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ARTICLE

A novel microdeletion syndrome involving 5q14.3-q15: clinical and molecular cytogenetic characterization of three patients

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Molecular karyotyping is being increasingly applied to delineate novel disease causing microaberrations and related syndromes in patients with mental retardation of unknown aetiology. We report on three unrelated patients with overlapping *de novo* interstitial microdeletions involving 5q14.3-q15. All three patients presented with severe psychomotor retardation, epilepsy or febrile seizures, muscular hypotonia and variable brain and minor anomalies. Molecular karyotyping revealed three overlapping microdeletions measuring 5.7, 3.9 and 3.6 Mb, respectively. The microdeletions were identified using single nucleotide polymorphism (SNP) arrays (Affymetrix 100K and Illumina 550K) and array comparative genomic hybridization (1 Mb Sanger array-CGH). Confirmation and segregation studies were performed using fluorescence *in situ* hybridization (FISH) and quantitative PCR. All three aberrations were confirmed and proven to have occurred *de novo*. The boundaries and sizes of the deletions in the three patients were different, but an overlapping region of around 1.6 Mb in 5q14.3 was defined. It included five genes: *CETN3*, *AC093510.2*, *POLR3G*, *LYSMD3* and the proximal part of *GPR98/MASS1*, a known epilepsy gene. Haploinsufficiency of *GPR98/MASS1* is probably responsible for the seizure phenotype in our patients. At least one other gene contained in the commonly deleted region, *LYSMD3*, shows a high level of central nervous expression during embryogenesis and is also, therefore, a good candidate gene for other central nervous system (CNS) symptoms, such as psychomotor retardation, brain anomalies and muscular hypotonia of the 5q14.3 microdeletion syndrome.
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Introduction

Molecular karyotyping by single nucleotide polymorphism (SNP) arrays and array-CGH allows high-resolution screening of the whole human genome for DNA copy number changes. When applied to cohorts of patients with mental

retardation (MR) and dysmorphic features, this approach detects causative microdeletions and microduplications in 10–15% of the patients, most of which are scattered across the genome.[1–3] For newly described copy number changes, *de novo* origin *versus* inheritance from an unaffected parent is used to help to differentiate between causative copy number changes and benign variants that affect more than 12% of the human genome.[4] Causative familial copy number changes with reduced penetrance have also been described, however.[2,5,6] Several patients with similar copy number changes and a similar clinical presentation are, therefore, needed to allow reliable genotype–phenotype correlation. Recently, some recurrent rearrangements have been described for, amongst others, chromosomal regions 1q21.1,[5] 1q41q42,[7] 2p15p16.1,[8] 16p13.11,[9,10] 18q21[11–13] and 17q21.31.[14–16]

Here, we report on three unrelated patients with overlapping microdeletions in chromosomal region 5q14.3-q15. After diagnosis of patient 3, a 'call for patients' within the German MRNET yielded patient 1. Patient 2 was then ascertained through the DECIPHER database (https://decipher.sanger.ac.uk). Clinical similarities between all three patients include severe MR, epilepsy and/or febrile seizures, and muscular hypotonia. In addition to the clinical characterization of the new syndrome, we delineate a critical region, presumed to contain haploinsufficient genes, that is involved in the aetiology of the observed overlapping phenotypic features.

Case reports

Ethical approval for this study was obtained from the ethics committees of all participating centres and informed consent for genetic investigations into mental retardation was obtained from participating families.

