Differences in the importance of microcephaly, dysmorphism, and epilepsy in the detection of pathogenic CNVs in ID and ASD patients

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Background. Autism spectrum disorders (ASD) and intellectual disabilities (ID) are heterogeneous and complex developmental diseases with significant genetic backgrounds and overlaps of genetic susceptibility loci. Copy number variants (CNVs) are known to be frequent causes of these impairments.However, the clinical heterogeneity of both disorders causes the diagnostic efficacy of CNV analysis to be modest. This could be resolved by stratifying patients according to their clinical features.

Aim. First, we sought to assess the significance of particular clinical features for the detection of pathogenic CNVs in separate groups of ID and ASD patients and determine whether and how these groups differ from each other in the significance of these variables. Second, we aimed to create a statistical model showing how particular clinical features affect the probability of pathogenic CNV findings.

Method. We tested a cohort of 204 patients with ID (N = 90)and ASD (N = 114) for the presence of pathogenic CNVs. We stratified both groups according to their clinical features. Fisher's exact test was used to determine the significance of these variables for pathogenic CNV findings. Logistic regression was used to create a statistical model of pathogenic CNV findings.

Results. The frequency of pathogenic CNV was significantly higher in the ID group than in the ASD group – 18 (19.78%) versus 8 (7%) ($p < 0.004$). Microcephaly showed a significant association with pathogenic findings in ID patients ($p < 0.01$) according to Fisher's exact test, whereas epilepsy showed a significant association with pathogenic findings in ASD patients ($p < 0.01$). The probability of pathogenic CNV findings when epilepsy occurred in ASD patients was more than two times higher than if epilepsy co-occurred with ID (29.6%/14.0%). Facial dysmorphism was a significant variable for detecting pathogenic CNVs in both groups (ID $p = 0.05$, ASD $p = 0.01$). However, dysmorphism increased the probability of pathogenic CNV detection in the ID group nearly twofold compared to the ASD group (44.4%/23.7%). The presence of macrocephaly in the ASD group showed a 25% probability of pathogenic CNV findings by logistic regression, but this was insignificant according to Fisher's exact test. The probability of detecting pathogenic CNVs decreases up to 1% in the absence of dysmorphism, macrocephaly, and epilepsy in the ASD group.

Conclusion. Dysmorphism, microcephaly, and epilepsy increase the probability of pathogenic CNV findings in ID and ASD patients. The significance of each feature as a predictor for pathogenic CNV detection differs depending on whether the patient has only ASD or ID. The probability of pathogenic CNV findings without dysmorphism, macrocephaly, or epilepsy in ASD patients is low. Therefore theefficacy of CNV analysis is limited in these patients.

1 **Differences in the importance of microcephaly, dysmorphism, and epilepsy in the detection**

2 **of pathogenic CNVs in ID and ASD patients**

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63 **Conclusion.** Dysmorphism, microcephaly, and epilepsy increase the probability of pathogenic

64 CNV findings in ID and ASD patients. The significance of each feature as a predictor for

65 pathogenic CNV detection differs depending on whether the patient has only ASD or ID. The

66 probability of pathogenic CNV findings without dysmorphism, macrocephaly, or epilepsy in ASD

67 patients is low. Therefore the efficacy of CNV analysis is limited in these patients.

68 **Introduction**

69 Intellectual disabilities (ID) and autism spectrum disorders (ASD) are relatively common and

70 their impacts on patients, their families, and society are well known (Tonnsen et al., 2016;

71 Matson & Shoemaker, 2009; Schaefer, 2016). Autism spectrum disorders involve a broad range

72 of conditions characterised by deficits in social skills, repetitive behaviours, impaired speech,

73 and nonverbal communication (Bourgeron, 2016; Schaefer, 2016). Intellectual disabilities

74 involve problems with general mental abilities, which affect intellectual functioning and adaptive

75 functioning (Quintela et al., 2017).

76 The rates of ID and ASD are approximately 3 in 100 and 1 in 68, respectively, in the worldwide

77 child population (Harripaul et al., 2017). Despite the fact that these conditions are distinct

78 entities, they share some characteristics: a preponderance of affected males (Lai et al., 2015;

79 Mitra et al., 2016; Jacquemont et al., 2014), a genetic background (Diaz-Beltran et al., 2017;

80 Gonzalez-Mantilla et al., 2016), and accompaniment by clinical features such as hyperactivity

81 (ADHD), epilepsy, speech impairment, and learning disabilities. In both disorders, skeletal

82 abnormalities of the skull—microcephaly, macrocephaly, dysmorphic features—are frequently

83 described (Viñas-Jornet et al., 2018; Matson & Shoemaker, 2009). Multiple studies have

84 demonstrated the genetic bases of ASD and ID, including chromosome abnormalities, single

85 genes, copy number variants (CNVs), and multifactorial inheritance (Krishnan et al., 2016; de la

86   Torre-Ubieta et al., 2016; Egger et al., 2014). Despite all efforts, the aetiology of these

87   conditions is still not fully understood (Stessman et al., 2017).

88   Portfolio methods commonly used to find the causes of ASD and ID involve conventional

89   karyotyping, fluorescent in situ hybridisation (FISH), multiplex ligation-probe dependent

90   amplification (MLPA), and chromosomal microarrays (CMA) – recently accepted as a frontline

91   method (Battaglia et al., 2013) – which have resulted in the discovery of many genetic variants

92   (de la Torre-Ubieta et al., 2016; Schaefer, 2016; Hehir-Kwa et al., 2013; Merikangas et al.,

93   2015).

