

Molecular characterization of 1q44 microdeletion in 11 patients reveals three candidate genes for intellectual disability and seizures.

Gaëlle Thierry, Claire Bénéteau, Olivier Pichon, Elisabeth Flori, Bertrand Isidor, Françoise Popelard, Marie-Ange Delrue, Laetitia Duboscq-Bidot, Ann-Charlotte Thuresson, Bregje van Bon, et al.

► **To cite this version:**

Gaëlle Thierry, Claire Bénéteau, Olivier Pichon, Elisabeth Flori, Bertrand Isidor, et al.. Molecular characterization of 1q44 microdeletion in 11 patients reveals three candidate genes for intellectual disability and seizures.. American Journal of Medical Genetics Part A, Wiley, 2012, 158A (7), pp.1633-40. 10.1002/ajmg.a.35423 . inserm-00706725

HAL Id: inserm-00706725

<https://www.hal.inserm.fr/inserm-00706725>

Submitted on 12 Jul 2012

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.

**1Molecular characterization of 1q44 microdeletion in eleven patients reveals three
2candidate genes for intellectual disability and seizures**

3

4Gaelle Thierry,^{1,18} Claire Bénéteau,^{1,18} Olivier Pichon,¹ Elisabeth Flori,² Bertrand Isidor,¹
5Françoise Popelard,³ Marie-Ange Delrue,⁴ Laetitia Duboscq-Bidot,⁵ Ann-Charlotte
6Thuresson,⁶ Bregje WM van Bon,⁷ Dorothee Cailley,⁴ Caroline Rooryck,⁴ Agathe Paubel,⁸
7Corinne Metay,⁹ Anne Dusser,¹⁰ Laurent Pasquier,¹¹ Mylène Béri,¹² Céline Bonnet,¹² Sylvie
8Jaillard,¹³ Christèle Dubourg,^{14,15} Bassim Tou,¹⁶ Marie-Pierre Quéré,¹⁷ Cecilia Soussi-Zander,⁶
9Annick Toutain,⁸ Didier Lacombe,⁴ Benoit Arveiler,⁴ Bert BA de Vries,⁷ Philippe Jonveaux,¹²
10Albert David,¹ Cédric Le Caignec^{1,5,*}

11

12¹CHU Nantes, Service de Génétique Médicale, Nantes, France

13²Service de Cytogénétique, Hôpital de Hautepierre, Strasbourg, France

14³Service de Pédiatrie, Centre Hospitalier Jean Monnet, Epinal, France

15⁴Service de Génétique Médicale, Hôpital Pellegrin; Université de Bordeaux, Maladies Rares :

16Génétique et Métabolisme (MRGM), EA 4576, Bordeaux, France

17⁵Inserm, UMR_S915, l'institut du thorax, Nantes, France

18⁶Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

19⁷Department of Human Genetics, Radboud University Nijmegen Medical Center, Nijmegen,

20The Netherlands

21⁸Service de Génétique Médicale, CHU, Tours, France

22⁹Plateforme Génétique Constitutionnelle CGH array, Hôpital Henri Mondor, Créteil, France

23¹⁰Service de Neurologie Pédiatrique, CHU Bicêtre, Le Kremlin Bicêtre, France

24¹¹Service de Génétique Médicale, CLAD Ouest, Hôpital Sud, CHU Rennes, Rennes, France

25¹²Laboratoire de génétique, CHU, Nancy, France

1

1¹³Laboratoire de Cytogénétique, CHU Pontchaillou, Rennes Cedex, France

2¹⁴Institut de Génétique et Développement, CNRS UMR 6061, Université de Rennes 1, IFR140
3GFAS, Faculté de Médecine, Rennes, France

4¹⁵Laboratoire de Génétique Moléculaire, CHU Pontchaillou, Rennes, France

5¹⁶Laboratoire de Génétique Moléculaire, Pharmacogénétique et Hormonologie, Hôpital
6Universitaire de Bicêtre, Secteur Paul Broca, Le Kremlin Bicêtre, France

7¹⁷CHU Nantes, Service de radiologie pédiatrique, Nantes, France

8¹⁸Both authors contributed equally to this work

9

10

11*Correspondence to: Cédric Le Caignec, MD, PhD, Service de Génétique Médicale, CHU, 9,
12quai Moncousu 44093 Nantes, France, Tél.: +33 2 40 08 42 84, Fax: +33 2 40 08 39 43, E-
13mail: cedric.lecaignec@chu-nantes.fr

14

15**Key Words:** 1q44, deletion, chromosome, HNRNPU, FAM36A, ncRNA, intellectual
16disability, corpus callosum, seizure

