

1 **Recurrent rearrangements in synaptic and neurodevelopmental genes**
2 **support the existence of shared biological pathways**
3 **between Schizophrenia, Autism and Mental Retardation**
4
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44 **Abstract**

45

46 **Context**

47 Comparative genomic hybridization (array-CGH) studies have suggested that rare copy
48 number variations (CNVs) at numerous loci are involved in the etiology of mental retardation
49 (MR), autism spectrum disorders (ASD) and schizophrenia.

50

51 **Objective**

52 The goal of the present paper was (i) to provide an estimate of the collective frequency of a
53 set of recurrent/overlapping CNVs in three different groups of patients as compared with
54 healthy controls and (ii) to assess whether each CNV is present in more than one clinical
55 category.

56

57 **Design, setting and population**

58 We have investigated 28 candidate loci previously identified by array-CGH studies for gene
59 dosage alteration in 247 subjects with MR, 260 with ASD, 236 with schizophrenia or
60 schizoaffective disorder and 236 healthy controls.

61

62 **Main outcome measures**

63 Collective and individual frequency of the analyzed CNVs in patients as compared with
64 controls.

65

66 **Results**

67 Recurrent or overlapping CNVs were found in patients at 40% of the selected loci. We show
68 that the collective frequency of CNVs at these loci is significantly increased in autistic

69 patients, patients with schizophrenia and patients with MR as compared with controls ($p=$
70 0.005 , $p < 0.001$ and $p = 0.001$ respectively, Fisher exact test). Individual significance ($p=$
71 0.02) was reached for association between autism and a 350 kb deletion located in 22q11 and
72 spanning the *PRODH* gene.

73

74 **Conclusions**

75 These results support the hypothesis that weakly to moderately recurrent CNVs, either
76 transmitted or occurring *de novo*, are causing or contributory factors for these diseases.

77 Second, we show that most of these CNVs, which contain genes involved in
78 neurotransmission or synapse formation and maintenance, are present in the 3 pathological
79 conditions, supporting the existence of shared biological pathways between these
80 neurodevelopmental disorders.

81

82 The development of microarray based technologies for comparative genomic hybridization
83 (array-CGH) analysis has enabled the detection of submicroscopic microdeletions or
84 microduplications also referred as copy number variations (CNVs). In the past months, this
85 approach has been widely used in neurological and psychiatric disorders including **mental**
86 **retardation (MR)¹⁻³, autism spectrum disorders (ASD)⁴⁻⁷ and schizophrenia⁸⁻¹¹**. These
87 studies have suggested that several genes involved in similar neurodevelopmental pathways
88 may be associated with these conditions. However, so far only rare structural variants,
89 sometimes present in a single patient have been identified. It is therefore difficult to decipher
90 which of these variations are causative, which are risk factors and which are only rare
91 polymorphisms unrelated to any pathological phenotype. It is usually considered that *de novo*
92 rearrangements are likely to be pathogenic, but this argument which is acceptable for rare
93 large rearrangements detectable by conventional cytogenetics, should be considered with
94 caution for smaller CNVs, for which a very high mutation rate is expected. Indeed, it has been
95 estimated that one *de novo* segmental deletion occurs in one per eight newborns and one
96 segmental duplication in one per 50 newborns, most of these rearrangements being benign
97 polymorphic variants¹². Therefore, the disease association of CNVs has to be tested
98 systematically by comparing the frequency of each candidate CNV in patients and controls.
99 Given the very low frequency of each CNV, this would require the study of huge series, only
100 achievable in the context of forthcoming meta-analyses. Another problem arises from the fact
101 that the ascertainment of most of the published samples, initially recruited for linkage studies,
102 is biased toward multiplex cases and that control samples, when present, are generally
103 composed of subjects not screened for the studied pathologies. The goal of the present paper
104 was (i) to provide an estimate of the collective frequency of a set of recurrent/overlapping
105 CNVs in three different groups of patients as compared with healthy controls (ii) to assess
106 whether each CNV is present in more than one clinical category.

