



HAL
open science

Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions

Björn Menten, Sarah Vergult, Danijela Krgovic, Bart L. Loeys, Stanislas Lyonnet, Agne Liedén, Britt Marie Anderlid, Freddie H Sharkey, Shelagh Joss, Geert Mortier

► To cite this version:

Björn Menten, Sarah Vergult, Danijela Krgovic, Bart L. Loeys, Stanislas Lyonnet, et al.. Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions. *European Journal of Human Genetics*, 2011, 10.1038/ejhg.2011.67 . hal-00633985

HAL Id: hal-00633985

<https://hal.science/hal-00633985>

Submitted on 20 Oct 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions**

2 Sarah Vergult^{a,*}, Danijela Krgovic^{b,*}, Bart Loeys^{a,g}, Stanislas Lyonnet^c, Agne Liedén^d, Britt-Marie
3 Anderlid^d, Freddie Sharkey^e, Shelagh Joss^f, Geert Mortier^g & Björn Menten^{a,°}

4 * These authors contributed equally

5 ^aCenter for Medical Genetics, Ghent University Hospital, Ghent, Belgium

6 ^bLaboratory of Medical Genetics, University Medical Centre Maribor, Maribor, Slovenia

7 ^cINSERM, Paris, France

8 ^dDepartment of Clinical Genetics and Institution of Molecular Medicine and Surgery, Centre of
9 Molecular Medicine, Karolinska Universitetssjukhuset, Stockholm, Sweden

10 ^eSouth East Scotland Cytogenetics Laboratory, Western General Hospital, Edinburgh, Scotland,
11 UK.

12 ^fWest of Scotland Regional Genetics Service, Institute of Medical Genetics, Yorkhill Hospital,
13 Glasgow, UK.

14 ^gDepartement for Medical Genetics, Antwerp, Belgium

15 [°] Corresponding author:

16 Björn Menten

17 Center for Medical Genetics

18 Ghent University Hospital

19 De Pintelaan 185

20 9000 Ghent

21 Belgium

22 Telephone number: +32-9-3325284

23 Fax number: +32-9-3326549

24 E-mail adres: Bjorn.Menten@ugent.be

25

26 Running title: 12q15q21.1 microdeletions

27

28 **ABSTRACT**

29 The introduction of array CGH in clinical diagnostics has led to the discovery of many new
30 microdeletion/microduplication syndromes. Most of them are rare and often present with a
31 variable range of clinical anomalies. Here we report three patients with a *de novo* overlapping
32 microdeletion of chromosome bands 12q15q21.1. The deletions are ~2.5 Mb in size with a
33 1.34 Mb common deleted region containing six RefSeq genes. All three patients present with
34 learning disability or developmental delay, nasal speech and hypothyroidism. In this paper we
35 will further elaborate on the genotype-phenotype correlation associated with this deletion and
36 compare our patients with previously reported cases.

37

38 **KEYWORDS**

39 12q15 microdeletion, nasal speech, hypothyroidism, developmental delay

40 **INTRODUCTION**

41 Array CGH has led to the identification of several microdeletion syndromes by screening
42 individuals with shared phenotypical characteristics, e.g. 12q14 microdeletion syndrome.¹ By
43 combining array CGH data from large cohorts of patients, the screening of overlapping
44 microdeletions is made possible, which in turn can lead to the identification of shared
45 phenotypic characteristics. Here we present the clinical and molecular data of three previously
46 unreported patients with submicroscopic overlapping deletions distal to the 12q14
47 microdeletion syndrome at chromosome bands 12q15q21.1. The deletions presented in this
48 study vary from 2.5 Mb to 2.59 Mb in size with a 1.34 Mb common deleted region containing
49 six RefSeq genes. To our knowledge, only seven patients have been reported with deletions in
50 this region.²⁻⁸ The deletion breakpoints in the literature vary from 12q13 to 12q23 and are

51 associated with growth retardation, developmental delay and dysmorphic features. The
52 previously reported cases were detected with standard cytogenetic techniques, except for the
53 patient of Tocyap *et al.* (2006)⁷, where localization of microsatellite markers was investigated
54 and the patient from Schluth *et al.* (2008)² which was fine mapped using a 1 Mb resolution
55 array. Although a variable clinical phenotype is present in all patients, the three patients in this
56 paper all present with developmental delay or learning disability, nasal speech and
57 hypothyroidism. The introduction of array CGH in clinical diagnostics allows the identification
58 of smaller deletions in the genome and simultaneous delineation of the exact breakpoints. This
59 has made the detection of smaller overlapping deletions possible, leading to a better
60 understanding of the genotype-phenotype correlation in these patients.