Patient 1

The female proband was the second of three children born to a healthy non-consanguineous couple of Turkish origin. The girl was born 11 days postterm following an uneventful pregnancy. At birth, the umbilical cord was wrapped around the neck. Her birthweight was 3420 g (25–50th centile/−0.37 SDS), length was 53 cm (50–75th centile/+0.36 SDS) and OFC was 38 cm (>97th centile/+2.15 SDS). Apgar scores were 9, 9, 10 and cord pH was 7.27. Nine hours after birth, the child was referred to the paediatric intensive care ward with muscular hypotonia and tachydyspnoea, where she subsequently developed infantile spasms. Cerebral MRI studies showed aplasia of the cerebellar vermis and posterior part of the corpus callosum, multiple plexus cysts and enlarged occipital horns of the lateral ventricles. Chromosome studies, screening for perinatal infections and metabolic disorders and ophthalmological investigation revealed no abnormalities. Extensive EEG studies at the age of 4 months showed hypsarrhythmia, which was unresponsive to ACTH therapy but a combination of ACTH and vigabatrin reduced the frequency of myoclonic seizures although rolling eye movements persisted. Echocardiography showed concentric myocardial hypertrophy (ventricular septum 6–7 mm, left ventricular posterior wall 6 mm). X-ray studies showed incomplete closure of thoracic vertebral arches Th2–Th10. Chronic constipation and increased sweating was noted. The child showed no psychomotor development. Due to the clinical suspicion of Aicardi syndrome, ophthalmological investigation was repeated at the age of 3 years, but revealed only simple bilateral optic atrophy. At the age of 6 years, the girl was without anticonvulsant medication and presented with occasional seizures, truncal hypotonia and bilateral pes equines. Her psychomotor development was severely retarded and still comparable to that of a neonate. She was fed with pureed food only, had normal measurements (length: 111 cm (10th centile/−1.1 SDS), OFC: 52.5 cm (90–97th centile/+1.1 SDS)) and suffered from frequent upper respiratory tract infections. She showed no facial dysmorphism, but her ears were relatively large and her eyebrows were relatively broad. She had bilateral transverse palmar creases, short halluces and three café-au-lait spots (one spot of 0.5×1.5 cm on the back, two spots of 2×3 cm in the inguinal region). This patient was mentioned within a larger series previously (patient 77).[3]

Patient 2

Patient 2 was born following a pregnancy in which a markedly increased nuchal translucency measurement had been noted. A CVS revealed a normal female karyotype and fetal anomaly ultrasound imaging was normal. She was born at 40 weeks gestation weighing 3300 g (25–50th centile/−0.41 SDS) with Apgar scores of 8,[1] 9.[5] There were severe feeding difficulties postnatally with a poor suckling ability and frequent vomiting and she failed to thrive. Her development was very delayed with a social smile from 5 months and truncal hypotonia. When reviewed at the age of 7 years she was attending a school for children with special educational needs. She was not yet able to sit independently but had recently begun to babble. Her overall developmental level was equivalent to a child of approximately 6 months. She had a history of several febrile seizures and was maintained on anticonvulsants although she had been seizure free for the previous 3 years. She had a striking visual preoccupation with stripes. She had marked brachycephaly with a low anterior hairline. Facial features included downslanting palpebral fissures and a philtral haemangioma, which had been present since birth (Figure 1f and g). Her growth parameters at the age of 8 years were as follows: height, 120.4 cm (9th centile/−1.58 SDS); weight, 21.3 kg (2nd–9th centile/BMI 14.7); OFC, 51.3 cm (2nd–9th centile/−0.38 SDS).

A cranial MRI scan in infancy showed prominence of the arachnoid spaces in the perivascular areas. An EEG showed

