifetime. This is supported by a previous case series report that found the prevalence of anxiety symptoms to be higher in adults over 26 than in adolescents and adults under 25 (Prasad *et al.* 2018). More comprehensive studies exploring anxiety symptoms across the lifespan of individuals with AS are needed.

Anxiety behaviours most likely to be endorsed were being clingy, not able to relax, trembles and having a nervous habit. Furthermore, 63% of the sample had a preferred caregiver, and nearly half were agitated when attention given to them by that preferred caregiver was disrupted. These behaviours in response to separation have previously only been reported anecdotally by clinicians or in case series reports. Based on these results, this set of behaviours may be more pervasive than previously thought and suggests an important area for future intervention research. Separation anxiety has been reported to occur more frequently and for longer durations in individuals with IDDs than in children who are typically developing (Green *et al.* 2015). One hypothesis for this is that reduced ability to independently navigate the world results in increased reliance on a caregiver, thereby increasing anxiety when that safe person is no longer present. In the case of profound ID especially, individuals may not have full awareness of the caregiver's ongoing existence when separation occurs (Janssen *et al.* 2002), which may further increase anxiety reactions. Another possible underlying cause for these behaviours in AS especially may be the high social drive often reported in AS (Heald *et al.* 2013). It may be that high social approach behaviours in AS may become more maladaptive as they get older in relation to preferred or known caregivers. Understanding the underlying causes for this distress is an important future goal for research in this area.

The percentage of caregivers reporting anxiety as a concern increased with the age of the individual with AS; well over half of adolescents and adults reported having anxiety concerns. Adolescents received the highest severity score for anxiety symptoms and were also more likely to experience agitation when losing the attention of their preferred caregiver or when someone else comes in between them and their preferred caregiver. Adolescence is a highly tumultuous time, and individuals with IDDs are not immune from the hormonal changes and resulting mood shifts common in neurotypical teens. Therefore, it is not surprising that these behaviours may increase in individuals with AS. Although anxiety levels did remain high in the adult group, both anxiety and aggressive behaviours trended downward from the adolescent to adult groups, suggesting that many of these behaviours may decrease as the individual ages.

Individuals with the deletion subtype had significantly lower developmental scores and were also significantly less likely to be reported as experiencing anxiety symptoms or behaviour challenges. This pattern replicates previously reported profiles suggesting higher cognitive functioning but also higher likelihood for increased psychopathology in nondeletion subtypes of AS (Gentile *et al.* 2010; Miodrag & Peters 2015; Wink *et al.* 2015). The additional missing genetic information that results from a deletion has been hypothesised to be the reason for differences in severity of phenotype across AS subtypes. However, the reasons for increased psychopathology in nondeletion subtypes are less well understood. It could be that for individuals with the more severely limiting deletion subtype, they may be less aware of their environment or are not cognitively capable of demonstrating higher order anxiety responses. It could also be that their behaviours are interpreted as resulting from something other than anxiety, possibly due to their lower functional abilities.

In this study, diagnosable anxiety disorders were not assessed for several reasons: (1) most current measures used to assess anxiety disorders are not appropriate for individuals with severe and profound IDs; (2) researchers wanted to assess a wider range of anxiety symptomology and behaviours than are often assessed when determining criteria for a diagnosable disorder; and (3) researchers wanted to gather data on as many individuals with AS as possible, both within and outside of the clinical setting; therefore, a parent report measure was determined to be the most versatile to reach the largest number of participants. It is important to note that understanding the frequency and severity of diagnosable anxiety disorders in this population may be a vital future goal to fully understand the co-morbidity of anxiety disorders in

AS. It is also important to consider that the clinician-derived items in the measure used for this study are not a validated measure of anxiety and may not fully reflect the spectrum of anxiety-related issues present in AS. More work is needed to develop an appropriate, validated measure of anxiety for this population.