61

62 **MATERIALS AND METHODS**

63 **Chromosome analysis**

64 Conventional karyotyping of G-banded metaphase chromosomes was performed on short-
65 term cultured lymphocytes using standard procedures. No visible structural anomalies were
66 detected in any of the three probands.

67 **Array CGH analysis**

68 Patient 1 & 2

69 Array CGH was performed on patient 1 & 2 using the Agilent SurePrint G3 Human CGH
70 Microarray Kit 4x 180K (Agilent Technologies, Santa Clara, CA, USA). The assay was performed
71 according to manufacturer's instructions with minor modifications. Array analysis on DNA

72 obtained from the patient described by Schluth *et al.* (2008)² was also performed, in order to
73 refine the size and breakpoint positions of the deletion in this patient.

74

75 Patient 3

76 Array CGH analysis was performed using the 244K catalogue oligonucleotide array with
77 complete genome coverage (median probe spacing ~9 kb) produced by Agilent Technologies.

78 **FISH analysis**

79 Patient 2

80 Patient 2 was tested with FISH for 22q11 and 17p11.2 (Smith-Magenis region) deletions at the
81 age of four yielding normal results. The 12q15q21.1 deletion detected on array was confirmed
82 using FISH with BlueFISH BAC probes RP11-101K2 and RP11-181D11 (BlueGnome, Cambridge,
83 UK) according to manufacturer's instructions. FISH analysis for this deletion in both parents
84 gave normal results.

85 Patient 3

86 Patient 3 was tested with FISH for the 22q11 deletion yielding a normal result. FISH analysis for
87 the 12q15q21.1 deletion was performed on metaphase slides from patient and parental
88 samples. BAC clones (RP11-90G3, RP11-934P3, RP11-89P15 and RP11-89M22) were selected
89 using the UCSC Genome Browser (<http://genome.ucsc.edu>) and the March 2006 assembly. The
90 12q15q21.1 deletion was confirmed in the patient. For both parents, FISH analysis yielded
91 normal results.

92

93 **CLINICAL REPORTS**

94 **Patient 1 (Belgium, Decipher Patient 253774)**

95 Patient 1 was born at 35 weeks gestation after an uneventful pregnancy to healthy non-
96 consanguineous parents. Birth weight was 2.63 kg (P25-50), length 49 cm (P90) and head
97 circumference 33 cm (P75). The vaginal delivery was long and difficult and vacuum extraction
98 was necessary. Apgar scores were 1 after 1 minute, 6 after 5 minutes and 8 after 10 minutes.
99 After birth he was initially cyanotic and showed unusual movements with the arms but
100 recovered quickly with adequate support. He stayed for 14 days in the neonatal care unit.
101 Clinical evaluation in the postnatal period revealed hypotonia and facial dysmorphism with
102 large anterior and posterior fontanel. During the first year he experienced feeding problems
103 with vomiting. His motor development was delayed. From 18 months on he received Bobath
104 therapy and started walking at 23 months. He started in normal preschool classes but needed
105 to switch to adapted classes around the age of 4-5 years. In childhood he underwent surgery
106 for phimosis, removal of tonsils and adenoids and repair of the right ear drum. He has always
107 been prone to upper airway infections. He receives substitution therapy for hypothyroidism
108 which was diagnosed at the age of 14 years. Several investigations in the past failed in finding
109 the cause of his psychomotor delay. Clinical examination at the age of 16 years 9 months
110 revealed a height of 175 cm (P25-P50), weight of 56.8 kg (P10-P25) and head circumference of
111 54 cm (P25). He presented with a rather high forehead with bitemporal narrowing. The
112 palpebral fissures were horizontal but small. Hypotelorism and mild synophrys were present.
113 The face was flat with a small chin. Both ears were rather large but normally placed. The
114 mouth was small and the palate narrow and high arched. Nasal speech was clearly present. His
115 back was straight and lacked the physiological curvatures of thoracic kyphosis and lumbar
116 lordosis. The back remained straight upon anteflexion. Bilateral cubitus valgus was noted.
117 Hands and fingers were long and slender with multiple fine dermatoglyphics in the hand