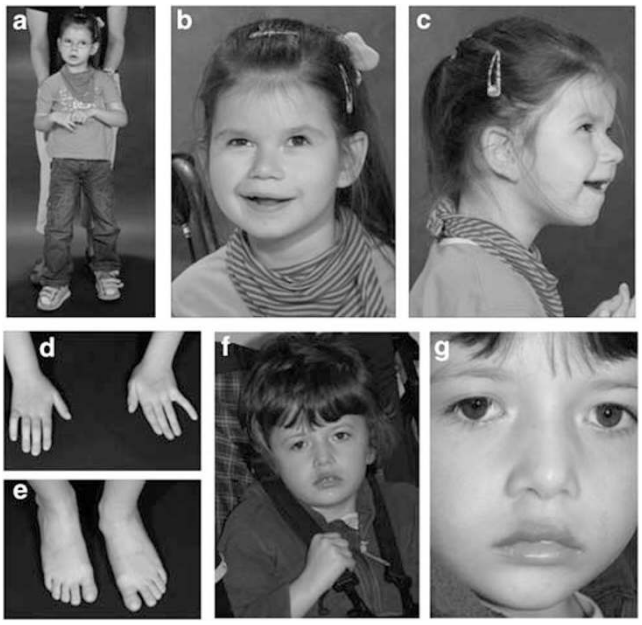

Figure 1 Variable mild dysmorphisms of patients 2 and 3. (**a**–**e**) Patient 3 at the age of 7 years showing minor anomalies, such as relatively large ears, and long and slender fingers. The seemingly narrowed right palpebral fissure is due to the patient's facial expression and thus not representative. (**f**, **g**) Patient 2 at the age of 5 years showing mildly downslanting palpebral fissures, downturned corners of the mouth and philtral haemangioma. Consent to the publication of clinical pictures of patient 1 could not be obtained.

high amplitude prominent rhythmical activity in the temporal regions with generalized bursts of spike and slow waves accompanied by some myoclonic jerking of the arms.

Patient 3

Patient 3 is a girl, the third-born child of non-consanguineous German parents with an unremarkable family history. After an uneventful pregnancy, she was born at term with normal measurements (weight: 3510 g (50th centile/ + 0.09 SDS), length: 51 cm (25th–50th centile/ −0.32 SDS), OFC: 35 cm (50th centile/ + 0.08 SDS)). Apgar scores were 9, 9, 10. No malformations or other abnormalities were apparent. According to her mother, she slept much more than her healthy brothers had done during the first months of life and she was very sensitive to noise. At the age of 4 months, psychomotor delay and severe muscular hypotonia were noted by a paediatrician. Her developmental milestones were delayed (head control: later than 1 year of age, unsupported sitting approximately at 2.5 years of age, no unsupported walking at the age of 7 years). She began uttering syllables (mamama, papapa) at the age of 3 years. At the age of 6 years, she had learned to say 'mama' in a directed fashion. Her language comprehension was reported as being better, and she could express basic needs using an electronic speaking aid.

At the age of 5 months, hyperopia (+ 5.5 dpt at the age of 5 years) and strabismus convergens were diagnosed. An EEG at the age of 9 months revealed no anomalies. Cranial MRIs (at 6 months and 2 years 3 months) demonstrated moderate atrophy of the supra- and infratentorial region, a slightly enlarged ventricular system and unspecific leucoencephalopathy. At the age of 4 years 9 months, the first atypical absences occurred. An EEG documented short focal seizures accompanied by atypical absences. Treatment with oxacarbazepine commenced. Later, complex partial seizures were reported. At the age of 5 years 3 months, one grand mal seizure occurred.

At the time of our first evaluation at the age of 5 years 3 months, she presented with severe MR and global developmental delay. Social interactions were very limited. Formal neuropsychological testing was not possible. Neurological examination revealed muscular hypotonia predominantly of the trunk. She could neither roll from her back to her front, nor move without support, although used her wheelchair adroitly.

On our second examination at 6 years 9 months of age, only mild dysmorphisms, such as simple ears, a slightly narrowed supraorbital region and slightly upslanting palpebral fissures were noted (Figure 1a–e). At the time of this examination, her growth parameters were as follows: height, 115 cm (10th centile/−1.53 SDS); weight, 21 kg (25th centile); OFC, 49.5 cm (10th–25th centile/ −1.61 SDS). Her mouth was frequently open and she hypersalivated. Hypotonia of the pharyngeal muscles had led to feeding problems and her diet, therefore, included only fluids or pureed food. She displayed teeth grinding and stereotypic dyskinetic hand movements. Her seizure medication had been changed to a combination of oxacarbazepine and levitiracetam. An EEG showed no epileptiform activity although short absence-like seizures and tonic seizures still occurred. Echocardiography and physical examination revealed no evidence of malformations. Genetic analyses with normal results included chromosome analyses (400–500 band level), Angelman syndrome and Rett syndrome testing.