107 **Material and Methods**

108

109 **Ascertainment and diagnoses**

110 **Patients with schizophrenia and subjects with mental retardation were ascertained at**
111 **Rouen Hospital from consecutive hospitalizations in patients with schizophrenia, or**
112 **from consecutive referral for phenotypic and genetic investigations in subjects with**
113 **intellectual disability. The ASD sample included patients ascertained from consecutive**
114 **consultations in four units specialized in autism diagnosis and evaluation** located at
115 Rouen, Dijon, Tours (France) and Messina (Italy) and patients directly referred by the French
116 Autism Foundation. Controls, all ascertained at Rouen, were screened with a standardized
117 data sheet derived from the SADS-LA¹³ and were required to be free of any psychotic
118 disorder or MR in themselves or in their first degree relatives. All psychiatric diagnoses were
119 established according to DSM4 criteria following review of case notes and direct examination
120 of subjects. **The schedule for affective disorder and schizophrenia (SADS-LA)¹³ was**
121 **used for the clinical assessment of all patients with schizophrenia or schizoaffective**
122 **disorder, the Autism Diagnostic Interview-Revised (ADI-R)¹⁴, the Autism Diagnostic**
123 **Observation Schedule-general (ADOS-G)¹⁵ or the Childhood Autism Rating Scale**
124 **(CARS)¹⁶ were used for 83% of ASD patients (100% of ASD patients with CNVs). IQs**
125 **were evaluated using standardized neuropsychological tests i.e. validated mental age-**
126 **appropriate Weschler scales (WPPSI, WISC or WAIS).**

127 The schizophrenia group included 189 subjects with a diagnosis of schizophrenia and 47 with
128 a diagnosis of schizoaffective disorder. **Post morbid IQs were available for 2/3 of patients**
129 **with schizophrenia; 18% of these subjects had an IQ < 70. The ASD group included 257**
130 **patients with autism and 3 with Asperger syndrome. The MR group included 12**
131 **patients with developmental language disorder.** All MR patients and 2/3 of ASD patients

132 were examined by an experienced clinical geneticist and were **also** screened for fragile X
133 mutation and karyotype abnormalities. Subjects with large chromosomal anomalies, fragile X
134 syndrome or other established syndromes were not included. Subjects with common
135 environmental etiologies of MR such as fetal alcohol syndrome or birth complications were
136 also excluded. Additional clinical features including intrauterine or postnatal growth
137 retardation, dysmorphic features or malformations were present in 8.5 % of ASD patients and
138 62 % of subjects with MR. Demographic characteristics of the sample, including a total of
139 979 Caucasian subjects from France or Italy, are summarized in Table 1.
140 Blood samples were drawn, after written informed consent, from all included subjects and
141 whenever possible from parents and affected relatives of patients. Ethics committee approval
142 was obtained from all regions where families were recruited.

143

144 **Candidate genes and QMPSF analysis**

145 A medline search with the words CNV, schizophrenia, autism and mental retardation allowed
146 us to select non exhaustively a set of 28 loci with microrearrangements characterized by prior
147 array-CGH analyses, often in a single patient. **This set included major candidate CNV loci**
148 **identified in ASD and SZ before April 2008 as well as 8 functionally related CNV loci**
149 **identified in MR (Table 2).** Each locus contained generally a single disease-associated
150 CNV, but in some cases overlapping CNVs with different boundaries had been described in
151 patients. The gene content of these loci ranged from 1 to 28. At each locus at least one
152 candidate gene had been previously suggested in the seminal reports and was retained for the
153 present analysis. Functionally, most of these candidate genes can be classified in two main
154 categories related to synapse formation and maintenance or neurotransmission.
155 Copy number variation at each locus was assessed by quantitative multiplex PCR of short
156 fluorescent fragments (QMPSF), a method based on the simultaneous amplification of several

157 short genomic fragments under quantitative conditions ¹⁷. **For each locus, amplicons were**
158 **designed in the coding sequence of selected candidate genes. All assays were grouped in**
159 **three multiplex PCR experiments including 10 short genomic fragments (range 101-**
160 **301bp) each. Primer sequences and PCR conditions are summarized in supplementary**
161 **Table S1. DNA fragments generated by QMPSF were separated on an ABI Prism 3100**
162 **sequencer (Applied Biosystem) and the resulting fluorescence profiles were analyzed**
163 **using the Gene Scan 3.7 Software (PE Applied Biosystems). For each patient, the**
164 **QMPSF profile was superimposed to that generated from a reference subject by**
165 **adjusting to the same level the peak obtained for a control amplicon corresponding to a**
166 **short exonic fragment of the PBGD gene.** When a copy number variation was detected,
167 further analyses aiming to confirm and delineate the size of the rearrangements were
168 performed using additional **dedicated QMPSF assays (supplementary figure S1)**, array-
169 CGH and/ or fluorescence in situ hybridization (FISH) analyses.

170

171 **Agilent 44K array**

172 Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH
173 microarray 4x44K (Agilent Technologies, Santa Clara, CA, USA). This array contains 44 290
174 60-mer oligonucleotide probes covering the whole genome with an average spatial resolution
175 of \approx 30-35 kb. 84% of the probes reside in intragenic regions, and over 30 000 genes are each
176 represented by at least one probe. All experiments were performed according to version 4.0
177 (June 2006) of the protocol provided by Agilent (“Agilent Oligonucleotide Array-Based CGH
178 for Genomic DNA Analysis”).