17

1ABSTRACT

2

3Patients with a submicroscopic deletion at 1q43q44 present with intellectual disability (ID),
4microcephaly, craniofacial anomalies, seizures, limb anomalies and corpus callosum
5abnormalities. However, the precise relationship between most of deleted genes and the
6clinical features in these patients still remains unclear. We studied 11 unrelated patients with
71q44 microdeletion. We showed that the deletions occurred de novo in all patients for whom
8both parents' DNA was available (10/11). All patients presented with moderate to severe ID,
9seizures and non-specific craniofacial anomalies. By oligoarray-based comparative genomic
10hybridization (aCGH) covering the 1q44 region at a high resolution, we obtained a critical
11deleted region containing two coding genes - *HNRNPU* and *FAM36A* - and one non-coding
12gene - *NCRNA00201*. All three genes were expressed in different normal human tissues,
13including in human brain, with highest expression levels in the cerebellum. Mutational
14screening of the *HNRNPU* and *FAM36A* genes in 191 patients with unexplained isolated ID
15did not reveal any deleterious mutations while the *NCRNA00201* non-coding gene was not
16analyzed. Nine of the 11 patients did not present with microcephaly or corpus callosum
17abnormalities and carried a small deletion containing *HNRNPU*, *FAM36A* and *NCRNA00201*
18but not *AKT3* and *ZNF238*, two centromeric genes. These results suggest that *HNRNPU*,
19*FAM36A* and *NCRNA00201* are not major genes for microcephaly and corpus callosum
20abnormalities but are good candidates for ID and seizures.

1

1

2INTRODUCTION

3

4 Intellectual disability (ID) represents the most frequent cause of severe handicap in
5 children and one of the main reasons for referral in clinical genetic practices. Causes of ID are
6 extremely heterogeneous and can result from chromosomal rearrangements, monogenic
7 disorders, and/or environmental factors. Despite clinical examination and extensive
8 complementary investigations, no etiology is identified in up to 50% of the patients with
9 moderate to severe ID [Chelly et al., 2006], hampering accurate genetic counseling and
10 clinical follow-up. During these last years, the advent of high-resolution microarray
11 techniques allowed for the detection of increasingly smaller rearrangements in patients with
12 ID. The method has greatly facilitated deciphering chromosomal disorders, enabling better
13 genotype – phenotype correlations and thus the identification of new genes responsible for ID.

14 A number of chromosomal regions scattered through the human genome are deleted in
15 patients with ID, among them the 1q43q44 region. This deletion syndrome was first described
16 by De Vries et al. [2001]. Patients present with ID, microcephaly, craniofacial anomalies,
17 seizures, limb anomalies and corpus callosum abnormalities. However, the precise
18 relationship between most of deleted genes and the clinical features in these patients still
19 remains unclear. Three studies have each proposed a different smallest region of overlap
20 (SRP) for corpus callosum abnormalities. A first critical deleted region described by Boland
21 et al. [2007] was 1.25 Mb in size and contained two candidate genes: *AKT3* and *ZNF238*.
22 Next, van Bon et al. [2008] identified a second distinct critical region of 0.36 Mb in size,
23 more telomeric than the first one, and containing four different candidate genes: *Clorf100*,
24 *ADSS*, *Clorf101* and *PNAS-4*. Caliebe et al. [2010] proposed a third interval of 0.44 Mb,
25 which is more telomeric than the other two, and which contained the *HNRNPU* gene. Finally,

1 the combined data from two recent studies, a first one performed on 22 patients [Ballif et al.,
2 2011] and a second one based on 7 patients [Nagamani et al., 2012] sharing 1q43q44
3 microdeletion, proposed three distinct SRO with different sizes implicated in corpus callosum
4 abnormalities (75 kb in size, including *ZNF238*), microcephaly (133 kb in size, including
5 *AKT3*) or seizures (100 kb in size, including *HNRNPU*, *FAM36A* and *NCRNA00201*
6 previously referred as *CIORF199*). We focused our study on 11 unrelated patients with ID
7 and seizures carrying a 1q44 interstitial microdeletion. We refined the SRO for ID and
8 seizures to three genes and explored each of these three genes to highlight their potential role
9 played in the phenotype.

10

11 PATIENTS AND METHODS

12

13 Patients

14

15 We studied 11 unrelated patients (eight females and three males) with a 1q44
16 microdeletion. The non-specific craniofacial anomalies are presented in Figure 1 and clinical
17 features are summarized in Table 1. The patients originated from Europe (France, Sweden,
18 Finland, Monaco, The Netherlands and Germany) and Senegal. No consanguinity or familial
19 genetic history was noted in the families. The pregnancies were uneventful.

20 All patients presented with moderate to severe ID, predominantly on verbal learning
21 disabilities. Milestones were delayed in all patients: sitting unsupported ranged from 6 months
22 to 3 years of age, walking unsupported from 2 to 5 years of age, and severe speech delay (6
23 patients aged from 4 years and 6 months to 17 years had no expressive speech and one patient
24 had severe speech delay and phonetic disorders). Neurological examination revealed axial
25 hypotonia in four cases. Five patients presented with stereotyped movements of the hands

1

1with voluntary use of their hands conserved and were suspected for Rett syndrome. Two
2patients had sleep disturbances, of which one was treated with Melatonin with good results.
3Four patients had autistic features and/or attention deficit disorder.