94   The overlap of genetic susceptibility loci has been described previously in diagnoses of both

95   disorders (Matson & Shoemaker, 2009; Lowther et al., 2017) together with other

96   neuropsychiatric disorders such as schizophrenia, Alzheimer's disease, Parkinson's disease, and

97   others. (Wolfe et al., 2017; Sokol et al., 2011; Catalá-López et al., 2014). It is assumed that the

98   incomplete penetrance and variable expression of CNVs are the causes of different clinical

99   phenotypes of the same CNV (Lowther et al., 2017). This situation usually causes patients with

100   ASD and ID to be grouped together based on overlapping clinical and genetic features, and so

101   the efficacy of genetics-based diagnostic methods is relatively low (Xu et al., 2018; Peycheva et

102   al., 2018, Schaefer & Mendelsohn, 2013, Chan et al. 2018). On the other hand, the yields of

103   these tests are strongly influenced by the clinical features that accompany ID and ASD (Ho et al.,

104   2016, Miller et al., 2010; Beaudet, 2013, Jacquemont et al., 2006).

105   We assessed the impact of particular clinical features (ADHD, epilepsy, growth defects,

106   congenital malformations, microcephaly, macrocephaly, and dysmorphism) on the detection of

107   pathogenic CNVs in separate ID and ASD groups and determined whether and how these groups

108   differ from each other.

109 **Materials**

110 A total of 204 patients of Caucasian descent with intellectual disabilities (ID) and autism

111 spectrum disorders (ASD) referred to genetic counselling were enrolled in this retrospective

112 study. All patients underwent rigorous examinations by paediatricians, neurologists,

113 psychiatrists, and geneticists, including metabolic tests and brain imaging. Metabolic disorders

114 were excluded by the Department of Clinical Biochemistry, Palacky University Olomouc, Czech

115 Republic, through biochemical screening. Peripheral blood samples were collected after genetic

116 counselling in the Department of Medical Genetics at the University Hospital Olomouc, Czech

117 Republic, during the years 2012–2018. Part of this counselling was the collection of informed

118 consent from parents or guardians of the patients in accordance with the Declaration of Helsinki.

119 The Institutional Review Board of the University Hospital and the Faculty of Medicine and

120 Dentistry, Palacky University Olomouc, granted a permit for this study (IRB number 96/17). The

121 patients were stratified into two groups – 90 patients with solely intellectual disabilities (ID) (51

122 male, 39 female) aged 5–35 years old, and 114 patients with autistic spectrum disorders (ASD)

123 (78 male, 36 female) aged 3–18 years old. The ASD cohort involved patients with (N = 96) and

124 without (N = 18) intellectual impairment, but they differ from the ID group in that they have

125 been diagnosed with autism, which was taken as the primary reason for the investigation. ASD

126 individuals were diagnosed with ASD by clinicians after performing the Autism Diagnosis

127 Observation Schedule. Subjects with pervasive developmental disorders and varying levels of

128 impairment were diagnosed with broad-spectrum disorder, which involves conditions such as

129 pervasive developmental disorders not otherwise specified (PDD-NOS) and Asperger's

130 syndrome.

131 **Methods**

132 The study design was retrospective. Patients' clinical data were collected from their most up-to-

133 date medical records to eliminate changes in the definitions of ID and ASD and to reflect the

134 actual diagnosis of each patient at that period of the time. General observations of the clinical

135 features in patients were made by genetic counsellors or specialists. Scored features were

136 ADHD; epilepsy; microcephaly (head circumference < 2nd percentile); macrocephaly (head

137 circumference > 98th percentile); facial dysmorphism (abnormalities of the eye slits, superciliary

138 arches, nose, lips, philtrum, ears, jaws, palatum durum, face shape, and

139 hairline); developmental defects of the heart, urogenital system, and brain; and growth

140 restrictions.

141 Patients with chromosomal aberrations and FMR1 mutations were excluded from the study.

142 DNA from peripheral blood isolated by the saline method was used for CNV analysis by MLPA

143 and/or CMA.

144 MLPA tests were performed with SALSA® MLPA® probes for testing subtelomeric regions

145 (P070 Subtelomeres Mix 2B, P036 Subtelomeres Mix 1), the most frequent microdeletion or

146 microduplication syndromes (P245 Microdeletion Syndromes-1A, P297 Microdeletion

147 Syndromes-2), autistic and X-linked intellectual disability susceptibility regions (P343 Autism-1,

148 P106 MRX). MLPA analysis adhered the protocol recommended by the manufacturer

149 (www.mlpa.com, 31 August 2018). Capillary electrophoresis (CE) was used for the

150 determination of PCR products using an ABI 3130 genetic analyser provided by the Gene

151 Mapper software (Applied Biosystems, Foster City, CA, USA). The Coffalyser program was

152 used for CNV calling (MRC-Holland, Amsterdam, the Netherlands).

153 Cytoscan HD (Affymetrix, Santa Clara, CA, USA) and CytoSNP-12 (Illumina, San Diego, CA,

154 USA) instruments were used for CMA analysis according to the manufacturers' protocols

155   (www.affymetrix.com, www.illumina.com, 31 August 2018). The data discussed in this

156   publication have been deposited in NCBI's Gene Expression Omnibus database (Edgar,

157   Domrachev & Lash, 2002) and are accessible using GEO Series accession number GSE132453.

158   The programs CHAS v1.2.2 (Affymetrix, Santa Clara, CA, USA) and Illumina KaryoStudio 1.3

159   (Genome Studio v2011.1) were used for CNV calling. Pathogenic CNVs were determined using

160   curated databases (ISCA, Decipher, SFARI, DGV) and with the acceptance of guidelines

161   (Kearney et al., 2011, Schaefer & Mendelsohn, 2013).