179

180

181

182 **Fluorescence in situ hybridization (FISH)**

183 FISH analyses were performed on metaphase spreads obtained from peripheral lymphocytes
184 from the patients. Selected Human genomic BAC clones were obtained from the BACPAC
185 resources center (<http://bacpac.chori.org>).

186

187 **DNA sequencing and paternity checking**

188 Sequence analysis of the coding exons of the *PRODH* gene was performed using primers and
189 PCR conditions previously described¹⁸ on an Applied Biosystem model 3100 automated
190 sequencer (PE Applied Biosystem). Paternity was checked by microsatellite typing.

191

192 **Determination of plasma proline level**

193 **In patients, plasma proline levels were determined after overnight fasting. All samples**
194 **were analyzed using ion exchange chromatography on a BIOTRONIK LC 3000 system.**

195

196 **Statistical analysis**

197 **Categorical variables were compared by Fisher's exact test. Two hypotheses were tested.**
198 **First, the distribution of the collective set of recurrent/overlapping CNVs found in each**
199 **group of patients was compared with that found in controls (3 tests). Second, the**
200 **distribution of each recurrent/overlapping CNV present in our population was**
201 **compared between each disease group and controls (33 tests). P-values were reported**
202 **without Bonferroni's correction.**

203

204

205

206

207 **Results**

208 **CNVs in the control group**

209 Among the 28 loci selected for this study (Table 2), only one CNV identical to a previously
210 described disease-associated one (i.e. a 22q11 350kb deletion spanning the *PRODH* and
211 *DGCR6* genes)¹⁹ was detected in the control group. This deletion, present in a single control
212 subject, had a low frequency (1/236) similar to that previously reported in Japanese²⁰ and
213 Canadian²¹ populations. A common CNV reciprocal to the expected one (i.e. a 350 kb
214 duplication) was also found at the same locus in 6/236 controls. This CNV, also present in
215 7/236 patients with schizophrenia, 4/260 ASD patients and 9/247 MR patients was clearly a
216 benign polymorphism. Another overlapping reciprocal CNV (i.e. a 490 kb duplication) was
217 detected at the *CHRNA7* locus located on chromosome 15q13.3. This CNV present in 2/236
218 controls, 1/236 patients with schizophrenia, 1/260 ASD patients and 1/247 MR was equally
219 unrelated to any pathological condition. **(Supplementary Table S2)**

220 **Diseases associated CNVs**

221 The proportion of recurrent/overlapping CNVs identified among the 28 selected loci in the
222 present sample totaling 743 affected subjects was 11/28 (40 %). Their collective frequency
223 was 10/236 (4 %) in patients with schizophrenia, 16/260 (6 %) in ASD patients and 13/247
224 (5.3 %) in MR patients vs 1/236 (0.4 %) in controls, therefore demonstrating a significant
225 excess of these CNVs in each disease group as compared with controls ($p=0.005$, $p<0.001$
226 and $p=0.001$ respectively, Fisher exact test) (Table 3a). Individual significance for
227 association with ASD was reached for the *PRODH/DGCR6* deletion (9/260 in ASD patients
228 vs 1/236 in controls, $p=0.02$). **None of the cases had more than one of these 28 CNVs.**

229 Among the four most prevalent CNVs, three, located in 22q11, 16p11 and 15q13, were
230 flanked by known regions of segmental duplication and resulted most likely from a non allelic
231 homologous recombination mechanism (NAHR). At the 2p16 locus, the *NRXNI* gene was

232 recurrently disrupted by a set of partially overlapping deletions spanning either the promoter
233 and first exons of neurexin1 alpha or the exons coding for the middle section of this protein as
234 well as for the proximal region of neurexin1 beta. These rearrangements occurred in a region
235 devoid of any segmental duplication and resulted from another mechanism distinct from
236 NAHR.

237 **Transmission and cosegregation in multiplex sibships**

238 **Among 27 families in which transmission was tested (69% of the total sample), it was**
239 **found that only eight CNVs (located in 8p23, 15q11-q13, 15q13, 16p11 and 22q13) had**
240 **occurred *de novo* (Table 3a). Paternal age was not significantly different between**
241 **families with *de novo* and inherited CNVs (27.2 ± 4.7 y vs 30.7 ± 4.6 y, NS, Mann**
242 **Whitney U test).** In most families, CNVs were transmitted from an apparently unaffected
243 (although not clinically nor neuropsychologically assessed) parent. This includes a partial
244 duplication of the X linked *GRIA3* gene, present in a young male autistic patient, which was
245 inherited from the unaffected mother. The 22q11 350 kb deletion spanning the
246 *PRODH/DGCR6* locus was also transmitted in **11/11** tested cases. *PRODH* encodes for the
247 proline dehydrogenase and *PRODH* deficiency is responsible for type 1 hyperprolinemia, a
248 condition often associated with both cognitive impairment and psychotic symptoms¹⁸.
249 However, hemizygous deletion of the *PRODH* gene is not sufficient *per se* to result in
250 hyperprolinemia since only 35-50 % of Velo-cardio-facial syndrome (VCFS) patients, all
251 bearing a single copy of *PRODH*, exhibit hyperprolinemia^{18,22}. Indeed, a reduction of more
252 than 50% of the enzymatic activity is generally required to produce hyperprolinemia¹⁸. The
253 presence of a mutation affecting enzyme activity²³ on the second allele is therefore necessary.
254 To examine this issue, the remaining *PRODH* allele was sequenced in all affected subjects
255 bearing the 350 kb deletion, and, when possible, plasma proline level was assessed. As shown
256 in Table 4, 14/15 patients harbored a genotype predicted to result in a reduction of at least