4 Seizures were observed in all patients. The age of the first seizure ranged from 6
5months to 2.5 years of age. Different types of seizures were observed: absences, generalized,
6tonic-clonic seizures and Lennox-Gastaut syndrome. Epilepsy required treatment in most
7patients, no pharmaco-resistant epilepsy was noted except for patient 3. Two patients
8developed status epilepticus history. Magnetic resonance imaging (MRI) revealed agenesis of
9corpus callosum in patient 2. The corpus callosum was normal in all other patients. MRI
10revealed other brain abnormalities: delayed myelinisation, generalized or subcortical atrophy,
11micropolygyria, moderate ventricular dilatation and moderate cerebellar hypoplasia. Only two
12patients (#1 and # 2) among the eleven presented with microcephaly. Both patients carried the
13larger deletions including the *AKT3* gene. This finding is consistent with other recent reports
14suggesting that *AKT3* is a strong candidate gene for microcephaly [Ballif et al., 2011;
15Nagamani et al., 2012].

16 Six patients developed general obesity during childhood (BMI around +4 SD, obesity
17grade 2). Five patients had short stature (heights between -2.5 and -3 SD). Six patients had
18small hands and broad, short and/or small feet with small toes. Craniofacial anomalies were
19present in all patients, but did not lead to a characteristic facial dysmorphism. Hypertelorism
20(4/10), strabismus (4/9), bulbous nose (3/10), long and flat philtrum (5/10) and abnormal ears
21(4/10) were frequently observed.

22 Occasionally, some malformations were observed: unilateral renal agenesis, congenital
23heart defects (atrial septal defect and pulmonary stenosis), and some skeletal anomalies (a
24butterfly vertebrae, a scoliosis).

25

1

1 Cytogenetic and aCGH studies

2

3 Informed consent for genetic analyses was obtained from parents of the patients
4 according to local ethical guidelines. Karyotyping **based on R or G banding** was performed
5 using standard methods on metaphase spreads from peripheral blood of the patients. Genomic
6 DNA was extracted from peripheral blood using standard protocols. Molecular karyotyping of
7 the 11 patients was initially conducted using different array platforms according to
8 manufacturers' instructions. Subsequently, we used a custom targeted 60K Agilent array to
9 fine map the breakpoints of the deletions with a median resolution of 240 bp. **Custom arrays**
10 **comprising 25,021 probes covered a 6 Mb in the 1q44 region including the *HNRNPU*,**
11 ***FAM36A* and *NCRNA00201* genes.** Arrays were analyzed with a Agilent scanner and the
12 Feature Extraction software (v. 10.5.1.1). Graphical overview was obtained using the
13 Genomic Workbench software (v.5.0). Deletion breakpoints were mapped to the UCSC
14 genome browser, hg19. A second independent method (fluorescence in situ hybridization
15 (FISH) with different probes, qPCR or MLPA) was used to confirm the deletions and for
16 parental inheritance in patients for whom DNA was available. Three individuals of Yoruba
17 Nigerian origin from the HapMap Project were obtained from the Coriell Institute [IHMC,
18 2005] and were also analyzed using the custom 60K array.

19

20 mRNA expression studies

21

22 We performed expression analyses for *HNRNPU*, *FAM36A* and *NCRNA00201* using
23 total RNA extracted from different human tissues (primer sequences available upon request).
24 RNAs were obtained from adult brain, heart, kidney, liver, cerebellum tissues and from fetal
25 brain tissue (Clontech). Real time quantitative reverse transcription PCR (RT-qPCR) was

1

1performed using the $\Delta\Delta\text{Ct}$ method [Livak et al., 2001] to assess expression level of the three
2target genes - *HNRNPU*, *FAM36A* and *NCRNA00201* – relative to the expression level of the
3 β -actin (*ACTB*) and cyclophilin E (*PPIE*) housekeeping genes. For a given target gene, the
4 ΔCt of each tissue was compared to the median of the ΔCt of the 6 tissues analyzed.

5

6Mutation screening

7

8 The coding exons and the exon-intron boundaries of the *HNRNPU* and *FAM36A* genes
9were sequenced in 9/11 patients with a 1q44 deletion using the Sanger technology and run on
10ABI 3130 (primer sequences available upon request). A series of 191 patients with
11unexplained isolated ID were used to search for point mutations in *HNRNPU* and *FAM36A*.
12Standard karyotyping was normal in all 191 patients. In addition, molecular karyotyping
13performed with a 44K Agilent array was normal in 112/191 patients while the other patients
14were not analyzed. PCR amplifications followed by high-resolution melting method (HRM)
15were performed to screen *HNRNPU* (exons 2-14). PCR amplifications followed by Sanger
16sequencing were performed to screen the 5' half of exon 1 of *HNRNPU* and the four exons of
17*FAM36A*. We failed to sequence the 3' half of exon 1 of *HNRNPU*.

