

1 **Tracing Autism Traits in Large Multiplex Families to Identify Endophenotypes of the**
2 **Broader Autism Phenotype**

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Abstract

Families comprising many individuals with Autism Spectrum Disorder (ASD) may carry a dominant predisposing mutation. Our aim was to use rigorous phenotyping of the ‘Broader Autism Phenotype’ (BAP) in large multiplex ASD families to identify endophenotypes of the BAP for future genetic studies. We evaluated ASD/BAP features using standardised tests and a semi-structured interview to assess social, intellectual, executive and adaptive functioning in 109 individuals, including two large multiplex families (Family A: 30; Family B: 34) and an independent sample of small families ($n=45$). Our protocol identified four psychological endophenotypes of the BAP that were evident in both samples, and showed high sensitivity (97%) and specificity (82%) for individuals classified with the BAP. The patterns of inheritance of these endophenotypes varied in the two large families, supporting their utility for identifying genes in autism.

Keywords: Broader autism phenotype, genetic, autism spectrum disorder, multiplex family

41 Autism Spectrum Disorder (ASD) is a neurodevelopmental condition that spans deficits in two
42 domains: social communication, and restricted interests or repetitive behaviours APA 2013.
43 Recent estimates from the Center for Disease Control and Prevention indicate prevalence of
44 ASD is one in 68 children aged 8 years¹ making ASD a critical international health problem.
45 Clinical and molecular research provides evidence for a genetic aetiology in ASD, yet despite
46 recent molecular advances the cause remains unidentified in the majority of cases.

47 Improved understanding of ASD has facilitated recognition of milder phenotypes. Early
48 clinical research identified autistic traits in relatives of children with ASD, described as the
49 ‘broader autism phenotype’ (BAP)^{2,3}. The BAP sits at the mildest end of the ASD spectrum and
50 includes a range of subtle behavioural and cognitive features that reflect the two core domains of
51 ASD. The *Diagnostic and Statistical Manual of Psychiatric Disorders –V* (DSM-V) stipulates
52 that a significant degree of impairment must be present to qualify for a diagnosis of ASD⁴,
53 whereas BAP traits lie on a continuum of normal population behaviours⁵. Monozygotic twins
54 demonstrate 30% concordance for ASD⁶, increasing to 92% if the BAP is considered, while
55 dizygotic twin concordance is ~10%⁷. Prevalence of the BAP in the general population is
56 unknown, whereas several studies have demonstrated higher rates of BAP traits (20-50%) among
57 relatives of children with ASD compared to controls, particularly in the areas of pragmatic
58 language^{8,9}, personality¹⁰, social cognition^{9,11,12} and executive function^{13,14}. Together these
59 findings support the notion of complex inheritance of ASD.

60 The overall diagnostic rate of ASD is now >30%, including monogenic and chromosomal
61 aetiologies^{15,16}. The remaining ~70% are likely to have a genetic basis, with polygenic
62 architecture in some, and unique *de novo* mutations in others¹⁷. Complementary techniques will
63 be necessary to unravel aetiology in unsolved cases. Typically, family studies combine many

64 small families (2-3 affected individuals), however, these are likely to be confounded by genetic
65 heterogeneity. Very large multiplex families (> 8 affected) where ASD traits appear dominantly
66 inherited are rare, but more genetically homogeneous. In other complex disorders, such as
67 epilepsy, phenotypic characterisation of such families has proved powerful in gene discovery¹⁸,
68 however this approach has received limited attention in ASD^{18,19}. In multiplex ASD families, the
69 identification of family members with BAP traits, or endophenotypes, may serve as markers of
70 carrier status^{19,20}. In turn, this may facilitate gene identification²¹.

71 Endophenotypes are measurable features within a disorder that are proposed to reduce its
72 complexity into more quantifiable elements²². They have been hypothesised to reflect more
73 aetiologically homogeneous subgroups within genetically heterogeneous conditions. There are
74 several BAP traits that may be considered “endophenotypes” from within the domains of
75 language, executive function, and social cognition²¹. In the context of a single large family where
76 numerous individuals demonstrate ASD or the BAP, recognition of BAP endophenotypes should
77 allow granular identification of an autism gene of dominant effect. This study is the first known
78 to the authors to apply this approach in autism.

79 The aim of our study was to analyse autistic traits within large multiplex families to
80 examine inheritance of ASD by identifying endophenotypes of the BAP. We achieved this aim
81 using an iterative process, first rigorously phenotyping many members of large multiplex
82 families to delineate the full range of BAP traits for potential endophenotypes. We then assessed
83 these traits in a separate sample of 20 small families, each with at least one member with ASD,
84 to independently validate the endophenotypes. We then applied these endophenotypes to two of
85 our fully characterised large multiplex families (from step 1) to assess their utility for examining
86 inheritance patterns. We hypothesised that (1) multiple individuals in large families would

87 demonstrate the BAP, (2) specific BAP endophenotypes would be identifiable across the
88 traditional BAP domains, and (3) these endophenotypes would vary in presentation between
89 large multiplex families.

90 **Methods**

91 *Large Multiplex Families*

92 Large multiplex families were primarily ascertained from the Barwon Autism Database as part of
93 a broader Collaborative Autism Study²³. For inclusion as a multiplex family, > 8 individuals with
94 a diagnosis or suspected diagnosis of ASD or the BAP were required. The two fully
95 characterised large multiplex families used to examine inheritance patterns using BAP
96 endophenotypes are referred to as ‘Family A’, ascertained from the Barwon Autism Database,
97 and ‘Family B’, who was self-referred. All available relatives were recruited, including those
98 with and without reported BAP traits. Informed consent was obtained from all participants or a
99 parent/guardian, following approval of the study by the Human Research Ethics Committees of
100 Barwon Health and the Royal Children’s Hospital, Melbourne.

101 **(Figure 1)**

102 *Protocol for Diagnosing ASD in Large Multiplex Families*

103 ASD diagnoses were confirmed using the Autism Diagnostic Observation Schedule-Generic²⁴
104 (ADOS-G), the Autism Diagnostic Interview-Revised²⁵ (ADI-R), or DSM-IV-TR criteria²⁶. For
105 adults, the structured Family History Interview² (FHI) was administered by NJB, while for
106 adolescents, a detailed developmental and medical history was obtained. Quantitative measures
107 of intellect, executive functions, adaptive behaviour and social functioning were also completed
108 (Table 1). Testing was undertaken over a number of days to minimise fatigue effects. A physical

109 examination was conducted for dysmorphic and neurocutaneous features and growth parameters.
 110 Standard genetic testing (karyotype, fragile X testing) and metabolic investigations were
 111 performed on probands.