Material and methods

Patient 1

Molecular karyotyping using a GeneChip Human Mapping 100K SNP array (Affymetrix) was performed as previously described. [3] This patient was mentioned within a larger series previously ([3] patient 77). The deletion was confirmed by two-colour FISH with locus-specific probe RP11-88D22 and a subtelomeric control probe on lymphocyte metaphase spreads of the patient and her parents as described previously.[3]

Patient 2

BAC array analysis using the Sanger 1 Mb array was performed as previously described.[17] FISH was performed

with multiple tile path RPCI11 clones obtained from BAC PAC resources (http://bacpac.chori.org) directly labelled with either Vysis Spectrum Orange-dUTP or Spectrum Green-dUTP (Vysis) as previously described[17] to size the deletion in the proband and to screen the parents.

Patient 3

Genomic DNA of Patient 3 was analysed using an Illumina Sentrix HumanHap 550-Duo v3Beadchip. Quality control demonstrated a call rate of 99.6%. Putative CNVs were identified using the QuantiSNP algorithm.[18] In brief, QuantiSNP is a Hidden-Markov Model-based algorithm for high-resolution CNV detection that uses log2 R ratio (LRR) values (a normalized measure of the signal intensity for each SNP) and B allele frequency (BAF) values (an allelic intensity ratio for each SNP) to generate CNV calls. As a measure of confidence, a log Bayes factor is computed for each CNV. We computed LRR and BAF values with the Beadstudio v3.1.3.0 genotyping module (Illumina Inc.) and analysed these with QuantiSNP using default settings. We discarded all CNV calls with a log Bayes factor value below 7, containing less than 5 SNPs or a genomic size below 10 kb. Putative CNVs were disregarded if they were covered entirely by known variants according to the Database of Genomic Variants (http://projects.tcag.ca/variation/) or contained no known genes. For qPCR analyses, we designed 3 amplicons localized in the coding regions of GPR98/*MASS1*, *CETN3* and *LYSMD3*. qPCR was performed on an ABI Prism 7900HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) using SYBR Green for detection. Reaction mixtures contained 0.2 μM of each primer and 5 μl of Power SYBR Green PCR Master Mix (Applied Biosystems). Each assay included DNA from four control persons and the patient DNA at a final concentration of 20 ng/μl in triplicate (total volume: 10 μl). The cycling conditions were as follows: initiation, 50°C for 2 min; denaturation, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and a combined annealing and extension step at 60°C for 60 s. To exclude the presence of unspecific products, a melting-curve analysis of the products was performed. The threshold cycles (C_t) values were normalized using the C_t value of three reference genes (*BNC1*, *CFTR* and *RNAseP* subunit p38) for each DNA sample. Relative quantification was performed by use of the comparative C_t method. Additionally, qPCR screening for the microdeletion was performed on the genomic DNA of 57 patients with MR and an overlapping clinical spectrum, for example, (febrile) seizures/pathological EEG, pathological cranial MRI, muscular hypotonia, autistic behaviour or stereotypies. FISH was performed using BlueFISH tile BAC probes RP11-12D3 and RP11-29K14 from the deleted region according to manufacturer's instructions (BlueGnome Ltd, Cambridge, United Kingdom) and as published previously.[2]

Results

Patient 1

Molecular karyotyping using a GeneChip Human Mapping 100K SNP array (Affymetrix) revealed a 5.69Mb deletion of 5q14.3-q15 containing 234 SNP probes (start SNP: rs10514301, genomic position/NCBI assembly 36: bp 87 975 410; end SNP rs9314105, bp 93 668 872; Figure 2a). FISH with BAC probe RP11-88D22 from the deleted region in patient 1 on metaphase preparations of the patient and her parents confirmed the *de novo* occurrence of the deletion (Figure 2d). The patient's karyotype according to ISCN 2005 is arr cgh 5q14.3q15(rs10514301 → rs9314105) × 1 dn.