Aggressive behaviours were a consistent predictor of anxiety in our sample. This finding is not surprising given that the co-occurrence of anxiety and externalising behaviour challenges has been documented as occurring at higher than expected rates in individuals with IDDs (Baker *et al.* 2010; Green *et al.* 2015). However, the relationship between anxiety and aggressive behaviours is not clear (Pruijssers *et al.* 2014). Are the challenging behaviours a coping mechanism for increased arousal occurring as a result of anxiety? Or does anxiety arise following consequences that occur in response to behavioural outbursts, which then give rise to additional behaviours? Challenging behaviours may also be a coping strategy to convey a need for help in managing a stressful situation (Clegg & Sheard 2002). Unfortunately, this cross-sectional analysis is not able to answer these questions.

Additionally, sleep difficulties were a significant predictor of anxiety across both samples. The association between sleep disturbances and anxiety disorders in paediatric populations is well documented (Alfano *et al.* 2007; Shanahan *et al.* 2014; McMakin & Alfano 2015). Sleeping disturbances are common in individuals with AS, and based on results, further research into the association of sleep disorders and anxiety behaviours among individuals with AS is warranted using validated measures appropriate for this population.

In individuals with severe conditions like AS, the difference between anxiety and challenging behaviours is even more blurred because of the lack of ability for most individuals with AS to communicate their thoughts and feelings. This challenge, along with their significant cognitive impairment, requires the use of proxy reporters for understanding anxiety and challenging behaviours. Proxy reporters will necessarily apply their own interpretations and lens to the behaviours, rendering a high subjectivity in the evaluation of what is happening for the individual with AS. Some of the behaviours that have been categorised or interpreted as anxiety may in reality be more primitive in nature; for example, behaviours in response to separation from a preferred caregiver may be frustration or agitation at the situation not being as desired rather than fear of separation.

More objective measures for assessing anxiety exist in the form of physiological biomarkers such as arousal, which can be assessed through respiration, skin conductance heart rate variability and startle response (Vos *et al.* 2010; Lyons *et al.* 2013). Physiological arousal as a measurement of anxiety has been successfully assessed in populations of individuals with IDDs (Klusek *et al.* 2013; Kushki *et al.* 2013; Roberts *et al.* 2018), but as of this writing, this type of study has not been conducted with individuals with AS. Theoretically, inclusion of more objective, physiological measures in conjunction with behavioural coding could help in discriminating whether some behaviours are a result of a fear response versus anger or frustration.

The items on the primary measure used in this study were chosen initially to provide richer clinical data to support caregivers in describing the challenges they perceive in their loved one in AS. The measure did have internal consistency in our sample and provides some direction with regard to the types of behaviours often perceived to be related to anxiety in AS. However, the items were primarily clinician derived and were not field tested or correlated with other existing measures, which reduces our ability to make more solid conclusions about anxiety in this population. The challenge of modifying or developing new robust behaviour measures for individuals with AS is no small matter. Clinical trials addressing key features in AS are becoming more common, and, as with many rare disorders, the lack of psychometrically sound outcome measures is a major challenge for the field. There is a significant need for validation studies on quality behavioural tools for assessing change over time or in response to treatment.

There are several other limitations to this study, which include lack of comprehensive, diagnostic measures of anxiety; the cross-sectional nature of the study; and the lack of information on several potentially important key variables, such as functional communication skills, co-morbid conditions such as seizures, pain or gastrointestinal issues, medication use and behaviour management techniques used by caregivers. Furthermore, the lack of available data for the Bayley-3 and Vineland-II in the survey sample

limits our ability to examine these as predictors of total anxiety. However, anxiety-related symptoms are a common and challenging aspect of the AS phenotype, one that deserves greater attention. This paper provides additional evidence of the frequency, severity and nature of behaviours thought to be related to anxiety in this population. Being able to identify better both the molecular and environmental contributions to these behaviours is an important goal for researchers and clinicians working to improve the quality of life of individuals with AS and their families. Future comprehensive studies are needed to better capture symptoms of anxiety in individuals with AS over time to better understand this associated feature.

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Conflict of Interest

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