118 palms. He was not able to fully stretch his fifth fingers. They were held in flexion. In the lower
119 limbs a mild asymmetry in length was noted. His legs appeared thin with hypoplastic calves.
120 The feet were also slender with hallux valgus. The knee tendon reflexes in the legs were brisk.
121 Radiographs showed mild scoliosis and a S-shaped configuration of the tibiae.

122

123 **Patient 2 (UK, Decipher Patient 251427)**

124 The second patient is an 11-year-old girl born to non-consanguineous healthy parents. At birth,
125 she weighed 2.85 kg (P10) and presented with Down syndrome-like features and feeding
126 problems. At the age of three she was diagnosed with acute lymphoblastoid leukemia (ALL),
127 but was in complete remission at the age of six. Aged nine she started thyroxine treatment due
128 to a persistently high TSH. The patient is now 11 years old, her height is on the 9th percentile
129 and her head circumference is 56.5 cm (>P98). Her facial features include midfacial hypoplasia,
130 straight eyebrows, high forehead, low-set ears and dorsally rotated auricles. She also has
131 hammer toes and hypoplastic fifth toenails. Her speech has a nasal quality and she requires
132 learning support in mainstream school due to dyslexia and dyspraxia. Recent psychological
133 assessment places her full scale IQ on the 7th centile (IQ=69) and her literacy skills three years
134 behind her chronological age while her numeracy skills have shown no progress in two years.

135 **Patient 3 (Sweden, Decipher Patient 248767)**

136 The third patient is a 21-year-old woman, the second child of four born to healthy, unrelated
137 parents. The siblings, two brothers and one sister, are all healthy. She was born after an
138 uneventful pregnancy with a birth weight of 3.5 kg (P50). She had a history of recurrent upper
139 airway infections and middle ear infections. Since preschool age she has had nasal speech due
140 to velopharyngeal insufficiency, and she has undergone several operations without success, as

141 her speech is still very nasal. She was tested with FISH for the 22q11 deletion, yielding a
142 normal result. Growth has been normal. She has a slender built, small low set ears and straight
143 eyebrows. There are no known malformations. She wears glasses due to a refraction error and
144 her hearing is normal. Up to seventh grade she followed regular school but after that, she has
145 received special schooling. Her IQ is within the low normal range (total IQ 74, verbal scores
146 higher than performance). In her late teens she developed anorexia nervosa. She recently gave
147 birth to a healthy baby girl, who has been tested negative for the 12q15q21.1 deletion present
148 in the mother. During the pregnancy, she developed hypothyroidism.

149

150 RESULTS

151 Conventional chromosome analysis did not reveal any structural rearrangements in any of the
152 patients. Using oligonucleotide arrays (Agilent), a deletion involving chromosome bands
153 12q15->q21.1 was revealed in all patients. The larger deletion in the patient from Schluth *et*
154 *al.*² was also finemapped on a 180k oligonucleotide array. In patient 2 the deletion was
155 confirmed with FISH using BAC probes RP11-101K2 and RP11-181D11. For patient 3, the
156 12q15q21.1 deletion was confirmed with FISH using BAC probes RP11-90G3, RP11-934P3,
157 RP11-89P15 and RP11-89M22. Figure 1 depicts the deletions found in all three patients and
158 the fine mapped deletion in the patient from Schluth *et al.* The deletions in patients 2 and 3
159 are similar in size and breakpoint location, while the deletion in patient 1 is slightly shifted
160 centromeric. Analysis of the parents revealed that all the deletions occurred *de novo*. The data
161 of these three patients were submitted to the DECIPHER database (Database of Chromosomal
162 Imbalance and Phenotype in Humans using Ensembl Resources,
163 <https://decipher.sanger.ac.uk/>).