Patient 2

BAC array analysis using the Sanger 1 Mb array showed a deletion of 5 clones localized in 5q14.3. (start clone: RP11-72L22, midpoint genomic position 86.4 Mb; end clone RP11-302L17, midpoint genomic position: 89.6 Mb; Figure 2c). FISH on lymphocyte metaphase preparations of the patient and her parents confirmed the *de novo* occurrence of the deletion and was used to define the breakpoints in the proband (Figure 2e). At the centromeric end the normal flanking clone was RP11-265O6 (bp 85 851 532 – bp 85 988 261) and the first deleted clone was RP11-291O24 (bp 86 206 067 – bp 86 341 427). At the telomeric end, the last deleted clone was RP11-62E10 (bp 90 044 836 – bp 90 139 366) and the normal flanking clone was RP11-351K10 (bp 90 139 366 – bp 90 265 224). The deletion size determined by the distance of the genomic end points of the deleted clones RP11-291O24 and RP11-62E10 was 3.93 Mb and the patient's karyotype is arr cgh 5q14.3(RP11-291O24 → RP11-62E10) × 1 dn.

Patient 3

Illumina HumanHap 550 BeadChip analysis revealed a 3.574 Mb heterozygous deletion containing 524 SNPs in 5q14.3-q15 (start SNP: rs10223241, genomic position: bp 88,448,144; end SNP rs17664587, bp 92 022 455; Figure 2b). qPCR analyses of patient 3 and her parents confirmed the deletion in patient 3 and demonstrated its *de novo* occurrence. qPCR screening for the microdeletion performed on the genomic DNA of 57 additional patients with an overlapping clinical spectrum (eg, MR and seizures, muscular hypotonia or stereotypies) identified no additional microdeletions.

FISH with two BAC probes from the region deleted in patient 3 (RP11-12D3, RP11-29K14) performed on lymphocyte metaphase preparations of the patient and her parents confirmed the *de novo* occurrence of the deletion (Figure 2f). The patient's karyotype is arr cgh 5q14.3q15 (rs10223241 → rs17664587) × 1 dn.