162   The significance of particular clinical features for the detection of pathogenic CNVs was

163   determined by Fisher's exact test. A statistical model was prepared using a forward/stepwise

164   logistic regression model to resolve how particular features affect the probability of pathogenic

165   CNV findings in both groups. Both tests were performed in each group (ID and ASD) by an

166   analytical company (ACREA, Prague, Czech Republic).

167   **Results**

168   Dysmorphism, microcephaly, and developmental defects (heart, urogenital system, and brain)

169   were significantly more abundant in patients with solely ID ($p < 0.5$), whereas ADHD was more

170   prevalent in the ASD group ($p < 0.5$) (Table 1).

171   We detected pathogenic CNVs in 26 patients (12.7%); altogether: ID = 18 (19.8%); ASD = 8

172   (7.0%). The frequency of pathogenic CNVs was significantly higher in the ID group than in the

173   ASD group ($p < 0.004$). This p-value decreased when 18 ASD patients without intellectual

174   disabilities were excluded ($p = 0.02$). The frequency of pathogenic CNVs in both groups (ID and

175   ASD) taken together and in each group separately and stratified according to discrete features are

176   shown in Table 2 and Figures 1, 2, and 3. Patients with pathogenic CNVs are described in

177   Supplement Table 1.

178 Fisher's exact test of the groups separated according to ID or ASD showed that microcephaly (p

179 = 0.01) in the ID group and epilepsy (p = 0.01) in the ASD group were significant for the finding

180 of CNVs, whereas dysmorphism is significant in both groups (ID p = 0.05, ASD p = 0.01). ASD

181 patients with macrocephaly and ADHD expressed a higher percentage of pathogenic variants,

182 though this was not as statistically significant as some growth defects in the ID group (p = ns).

183 Developmental defects of the heart, urogenital system, and brain did not achieve statistical

184 significance in either group (p = ns). The summary in Table 3 shows the achieved significance of

185 particular clinical features to pathogenic CNV findings in the ASD and ID groups using Fisher's

186 exact test.

187 We created a statistical model and calculated the probability of pathogenic CNV findings in ASD

188 and ID patients with each significant feature from Fisher's exact test. Macrocephaly in ASD

189 patients was added to the model based on the close percentage of pathogenic CNVs

190 to dysmorphism. In the ID group, microcephaly was significant only when separate and fell out

191 of the overall model. Dysmorphism achieved a 44.4% probability of pathogenic CNV findings in

192 the ID group, in contrast with the ASD group, which reached only 23.7%. These differences

193 were nearly twice as high for the ID group compared to the ASD group (p = 0.052).

194 The risk of pathogenic CNVs in patients with epilepsy was 29.6% in the ASD group and 14% in

195 the ID group, meaning the risk was more than twice as high for the ASD group than for the ID

196 group (p = 0.003). This difference was significant and it implies that for the detection of

197 pathogenic CNVs in patients with epilepsy it is important to know whether this patient is

198 classified as ID or ASD (Figure 4).

199 Added macrocephaly showed a 25% pathogenic CNV probability in ASD patients, which is

200 higher than dysmorphism (23.7%).

201   The absence of dysmorphism, macrocephaly, and epilepsy decreases the probability of detecting

202   pathogenic CNVs to 1% in the ASD group (Figures 5). The calculation of logistic regression and

203   formulas for the calculation of pathogenic CNV probability in the ASD group are shown in

204   Supplement Table 2 and Figure 6. No pathogenic CNVs were found in autistic patients without

205   intellectual disability. Apart from one case of epilepsy, no microcephaly, macrocephaly,

206   or dysmorphism were found in this subgroup, nor were any pathogenic CNVs. Although it would

207   be interesting to compare this subgroup with the ID cohort, this could not be accurately

208   calculated due to the cohort size (N = 18).

209   **Discussion**

210   Autism spectrum disorders (ASD) and intellectual disabilities (ID) are overlapping genetically

211   conditioned developmental diseases that are frequently accompanied by ADHD, epilepsy,

212   microcephaly, macrocephaly, dysmorphism, developmental defects, and/or growth defects

213   (Whittington et al., 2018; Li et al., 2018; Mulle et al., 2014, Bourgeron, 2016; Schaefer, 2016;

214   Quintela et al., 2017). Copy number variants (CNVs) have been identified as one of the possible

215   cause of these diseases (Miller et al., 2010, Battaglia et al., 2013, de la Torre-Ubieta et al., 2016;

216   Schaefer, 2016; Hehir-Kwa et al., 2013; Merikangas et al., 2015). CNV analysis is recommended

217   in patients with ASD and/or ID as a frontline test, and the efficacy of this approach is 8.7%–

218   14.7% in patients with ID and 12% in patients with ASD (Xu et al., 2018; Peycheva et al., 2018,

219   Miller et al., 2010). The yields of CNV analyses are influenced by the accompanying clinical

220   features in ID/ASD patients (Ho et al., 2016).

221   Pathogenic CNVs have been detected in higher percentages (15%–20%) in ID and ASD cohorts

222   when additional clinical features (e.g. micro-/macrocephaly, dysmorphism, developmental and

223   growth defects) are present (Miller et al., 2010; Beaudet, 2013, Jacquemont et al., 2006). In some

224   studies, dysmorphia and/or microcephaly increased the percentage of pathogenic CNVs up to

225   45.8% in ID and ASD cohorts (Jacquemont et al., 2006, Chan et al. 2018, Miles et al. 2011). In

226   published results, patients with ASD and ID are usually grouped based on overlapping clinical

227   and genetic features, but we were interested in the impact of each clinical feature on each group

228   separately (ID, ASD) (Miller et al., 2010; Beaudet, 2013, Jacquemont et al., 2006, Chan et al.