18

19RESULTS

20

21Cytogenetic and aCGH results

22

23 Following normal standard karyotyping, a 1q44 microdeletion was identified in 11
24patients with moderate to severe ID, craniofacial anomalies and seizures using different high-
25resolution array platforms. No other pathogenic genomic imbalances were identified in the

1

1patients. All deletions were confirmed by FISH, qPCR or MLPA. Parental analyses
2demonstrated de novo deletions in all families when both parents' DNA was available. Using
3a custom targeted aCGH method, we showed that the sizes of the deletions were variable,
4ranging from 626 Kb to 2.57 Mb (supplementary **Table I**). The size of the SRO was 188 Kb
5and encompassed four genes: *HNRNPU*, *FAM36A*, *NCRNA00201*, and *EFCAB2*. The
6identification of a normal individual with a partial deletion of the *EFCAB2* gene **led us to**
7**consider as unlikely causative this gene** as a cause of ID, thus refining the SRO to three genes:
8*HNRNPU*, *FAM36A*, and *NCRNA00201* (Fig. 2). In a previous study, Matsuzaki et al. [2009]
9identified a deletion involving *HNRNPU* in three HAPMAP individuals of Yoruba Nigerian
10origin. In contrast, we obtained normal results with our targeted 60K array, excluding a
11deletion in the 1q44 region in these individuals and, thus, demonstrating that the deletions
12identified by Matsuzaki et al. [2009] were false positive results. Therefore, no deletion
13involving one of these three genes located in this 1q44 region has been observed within
14individuals of the general population (www.tcag.org).

15

16**mRNA expression studies**

17

18 We showed that *HNRNPU*, *FAM36A* and *NCRNA00201* were expressed in 6 different
19tissues (adult brain, heart, kidney, liver, cerebellum tissues and fetal brain tissue), with the
20strongest expression in the cerebellum (Fig. 3). The highest level of transcripts was obtained
21for *NCRNA00201* in the cerebellum. As the strongest expression of these three genes were
22detected in cerebellum, we analyzed the expression of two control genes: *SULF1* and
23*SLCO5A1*. We obtained low expression levels in the cerebellum compared to other tissues for
24these genes, thus excluding a potential bias in our sample of RNA extracted from the
25cerebellum (data not shown).

2

9

1

1

2Mutation screening

3

4 Direct sequencing of *HNRNPU* and *FAM36A* did not reveal any deleterious point
5 mutations in the remaining allele of the patients with a 1q44 deletion, rendering unlikely a
6 recessive mode of inheritance. Neither did we detect any deleterious mutations in these two
7 genes in our series of 191 patients with unexplained ID. Two identified exonic variants in
8 *HNRNPU* (exon 6 c.1215G>A, synonymous; exon 14 c.2437C>G, p.Gln813Glu) and one in
9 *FAM36A* (exon 4 c.340G>A, p.Gly114Ser) were predicted to be benign using the PolyPhen
10 software.

11

12DISCUSSION

13

14 In this study, all eleven patients carrying a 1q44 microdeletion presented with
15 moderate to severe ID, seizures and non-specific craniofacial anomalies, corresponding to a
16 non-recognizable phenotype with ID. The aCGH data allowed us to fine map a SRO for
17 moderate to severe ID and seizures. However, since other reports described patients with
18 deletions in the 1q43q44 bands that did not include the SRO defined in the present study,
19 there may be a number of additional genes that when haploinsufficient can cause ID in these
20 patients.

21

22 Two recent studies, a first one performed on 22 patients [Ballif et al., 2011] and a
23 second one based on 7 patients [Nagamani et al., 2012] sharing 1q43q44 microdeletion,
24 clarified the phenotype/genotype correlation and proposed three distinct SRO. The first SRO
25 encompassing *ZNF238* was associated with corpus callosum abnormalities, the second SRO
including *AKT3* caused microcephaly in most patients while the third SRO containing the

1 three genes *FAM36A*, *HNRNPU* and *NCRNA00201* was associated with seizures. In our
2 study, 2/11 patients (#1 and # 2) carried a deletion of both *ZNF238* and *AKT3*. Both patients
3 presented with microcephaly which was consistent with a role of *AKT3* in microcephaly. Only
4 patient 2 presented with a corpus callosum agenesis. Incomplete penetrance associated with
5 deletion of *ZNF238* could explain the lack of corpus callosum abnormality in patient 1.
6 Finally, the third previously published SRO associated with seizures in Ballif et al. [2011]
7 overlapped with our 188 Kb SRO associated with ID, seizures and craniofacial anomalies.

8 *FAM36A* encodes a hypothetical protein and, to date, its biological role is still
9 unknown.

10 *HNRNPU* is a protein-coding gene comprising 14 exons, which are highly conserved
11 during evolution. The HNRNPU protein is able to bind RNAs and mediates different aspects
12 of their metabolism and transport [Dreyfuss et al., 2002; Krecic and Swanson, 1999]. Mice
13 with a homozygous hypomorphic mutation in *HNRNPU* are severely retarded in both growth
14 and development indicating that this gene is essential for embryonic development [Roshon et
15 al., 2005]. Although ubiquitously expressed, we detected the highest expression level for
16 *HNRNPU* in human cerebellum, a tissue which plays an essential role in cognition.
17 Interestingly, *HNRNPU* is involved in later stages of differentiation of cerebellar neurons via
18 the regulation of DNA topoisomerase II β activity [Kawano et al., 2010]. Thus,
19 haploinsufficiency for *HNRNPU* may lead to ID in our patients, even in the absence of
20 clinical cerebellar anomalies. *CDH15* is such an example where a gene is strongly expressed
21 in the cerebellum and mutations for which are associated with ID with no features of
22 cerebellar dysfunction [Bhalla et al., 2008].