112 Across Family A and B, 65 individuals were recruited: 16 children (2 -12 years), 9
 113 adolescents (13-17 years) and 40 adults (18-79 years) spanning 4 generations. Of these, 16/65
 114 met criteria for a diagnosis of ASD. Family B also reported a deceased family member who had
 115 a diagnosis of ASD (generation V) and an additional family member with ASD (generation 4)
 116 who was not recruited. Scrambled pedigrees of affected status are presented to preserve
 117 participant anonymity (Fig.1). In each family, a matriarch was identified. Individuals directly
 118 related to each matriarch are classified as ‘core family’; others are referred to as ‘married-in’.
 119 Family A comprised 30 individuals, including 7 diagnosed with ASD (6/9 children, 1/6 adults;
 120 Fig.1a). Nineteen were core family; 11 were married-in. In Family B, we fully phenotyped 35
 121 individuals, not including the matriarch (who was not assessed). Three children and three
 122 adolescents participated in a limited range of phenotyping activities and as such, these
 123 individuals were excluded from final analyses. Nine had ASD (5/7 children, 1/5 adolescents,
 124 3/22 adults; Fig.1b); 31 were core family, and four were married-in.

Protocol Item	Participants with ASD		Participants without ASD		
	Child or Adolescent ≥4.5-17yr	Adult ≥18yr	Child <13yr	Adolescent ≥13–17yr	Adult ≥18yr
ADI-R + ADOS-G <i>or</i> DSM-IV interview + ADOS-G	+	±	-	-	-
Detailed developmental, medical, psychiatric and behavioural history	+	+	+	+	+
Family History Interview	-	+	-	-	±

Standardised testing of cognition and executive function ^a	±	+	+	+	±
Questionnaires of adaptive behaviour ^b	+	+	+	+	±
Broader Autism Phenotype Interview, the Faux Pas Task, Cartoon Task and Pragmatic Rating Scale	-	±	-	+	+
Physical Examination	+	+	+	+	+
High resolution molecular karyotype, Fragile X testing, metabolic investigations	+	-	-	-	-

ASD Autism Spectrum Disorder, *ADI-R* Autism Diagnostic Interview-Revised, *ADOS-G* Autism Diagnostic Observation Schedule-Generic, *DSM-IV* Diagnostic and Statistical Manual of Mental Disorders (4th edition).

+ all individuals completed this assessment; - no individuals completed this assessment; ± only some individuals completed this assessment

^aWechsler Abbreviated Scale of Intelligence and subtests of the Delis-Kaplan Executive Function System

^bThe Adaptive Behavioural Assessment System (2nd edition) and The Behavioural Rating Inventory of Executive Function

125 **Table 1. Protocol for diagnosing ASD and phenotyping the BAP in large multiplex families**

126

127 *Protocol for Phenotyping the BAP in Large Multiplex Families*

128 We employed a mixed methods approach to rigorously assess the BAP, including an evaluation

129 of general intellect, executive functions, adaptive behaviour, social cognition and language

130 pragmatics (Table 1). A purpose developed semi-structured interview, the Broader Autism

131 Phenotype Interview (BAPI), was also administered by three clinicians with expertise in

132 neurobehavioural disorders (NJB, SJW, IES) to all individuals ≥ 13 years, to determine the

133 presence, nature and extent of BAP features. Questions focused on the participant's life story,

134 personal qualities, relationships, social functioning, and developmental, medical, psychiatric and

135 vocational history. During the interview we included one or two "intentional errors" to elicit

136 pragmatic elements of the BAP, such as terse speech⁹.

137 Full scale (FSIQ), verbal (VIQ) and performance (PIQ) intelligence quotients were
138 derived with the four subtest Weschler Abbreviated Scale of Intelligence²⁷ (WASI; $M=100$,
139 $SD=15$). Executive functions were measured with seven subtests of the Delis-Kaplan Executive
140 Function System²⁸ (D-KEFS; $M=10$, $SD=3$). The second edition of the Adaptive Behavioural
141 Assessment System²⁹ (ABAS-II) and the Behavioural Rating Inventory of Executive Function³⁰
142 (BRIEF) were used to assess adaptive functioning (Table 1).

143 Social discourse was assessed using an adapted Faux Pas Task^{31,32} (FPT). The task
144 included four faux pas stories and four control stories³³ (maximum score=40, $M=37$, $SD=4$). In
145 addition, the Goldman-Eisler Cartoon task³⁴ was used to explore previous observations of overly
146 detailed speech and longer pauses between words in the BAP⁹. This task measures discourse
147 production by eliciting a description of an eight frame captionless cartoon, “The Cowboy Story”,
148 over three successive trials³⁵. Control individuals show increased verbal fluency with successive
149 trials compared with decreased fluency in individuals with communication deficits³⁴. Following
150 all assessments, the Pragmatic Rating Scale (PRS) was independently completed by the three
151 interviewers and consensus ratings reached. A score ≥ 4 defined pragmatic impairment¹⁰. After
152 independent review of all qualitative and quantitative data by NJB, SJW, and IES the presence of
153 the BAP was determined by consensus.

154 ***Small Families***

155 We recruited an independent sample of 45 individuals from 20 small families with at least one
156 member diagnosed with ASD, via advertisements and from the Barwon Autism Database. All
157 participants provided written informed consent, as described above. Inclusion criteria were: (i)
158 no diagnosis of ASD (based on DSM-IV or DSM-V criteria), (ii) ≥ 1 family member with ASD
159 (based on DSM-IV or DSM-V criteria), and (iii) >12 years of age. Individuals were classified as

160 having the BAP if they met ≥ 2 criteria for a BAP diagnosis on the Broader Autism Phenotype
161 Rating Scale² (BAPRS). Individuals were classified as unaffected if they did not meet criteria for
162 any BAP traits or a diagnosis of ASD. This identified 30 individuals with the BAP (4
163 adolescents, 26 adults) in the 20 families, ranging in age from 14-71 years, and 11 unaffected
164 adult family members ranging in age from 18-53 years. Four adult individuals showed only one
165 BAP trait on the BAPRS and thus, were excluded from analyses based on the above criteria.

166 In 27 individuals, average total scores were available for the Broader Autism Phenotype
167 Questionnaire (BAPQ), and in 31 individuals, FSIQ, Verbal Comprehension (VCI) and
168 Perceptual Reasoning (PRI) indices had been derived with the WASI-II³⁶ ($M=100$, $SD=15$). As
169 shown in Table 2, all individuals were within the normal range based on FSIQ, with no
170 significant differences between unaffected and BAP individuals for age or intellect (all $p < .250$).
171 Consistent with expectations, there was a trend for higher scores on the BAPQ in the BAP group,
172 with a medium effect size ($t(24.56) = -1.96$, $p = .062$, $d = 0.70$).