257 70% of enzymatic activity. **Among the 10 subjects in whom plasma proline level was**
258 **assessed, 7 had mild to severe hyperprolinemia and 3 had plasma proline level at the**
259 **upper boundary of normal values.**

260 **Cosegregation of the CNV with pathological conditions was examined in 4 multiplex**
261 **families in which DNA from affected siblings was available. In family 144, the two sibs**
262 **with schizophrenia both harbored the *PRODH/DGCR6* deletion. In family 11695, the**
263 **two MR sibs both harbored the 2p16.3 deletion. In family 14390, the 16p11 deletion was**
264 **present in the proband with developmental language disorder but not in his mentally**
265 **retarded sib.** In family 33, the 16p11 duplication was present in two sibs with schizophrenia
266 as well as in a non affected sibling, but not in a third sibling with schizophrenia.

267 **Disease specificity**

268 Combining the results of the present study and those of previous reports, we found that, with
269 **the possible exception of the maternally derived 15q13 duplication associated with ASD,**
270 none of the observed rearrangement was disease-specific. The 22q11¹⁹ and 2p16¹ deletions
271 were found in the three conditions, **whereas the 22q13 deletion found in two ASD patients**
272 **had already been described in ASD and MR^{4,24-26}.** The 16p11 and 8p23 rearrangements
273 previously described in ASD^{4,5}, were both found in SZ and also in MR patients for the 16p11
274 rearrangements. The Xp11.4 duplication spanning the *TSPAN7* gene, previously described in
275 MR and ASD^{4,27}, was found in a patient with SZ, as well as the 17q21 duplication
276 previously described in a patient with MR²⁸. Two different sized 15q13 duplications
277 encompassing *APBA2* were found in one ASD and one SZ patient. A partial duplication of the
278 *GRIA3* gene, encompassing the promoter region and the exons coding for the proximal region
279 of *GRIA3* was detected in a single autistic patient. Although slightly different by its size and
280 by the number of duplicated exons, this partial duplication is reminiscent from that recently
281 reported in a patient with MR²⁹. At the 8p23 locus, both gain and loss of material were found,

282 as well as at the 16p11 and 17q21 loci, as recently described^{28,30,31}. **This suggests that**
283 **dosage-sensitive genes, whose expression is finely tuned, are located within these**
284 **rearranged segments.**

285 **Comorbidity**

286 **From a phenotypic point of view, it is noteworthy that 3 out of 9 patients with**
287 **schizophrenia bearing a candidate CNV had mild MR in an IQ assessment obtained**
288 **after the onset of their psychotic symptoms (Table 3b). Although postmorbid IQ is likely**
289 **to constitute an underestimate of the premorbid level of cognitive functioning in patients**
290 **with schizophrenia, cognitive deficits testified by severe learning disorder were already**
291 **noted in these 3 patients during childhood before the onset of their psychotic symptoms,**
292 **thus supporting the diagnosis of comorbidity between MR and SZ. With the exception of**
293 **two subjects who had normal cognitive functioning (high-functioning autism), all tested**
294 **CNV bearing autistic patients had IQs falling in the range of mental retardation, albeit**
295 **one of them had only mild cognitive dysfunction (Table 3b).**

296

297 **Comments**

298 After a first wave of CNV discovery by array-CGH analyses in neuropsychiatric disorders,
299 this study for the first time examines the involvement of a limited number of candidate loci in
300 large samples of patients with different clinical diagnoses. Two strengths of our study design
301 are the inclusion of controls carefully screened for the studied pathologies and of series of
302 patients mostly ascertained through consecutive admissions or consultations and therefore
303 including subjects belonging either to simplex or multiplex families. Given the expected rarity
304 of each variant, our first goal was not to test the association of every individual CNV with SZ,
305 ASD or MR but to determine whether these variants were collectively more frequent in
306 patients with these diseases than among healthy controls. This aim was successfully achieved