23 The third gene within the SRO of our study, *NCRNA00201*, encodes a long non-
24 coding RNA (lncRNA). The majority of lncRNA has very high levels of expression in the
25 central nervous system in a cell-type specific manner, of which some have already been

1

1 implicated in neurological and developmental disorders [for a review Qureshi et al., 2010]. It
2 is assumed that they regulate gene expression notably via chromatin remodeling at their
3 originate locus (in cis) and/or elsewhere in the genome (in trans). This property considerably
4 increases the difficulty to identify their triggers and to understand their physiological roles.
5 By RT-qPCR, we detected the highest expression level for *NCRNA00201* in human
6 cerebellum, making it a good candidate. Moreover, since our three deleted genes showed
7 relatively high expression in the cerebellum when compared to other tissues, we might
8 hypothesize that an epistatic effect of at least two genes from this locus could be responsible
9 for our patients' phenotype. The lack of knowledge and the difficulty to interpret the variants
10 identified in a non-coding gene explain why we did not sequence *NCRNA00201* in our series
11 of patients with ID. *NCRNA00201* still remains a good candidate to explain ID but functional
12 analyses are needed to clarify the implication of this gene in the phenotype.

13 Taken together, our aCGH, expression and sequencing data highlight a critical region
14 containing three good candidate genes for non-syndromic ID and seizures. These results will
15 be important for clinicians in genetic counseling.

16

17 **ACKNOWLEDGMENTS**

18

19 We are grateful to the patients and their families who participated in this study, to Rémi
20 Houlgatte, Catherine Chevalier from Plateforme génomique intégrative de Nantes, to Jean
21 Mosser from Biogenouest de Nantes, France, and to Lucie Tosca and Sophie Brisset from the
22 cytogenetic laboratory, Hôpital Antoine Beclère. GT was supported by La Fondation pour la
23 Recherche Médicale.

24

25 **CONFLICT OF INTEREST**

1

1

2The authors declare no conflict of interest.

3

4**REFERENCES**

5

6Ballif BC, Rosenfeld JA, Traylor R, Theisen A, Bader PI, Ladda RL, Sell SL, Steinraths M,
7Surti U, McGuire M, Williams S, Farrell SA, Filiano J, Schnur RE, Coffey LB, Tervo RC,
8Stroud T, Marble M, Netzloff M, Hanson K, Aylsworth AS, Bamforth JS, Babu D, Niyazov
9DM, Ravnan JB, Schultz RA, Lamb AN, Torchia BS, Bejjani BA, Shaffer LG. 2011. High-
10resolution array CGH defines critical regions and candidate genes for microcephaly,
11abnormalities of the corpus callosum, and seizure phenotypes in patients with microdeletions
12of 1q43q44. *Hum Genet* [Epub ahead of print].

13

14Bhalla K, Luo Y, Buchan T, Beachem MA, Guzauskas GF, Ladd S, Bratcher SJ, Schroer RJ,
15Balsamo J, DuPont BR, Lilien J, Srivastava AK. 2008. Alterations in CDH15 and KIRREL3
16in patients with mild to severe intellectual disability. *Am J Hum Genet* 83:703-13.

17

18Boland E, Clayton-Smith J, Woo VG, McKee S, Manson FD, Medne L, Zackai E, Swanson
19EA, Fitzpatrick D, Millen KJ, Sherr EH, Dobyns WB, Black GC. 2007. Mapping of deletion
20and translocation breakpoints in 1q44 implicates the serine/threonine kinase AKT3 in
21postnatal microcephaly and agenesis of the corpus callosum. *Am J Hum Genet* 81:292-303.

22

23Caliebe A, Kroes HY, van der Smagt JJ, Martin-Subero JJ, Tönnies H, van 't Slot R,
24Nivelstein RA, Muhle H, Stephani U, Alfke K, Stefanova I, Hellenbroich Y, Gillessen-
25Kaesbach G, Hochstenbach R, Siebert R, Poot M. 2010. Four patients with speech delay,

1

1seizures and variable corpus callosum thickness sharing a 0.440 Mb deletion in region 1q44
2containing the HNRPU gene. *Eur J Med Genet* 53:179-85.

3

4Chelly J, Khelifaoui M, Francis F, Chérif B, Bienvenu T. 2006. Genetics and pathophysiology
5of mental retardation. *Eur J Hum Genet* 14:701-13.

6

7De Vries BB, Knight SJ, Homfray T, Smithson SF, Flint J, Winter RM. 2001.
8Submicroscopic subtelomeric 1qter deletions: a recognisable phenotype? *J Med Genet*
938:175-8.

10

11Dreyfuss G, Kim VN, Kataoka N. 2002. Messenger-RNA-binding proteins and the messages
12they carry. *Nat Rev Mol Cell Biol* 3:195-205.

13

14IHM (International HapMap Consortium). 2005. A haplotype map of the human genome.
15*Nature* 437:1299-320.