	Unaffected	BAP
Number of participants (female)	11 (6)	30 (19)
Mean age (range)	41.09 (18-53)	39.50 (14-53)
Mean BAPQ (SD)*	2.43 (0.38)	2.92 (0.92)
Mean FSIQ (SD)**	108 (14)	111 (13)
Mean VCI (SD)**	106 (18)	109 (15)
Mean PCI (SD)**	109 (7)	110 (15)

*data available for unaffected ($n=9$) and BAP ($n=18$); ** data available for unaffected ($n=10$) and BAP ($n=21$). BAPQ=Broader Autism Phenotype Questionnaire; FSIQ=Full Scale Intelligence Quotient; VCI=Verbal Comprehension Index; PRI=Perceptual Reasoning Index

173 **Table 2. Demographics of the small families sample**

174 ***Procedure***

175 We used an iterative process to characterise, refine and assess endophenotypes of the BAP in our
176 two separate samples, as summarised in Fig.2.

177 *Step 1: Identification of Potential BAP Endophenotypes in Large Multiplex Families*

178 Using a grounded theory approach, BAP traits were initially identified from a detailed literature
179 review targeting the theoretical domains described in the seminal work of Bolton (1994), on
180 which the conceptualisation of the BAP is largely based. The domains included speech, literacy,
181 pragmatics, relationships, and circumscribed interests, which were explored in-depth using our
182 BAP phenotyping protocol (described above) in members of unrelated large multiplex families
183 primarily ascertained through the Collaborative Autism Study³³. This in-depth characterisation
184 was phenomenologically based³⁷, whereby the number of traits within each domain was fully
185 expanded through administration of the semi-structured interview (BAPI) with separate family
186 members until no further traits were identified (saturation) to capture the entire range of BAP
187 traits (Table 3).

188 **(Figure 2)**

189 Initial phenotyping produced an exhaustive list of 36 BAP traits. Ordinal ratings of these
190 traits were then assigned to capture subtle variations in their presentation, with severity rated on
191 a scale of 0=absent, 1=mild, 2=moderate, and 3=severe. The presence of traits through each
192 individual's developmental history was also evaluated where available. Exploratory hierarchical
193 cluster analysis was then performed to identify potential BAP endophenotypes. We used Ward's
194 method with Euclidean squared distances based on z-scores to progressively group traits by
195 minimising the variability within clusters and maximising the variance between clusters³⁸.

196 Interpretation of cluster groupings was informed by the relative similarity and dissimilarity in the
197 linkage output combined with clinical judgement, leading to the initial identification of five
198 endophenotypes. Inspection of these endophenotypes revealed a consistent rating of 0 for two of
199 the 36 traits across all interviews, leading to their removal. One further trait reflecting
200 inflexibility to intentional errors was removed due to challenges reliably assessing it across
201 interviewers, resulting in a final set of 33 BAP traits (Table 3).

202 *Step 2: Validation of BAP Endophenotypes in Small Families*

203 In the small families sample, an independent expert in ASD assessment (CG) interviewed and
204 rated 45 participants on the 33 BAP traits based on all qualitative and quantitative data, with a
205 subset (9%) rated via consensus between CG, IES and SJW to ensure consistency in ratings
206 across both samples and to clarify borderline cases. As above, Ward's hierarchical cluster
207 analysis was used to examine natural trait groupings. This led to the identification of four
208 endophenotypes that showed a high degree of similarity to the initial five cluster solution.

209 To account for a variable number of traits in each cluster we computed proportional
210 scores, whereby scores on each trait (range 0-3) were summed and divided by the maximum total
211 score for that cluster, to produce four cluster scores for each individual. An ROC curve was
212 plotted for each cluster in the small families sample to identify optimum cut-off scores for
213 determining endophenotypic status using Youden's Index to allow mildly affected individuals to
214 be included^{39,40}. The highest score was used to represent the most prominent endophenotype for
215 each individual, calculated as the difference between the observed endophenotype (i.e., cluster)
216 score and the threshold score for the endophenotype (i.e., cut-off score).

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BAP domains	Expanded traits
Communication (speech and literacy)	Reduced capacity for clear narrative Difficulty answering open ended questions Reduced quantity of verbal output Speech has little variation in tone (i.e. monotonous) Unusual speech volume Precise articulation and language Use of an accent*
Social communication (pragmatics and relationships)	An unusual or awkward greeting style A limited capacity to develop rapport with assessors Unusual eye gaze Awkward social interactions Making inappropriate or awkward comments either on history or during assessments Tangential pragmatic style Terse pragmatic style Tendency to monologue rather than participate in reciprocal conversation Opinionated in conversation Overly technical language Little appreciation of humour (during the Cartoon task) Inflexible to intentional errors* Tendency to anger easily Narcissistic personality style Self perception incongruent with views of others Aloof personality style Difficult or limited interpersonal relationships Reduced affection Reduced emotional empathy Reduced cognitive empathy Excessive worry
Circumscribed interests	Preference for structure in activities of daily living Fastidious regarding personal appearance Fastidious cleaning Hobby or interest of unusual intensity, or restricted range of interests relative to peers Large collections or hoarding of items Focus on technicalities or minutiae Recurrent thoughts (distressing)* Recurrent thoughts (not distressing)

*BAP trait was removed from the final list (see text for details).

219 **Table 3. Identification of BAP traits through expansion of the BAP domains**

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221 *Step 3: Assessment of BAP Endophenotypes in Family A and B*

222 A team member who had not been involved in the phenotyping of Family A and B (Step
223 1) performed the endophenotype analysis (KT). Proportional scores for the four endophenotypes
224 were calculated, and family members classified as having the endophenotype if their
225 proportional score was greater than or equal to the cut-off scores identified in the small families
226 analysis (step 2). As above, the highest score (observed endophenotype score – threshold
227 endophenotype score) for any endophenotype was used to represent an individual’s most
228 prominent endophenotype. A discriminant function analysis was then used to determine the
229 sensitivity and specificity of the endophenotype approach to identifying the presence of the BAP
230 in these families. In addition, endophenotype results were correlated with measures of intellect,
231 executive, social and adaptive functions using conservative non-parametric Spearman’s
232 correlations (r_s).

233 **Results**

234 *Hypothesis 1: Multiple individuals in large families demonstrate the BAP*

235 Based on our rigorous protocol for phenotyping the BAP in large multiplex families, we
236 identified 32 members with the BAP across Family A and B. Of the 23 members in Family A
237 who did not have an ASD diagnosis, we detected the BAP in 17 (74%) individuals, with 6
238 individuals unaffected. In Family B, we detected 15 (63%) individuals with the BAP, with 9
239 individuals unaffected (Table 4).