307 and, in addition, we were able to obtain suggestive statistical significance for the association
308 between the 22q11 350 kb deletion and ASD. This deleted segment, located within the
309 chromosomal region deleted in VCFS, a contiguous gene syndrome known to be associated
310 with a high frequency of MR, ASD and psychosis, contained two genes *PRODH* and *DGCR6*.
311 Although we cannot exclude an involvement of *DGCR6* in the neuropsychiatric phenotype of
312 the subjects bearing this CNV, previous work from our group strongly suggests that *PRODH*
313 is the prime candidate. We had previously shown that hyperprolinemia, resulting from the
314 partial or total inactivation of this enzyme (i) may lead to MR and autism in patients with type
315 1 hyperprolinemia¹⁸ (ii) is a risk factor for schizoaffective disorder³² (iii) is inversely
316 correlated with IQ in the VCFS¹⁸. Here, we show that all but one patients harboring this
317 deletion were in fact compound heterozygotes, also bearing mutations affecting enzymatic
318 activity on the second allele. It results in a loss of at least 70% of the predicted *PRODH*
319 residual activity in 14/15 subjects and in hyperprolinemia in **7/10 assessed subjects**.
320 Second, we show that both *de novo* CNVs and CNVs inherited from an apparently healthy
321 parent can be found in patients. For transmitted CNVs, the mode of inheritance of the disease
322 was, in some cases recessive (e.g. hyperprolinemia related to the 22q11 deletion) or implied
323 the transmission of an X linked gene by a woman to her son (*GRIA3*). **Consistent with**
324 **previous reports^{4,8,33}, the 16p11 rearrangements were inherited from an apparently**
325 **non affected parent in three families. These CNVs, whose estimated frequency in**
326 **Icelandic population was 5/18,834 (0.03%) for the duplication and 2/18,834 (0.01%) for**
327 **the deletion³¹, should therefore be considered as risk factors rather than fully causative**
328 **variations. The presence of affected siblings that do not share the CNV, already noted in**
329 **a previous report³³, does not necessarily rule out the causative implication of these**
330 **CNVs, but raises the question of intra-familial genetic heterogeneity. This hypothesis,**
331 **which is not un- plausible for these frequent disorders often characterized by assortative**

332 **mating, remains speculative since the parents and their relatives were not**
333 **psychiatrically nor cognitively assessed in these families.**

334 Third and more importantly, our study confirms and extends the conclusions of several recent
335 reports suggesting that a large number of candidate CNVs are not disease-specific but are
336 involved in the expression of different behavioral phenotypes including MR, ASD and SZ.
337 This implies the existence of shared biological pathways between these three
338 neurodevelopmental conditions. These pathways affect chiefly synapse formation and
339 maintenance as well as neurotransmission with a special emphasis on glutamate and GABA.
340 The dysfunction of specific neuronal networks underlying the peculiar symptomatology of
341 each clinical condition most likely depends on additional genetics, epigenetics and
342 environmental factors which remain to be characterized. From a clinical point of view, it
343 should be stressed that despite the diversity of categorical diagnoses, many subjects harboring
344 these CNVs shared some clinical features: **1/3 of patients with schizophrenia and 80% of**
345 **autistic patients with CNVs** had a level of cognitive functioning which falls in the range of
346 mental retardation. This is in accordance with previous reports showing that that point
347 prevalence of schizophrenia is increased by a factor of 3 in subjects with intellectual
348 disabilities³⁴ and that 3/4 of autistic patients have mental retardation³⁵. **However, it should**
349 **be remembered that (i) because attention and communication are markedly impaired in**
350 **children with autism, assessment of their IQs (even performance IQs in non verbal**
351 **subjects) are notoriously unreliable and (ii) these results were not obtained in a single**
352 **community based population, but in three disease groups ascertained according to**
353 **different schemes, a factor whose exact impact is difficult to appreciate but which is**
354 **likely to have implications related to the phenotypic severity of these subject.**
355 Finally, we would like to point out the utility of targeted methods for CNV analysis, such as
356 the QMPSF method, as a cost effective alternative to a-CGH for the screening of candidate

357 loci in large case-control cohorts. We plan to conduct extensive resequencing of these
358 candidate genes in order to further validate their role in these conditions.

359 **Note added in proof**

360 **Since the submission of this manuscript additional reports documenting shared CNVs**
361 **between MR, ASD and /or schizophrenia have been published**^{9,36,37}.