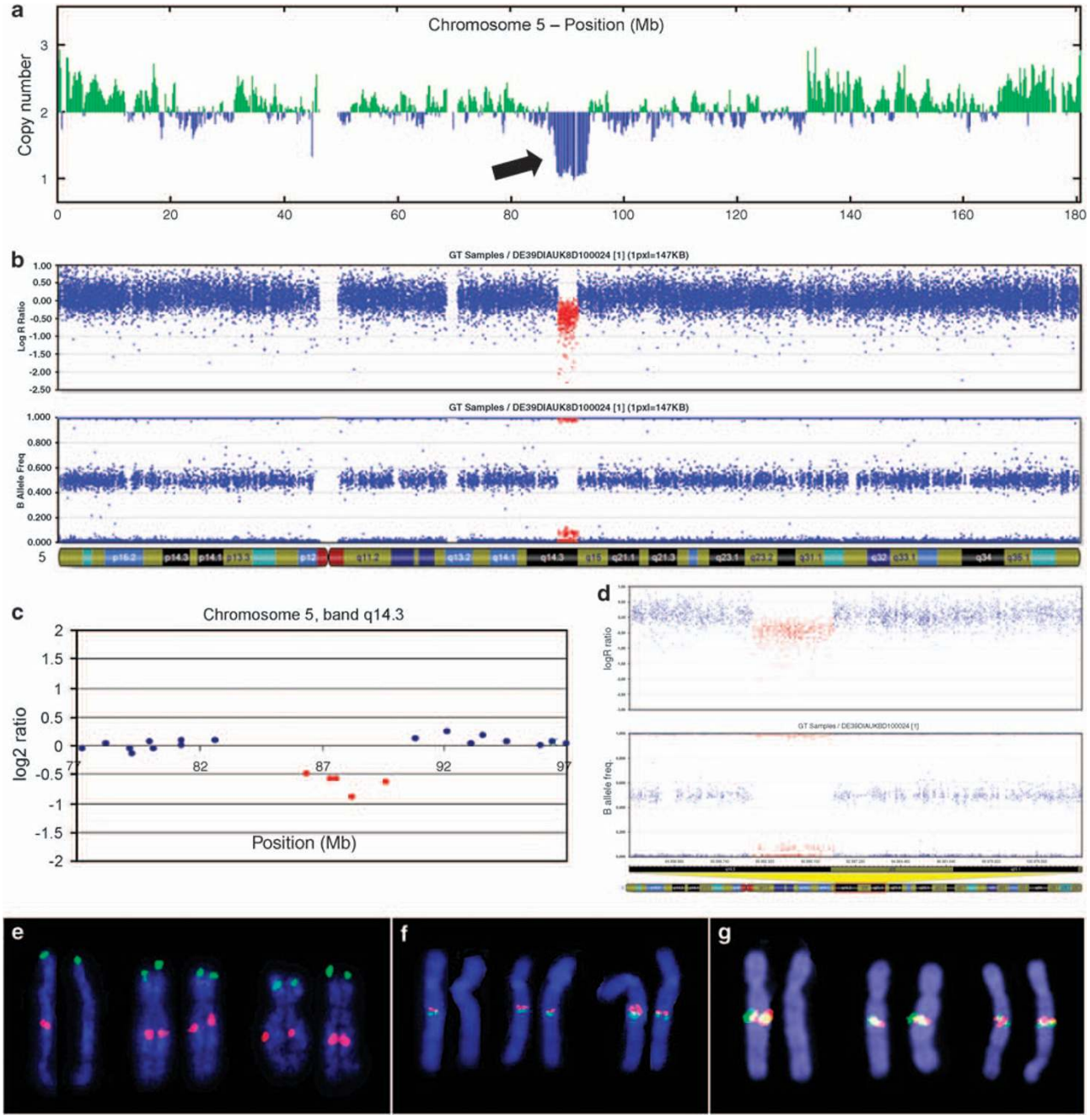

Figure 2 Summary of the molecular karyotyping and FISH results in patients 1, 2, and 3. (**a**) Patient 1: Affymetrix GeneChip 100K Mapping SNP array copy number plot of the entire chromosome 5 showing the 5.6 Mb deletion in 5q14.3–q15 containing 234 SNPs. (**b**) Patient 3: Illumina HumanHap 550 BeadChip hybridisation plot of chromosome 5 with a 3.6 Mb deletion involving 5q14.3–q15 and containing 524 SNPs. (**c**) Patient 2: Partial chromosome 5 plot (Sanger 1 Mb array) of the deletion in 5q14.3 containing 5 clones. (**d**) Patient 3: Partial chromosome 5 plot of the deletion 5q14.3–q15 and containing 524 SNPs. (**e**) Patient 1: Representative FISH results of clone RP11-88D22 (red) and subtelomeric control clone (green) in the patient (left), her mother (middle) and her father (right). (**f**) Patient 2: Representative FISH results of clones RP11-291O24 (red) and RP11-62E10 (green) in the patient (left), her mother (middle) and her father (right). (**g**) Patient 3: Representative FISH results of clones RP11-12D3 (red) and RP11-29K14 (green) in the patient (left), her mother (middle) and her father (right). In all cases, the deletions were confirmed by FISH and found to be of *de novo* origin.

Commonly deleted region in patients 1–3

The region of overlap of all three deletions encompasses 1.64 Mb in 5q14.3 (from genomic position 88.45Mb to 90.09 Mb). It contains five genes (*CETN3*, *AC093510.2*, *POLR3G*, *LYSMD3* and the proximal part of *GPR98/MASS1*) and a pseudogene (Figure 3). There were no gene interruptions at any of the six breakpoints.