240 Intellectual function was directly assessed in 54/63 (86%) individuals. Overall,
241 participants were of average or greater intelligence. Average FSIQ was observed in 32/54 (59%)
242 of individuals, while 20/54 (37%) demonstrated superior or very superior FSIQ (Table 4). We
243 performed group-level comparisons of cognitive, social and adaptive functions between family

244 members with and without the BAP using non-parametric and parametric tests (Mann-Whitney
245 U and t-tests respectively), with the more conservative parametric tests reported here as there
246 were no differences between these approaches. On average, individuals with the BAP
247 demonstrated poorer pragmatic language, with significantly higher mean PRS scores ($M=8.75$,
248 $SD=7.08$) compared to unaffected individuals ($M=2.00$, $SD=3.05$), $t(40.99)=-4.42$, $p<0.001$. No
249 significant differences were observed for general intellect (FSIQ, VIQ, PIQ), the FPT, executive
250 (D-KEFS) or adaptive function measures (ABAS-II, BRIEF).

251 ***Hypothesis 2: Specific BAP endophenotypes exist across BAP domains***

252 Based on our iterative characterisation process, four distinct endophenotypes of the BAP were
253 reliably identified. Based on the natural grouping of traits, these reflected ‘socially unaware’,
254 ‘pedantic’, ‘aloof’, and ‘obsessive’ endophenotypes (Table 5). At the highest level of the
255 dendrogram of the 33 BAP traits there was a clear split, whereby traits of the socially unaware
256 and pedantic endophenotypes were more similar to each other and more dissimilar to the
257 combination of traits of the aloof and obsessive endophenotypes. There was a significant
258 difference between the mean proportional scores of the unaffected and BAP groups, with the
259 BAP group demonstrating significantly higher scores on all four endophenotypes (all $p>0.015$).

260 Analysis of ROC curves indicated relatively good discrimination within the small
261 families for the socially unaware, aloof and obsessive endophenotypes (all $AUC > 0.73$, all
262 $p<.025$), and acceptable discrimination for the pedantic endophenotype ($AUC=0.68$, $p=0.077$).
263 Although we note that Box’s M was violated in the discriminant function analysis (likely due to
264 variation in the sample sizes), combined, the four endophenotypes captured 93% of cases
265 (Wilk’s $\lambda=0.47$, $\chi^2=27.83$, $p<.001$). In particular, the endophenotypes showed high sensitivity

266 for the BAP group (97%), characterised by higher proportional scores, and good specificity for
 267 the unaffected group (82%), with lower proportional scores (Table 5).

268

	Number of participants (female)	Mean age (range)	Cognitive data (<i>n</i>)	FSIQ Mean (SD)	VIQ Mean (SD)	PIQ Mean (SD)
Family A						
ASD	7 (1)	11.43 (4 – 34)	5	107 (16)	97 (15)	101 (30)
Unaffected	6 (5)	25.83 (2 – 50)	5	127 (17)	130 (17)	117 (15)
BAP	17 (8)	49.18 (13 – 79)	16 ^c	119 (13)	117 (13)	115 (12)
Total	30 (14)	35.7 (2 – 79)	26	118 (15)	116 (17)	113 (18)
Family B						
ASD	9 (3)	15.00 (8 – 20)	5	95 (20)	89 (28)	105 (16)
Unaffected	9 (7)	37.33 (10 – 73)	9	110 (14)	111 (14)	108 (11)
BAP	15 (10)	47.40 (15 – 73)	15	102 (17)	105 (18)	99 (17)
Total	33 (20)	35.06 (6 – 73)	29	103 (17)	104 (19)	102 (15)
Both families	63 (34)	35.37 (2 – 79)	55 ^c	110 (18)	110 (19)	107 (17)

269 *Note.* Average FSIQ = 80-119; Superior FSIQ = ≥ 120

270 ^aOne individual only completed VIQ and select executive functioning subtests

271 **Table 4. Intellectual functioning in Family A and B by diagnostic classification**

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Mean Proportional Score (SD)		Cut-off score	BAP traits ¹
Unaffected (n=11)	BAP (n=30)		
‘Socially unaware’: Poor self-regulation and reciprocity in conversation			
0.69 (0.06)	0.20 (0.18)**	>0.17	<ul style="list-style-type: none"> • Reduced capacity for clear narrative • Difficulty answering open ended questions • Making inappropriate or awkward comments either on history or during assessments • Tangential pragmatic style • Tendency to monologue rather than participate in reciprocal conversation • Tendency to anger easily • Reduced quantity of verbal output
‘Pedantic’: Self-focused and technical in interactions			
0.04 (0.05)	0.11 (0.12)*	>0.14	<ul style="list-style-type: none"> • An unusual or awkward greeting style • Unusual eye gaze • Speech has little variation in tone (i.e. monotonous) • Unusual speech volume • Precise articulation and language • Terse pragmatic style • Overly technical language • Narcissistic personality style • Focus on technicalities or minutiae • Fastidious regarding personal appearance • Self perception incongruent with views of others
‘Aloof’: Difficulties relating to other’s emotions and expressing own emotions			
0.12 (0.07)	0.31 (0.16)***	>0.20	<ul style="list-style-type: none"> • Aloof personality style • Difficult or limited interpersonal relationships • Reduced emotional empathy • A limited capacity to develop rapport with assessors • Reduced affection • Awkward social interactions • Opinionated in conversation • Reduced cognitive empathy • Little appreciation of humour (during the Cartoon task)
‘Obsessive’: Regimented approach to life and tendency to ruminate			
0.13 (0.10)	0.27 (0.18)***	>0.25	<ul style="list-style-type: none"> • Hobby or interest of unusual intensity, or restricted range of interests relative to peers • Large collections or hoarding of items • Fastidious cleaning • Preference for structure in activities of daily living • Recurrent thoughts that are not distressing • Excessive worry

* $p < .05$, ** $p < .01$, *** $p < .001$

277 ***Hypothesis 3: BAP endophenotypes vary in large multiplex families***

278 Applying the above endophenotype thresholds to the proportional scores of the 33 BAP traits for
279 members of Family A and B led to the identification of all individuals classified as having the
280 BAP. Two additional BAP cases were identified in Family B based on the presence of above
281 threshold endophenotype scores, indicating good utility of this approach (Fig.3). One individual
282 was excluded from this analysis due to incomplete data (III-7). Across both families, the aloof
283 endophenotype was most commonly observed (62%), followed by obsessive (60%), pedantic
284 (55%) and socially unaware (48%). Approximately one quarter of family members met criteria
285 for only one endophenotype, 15% met criteria for two, and the remainder met criteria for 3-4
286 (62%) (Fig.3). The dominant endophenotype across both families, as determined by the highest
287 score, was aloof (47%), followed by obsessive (26%), socially unaware (18%) and pedantic
288 (9%).