362

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364

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570 **Table legends**

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572 **Table 1:** Demographic and clinical features of the sample

573 ASD: autism spectrum disorder

574 SZ: schizophrenia and schizoaffective disorder

575 MR: mental retardation

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577 **Table 2:** Candidate regions and genes selected for QMPSF analysis

578 CR: Chromosomal rearrangement

579 M: mutation

580 TS: Tourette syndrome

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582 **Table 3:** Recurrent CNVs and clinical features in ASD, SZ and MR patients

583 a: diseases associated CNVs, b: clinical features of patients with CNVs

584 M: male; F: female

585 HFA: High-functioning autism

586 SZ: schizophrenia

587 SZ AFF: schizoaffective disorder

588 MR: mental retardation

589 Mod: moderate

590 DLD: developmental learning disorder

591 Del: deletion; dup: duplication

592 HRK: High resolution karyotype

593 D: dysmorphism; E: epilepsy; M: microcephaly; IUGR: intrauterine growth retardation

594 NA: not assessed

595 * deletion also present in a sibling with schizophrenia

596 ** duplication present in a schizophrenic and a healthy sibling, and not present in a

597 schizoaffective sibling

598 *** deletion not present in a sibling with mental retardation

599 **** **deletion present in the proband and an affected sibling**

600 ¶ maternally derived

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602 **Table 4:** Genotype and plasma proline level of the *PRODH* deletion bearing patients

603 **Predicted PRODH residual activity was evaluated for each genotype according to the**

604 **functional data published by Bender et al.** ²³. Mutations with severe effect on PRODH

605 activity appear in bold, mutations with moderate effect in normal character and mutations

606 with unknown effect in italic. Abnormal plasma proline values appear in bold. **Fasting**

607 **abnormal plasma proline values: age >18 years: males > 377 $\mu\text{mol/L}$, females > 316**

608 **$\mu\text{mol/L}$; 5 years>age <18 years: > 270 $\mu\text{mol/L}$; age < 5 years: > 235 $\mu\text{mol/L}$.**

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610 ND: not determined. WT: wild type. HFA: High-functioning autism

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616 Table 1

	age (y)	% Male	% Familial	% syndromic	Geographic ancestry
ASD (n= 260)	11.8 ± 7.6	80	9.6	8.5	France and Italy (n=29)
MR (n= 247)	13.2 ± 11.6	64.8	34	62	North western France
SZ (n= 236)	38 ± 11	66.8	23.7	/	North western France
Controls (n= 236)	39.5 ± 16.8	43.8	/	/	North western France

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633 Table 2

Chromosomal

location	Type	Candidate Genes	Pathways	Function	Evidence	Disorder	Selected references
2p16.3	loss	NRXN1	synapse formation and maintenance	cell adhesion molecule	CR	ASD, SZ, MR	1, 4, 7, 8, 10, 38, 39
2q12.3-q14.2	loss/gain	DPP10	neurotransmission	dipeptidyl peptidase	CR	ASD	4
2q33.3-q34	loss	ERBB4	synapse formation and maintenance	NRG1 receptor	CR, M	SZ	8
3p26-p25	loss/gain	CNTN4	synapse formation and maintenance	Axon-associated cell adhesion molecule	CR, M	ASD, MR	40, 41
3p26.1-p25.2	loss	GRM7	neurotransmission	glutamate receptor	CR	SZ	8
3q26.31	gain	NLGN1	synapse formation and maintenance	cell adhesion molecule	CR	MR	42
		GABRG1, GABRA4,					
4p14-q21.1	gain	GABRA2	neurotransmission	GABA receptor subunits	CR	ASD	4, 43
5p13	loss	SLC1A3	neurotransmission	excitatory amino acid transporter	CR, M	SZ	8
7q31.1	loss	ST7	other	tumor suppressor	CR, M	ASD	4
7q35-q36	loss	CNTNAP2	synapse formation and maintenance	contactin associated protein	CR, M	ASD, SZ, MR, TS	44
8p22-p11	loss	NRG1	synapse formation and maintenance	signaling protein	CR	MR	45
8p23	gain	DLGAP2	neurotransmission	associated to NMDA receptor and K ⁺ channels	CR	ASD	4
8q24-qter	gain	PTK2	neuronal migration and growth	focal adhesion kinase	CR	SZ	8
11q21	loss	DLG2	synapse formation and maintenance	synaptic scaffolding protein	CR	SZ	8
12q14.3	loss	GRIP1	neurotransmission	glutamate receptor interacting protein	CR	MR	1, 3
		GABRA5, GABRB3,					
15q11-q14	gain	GABRG3	neurotransmission	GABA receptor subunits	CR	ASD, MR	46, 47
15q13	gain	APBA2	neurotransmission	synaptic exocytosis	CR	ASD, SZ	6, 10
15q13.3	loss	CHRNA7	neurotransmission	acetylcholine receptor	CR	MR	48
16p11.2	loss/gain	DOC2A	neurotransmission	neurotransmitter release regulation	CR	ASD, SZ	4, 6, 8, 31, 33