229   2018).

230   We are able to confirm that dysmorphism increases the probability of pathogenic

231   CNV detection in ID and ASD patients (Miller et al., 2010; Beaudet, 2013, Jacquemont et al.,

232   2006). The calculated probability of pathogenic CNVs in patients with dysmorphism was 44.4%

233   and 23.7% in the ID cohort and ASD cohort, respectively, meaning the risk was nearly twice as

234   high for the ID group than for the ASD group (p = 0.052). Dysmorphic features have proven to

235   be the most prominent predictor for pathogenic CNVs in the ID group.

236   Defects of the skull, namely size, are important clinical features for findings of pathogenic CNVs

237   in ID and/or ASD patients (Qiao, 2009; Hultman et al., 2010; Shaw-Smith et al., 2004;

238   Bernardini et al., 2010, Blanken et al., 2018; Klein, Sharifi-Hannauer & Martinez-Agosto, 2013).

239   The co-segregation of microcephaly and dysmorphism has frequently been associated with the

240   presence of pathogenic CNVs in groups of ID children (Qiao, 2009; Hultman et al., 2010; Shaw-

241   Smith et al., 2004; Bernardini et al., 2010), whereas macrocephaly might be associated with

242   certain subtypes of autism (Blanken et al., 2018; Klein, Sharifi-Hannauer & Martinez-Agosto,

243   2013). Our study confirmed that microcephaly is more frequently found in ID patients than in

244   ASD patients and that the probability of finding pathogenic CNVs is significantly higher in the

245   ID group than in the ASD group.

246   We cannot prove that macrocephaly is significantly associated with pathogenic CNV findings in

247   both groups (ID p = 0.338; ASD p = 0.256). Even though the difference was not significant,

248   macrocephaly was included in the statistical model of logistic regression in the ASD group in

249   order to situate our work in relation to previously published works (Blanken et al., 2018; Klein,

250   Sharifi-Hannauer & Martinez-Agosto, 2013). Macrocephaly increased the probability of

251   pathogenic CNV detection in affected ASD children up to 25%, more

252   than dysmorphism (23.7%). The explanation for this is that isolated macrocephaly has low

253   predictive value, but in combination with other traits it could profoundly increase the probability

254   of pathogenic CNV detection.

255   There are still controversies over the association between autism and epilepsy (Amiet et al.,

256   2013; Lee, Smith & Paciorkowski, 2015; Berg & Plioply, 2012). A recent population-based

257   study found that 44% of children with ASD received a subsequent diagnosis of epilepsy, and

258   54% of children with epilepsy received a subsequent diagnosis of ASD (Jokiranta et al., 2014).

259   The role of pathogenic CNVs in epilepsy has been previously described (Olson et al., 2014,

260   Viscidi et al., 2013). We proved that epilepsy is significant for pathogenic CNV findings in ASD

261   patients but was insignificant in patients with ID, even though the proportion of patients with

262   epilepsy was the same in both groups.

263   ASD and ID are frequently accompanied by ADHD, developmental and growth defects,

264   epilepsy, micro-/macrocephaly, and/or dysmorphism (Whittington et al., 2018; Li et al., 2018;

265   Mulle et al., 2014, Bourgeron, 2016; Schaefer, 2016; Quintela et al., 2017).

266   ADHD manifested more frequently in the ASD cohort than in the ID cohort. However, ADHD,

267   similar to developmental (heart, urogenital system, and brain) and growth defects, is a variable

268   that appeared to be insignificant in the detection of pathogenic CNVs in both groups in our work.

269   We assume that causes other than pathogenic CNVs underlie ADHD (Kim et al. 2017).

270   Interestingly, developmental (heart, urogenital system, and brain) and growth defects are part of

271   some syndromes that accompany ASD and/or ID, but both were insignificant in their effects on

272   findings of pathogenic CNVs in our patients (Whittington et al., 2018; Li et al., 2018; Mulle et

273   al., 2014, Bourgeron, 2016; Schaefer, 2016; Quintela et al., 2017).

274   Finally, Ho et al. (2016) tested more than 10,000 patients with neurodevelopmental diseases,

275   namely developmental delays / intellectual disabilities and autism spectrum disorders, and noted

276   that the percentage of pathogenic CNVs in patients with neurodevelopmental disorders increases

277   when autistic patients are excluded (28.1% vs 33.0%). This supports our results. The probability

278   of pathogenic CNVs was calculated to be 1% for ASD patients without dysmorphism, epilepsy,

279   or macrocephaly. The result implies that the relevance of CNV analysis is significantly

280   influenced by accompanying features, especially in ASD patients. This fact generally goes

281   against the accepted recommendation of CNV analysis in patients with ASD but without

282   additional clinical features (Ho et al., 2016).

283   We confirmed the impact of dysmorphic features and microcephaly and added epilepsy as a

284   predictor for the finding of pathogenic CNVs and highlighted the differing impacts of each

285   clinical feature on each group (ID, ASD) in searching for pathogenic CNVs. In addition, we

286   identified low rates of pathogenic variants in patients with ASD but without intellectual

287   disability, dysmorphism, macrocephaly, or epilepsy. However, our results did not asses the

288   influence of other variables that could affect CNV rates (e.g. gender).