289 Family A appeared to have two endophenotype profiles, with one characterised by the
290 presence of a single endophenotype (35%) seen in individuals who were mostly married-in
291 (67%), contrasting with the second profile (41%) of all four endophenotypes, most evident in
292 core family members (72%) (Fig.4). Overall, the obsessive endophenotype occurred most
293 frequently (77%), followed equally by pedantic (65%) and aloof (65%), and then socially
294 unaware (53%). The co-occurrence of the obsessive and pedantic endophenotypes was relatively
295 common, seen in 29% of married-ins and core family members. Overall, there was a range of
296 dominant endophenotypes across individuals, with aloof the most frequent (35%) particularly in
297 core family members (83%).

298 Contrasting with Family A, Family B had more individuals (70%) with multiple
299 endophenotypes, in both married-in and core family members (Fig.4). All four endophenotypes

300 were again most frequently observed in core family members, indicative of a more severe BAP
301 presentation. Unlike Family A, however, the aloof endophenotype occurred most frequently in
302 Family B (88%), followed by obsessive (71%), pedantic (65%), and socially unaware (65%).
303 The aloof endophenotype was also identified as dominant (59%), evident in 70% of core family
304 members.

305 **(figure 3)**

306 **(figure 4)**

307 *Correlates of the BAP Endophenotypes*

308 Across both families, no sex or age differences were observed for any of the
309 endophenotypes (all $p < .200$). Overall, a more severe BAP presentation (indicated by a greater
310 number of endophenotypes) was associated with reduced social adaptive functioning on both
311 self-report and objective measures of social communication (Table 6). In particular, a more
312 severe BAP presentation showed a strong correlation with more severe pragmatic language
313 difficulties, with scores for each endophenotype also significantly correlated. A similar
314 relationship was evident for the ability to detect a faux pas in social discourse and self-reported
315 social functioning, particularly for family members with the socially unaware endophenotype
316 (Table 6).

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Domain	Task	Endophenotypes				Total number
		Socially unaware	Pedantic	Aloof	Obsessive	
Social	PRS	0.83**	0.73**	0.76**	0.45**	0.86**

communication	FPT	-0.43**	-0.28	-0.24	-0.18	-0.40**
Intellect	FSIQ	-0.36*	-0.10	-0.31*	-0.02	-0.28
	VIQ	-0.29	-0.05	-0.31*	-0.03	-0.28
	PIQ	-0.36*	-0.13	-0.19	-0.02	-0.26
Executive functions	Trails (numbers) ^a	-0.32*	-0.20	-0.29	0.17	-0.19
	Trails (switch) ^a	-0.24	-0.23	-0.27	-0.22	-0.34*
	Design fluency (switch) ^a	-0.27	-0.25	-0.15	-0.09	-0.34*
	Design fluency (composite) ^a	-0.27	-0.25	-0.25	-0.03	-0.32*
	Tower task (achievement) ^a	-0.40**	-0.19	-0.27	-0.22	-0.26
	Sorting (confirmed)	-0.33*	-0.23	-0.32*	-0.22	-0.33*
	Sorting (free sort)	-0.31	-0.15	-0.24	-0.37*	-0.37*
Adaptive function	Social index (self-report)	-0.46*	-0.35	-0.19	-0.31	-0.43*

* $p < .05$; ** $p < .01$

^anonverbal executive function subtests; PRS= Pragmatic Rating Scale; FPT=Faux Pas Test; FSIQ=Full Scale Intelligence Quotient; VIQ = Verbal Intelligence Quotient; PIQ = Performance Intelligence Quotient

320 **Table 6. Correlations between endophenotypes and quantitative measures in Family A and**
 321 **B**

322 For the cognitive measures, a more severe BAP presentation was associated with reduced
 323 executive functioning, particularly for nonverbal measures of cognitive flexibility (switching and
 324 fluency; Table 6). A pattern of weaker correlations was also evident for specific endophenotypes,
 325 including lower IQ in the socially unaware and aloof endophenotypes (Table 6).

326 **Discussion**

327 We studied the BAP to highlight the phenotypic variation within and between high-risk ASD
 328 families, to improve identification of individuals crucial for accurate molecular genetic analysis.
 329 We identified multiple individuals with the BAP in large multiplex families using rigorous
 330 phenotyping and a new endophenotyping approach, which was validated in an independent
 331 sample of small ASD families. The results of this work show that specific BAP endophenotypes
 332 exist across the traditional BAP domains of social relationships, communication, and

333 circumscribed interests and behaviour, providing a more nuanced way to detect subtle features of
334 the BAP. Moreover, these endophenotypes show different patterns of inheritance in two large
335 multiplex families, supporting their use to identify autism genes of dominant effect.

336 Despite major advances in ASD genetics, aetiology in the majority of cases remains
337 unknown. The research model employed here to phenotype rare large multiplex families reveals
338 a pattern consistent with autosomal dominant inheritance of ASD/BAP traits that would not have
339 been captured without such rigorous phenotyping. Fifteen individuals (23%) met criteria for
340 ASD and 33 (51%) the BAP, including some married-in individuals. Our promising
341 endophenotype analysis provides further insight into specific profiles of the BAP and its varied
342 presentation. Traditionally, ASD family studies include 2-3 affected individuals^{41,42}. For
343 example, four candidate ASD genes were identified in seven ASD/BAP pedigrees with ≥ 3
344 affected individuals⁴³. Larger multiplex families remain scarce in the literature^{20,44}. Here, we
345 identified more subtle indicators of carrier status in two large families, using a robust
346 endophenotyping method with good sensitivity and specificity to detect the BAP in two
347 independent samples.

348 ***Endophenotypes of the BAP***

349 Over the last 20 years, the BAP has emerged as strongly associated with ASD. The BAP is
350 considered a marker of carrier status of genes that may contribute to autism risk^{21,45}. Here we
351 aimed to dissect the BAP into endophenotypes to understand the phenotypic variation within and
352 between families. Importantly, each endophenotype cluster was characterised by a combination
353 of communication, personality and behavioural indicators showing how specific traits across the
354 traditional BAP domains may group together to form distinct endophenotypes or ‘profiles’. As
355 summarised in Table 7, these profiles capture identifiable ‘personas’ that have core

356 characteristics with high face validity. These profiles also vary with functional correlates in
 357 distinct ways, supporting their construct validity. For example, the aloof endophenotype was
 358 characterised by a lack of innate social motivation or ability to meaningfully connect and
 359 empathise with others, associated with decreased theory of mind, lower executive and
 360 intellectual functioning. One individual dominant for the aloof endophenotype described social
 361 interactions as “a means to an end”. In contrast, the pedantic endophenotype was primarily
 362 characterised by detail-oriented traits, showing no associations with intellectual, executive or
 363 adaptive functions. Unsurprisingly, given the importance of social communication deficits in
 364 ASD and the BAP, all endophenotypes were associated with poor social communication, with
 365 the socially unaware endophenotype most broadly affected across social, intellectual, executive
 366 and adaptive function domains (Table 7).

