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

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

ABSTRACT

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

INTRODUCTION

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

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

the combined data from two recent studies, a first one performed on 22 patients [Ballif et al., 2011] and a second one based on 7 patients [Nagamani et al., 2012] sharing 1q43q44 microdeletion, proposed three distinct SRO with different sizes implicated in corpus callosum abnormalities (75 kb in size, including *ZNF238*), microcephaly (133 kb in size, including *AKT3*) or seizures (100 kb in size, including *HNRNPU*, *FAM36A* and *NCRNA00201* previously referred as *C1ORF199*). We focused our study on 11 unrelated patients with ID and seizures carrying a 1q44 interstitial microdeletion. We refined the SRO for ID and seizures to three genes and explored each of these three genes to highlight their potential role played in the phenotype.

PATIENTS AND METHODS

Patients

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

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

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

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

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

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

Cytogenetic and aCGH studies

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

mRNA expression studies

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

performed using the ΔΔCt method [Livak et al., 2001] to assess expression level of the 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.

Mutation screening

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

RESULTS

Cytogenetic and aCGH results

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

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

mRNA expression studies

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

Mutation screening

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

DISCUSSION

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

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

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

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

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

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

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

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

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CONFLICT OF INTEREST

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2The authors declare no conflict of interest.

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LEGENDS TO FIGURES

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

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

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 ΔΔCt 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	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	cryptorchidy, mild nail hypoplasia fingers	operated talipes valgus and flat feet	clinodactyly of fifth fingers, butterfly vertebra	erythroderma	cryptorchidy	genu valgum, valgus and flat feet, hyperlordosis, articular hyperlaxity		right renal agenesis	

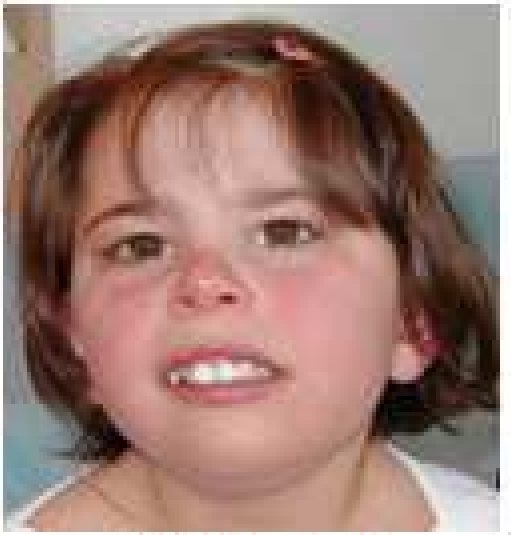
Supplementary TABLE I. Boundaries and sizes of the deletions identified by array CGH

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	chr1:243127162-243127221	A_18_P18165872	chr1:243169028-243169078	A_16_P15516910	chr1:245427809-245427868	A_16_P56350629	chr1:245428632-245428691	2258840	2301470
2	A_18_P18170199	chr1:243981429-243981487	A_16_P56348234	chr1:243981657-243981707	A_18_P18179048	chr1:246550358-246550417	A_16_P563519972	chr1:246550861-246550920	2568760	2569433
3	A_16_P15514696	chr1:244368968-244369027	A_16_P00291606	chr1:244369040-244369093	A_16_P00292517	chr1:245162815-245162874	A_16_P15516265	chr1:245162911-245162970	793834	793943
4	A_16_P00291762	chr1:244463571-244463630	A_18_P18170664	chr1:244463644-244463696	A_16_P563500560	chr1:245372348-245372405	A_18_P18173616	chr1:245373000-245373057	908709	909427
5	A_18_P181717703	chr1:244463112-244463156	A_18_P18171183	chr1:244463483-244463537	A_16_P00293234	chr1:245628096-245628148	A_18_P10584150	chr1:245628202-245628246	1164665	1165090
6	A_16_P15515033	chr1:244511664-244511723	A_16_P00291804	chr1:244512179-244512226	A_16_P00294898	chr1:246707893-246707952	A_16_P15520201	chr1:246707983-247708042	2195773	2196319
7	A_16_P00291835	chr1:244535687-244535746	A_16_P15515090	chr1:244536041-244536093	A_16_P56350678	chr1:245466381-245466440	A_16_P15516988	chr1:245466879-245466938	891827	931192
8	A_16_P56349373	chr1:244668802-244668861	A_16_P15515367	chr1:244669875-244669934	A_18_P10586547	chr1:246173948-246173007	A_16_P35539723	chr1:246173423-246173482	1503132	1504562
9	A_16_P00292062	chr1:244764867-244764926	A_16_P00292063	chr1:244765330-244765375	A_16_P56350651	chr1:245443262-245443321	A_16_P15516929	chr1:245446121-245446180	677991	681244
10	A_18_P181724450	chr1:244890983-244891027	A_18_P18172462	chr1:244891117-244891162	A_18_P18175051	chr1:245527469-245527024	A_16_P15517164	chr1:245527049-245527100	635907	636022
11	A_16_P56350022	chr1:244974578-244974632	A_16_P56350024	chr1:244974721-244974774	A_18_P10588331	chr1:246332610-246332669	A_18_P18177727	chr1:246332753-246332812	1357948	1358234
CNP	A_18_P18171833	chr1:245197522-245197581	A_16_P56350270	chr1:245197522-245197581	A_16_P15517963	chr1:245823420-245823479	A_16_P15517964	chr1:245823589-245823648	625957	626008