164

165 **DISCUSSION**

166 To date, only seven patients with larger deletions containing this region have been reported in
167 the literature.²⁻⁸ The deletion breakpoints vary from 12q13 to 12q23 and the phenotype differs
168 from patient to patient depending on the size of the deletion. Phenotypical features include
169 intra-uterine growth retardation (6/7), developmental delay (5/7), delayed walking (4/7) and
170 dysmorphic features. Low-set ears and a high arched palate were present in respectively 6/7
171 and 3/7 patients.²⁻⁸ Here we report three new cases with an overlapping submicroscopic
172 deletion of 12q15q21.1 with a common deleted region of 1.34 Mb. These patients present
173 with developmental delay (3/3), nasal speech (3/3) and hypothyroidism (3/3) in addition to
174 other congenital anomalies (Table 1-2).

175 Patients 2 and 3 appear to share the same proximal and distal breakpoint (Figure 1). The
176 analysis of the genomic structure of that region did not reveal the presence of any segmental
177 duplications or low copy repeats (LCR). However, a common HERVH element is present at both
178 sides of the deletion for both patients. Recently Vissers *et al.* indicated that rare pathogenic
179 CNVs do not seem to occur in random genomic sequences, but may favor locations with a high
180 content of specific architectural features.⁹ The presence of this microhomology at the
181 breakpoint junctions in patient 2 & 3, is suggestive for a micro-homology mediated
182 rearrangement.

183

184 In the smallest region of overlap (SRO), six RefSeq genes are located: *CNOT2*, *KCNMB4*, *PTPRB*,
185 *PTPRR*, *TSPAN8* and *LGR5*. Little is known about these genes, and none of them has been
186 implicated in palatal development.

187 *CNOT2*, a homeobox gene, is part of the CCR4-NOT complex.^{10,11} This complex functions as a
188 general transcription regulation complex and is ubiquitously expressed. Abdelkhalek *et al.*

189 (2004) showed that in mouse embryos loss of the orthologue *Not* results in abnormal
190 notochord formation in and caudal to the posterior trunk.¹²

191 The second deleted gene, *KCNMB4*, encodes a regulatory subunit of the calcium-activated
192 potassium KCNMA1 (maxiK) channel.^{13,14}

193 Both *PTPRB* and *PTPRR* belong to the protein tyrosine phosphatase (PTP) family. This family of
194 enzymes consists of key players in a variety of cellular processes including cell growth,
195 differentiation, metabolism, cell cycle, cell-cell communications, cell migration, gene
196 transcription, ion channels, immune response and survival.¹⁵ Malfunction of PTP activity is
197 associated with a number of human disorders including cancers, diabetes, rheumatoid arthritis
198 and hypertension.¹⁶ Studies of *PTPRR*-deficient mice showed that *PTPRR* isoforms¹⁷ are
199 physiological regulators of MAPK phosphorylation. These mice displayed impaired motor
200 coordination and defects in their balance skills, reminiscent of a mild ataxia due to altered
201 MAPK signaling.¹⁸

202 The protein encoded by *TSPAN8* is a member of the transmembrane 4 superfamily, also known
203 as the tetraspanin family. Tetraspanin 8 affects protein trafficking and is known to influence a
204 wide variety of physiological processes.¹⁹ GWA studies for schizophrenia and bipolar disorder
205 revealed association between *TSPAN8* and both psychiatric disorders.²⁰

206 Finally, *LGR5* encodes a leucine-rich repeat-containing, G-protein coupled receptor. LGRs are
207 structurally similar to receptors for gonadotropins and thyrotropin.^{21,22} The *LGR5* knock-out
208 mouse model is associated with neonatal lethality, gastrointestinal distention and
209 ankyloglossia.²³

210 Although all three patients present with nasal speech, none of the genes in the SRO have a
211 clear function in palate formation.