16

17Kawano S, Miyaji M, Ichiyasu S, Tsutsui KM, Tsutsui K. 2010. Regulation of DNA
18Topoisomerase IIbeta through RNA-dependent association with heterogeneous nuclear
19ribonucleoprotein U (hnRNP U). *J Biol Chem* 285:26451-26460.

20

21Krecic AM, Swanson MS. 1999. hnRNP complexes: composition, structure, and function.
22*Curr Opin Cell Biol* 11:363-71.

23

24Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time
25quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* 25:402-8.

1

1

2Matsuzaki H, Wang PH, Hu J, Rava R, Fu GK. 2009. [High resolution discovery and](#)
3[confirmation of copy number variants in 90 Yoruba Nigerians.](#) Genome Biol 10:R125.

4

5Nagamani SC, Erez A, Bay C, Pettigrew A, Lalani SR, Herman K, Graham BH, Nowaczyk
6MJ, Proud M, Craigen WJ, Hopkins B, Kozel B, Plunkett K, Hixson P, Stankiewicz P, Patel
7A, Cheung SW. 2012. Delineation of a deletion region critical for corpus callosal
8abnormalities in chromosome 1q43-q44. Eur J Hum Genet 20:176-9.

9

10Qureshi IA, Mattick JS, Mehler MF. 2010. [Long non-coding RNAs in nervous system](#)
11[function and disease.](#) Brain Res 18;1338:20-35.

12

13Roshon MJ, Ruley HE. 2005. [Hypomorphic mutation in hnRNP U results in post-](#)
14[implantation lethality.](#) Transgenic Res 14:179-192.

15

16van Bon BW, Koolen DA, Borgatti R, Magee A, Garcia-Minaur S, Rooms L, Reardon W,
17Zollino M, Bonaglia MC, De Gregori M, Novara F, Grasso R, Ciccone R, van Duyvenvoorde
18HA, Aalbers AM, Guerrini R, Fazzi E, Nillesen WM, McCullough S, Kant SG, Marcelis CL,
19Pfundt R, de Leeuw N, Smeets D, Sistermans EA, Wit JM, Hamel BC, Brunner HG, Kooy F,
20Zuffardi O, de Vries BB. 2008. Clinical and molecular characteristics of 1qter microdeletion
21syndrome: delineating a critical region for corpus callosum agenesis/hypogenesis. J Med
22Genet 45:346-54.

1

1

2 LEGENDS TO FIGURES

3

4 **Figure 1.** Facial phenotypes of six patients with interstitial 1q44 deletion showing non-
5 specific craniofacial anomalies.

6

7 **Figure 2. A.** Map of the deletions in chromosomal band 1q44 identified by aCGH. Black
8 horizontal bars indicate the deletions in the 11 patients with ID and seizures. Grey horizontal
9 bar (CNP) indicates the deletion that we have identified in a healthy individual. The RefSeq
10 genes located in the genomic region are indicated. The vertical region shaded in red indicates
11 the smallest region of overlap (SRO) implicated in ID and seizures from our study; in yellow,
12 the SRO implicated in microcephaly; in blue, the SRO implicated in corpus callosum
13 abnormalities. **B.** Detailed map of the proposed critical region for ID and seizures, which
14 contains three candidate genes: *HNRNPU*, *FAM36A* and *NCRNA00201*. The *EFCAB2* gene
15 was considered as unlikely causative since we have identified it in a healthy individual.
16 Horizontal red bars indicate the deletions reported in the Database of Genomic Variants
17 (www.tcag.org). Three variants involving at least one of the three candidate genes (*HNRNPU*,
18 *FAM36A* and *NCRNA00201*) are reported in the Database of Genomic Variants have been
19 identified in three HAPMAP individuals of Yoruba Nigerian origin [Matsuzaki et al., 2009].
20 However, we obtained normal results with our targeted 60K array, excluding a deletion in the
21 1q44 region in these individuals and, thus, demonstrating that the deletions identified by
22 Matsuzaki et al. [2009] were false positive results.

23

Figure 3. Expression patterns of *HNRNPU*, *FAM36A* and *NCRNA00201* in a panel of human tissues.

cDNA were obtained using the MMLV reverse transcriptase (Invitrogen) with random primers from 1 µg of human total RNA of five adult and four fetal tissues. Real-time PCR was performed in triplicates using Takara SYBR premix on Light Cycler 480 (Roche diagnostics). The $\Delta\Delta\text{Ct}$ method was used to assess expression level of three target genes - *HNRNPU*, *FAM36A* and *NCRNA00201* – relative to the expression level of the β -actin (*ACTB*) and cyclophilin E (*PPIE*) housekeeping genes. For a given target gene, the ΔCt of each tissue was compared to the median of the ΔCt of the 6 tissues analyzed.