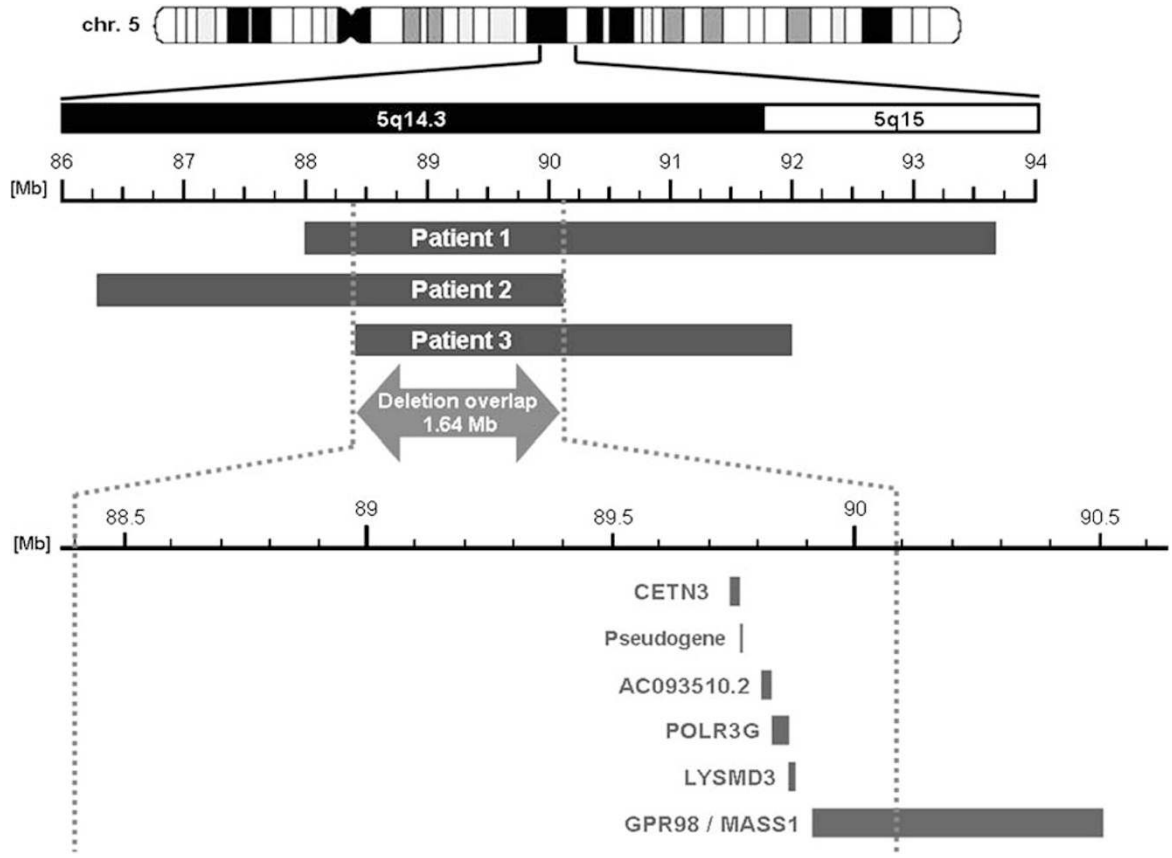

Figure 3 Schematic representation of the identified deletions involving 5q14.3-q15 in patients 1–3 (middle) and the genomic region of overlap with its gene content (bottom).

Discussion

We describe a new microdeletion syndrome caused by microdeletions within chromosomal bands 5q14.3-q15, which overlap in 5q14.3. All three patients display a common phenotype comprised of severe MR with an absence of speech and walking abilities, muscular hypotonia, seizure disorders and variable brain anomalies (Table 1). There was no consistent or striking facial dysmorphism; but mild anomalies.