212 Recently Curry *et al.* reported a patient with a homozygous 812-902 kb deletion at 12q21.1
213 containing *TSPAN8* and *LGR5*.²⁴ The phenotype of this patient consisted of mild facial
214 dysmorphism, bifid uvula, peripheral pulmonic stenosis and developmental delay. Both the
215 normal parents and two normal siblings carried the heterozygous deletion. Surprisingly, the
216 normal younger brother also had the homozygous deletion. Since the heterozygous deletion of
217 *TSPAN8* and *LGR5* were seen in both the normal parents and two normal siblings, it seems that
218 a heterozygous deletion does not yield an abnormal phenotype. This indicates that although
219 LGRs have a structural resemblance to TSH, deletion of *LGR5* is not causal for the
220 hypothyroidism seen in the patients reported here.

221 According to Schluth *et al.*, the 12q15 region may be associated with intra-uterine growth
222 retardation and failure to thrive.² Since these features were not observed in our patients, it is
223 likely that they are associated with the 12q15 region proximal to the deletions reported here.

224 Cardiac defects, syndactyly and some specific facial features were seen in previously reported
225 patients and seem to be linked to chromosome band 12q21.2.² The absence of these
226 characteristics in our cases supports this hypothesis.

227 Patient 1 and the patient from Schluth *et al.* share some facial features not seen in the two
228 other patients (eg. micrognathia and long philtrum). Hence, these can be caused by the
229 overlapping region not shared by patient 2 and 3.

230 Nasal speech and hypothyroidism were not reported in any of the previous cases. However, in
231 our patients, nasal speech and hypothyroidism seem to be associated with deletion of the
232 smallest region of overlap. This emphasizes the need for more patients to establish a better
233 genotype-phenotype correlation.

234 To conclude, we report on three patients with overlapping deletions of 12q15q21.1. Gathering
235 more patients with the same or smaller deletions will contribute to a better understanding of
236 these genes and the pathophysiology of nasal speech and hypothyroidism in this microdeletion
237 syndrome.

238

239 **ACKNOWLEDGEMENTS**

240 We are grateful to all patients, their families and the clinicians involved for their cooperation.
241 We would like to thank Lies Vantomme and Shalina Baute for their expert technical assistance
242 and Filip Pattyn for continuous support. Sarah Vergult is supported by a Ph.D. fellowship of the
243 Research Foundation - Flanders (FWO). Bart Loeys is senior clinical investigator of the Research
244 Foundation - Flanders (FWO). This work was supported by grant SBO60848 from the Institute
245 for the Promotion of Innovation by Science and Technology in Flanders (IWT) and a
246 Methusalem grant of the Flemish Government. This article presents research results of the
247 Belgian program of Interuniversity Poles of attraction initiated by the Belgian State, Prime
248 Minister's Office, Science Policy Programming (IUAP).

249 **CONFLICT OF INTEREST**

250 The authors declare no conflict of interest.

251 **REFERENCES**

- 252 1. Menten B, Buysse K, Zahir F *et al*: Osteopoikilosis, short stature and mental retardation
253 as key features of a new microdeletion syndrome on 12q14. *J Med Genet* 2007; **44**:
254 264-268.

- 255 2. Schluth C, Gesny R, Borck G *et al*: New case of interstitial deletion 12(q15-q21.2) in a
256 girl with facial dysmorphism and mental retardation. *Am J Med Genet A* 2008; **146A**:
257 93-96.
- 258 3. Meinecke P, Meinecke R: Multiple malformation syndrome including cleft lip and
259 palate and cardiac abnormalities due to an interstitial deletion of chromosome 12q. *J*
260 *Med Genet* 1987; **24**: 187.
- 261 4. Watson MS, McAllister-Barton L, Mahoney MJ, Breg WR: Deletion (12)(q15q21.2). *J*
262 *Med Genet* 1989; **26**: 343-344.
- 263 5. Perez Sanchez C, Ayensa F, Lloveras E *et al*: Prenatal diagnosis of an interstitial 12q
264 chromosome deletion. *Ann Genet* 2004; **47**: 177-179.
- 265 6. James PA, Oei P, Ng D, Kannu P, Aftimos S: Another case of interstitial del(12) involving
266 the proposed cardio-facio-cutaneous candidate region. *Am J Med Genet A* 2005; **136**:
267 12-16.
- 268 7. Tocyap ML, Azar N, Chen T, Wiggs J: Clinical and molecular characterization of a
269 patient with an interstitial deletion of chromosome 12q15-q23 and peripheral corneal
270 abnormalities. *Am J Ophthalmol* 2006; **141**: 566-567.
- 271 8. Yamanishi T, Nishio J, Miya S *et al*: 12q interstitial deletion with bilateral cleft lip and
272 palate: case report and literature review. *Cleft Palate Craniofac J* 2008; **45**: 325-328.
- 273 9. Vissers LE, Bhatt SS, Janssen IM *et al*: Rare pathogenic microdeletions and tandem
274 duplications are microhomology-mediated and stimulated by local genomic
275 architecture. *Hum Mol Genet* 2009; **18**: 3579-3593.