Endophenotype	Core Characteristic	Associated Functional Domains			
		Social	Intellect	Executive	Adaptive
Socially unaware	Poor self-regulation and reciprocity in conversation	✓	✓	✓	✓
Pedantic	Self-focused and technical in interactions	✓			
Aloof	Difficulties expressing and relating to other’s emotions	✓	✓	✓	
Obsessive	Regimented approach to life and tendency to ruminate	✓		✓	

367 **Table 7. Summary of the BAP endophenotypes and their functional correlates**

368

369 By clustering traits across traditional BAP domains, endophenotype profiles may
 370 improve detection of the BAP and thus, advance gene discovery. Specifically, in contrast with a
 371 traditional domain approach, where an individual may show mild BAP features across all
 372 domains but fail to meet criteria, an endophenotype approach allows individuals with autism

373 susceptibility genes to be captured by meeting threshold criteria for a specific profile.
374 Importantly, replication and validation of the proposed BAP endophenotypes is needed, using
375 targeted assessments to further validate and refine the traits characterising each endophenotype.
376 This, in turn, will provide the foundation for more efficient assessment protocols, and more
377 sophisticated and granular mapping of psychological and neural correlates where results have
378 been mixed to date⁴⁶.

379 ***Careful endophenotyping will enable genetic insights***

380 Consistent with previous literature, phenotypic heterogeneity was evident in both families at the
381 endophenotype level suggesting a single familial mutation may produce a phenotypic spectrum,
382 with other genetic, epigenetic and environmental factors influencing expression. With the
383 advancement of high-throughput next generation sequencing technologies, meticulous
384 phenotypic characterisation of both affected and apparently unaffected individuals remains
385 essential for accurate data interpretation. In other words, identification of subtle endophenotypes,
386 such as the four identified here, are crucial for advancing gene discovery programs.

387 Although multiplex families with ASD are genetically homogeneous, our phenotyping
388 analysis suggests possible bi-linear inheritance of the BAP in both families. Therefore, multiple
389 risk alleles may contribute to ASD/BAP in later generations, consistent with recent genetic and
390 phenotyping evidence^{47,48}. The importance of unique *de novo* genetic changes in both sporadic
391 (or ‘simplex’), ASD¹⁷, and small multiplex ASD families⁴⁴ has become increasingly apparent.
392 However, with at least seven individuals with ASD and many more with the BAP in our families,
393 there is less likelihood of *de novo* changes contributing to each phenotype. It is much more likely
394 that there is a single genetic variant of major phenotypic effect in each family, with the

395 possibility that there are additional *de novo* genetic changes in some individuals that contribute
396 to phenotypic severity.

397 ***Limitations***

398 The intensive nature of the study meant that clinicians were not blinded to family relationships,
399 potentially leading to investigator bias. However, our diagnostic method of consensus between
400 experienced clinicians aligns with current best practice for ASD/BAP diagnosis and was
401 informed by quantitative and qualitative measures. We selected a relatively low threshold for
402 BAP classification, leading to the identification of many affected individuals. However, this
403 approach is justified in a family with a clear genetic liability for ASD and was validated by the
404 finding of consistent data-driven endophenotypes in the small families. Successful gene
405 identification in future work requires capture of all individuals who may carry the putative
406 variant, with the approach outlined here enabling more robust gene identification work.

407 **Conclusion**

408 Despite significant advances in unravelling the heterogeneity of ASD, in most cases, the
409 underlying genetic aetiology remains unknown in part due to difficulties identifying
410 endophenotypes and potential carriers. We used a rigorous phenotyping approach to characterise
411 the BAP in two large multiplex families with dominant inheritance of ASD and the BAP. Further
412 phenotypic delineation identified four endophenotypes, showing differentiation of BAP features
413 beyond traditional domain approaches. This endophenotype approach advances our
414 understanding of the phenotypic spectrum to improve detection of the BAP in research and
415 clinical practice, facilitating gene discovery, neuroimaging investigations, and psychological
416 studies.

417

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517 Multiplex Families. *Am J Hum Genet* **99**, 540-554, doi:10.1016/j.ajhg.2016.06.036 (2016).

518 **Competing Interests:** The authors declare no conflicts of interest

519 **Figure Legends**

520 **Figure 1.** Scrambled pedigrees for Family A (panel A) and Family B (panel B) at recruitment.
521 Individuals with a diagnosis of ASD are marked in black, and individuals recruited from the
522 broader families are marked in yellow. White diamonds are individuals who were not recruited
523 but are represented here to preserve the pedigree lines.

524

525 **Figure 2.** Iterative process used to identify and assess BAP endophenotypes.

526

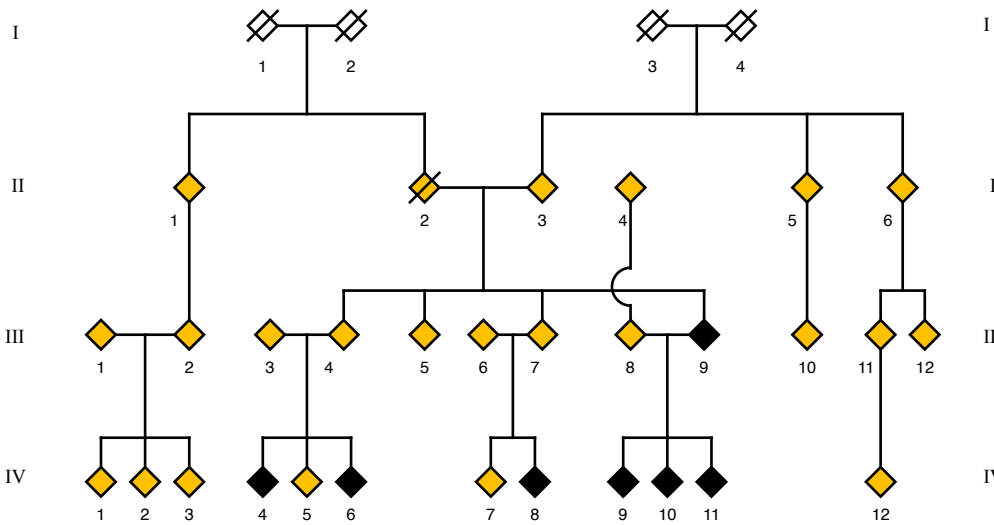
527 **Figure 1.** Scrambled pedigrees for Family A (panel A) and Family B (panel B) showing
528 phenotypes and endophenotypes. All individuals with ≥ 1 endophenotype had the BAP, with the
529 exception of two individuals from Family B (III-3 and IV-9) marked with an asterisk. These
530 individuals were clinically determined as unaffected (Family B III-3 and IV-9) but had above
531 threshold endophenotype scores based on ROC curves. Family members who were not
532 phenotyped are not shown to preserve the anonymity of these families.