17q21	loss/gain	MAPT	other	microtubule associated protein	CR	MR	28, 30
22q11	loss	PRODH	neuromodulation	proline dehydrogenase	CR, M	ASD, SZ, MR	19
22q13	loss	SHANK3	synapse formation and maintenance	synaptic scaffolding protein	CR, M	ASD, MR	4, 24-26
Xp22.3	loss/gain	NLGN4	synapse formation and maintenance	cell adhesion molecule	CR, M	ASD, TS, MR	4, 49-52
Xp11.4	gain	TSPAN7	neuronal migration and growth	transmembrane component	CR, M	ASD, MR	4, 27
Xp11.4	loss	CASK	synapse formation and maintenance	synaptic scaffolding protein	CR	MR	2
Xp22.1-p21.3	loss	IL1RAPL1	other	interleukin receptor	CR, M	ASD, MR	4, 53
Xq25	gain	GRIA3	neurotransmission	Kainate receptor subunit	CR	MR	29
Xq28	gain	MECP2	other	methylated CpG binding protein	CR, M	MR	42, 54

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644 Table 3a

Patients		CNV									
ID	diagnosis	chromosomal location	Size	Last outer margin (bp)	First inner margin (bp)	Last inner margin (bp)	First outer margin (bp)	genes involved	type	Confirmation	transmission
113	SZ	Xp11.4	56 kb	38,326,267	38,376,483	38,432,836	38,549,143	TSPAN7	dup	QMPSF, a-CGH	unknown
313	SZ	8p23	110 kb	1,436,299	1,481,035	1,590,831	1,642,837	DLGAP2	dup	QMPSF, a-CGH	unknown
185	SZ	15q13	994 kb	26,999,744	27,000,694	27,994,706	28,109,371	APBA2, TJP1, NDNL2	dup	QMPSF, a-CGH	unknown
144.1	SZ AFF	22q11	350 kb					PRODH, DGCR6	del	QMPSF	unknown *
223	SZ	2p16.3	107 kb	50,704,195	51,006,556	51,114,057	51,433,167	NRXN1alpha exons 1,2	del	QMPSF, a-CGH	unknown
220	SZ	2p16.3	<532 kb	50,172,024	50,420,164	50,420,164	50,704,195	NRXN1alpha/beta	del	QMPSF, a-CGH	unknown
33	SZ	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	maternally inherited**
136	SZ	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	de novo
146	SZ AFF	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF	unknown
151	SZ	17q21	627 kb	40,869,151	41,073,486	41,700,762	42,143,048	MAPT and 2 genes	dup	QMPSF, a-CGH	unknown
T 35	autism	2p16.3	<427 kb	51,006,556	51,114,057	51,114,057	51,433,167	NRXN1alpha exons 1,2	del	QMPSF, a-CGH, FISH	paternally inherited
45431	autism	2p16.3	107 kb	50,704,195	51,006,556	51,114,057	51,433,167	NRXN1 alpha exons 1,2	del	QMPSF, a-CGH	maternally inherited
47604	autism	22q13	2,26 Mb	47,124,905	47,265,476	49,525,071	-----	SHANK3 and 28 genes	del	QMPSF, a-CGH	de novo
Si22	autism	22q13	ND					SHANK3	del	QMPSF, MLPA	de novo
60478	autism	15q11-q13	4 Mb					GABRA5, GABRB3, GABRG3 and 17 genes	dup	QMPSF, FISH	de novo ¶
T 34	autism	Xq25	1,42 Mb	120,669,107	120,746,477	122,166,142	122,316,448	GRIA3 exons 1-4	dup	QMPSF, a-CGH, FISH	maternally inherited

12746	HFA	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
12452	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
13899	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	unknown
44737	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
45435	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	unknown
45856	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
46261	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
47766	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
Si30	autism	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
44813	HFA	15q13	3,8 Mb	26,198,996	26,999,744	30,796,716	30,812,300	APBA2, CHRNA7 and 16 genes	dup	QMPSF, a-CGH	maternally inherited
12363	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
11780	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	paternally inherited
9680	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
14684	MR	22q11	350 kb					PRODH, DGCR6	del	QMPSF	maternally inherited
11695	MR	2p16.3	<427 kb	51,006,556	51,114,057	51,114,057	51,433,167	NRXN1alpha exon 1	del	QMPSF, a-CGH	maternally inherited ****
14921	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	de novo
10417	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	maternally inherited
13165	MR	16p11	500 kb					DOC2A and 24 genes	dup	QMPSF, FISH	maternally inherited
14390	MR	16p11	500 kb					DOC2A and 24 genes	del	QMPSF, FISH	de novo ***
13907	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMPSF, a-CGH	unknown
11919	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMPSF, a-CGH	unknown