- 276 10. Collart MA: Global control of gene expression in yeast by the Ccr4-Not complex. *Gene*
277 2003; **313**: 1-16.
- 278 11. Denis CL, Chen J: The CCR4-NOT complex plays diverse roles in mRNA metabolism.
279 *Prog Nucleic Acid Res Mol Biol* 2003; **73**: 221-250.
- 280 12. Abdelkhalek HB, Beckers A, Schuster-Gossler K *et al*: The mouse homeobox gene Not is
281 required for caudal notochord development and affected by the truncate mutation.
282 *Genes Dev* 2004; **18**: 1725-1736.
- 283 13. Behrens R, Nolting A, Reimann F, Schwarz M, Waldschutz R, Pongs O: hKCNMB3 and
284 hKCNMB4, cloning and characterization of two members of the large-conductance
285 calcium-activated potassium channel beta subunit family. *FEBS letters* 2000; **474**: 99-
286 106.
- 287 14. Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW: Cloning and functional
288 characterization of novel large conductance calcium-activated potassium channel beta
289 subunits, hKCNMB3 and hKCNMB4. *The Journal of biological chemistry* 2000; **275**:
290 6453-6461.
- 291 15. Hunter T: Signaling--2000 and beyond. *Cell* 2000; **100**: 113-127.
- 292 16. Zhang ZY: Protein tyrosine phosphatases: prospects for therapeutics. *Curr Opin Chem*
293 *Biol* 2001; **5**: 416-423.
- 294 17. Hendriks WJ, Dilaver G, Noordman YE, Kremer B, Fransen JA: PTPRR protein tyrosine
295 phosphatase isoforms and locomotion of vesicles and mice. *Cerebellum* 2009; **8**: 80-88.

- 296 18. Chirivi RG, Noordman YE, Van der Zee CE, Hendriks WJ: Altered MAP kinase
297 phosphorylation and impaired motor coordination in PTPRR deficient mice. *J*
298 *Neurochem* 2007; **101**: 829-840.
- 299 19. Kuhn S, Koch M, Nubel T *et al*: A complex of EpCAM, claudin-7, CD44 variant isoforms,
300 and tetraspanins promotes colorectal cancer progression. *Molecular cancer research* :
301 *MCR* 2007; **5**: 553-567.
- 302 20. Scholz CJ, Jacob CP, Buttenschon HN *et al*: Functional variants of TSPAN8 are
303 associated with bipolar disorder and schizophrenia. *Am J Med Genet B Neuropsychiatr*
304 *Genet*; **153B**: 967-972.
- 305 21. Hsu SY, Liang SG, Hsueh AJ: Characterization of two LGR genes homologous to
306 gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G
307 protein-coupled, seven-transmembrane region. *Mol Endocrinol* 1998; **12**: 1830-1845.
- 308 22. Hsu SY, Kudo M, Chen T *et al*: The three subfamilies of leucine-rich repeat-containing G
309 protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling
310 mechanism for LGR7. *Mol Endocrinol* 2000; **14**: 1257-1271.
- 311 23. Morita H, Mazerbourg S, Bouley DM *et al*: Neonatal lethality of LGR5 null mice is
312 associated with ankyloglossia and gastrointestinal distension. *Mol Cell Biol* 2004; **24**:
313 9736-9743.
- 314 24. Curry CJ, Mao R, Aston E *et al*: Homozygous deletions of a copy number change
315 detected by array CGH: a new cause for mental retardation? *Am J Med Genet A* 2008;
316 **146A**: 1903-1910.