533

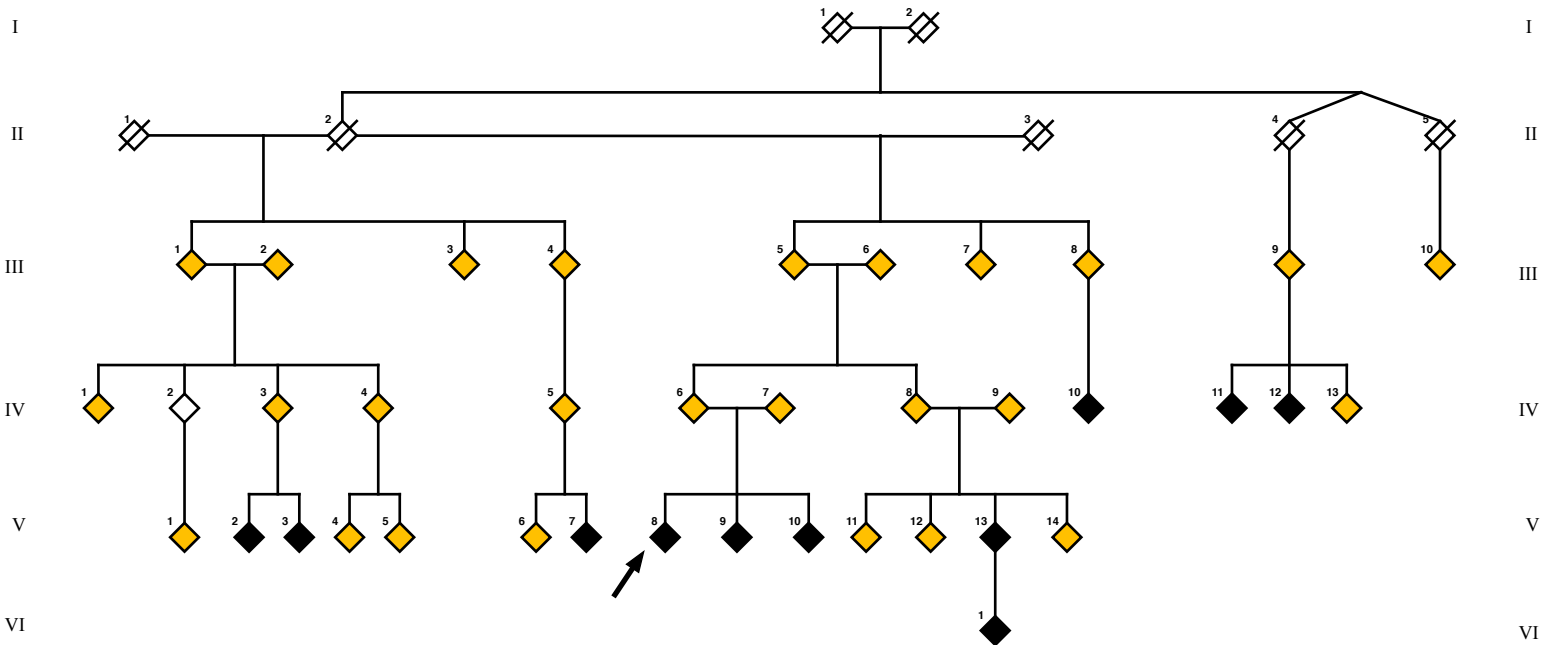
534 **Figure 4.** Number of BAP endophenotypes present in Family A and B. Individuals married-in to
535 Family A tend to have a single endophenotype, indicating a more mild BAP presentation, in
536 contrast with core family members who have multiple endophenotypes (obsessive most
537 frequent). In Family B, married-in and core family members tend to have more than one
538 endophenotype, with the aloof endophenotype most frequent.