289   **Conclusion:**

290   The presences of dysmorphism, microcephaly, and epilepsy increased the detection rate of

291   pathogenic CNVs in patients with ID and ASD in our study, but the significance of each feature

292   is different for each group. Microcephaly is a significant predictor for the risk of pathogenic

293   CNVs in patients with intellectual disabilities but not in patients with autism spectrum disorders,

294   whereas epilepsy is a significant predictor for the risk of pathogenic CNVs in patients with ASD

295   but not in patients with ID. Dysmorphism is a significant predictor in both groups, but is nearly

296   twice as high in patients with ID than patients with ASD. Macrocephaly may increase the

297   probability of pathogenic CNV findings in ASD patients. ID and ASD patients with ADHD or

298   developmental and growth defects as well as some ASD patients without dysmorphism, epilepsy,

299   or macrocephaly have a very low probabilities of pathogenic CNVs, which is contrary to the

300   generally accepted recommendation of CNV analysis in patients with ID and ASD.

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481

Figure 1

Frequency of pathogenic CNVs in both groups (ID and ASD) together stratified according to the clinical features

*heart, urogenital and brain , ADHD – hyperactivity, CNVs - copy number variants
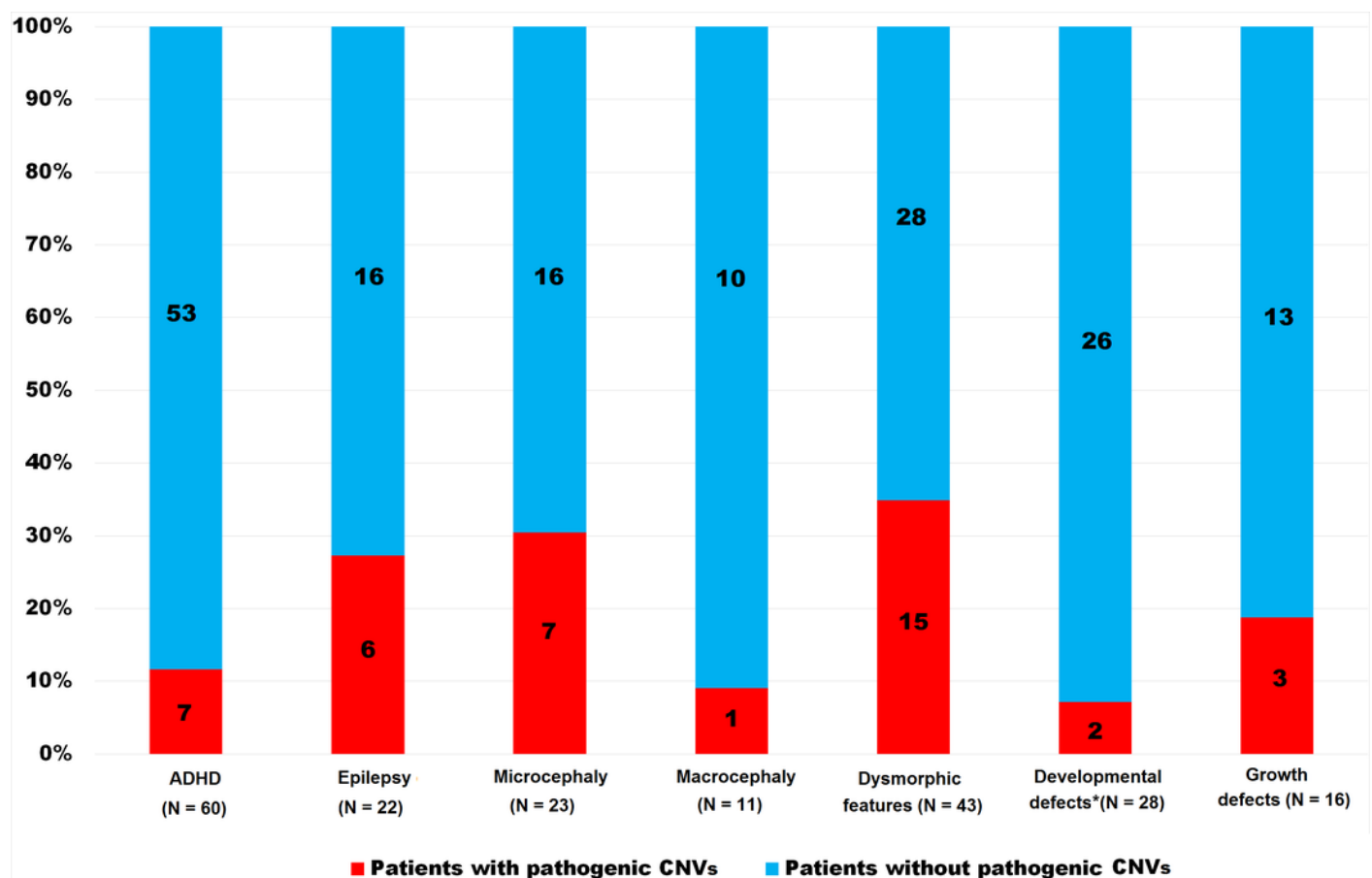
Figure 2

Frequency of pathogenic CNVs in ID group stratified according to the clinical features