TABLE I. Clinical features and array CGH data of the 11 patients with 1q44 microdeletion

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Total
Current age (years)	3 ^{10/12}	5 ^{1/12}	4 ^{6/12}	6/12	12 ^{9/12}	2 ^{6/12}	13 ^{6/12}	10 ^{6/12}	17	4 ^{10/12}	9 ^{10/12}	
Coordinates of the deletions (in Mb using hg19 genome build)	243.1-245.4	243.9-246.5	244.3-245.1	244.4-245.3	244.4-245.6	244.5-246.7	244.5-245.4	244.6-246.1	244.7-245.4	244.8-245.5	244.9-246.3	
Size of the deletions 1q44 (Mb)	2.26	2.56	0.79	0.90	1.16	2.19	0.89	1.5	0.68	0.63	1.35	
Parental inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	Mother normal, father NA	de novo	de novo	de novo	10/11
Distinctive facial features												
hypertelorism	-	+	-	-	+	+	+	NA	-	-	-	4/10
bulbous nose	+	+	+	+	-	-	+	NA	-	-	-	5/10
long philtrum	-	-	-	-	-	+	+	NA	-	+	+	4/10
philtrum flat/ absent cupidon bows	+	-	-	-	+	+	-	NA	+	-	+	5/10
thick lips	+	-	-	+	-	+	-	NA	-	-	+	4/10
abnormal ears	-	-	-	+	+	-	-	NA	-	+	+	4/10
flat occiput	-	+	-	-	-	-	-	NA	-	+	+	3/10
Measurement abnormalities (SD)												
IUGR birth weight	-2	-1	-2	+1	NA	0	0	-1	-1,5	-0,5	+0,5	
postnatal growth delay	-1	-3	-3	-1	-1,8	-2,5	-0,5	-1,5	0	-2,5	-0,8	
OFC	-4	-3	-2,6	-1	-0,5	-1,2	-1	1,5	0	0	-1	
BMI	+1	+4	+3	NA	+4	+0,5	+1,5	+3,5	+1,8	+4	+4	
small fingers and/or toes	-	+	+	-	+	+	-	-	-	+	+	6/11
Strabismus	-	NA	+	NA	+	-	-	-	+	+	NA	4/8
Developmental delay/mental retardation												
	severe	severe	severe	severe	severe	moderate	severe	moderate to severe	severe	severe	severe	
age of the sitted station (years)	3	NA	NA	NA	NA	6/12	10/12	11/12	10/12	NA	10/12	
age of walk	not acquired	5 (with support)	3 ^{10/12}	NA	3 ^{10/12}	2	1 ^{9/12}	1 ^{10/12}	2	4 ^{10/12}	2	
no expressive speech	+	-	+	NA	-	-	+	-	+	-	+	5/10
hypotonia	+	NA	+	-	-	+	-	-	+	NA	-	4/9
stereotyped movements of the hands	+	-	+	-	-	-	+	-	+	+	-	5/11
sleep disorders	-	NA	-	NA	-	-	-	-	+	+	-	2/9
autistic features and/or attention deficit disorders	-	NA	-	NA	-	-	+	-	+	+	+	4/9
Epileptic seizures	+	+	+	+	+	+	+	+	+	+	+	11/11
Cerebral MRI												
	generalised atrophy, moderate micropolygyria	micropolygyria, delayed myelinisation	normal	Myelinisation delay and generalised atrophy	normal	small-sized frontal angioma	Cerebellar hypoplasia	moderate cerebellar hypoplasia	delayed myelinisation, moderate subcortical atrophy normal	normal	moderate ventricular dilatation	
corpus callosum	normal	agenesis	normal	normal	normal	normal	normal	normal	normal	normal	normal	
Other features and malformations												
	spaced teeth, sparse hair, vertical striated nails	cardiopathy, scoliosis, dry skin, articular hyperlaxity	articular hyperlaxity	cryptorchidly, mild nail hypoplasia fingers	operated talipes valgus and flat feet	clinodactyly of fifth fingers, butterfly vertebra	erythroderma	cryptorchidly	genu valgum, valgus and flat feet, hyperlordosis, articular hyperlaxity		right renal agenesis	

Supplementary TABLE I. Boundaries and sizes of the deletions identified by array CGH