539

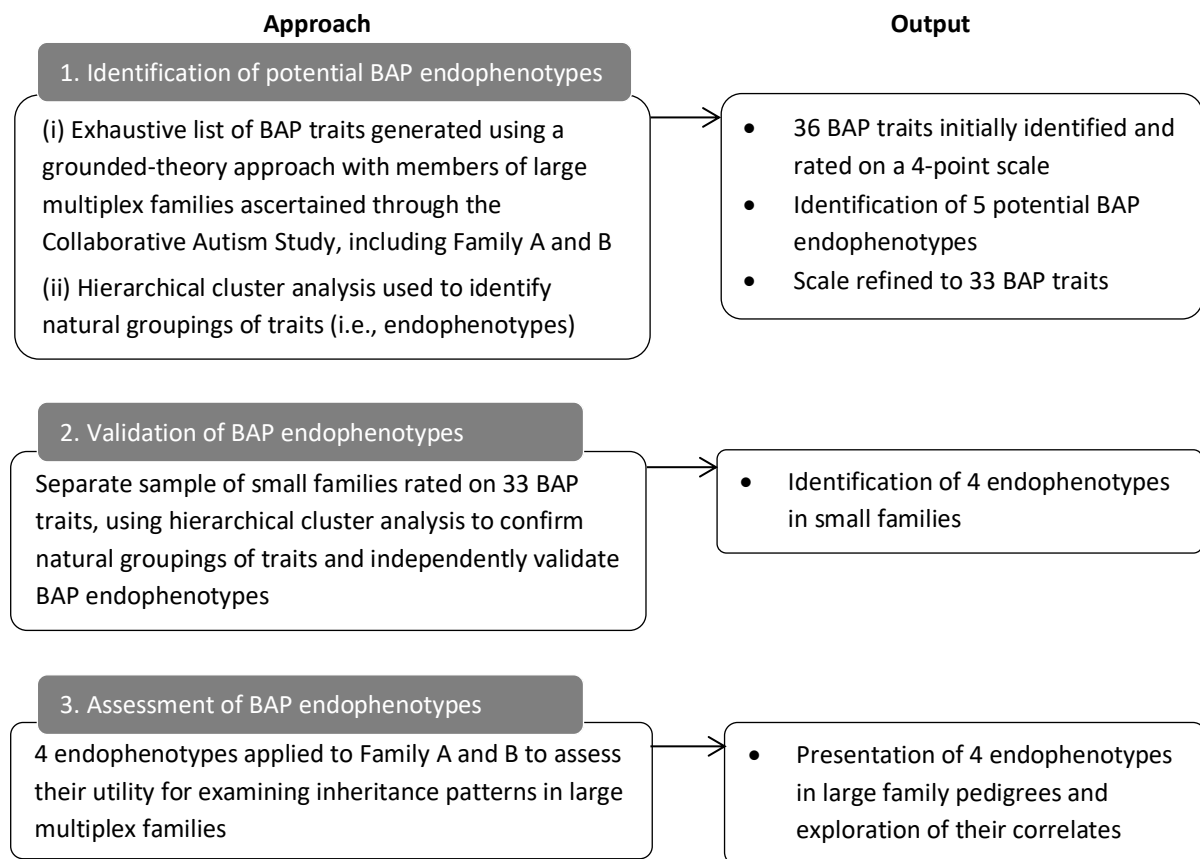
(a) Family A Pedigree



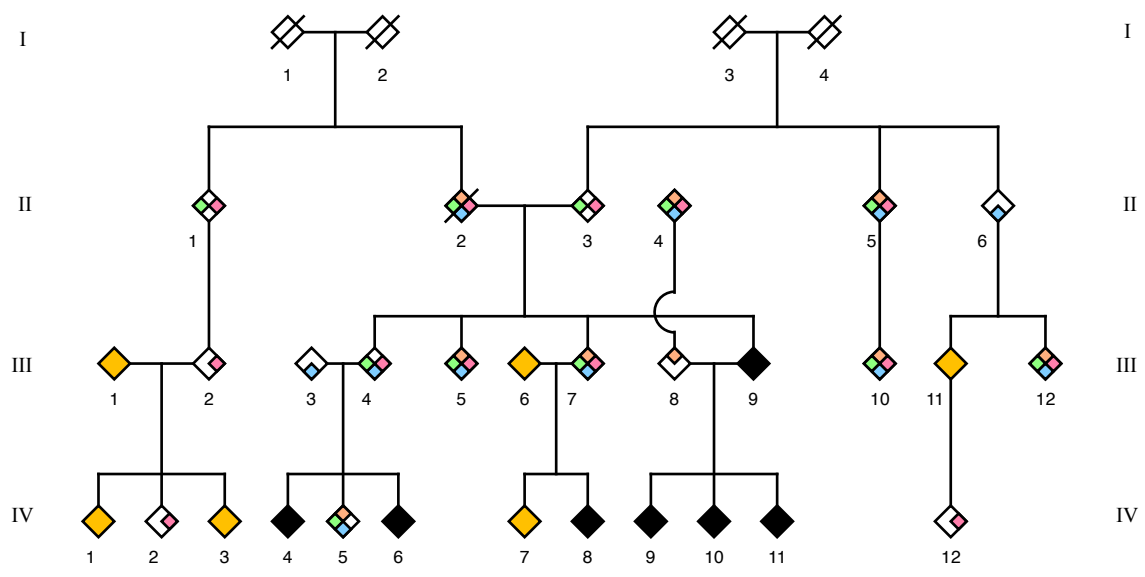
(b) Family B Pedigree



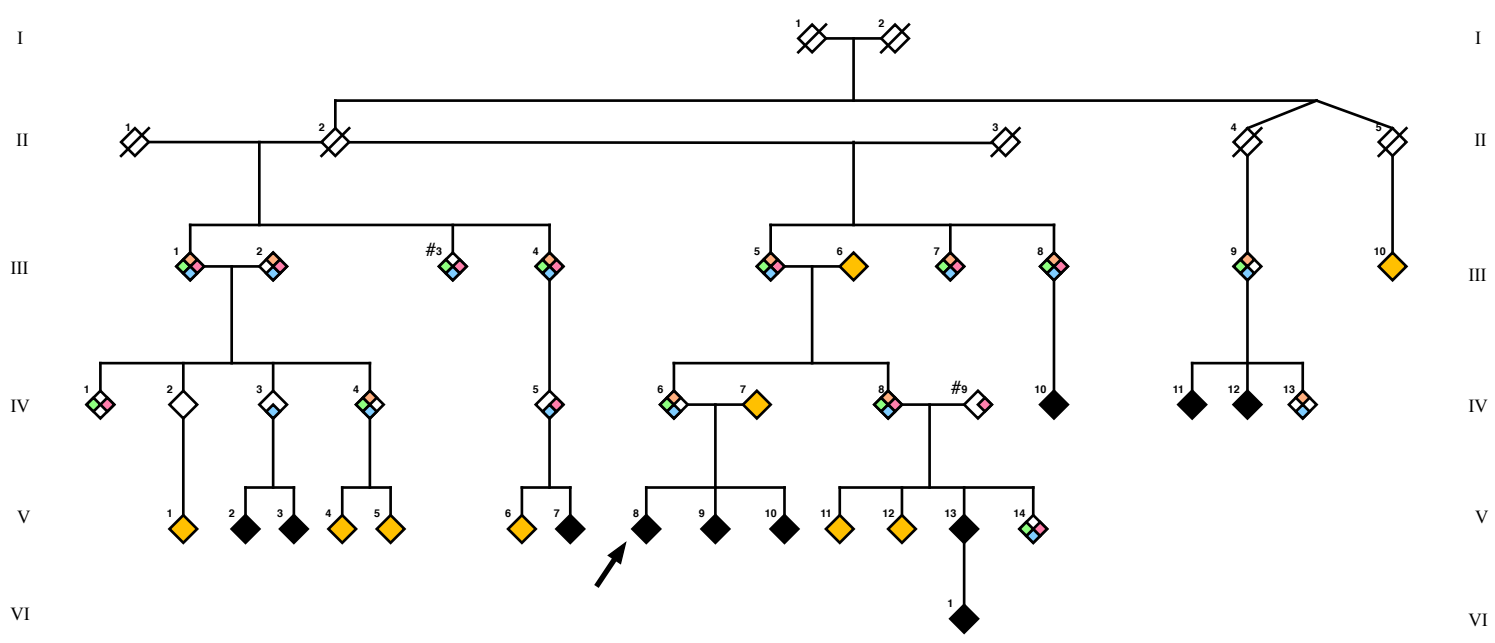
◆ Autism Spectrum Disorder ◆ Unaffected at recruitment ◇ Not recruited



(a) Family A Pedigree



(b) Family B Pedigree



- Pedantic
- Obsessive
- Socially unaware
- Aloof
- Autism Spectrum Disorder
- Unaffected
- Not recruited