*heart, urogenital and brain , ADHD – hyperactivity, CNVs - copy number variants
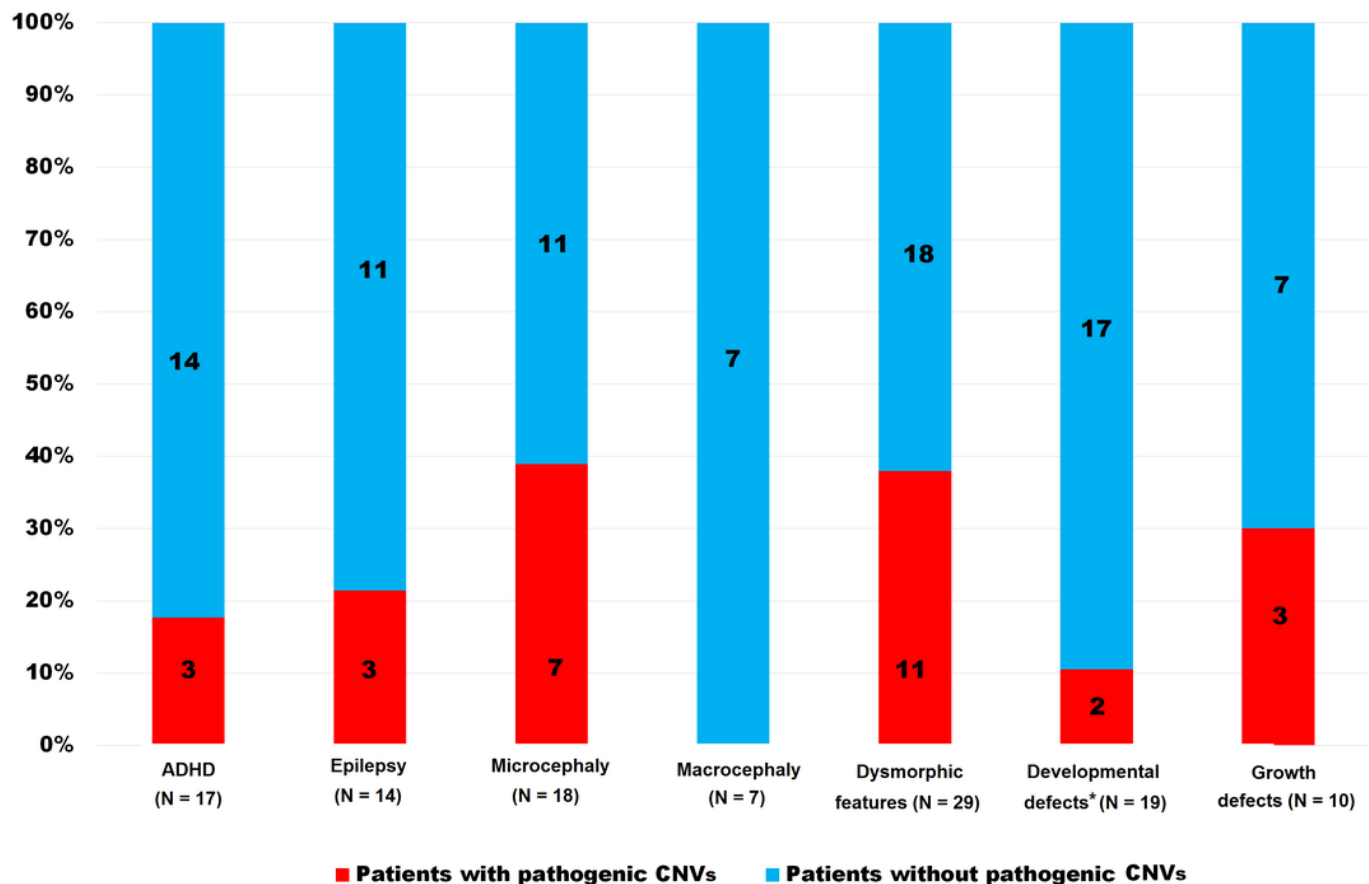
Figure 3

Frequency of pathogenic CNVs in ASD group stratified accordingto the clinical features

*heart, urogenital and brain , ADHD – hyperactivity, CNVs - copy number variants
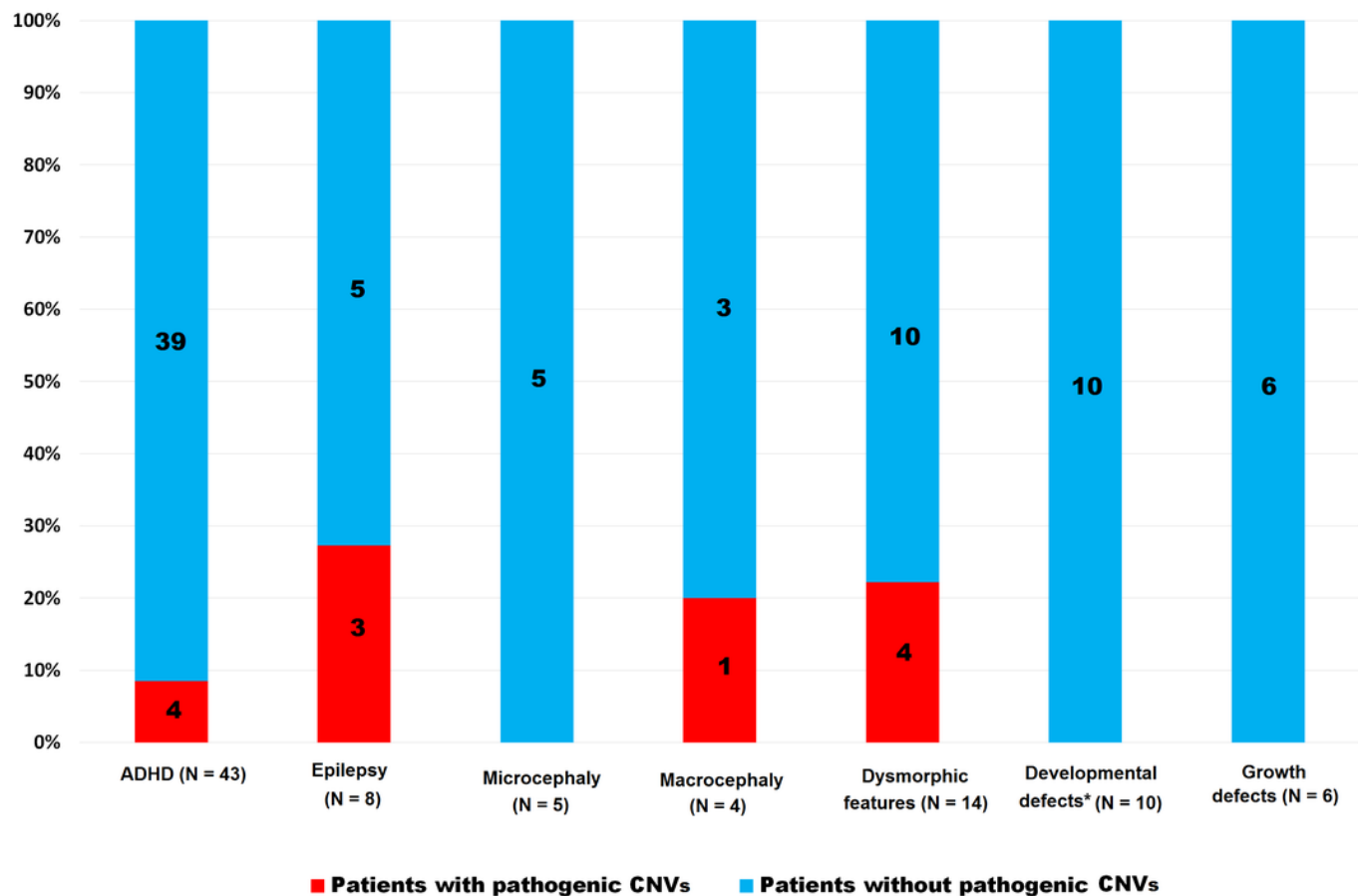
Figure 4