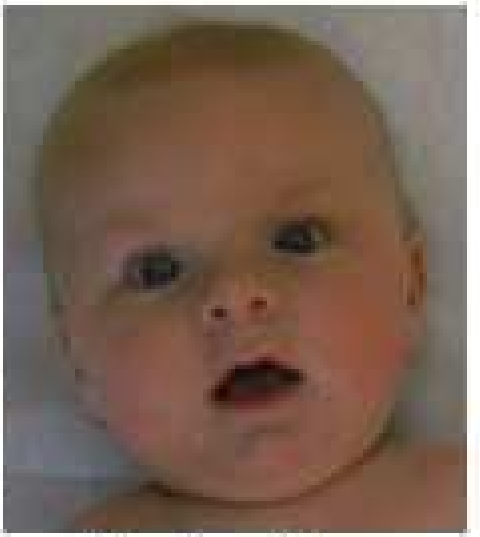
Figure 1



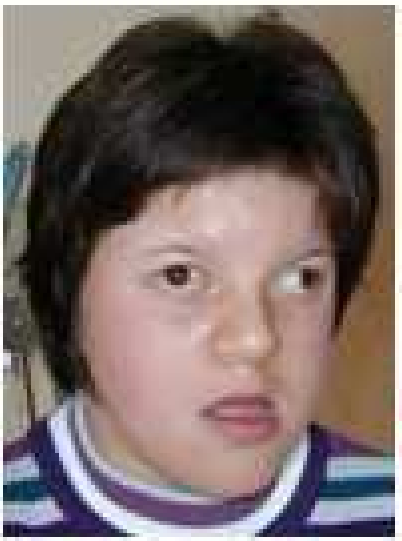
Patient 1



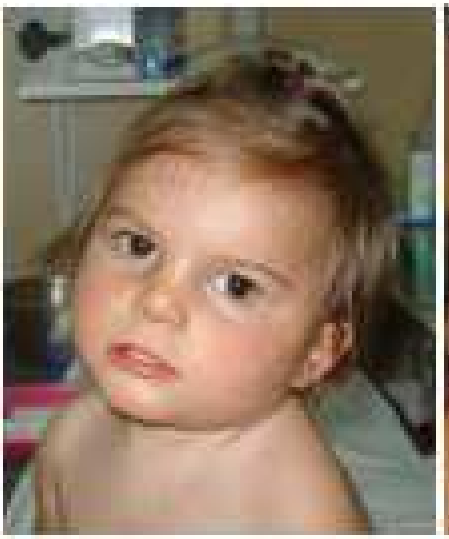
Patient 3



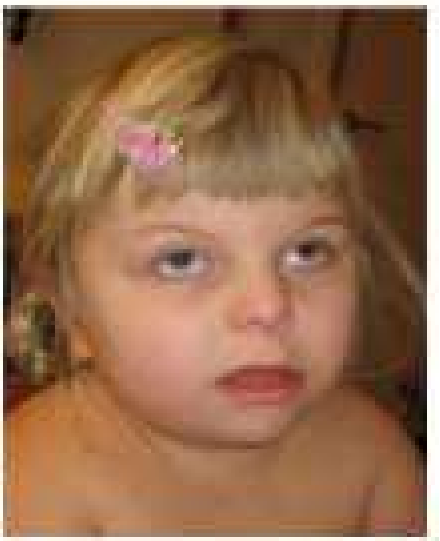
Patient 4



Patient 5



Patient 6



Patient 10

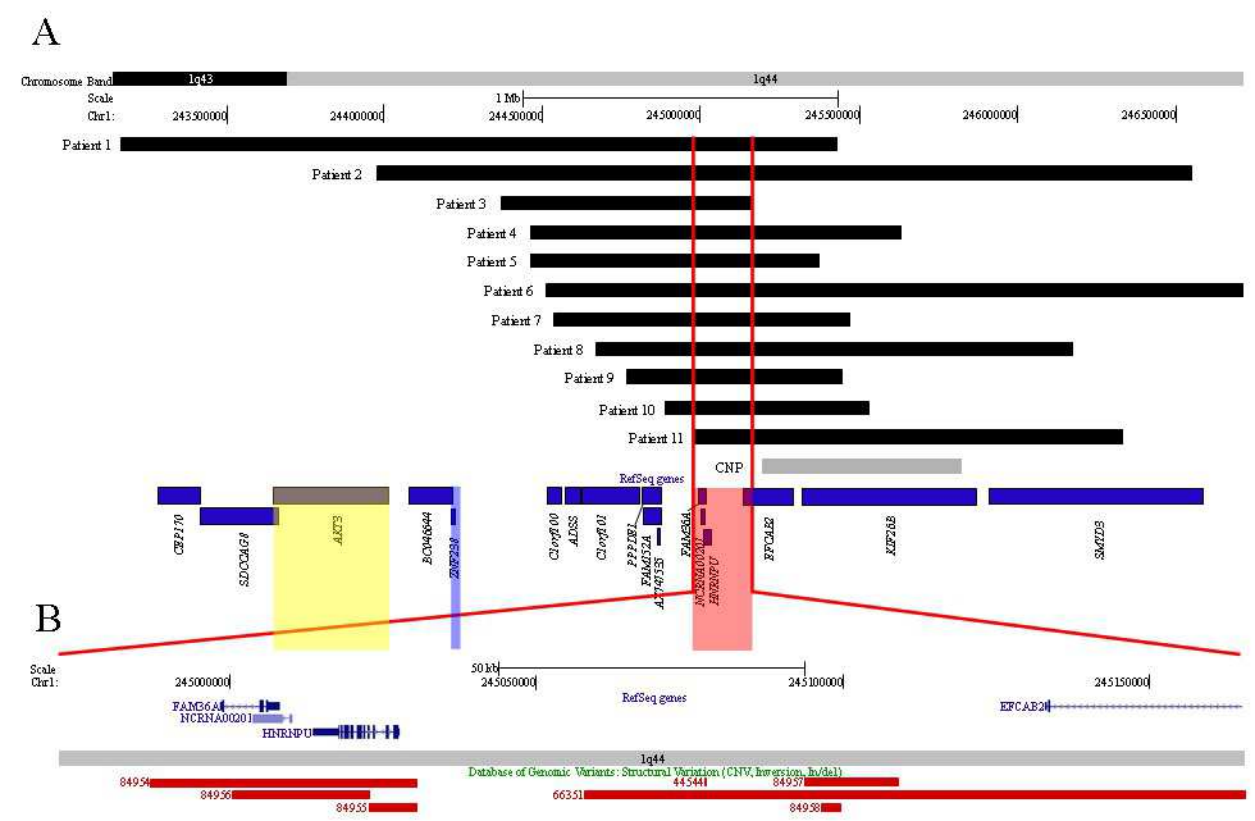
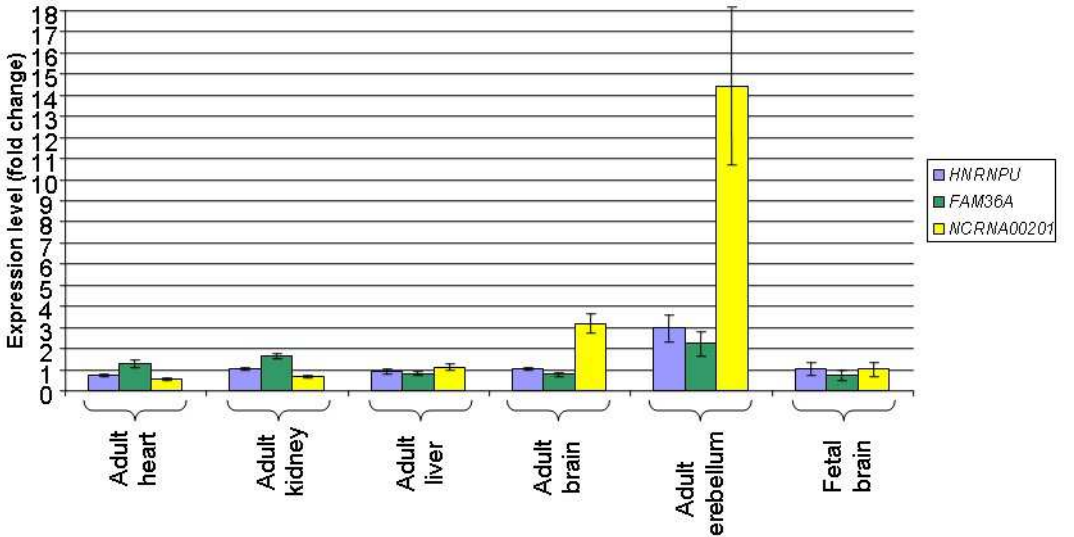

A
Chromosome Band
1q43
1q44
Scale
Chr1:
1 Mb
243500000
244000000
244500000
245000000
245500000
246000000
246500000
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
Patient 9
Patient 10
Patient 11
CNP
RefSeq genes
CEP170
SDCCAG8
AKT3
BC040644
ZNF238
C1orf100
ADSS
C1orf101
PPP1R1
FAM52A
AK074335
FAM36A
NCRNA00201
HNRNPU
EFCAB2
KIF26B
SMYD3
B
Scale
Chr1:
50 kb
245000000
245050000
245100000
245150000
RefSeq genes
FAM36A
NCRNA00201
HNRNPU
EFCAB2
1q44
Database of Genomic Variants: Structural Variation (CNV, Inversion, In/del)
849547
445441
849548
663511
849549
849550

Figure 3


Expression level (fold change)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
HNRNPU
FAM36A
NCRNA00201
Adult heart
Adult kidney
Adult liver
Adult brain
Adult cerebellum
Fetal brain