9930	MR	15q13	1,57 Mb	28,109,371	28,725,507	30,298,096	30,701,373	CHRNA7 and 5 genes	del	QMP5F, a-CGH	de novo
12988	MR	8p23	3 Mb					DLGAP2 and 23 genes	del	QMP5F, HRK	de novo

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653 Table 3b

Patients						Clinical features	
ID	diagnosis	sex	age (y)	family	CNV	Cognitive features	Associated features
				history			
113	SZ	M	52	sporadic	dup(Xp11.4)	IQ, 105	
313	SZ	M	32	familial	dup(8p23)	IQ, 67	D
185	SZ	M	48	sporadic	dup(15q13)	IQ, 73	
144.1	SZ AFF	F	42	familial	del(22q11)	IQ, 48	
223	SZ	M	38	sporadic	del(2p16.3)	IQ, 94	
220	SZ	M	25	unknown	del(2p16.3)	NA	
33	SZ	F	53	familial	dup(16p11)	IQ, 56	
136	SZ	F	41	sporadic	dup(16p11)	IQ, 75	
146	SZ AFF	M	32	familial	dup(16p11)	IQ, 95	
151	SZ	M	53	sporadic	dup(17q21)	IQ, 90	
T 35	autism	F	38	sporadic	del(2p16.3)	IQ, 66	
45431	autism	M	10	sporadic	del(2p16.3)	NA	
47604	autism	M	8	sporadic	del(22q13)	IQ<40	
Si22	autism	M	9	sporadic	del(22q13)	IQ<40	D, E
60478	autism	M	15	sporadic	dup(15q11-q13)	IQ<40	E
T 34	autism	M	10	sporadic	dup(Xq25)	NA	
12746	HFA	M	6	sporadic	del(22q11)	IQ, 74	
12452	autism	F	8	sporadic	del(22q11)	IQ<40	
13899	autism	M	11	sporadic	del(22q11)	IQ<40	
44737	autism	M	7	sporadic	del(22q11)	IQ<40	
45435	autism	F	31	sporadic	del(22q11)	IQ<40	
45856	autism	M	38	sporadic	del(22q11)	IQ<40	
46261	autism	F	11	sporadic	del(22q11)	NA	
47766	autism	M	8	sporadic	del(22q11)	NA	
Si30	autism	F	5	sporadic	del(22q11)	IQ<40	D, E
44813	HFA	M	8	familial	dup(15q13)	NA	
12363	MR	M	11	sporadic	del(22q11)	Mild MR	D
11780	MR	M	3	sporadic	del(22q11)	Mod MR	D
9680	MR	F	11	familial	del(22q11)	Mod MR	D
14684	MR	M	6	familial	del(22q11)	DLD	
11695	MR	F	10	familial	del(2p16.3)	Mod MR	

14921	MR	M	4	familial	del(16p11)	Mild MR	D
10417	MR	M	14	familial	del(16p11)	Mild MR	IUGR, D
13165	MR	F	4	sporadic	dup(16p11)	Mild MR	D
14390	MR	F	9	familial	del(16p11)	DLD	
13907	MR	F	9	sporadic	del(15q13)	Mild MR	M, D
11919	MR	F	7	familial	del(15q13)	Mild MR	
9930	MR	M	12	familial	del(15q13)	Mild MR	D
12988	MR	M	8	sporadic	del(8p23)	Mild MR	M, D, E

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674 Table 4

ID	diagnosis	sex	age	plasma	genotype	predicted PRODH
				proline ($\mu\text{mol/L}$)		residual activity
144.1	SZAFF	F	42	538	DEL/ R453C+ R185W	2%
144.2	SZ	F	39	338	DEL/ Q19P	30%
12 452	Autism	M	6	287	DEL/ R185W	25%
12 746	HFA	F	8	214	DEL/ Q19P+ A58T	$\leq 30\%$
13 899	Autism	M	11	312	DEL/ Q19P	$\leq 25\%$
44 737	Autism	M	7	ND	DEL/ T275N+ V427M	$\leq 20\%$
45 435	Autism	F	31	ND	DEL/ R185W+ Q19P	$\leq 25\%$
45 856	Autism	M	38	ND	DEL/ Q19P+ P30S	$\leq 30\%$
46 261	Autism	F	11	243-283	DEL/ R185W+ Q19P	$\leq 25\%$
47 766	Autism	M	8	ND	DEL/ WT	50%
Si30	Autism	F	5	422-1883	DEL/ R453C+ Q19P+ A58T + V427M	$\leq 2\%$
12363	MR	M	11	512	DEL/ R185W	25%
11780	MR	M	3	ND	DEL/ R185W+ Q19P	$\leq 25\%$
9680	MR	F	11	299	DEL/ Q19P+ P30S	$\leq 30\%$
14684	MR	M	6	238	DEL/ R185W+ Q19P	$\leq 25\%$

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