317

318

319

320 **TITLES AND LEGENDS TO FIGURES**

321 Figure 1 A. Overview of the 12q15q21.1 microdeletions in patients 1-3 and the finemapped
322 deletion in the patient from Schluth *et al.* Breakpoint locations were uploaded into the UCSC
323 Genome Browser (March 2006 assembly). Patient 1: arr 12q15q21.1 (67640963-70139285)x1
324 dn; Patient 2: arr 12q15q21.1(68782918-71372314)x1 dn; Patient 3: arr
325 12q15q21.1(68802240-71372314)x1 dn; Patient from Schluth *et al.*: arr 12q15q21.2(66869019-
326 77078118)x1 dn. The smallest region of overlap (SRO) indicated by the dashed lines, is 1.34 Mb
327 in size and contains six RefSeq genes. B. Overview of the SRO.

328

329 **Figure 2** Photographs of patients 1-3. (a) & (b) Patient 1 at the age of 17 years. (c) & (d) Patient
330 2 at the age of 11 years. Patient 3 at the age of 3 (e), 6 (f) and 19 years (g-h).

331

332 **Table 1** Array CGH analysis results and phenotypic features of patients 1-3 and the patient
 333 obtained from Schluth *et al.* (2008).
 334

	Schluth <i>et al.</i>	Patient 1 (Belgium)	Patient 2 (UK)	Patient 3 (Sweden)
Array CGH Analysis				
Proximal breakpoint (bp)	66,869,019	67,640,963	68,782,918	68,802,240
Distal breakpoint (bp)	77,078,118	70,139,285	71,372,314	71,372,314
Location of deletion	12q15q21.2	12q15q21.1	12q15q21.1	12q15q21.1
Size of deletion	10.21 Mb	2.50 Mb	2.59 Mb	2.57 Mb
Heredity	de novo	de novo	de novo	de novo
Characteristics				
Gender	F	M	F	F
General				
Birth weight (kg)	2.1 (P3)	2.63 (P25-50)	2.85 (P10)	3.5 (P50)
Head circumference (cm)	32	33		
Developmental delay	+	+	+	+
Feeding problems	-	+	+	-
Hypotonia	+	+	-	-
Hypothyroidism	NI	+	+	during pregnancy
Facial				
Flat face/midfacial hypoplasia	-	+	+	+
Large anterior fontanel	+	+	-	NI
High forehead	+	-	+	+
Hyper/hypotelorism	hyper	hypo	-	-
Straight eyebrows	-	+	+	+

Low-set ears	+	-	+	+
Micrognathia	+	+	-	-
Long philtrum	+	+	-	-
Hands and feet				
Hammer toes	-	+	+	-
Stature				
Slender	+	+	-	+
Speech				
Delayed speech	+	+	-	-
Nasal speech	-	+	+	+
Developmental				
Psychomotor development delay	+	+	+	+
Walking delay	+	+	-	-
Others				
Refraction error	-	-	+	+
Otitis media	-	+	-	+

335 NI: not investigated

336

337 **Table 2** Additional phenotypic characteristics seen in patients 1-3 and the patient from Schluth *et al.*
 338 (2008)

Patient	Additional Phenotypic Characteristics
Schluth <i>et al.</i>	IUGR ¹ , macrostomia, thin upper lip, downturned mouth, upward slanting palpebral fissures, strabismus, crescent-shaped eyes, anteverted nose, brachydactyly of hands, syndactyly 2/3 of toes, sacrococcygeal tuft of hair, pectus excavatum, ventricular septal defect, hypercalcemia, gastro-esophageal reflux
Patient 1 (Belgium)	Small palate, high arched palate, retrognathia, craniofacial dysmorphism, narrow forehead, fine sparse hair, synophrys, small nose, broad/depressed nasal bridge, arachnodactyly, slender hands/feet, shortness of one leg, flat back, recurrent vomiting
Patient 2 (UK)	Clinodactyly, dorsally rotated auricles, cutis marmorata, acute lymphoblastic leukaemia (ALL)
Patient 3 (Sweden)	Eating disorder, respiratory infections

339 ¹Intra-uterine growth retardation

340

341