Differences between ID and ASD patients with epilepsy in pathogenic CNVspresentation

ID - intellectual disabilities patients, ASD - autism spectrum disorders patients, CNVs - copy number variants
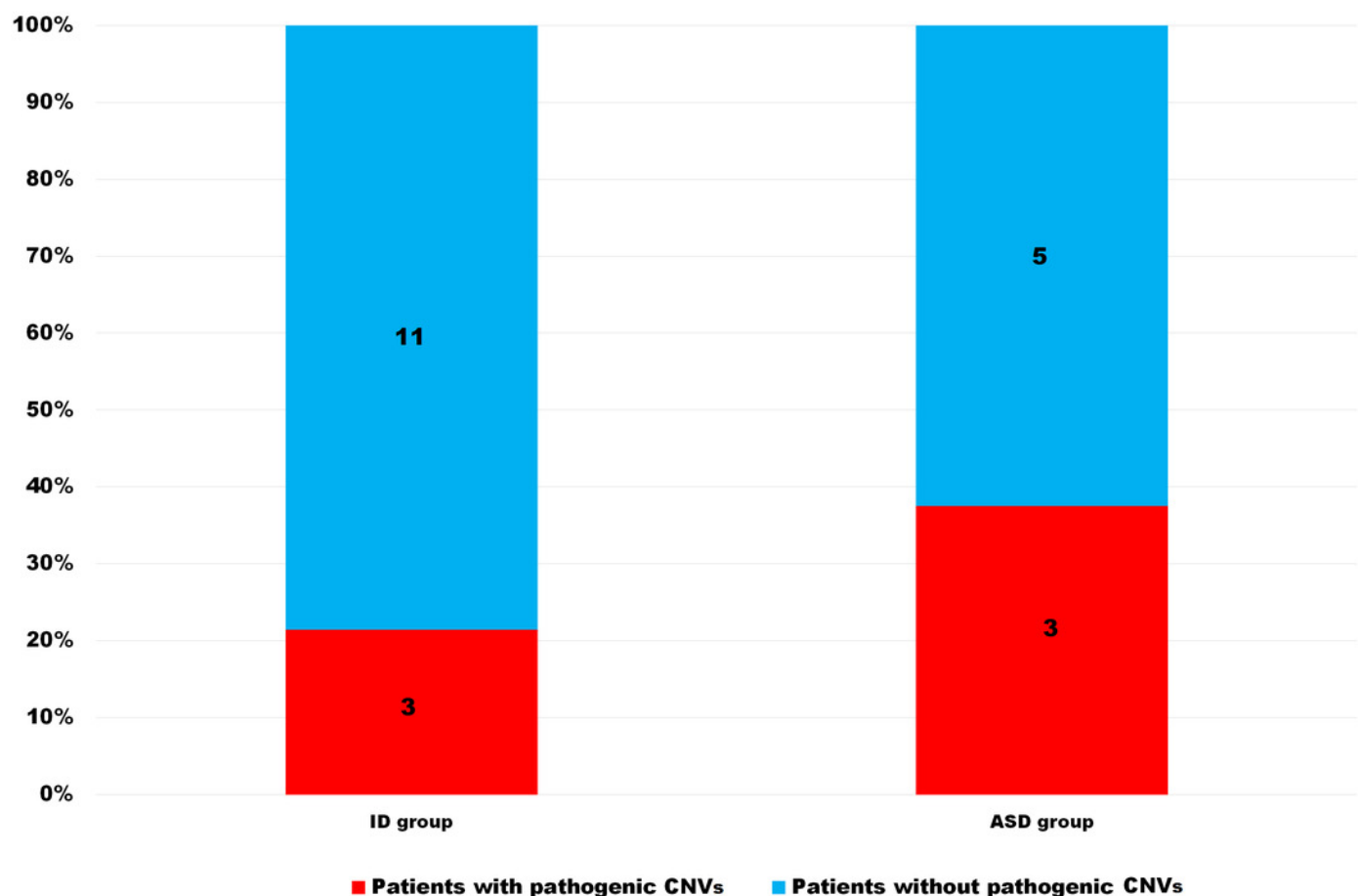
Figure 5