Patient ID	Last centromeric normal probe		First centromeric deleted probe		Last telomeric deleted probe		First telomeric normal probe		Size of the deletions	
	Agilent probe number	Genomic position	Agilent probe number	Genomic position	Agilent probe number	Genomic position	Agilent probe number	Genomic position	minimum size	maximum size
1	A_18_P10580799	chr1:24312716 2-243127221	A_18_P18165872	chr1:24316902 8-243169078	A_16_P15516910	chr1:24542780 9-245427868	A_16_P56350629	chr1:245428632- 245428691	2258840	2301470
2	A_18_P18170199	chr1:24398142 9-243981487	A_16_P56348234	chr1:24398165 7-243981707	A_18_P18179048	chr1:24655035 8-246550417	A_16_P563519972	chr1:246550861- 246550920	2568760	2569433
3	A_16_P15514696	chr1:24436896 8-244369027	A_16_P00291606	chr1:24436904 0-244369093	A_16_P00292517	chr1:24516281 5-245162874	A_16_P15516265	chr1:245162911- 245162970	793834	793943
4	A_16_P00291762	chr1:24446357 1-244463630	A_18_P18170664	chr1:24446364 4-244463696	A_16_P563500560	chr1:24537234 8-245372405	A_18_P18173616	chr1:245373000- 245373057	908709	909427
5	A_18_P181717703	chr1:24446311 2-244463156	A_18_P18171183	chr1:24446348 3-244463537	A_16_P00293234	chr1:24562809 6-245628148	A_18_P10584150	chr1:245628202- 245628246	1164665	1165090
6	A_16_P15515033	chr1:24451166 4-244511723	A_16_P00291804	chr1:24451217 9-244512226	A_16_P00294898	chr1:24670789 3-246707952	A_16_P15520201	chr1:246707983- 247708042	2195773	2196319
7	A_16_P00291835	chr1:24453568 7-244535746	A_16_P15515090	chr1:24453604 1-244536093	A_16_P56350678	chr1:24546638 1-245466440	A_16_P15516988	chr1:245466879- 245466938	891827	931192
8	A_16_P56349373	chr1:24466880 2-244668861	A_16_P15515367	chr1:24466987 5-244669934	A_18_P10586547	chr1:24617294 8-246173007	A_16_P35539723	chr1:246173423- 246173482	1503132	1504562
9	A_16_P00292062	chr1:24476487 7-244764926	A_16_P00292063	chr1:24476533 0-244765375	A_16_P56350651	chr1:24544326 2-245443321	A_16_P15516929	chr1:245446121- 245446180	677991	681244
10	A_18_P181724450	chr1:24489098 3-244891027	A_18_P18172462	chr1:24489111 7-244891162	A_18_P18175051	chr1:24552646 9-245527024	A_16_P15517164	chr1:245527049- 245527100	635907	636022
11	A_16_P56350022	chr1:24497457 8-244974632	A_16_P56350024	chr1:24497472 1-244974774	A_18_P10588331	chr1:24633261 0-246332669	A_18_P18177727	chr1:246332753- 246332812	1357948	1358234
CNP	A_18_P18171833	chr1:24519752 2-245197581	A_16_P56350270	chr1:24519752 2-245197581	A_16_P15517963	chr1:24582342 0-245823479	A_16_P15517964	chr1:245823589- 245823648	625957	626008

Figure 1



Patient 1



Patient 3



Patient 4



Patient 5

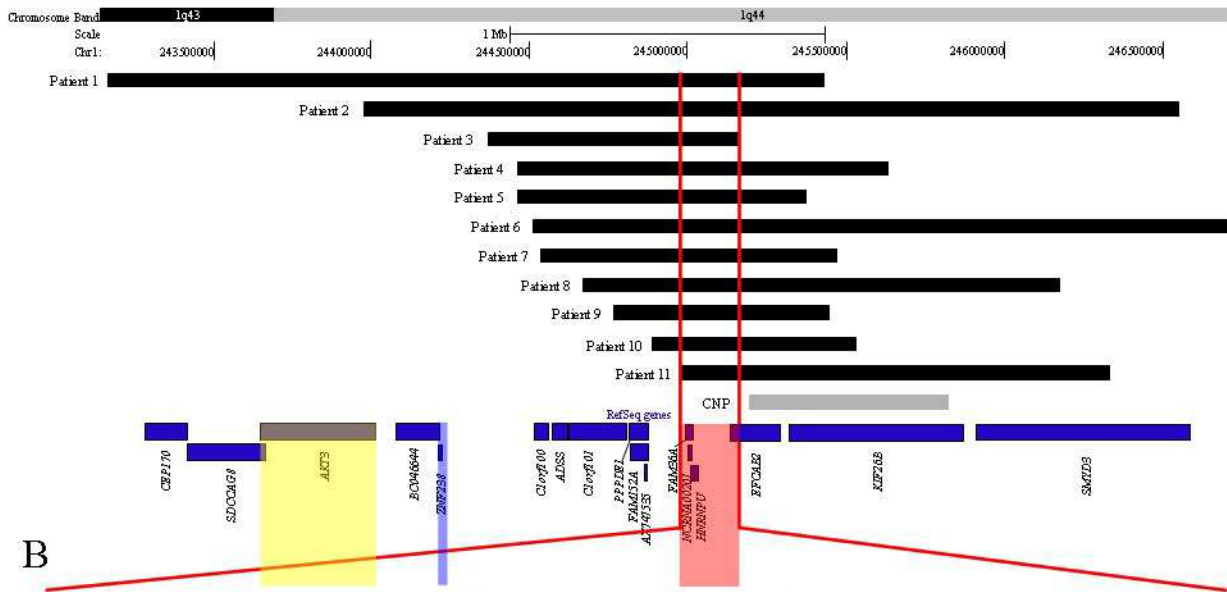


Patient 6



Patient 10

A



B

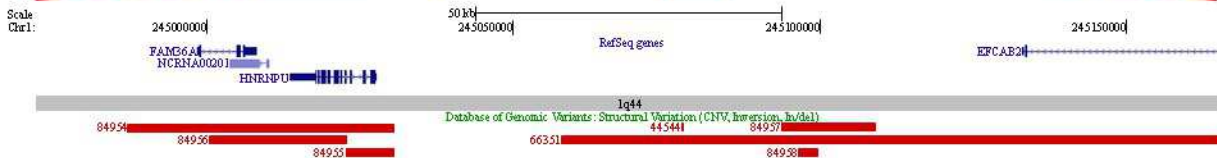


Figure 3