Probability of pathogenic CNV in presence (A) /absence (B) of facial dysmorphia , epilepsy andmacrocephaly in ASD patients according to logistic regression

A – probability of pathogenic CNV in ASD patients with facial dysmorphia , epilepsy and macrocephaly, B – probability of pathogenic CNV absence in ASD patients without facial dysmorphia , epilepsy and macrocephaly
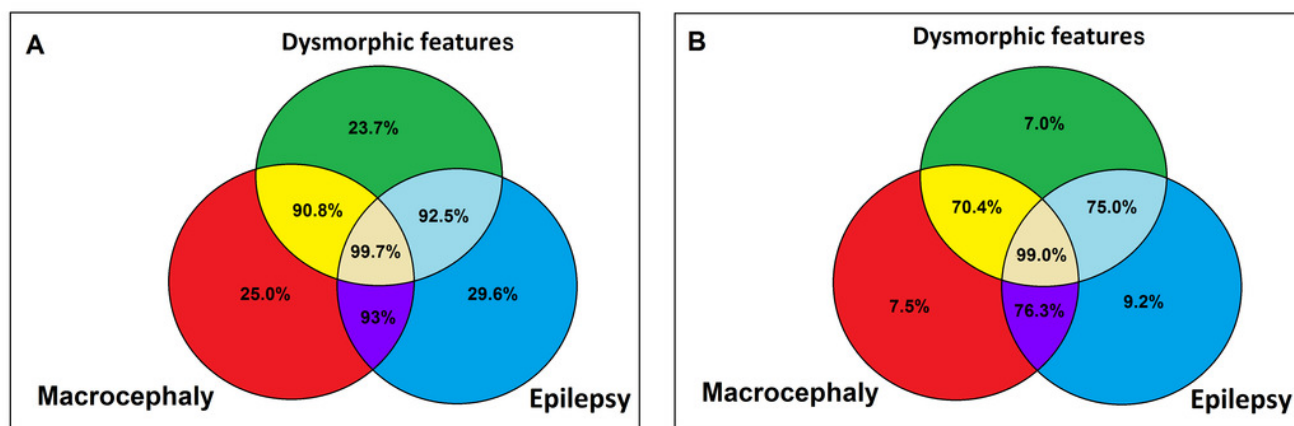
Figure 6

Formula for pathogenic CNVprobability calculation in ASD patients with epilepsy, macrocephaly anddysmorphism

β estimation of parameter from logistic regression

x presence/absence of each clinical feature (epilepsy, macrocephaly, dysmorphism)

$$P[Y(\boldsymbol{x}) = 1] = \frac{\exp(\boldsymbol{\beta}'\boldsymbol{x})}{1 + \exp(\boldsymbol{\beta}'\mathbf{x})}$$

$$P[Y(\boldsymbol{x}) = 1] = \frac{\exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3)}{1 + \exp(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3)}$$

Number of ID, ASD patients with each clinical features

*heart, urogenital and brain, ADHD – hyperactivity, ID - intellectual disabilities patients, ASD - autism spectrum disorders patients

Clinical features	Number of patients in ID group (N = 90)	Number of patients in ASD group (N = 114)	*p*-value
ADHD	17 (18.9%)	43 (37.7%)	*p* = 0.003
Epilepsy	14 (15.6%)	8 (7.0%)	ns
Microcephaly	18 (20.0%)	5 (4.4%)	*p* = 0.001
Macrocephaly	7 (7.8%)	4 (3.5%)	ns
Dysmorphic features	29 (32.0%)	14 (12.3%)	*p* = 0.001
Developmental defects*	18 (20.0%)	10 (8.8%)	*p* = 0.025
Growth defects	10 (11.1%)	6 (5.3%)	ns

Number ofpathogenic CNVs in ID and ASD patients with each clinical feature s

*heart, urogenital and brain , ADHD – hyperactivity, ID - intellectual disabilities patients, ASD - autism spectrum disorders patients, CNVs - copy number variants

Clinical features	Pathogenic CNVs in ID group N = 90	Pathogenic CNVs in ASD group N = 114	Pathogenic CNVs in both groups
ADHD (N = 60)	17.65% (3/17)	9.30% (4/43)	11.67% (7/60)
Epilepsy (N = 22)	21.43% (3/14)	37.50% (3/8)	27.27% (6/22)
Microcephaly (N = 23)	38.89% (7/18)	0.00% (0/5)	30.43% (7/23)
Macrocephaly (N = 11)	0.00% (0/7)	0.25% (1/4)	9.09% (1/11)
Dysmorphic features (N = 43)	37.93% (11/29)	28.57% (4/14)	34.88% (15/43)
Developmental defects* (N = 28)	11.11% (2/18)	0.00% (0/10)	7.14% (2/28)
Growth defects (N = 16)	30.00% (3/10)	0.00% (0/6)	18.75% (3/16)

Achieved significance of particular comorbidities topathogenic CNV finding in ASD and ID group by Fisher's exact test

*heart, urogenital and brain , ADHD – hyperactivity, ID - intellectual disabilities patients, ASD - autism spectrum disorders patients

Clinical features	*p*-value in ID group	*p*-value in ASD group
ADHD	ns	ns
Epilepsy	ns	0.01
Microcephaly	0.01	ns
Macrocephaly	ns	ns
Dysmorphic features	0.05	0.01
Developmental defects*	ns	ns
Growth defects	ns	ns