The region commonly deleted in all three patients within 5q14.3 is approximately 1.64 Mb in size. It contains only five genes: *CETN3* (Centrin-3), *AC093510.2*, *POLR3G* (polymerase (RNA) III (DNA directed) polypeptide G), *LYSMD3* (LysM, putative peptidoglycan-binding, domain containing 3) and the proximal part of *GPR98/MASS1* (G-protein coupled receptor 98 precursor/monogenic audiogenic seizure susceptibility protein 1 homologue). The proximal 1.28 Mb of the common deleted region contain no known genes. However, they contain several regions with medium to high conservation, which may be, for example, regulatory elements of adjacent, non-deleted genes and thus may play an aetiological role. Three of the genes of the common deleted region have an unspecific (*POLR3G*, *CETN3*) or as yet unknown (*AC093510.2*) expression pattern. *POLR3G and CETN3* are expressed only weakly in murine brain[19] However, two of the genes in the minimally deleted region are good candidates for the CNS phenotype of the 5q14.3 microdeletion syndrome patients. GPR98/Mass1 is highly expressed in the developing CNS of zebrafish embryos, particularly in the hypothalamus, epiphysis and the rhombic lips.[20] Mouse embryos show a high level of expression restricted to the eyes and the developing CNS, particularly in the ventricular zone, home of neural progenitor cells during embryonal neurogenesis.[21] *GPR98/MASS1,* a known epilepsy gene, contains both the epilepsy-associated repeat (EAR) and the epitempin (EPTP) repeat.[22] *GPR98/MASS1* knockout mice show audiogenic seizure susceptibility.[23] Loss of function mutations of *GPR98/MASS1* have been demonstrated in an autosomal-dominant family with familial febrile seizures.[24] All affected family members had normal mental and motor development and a brain MRI showed no abnormal findings.

LysmD3 is a potential single-pass membrane glycoprotein containing a LysM domain, which has been proposed as being a general peptidoglycan-binding module. LysmD3 shows an expression pattern concordant with CNS phenotypes. In early zebrafish embryos, it is expressed in the notochord, which serves both as the major skeletal

Table 1 Phenotype of patients 1, 2 and 3 carrying microdeletions of 5q14.3-q15

Features	*Patient 1*	*Patient 2*	*Patient 3*
Severe mental retardation	+	+	+
Muscular hypotonia with feeding difficulties	+	+	+
(Febrile) seizures	+	+	+
Cranial MRI findings	Aplasia of cerebellar vermis and posterior corpus callosum, multiple plexus cysts	Prominence of the arachnoid spaces in the perivascular areas	Atrophy of the supra- and infratentorial region, mildly enlarged ventricular region, unspecific leucoencephalopathy
Optic atrophy	+	−	−
Hyperopia and strabismus convergens	−	−	+
Myocardial hypertrophy	+	−	−
Transverse palmar creases	+	−	−
Long fingers	+	−	+
Café-au-lait spots	+	−	−
Brachycephaly	−	+	−
Low anterior hairline	−	+	−
Facial dysmorphism	−	mild	mild
Large ears	+	−	+
Visual preoccupation with stripes	−	+	−
Sensitivity to noise	−	−	+
Frequent infections	+	−	−
Chronic constipation	+	−	−
Foetal increased nuchal translucency	n.n.	+	n.n.

n.n.: not noted.

element of the embryo and as a signalling source for the pattern formation within the neurectoderm, the paraxial mesoderm and other tissues. In later stages, high level expression of LysmD3 seems to be restricted to the CNS [25–27] However, none of the four members of the LysmD family have been implied in human disease and no data from knockout models are available.

Haploinsufficiency of *GPR98/MASS1* is the probable cause for the seizure phenotype in the 5q14.3 microdeletion syndrome patients presented here, because loss of function mutations have already been shown to cause seizures in humans.[24] However, the carriers of these mutations had a normal psychomotor development and no abnormal findings on brain MRI. It may be hypothesized that haploinsufficiency of other genes within the *de novo* deletions is the cause for the additional CNS symptoms of the microdeletion patients, such as mental retardation and variable structural brain anomalies. Given its embryonic pattern of expression in animal models, *LYSMD3* may be one of those haploinsufficient genes.

MEF2C is deleted in patients 1 and 2 only. It is also expressed in the brain, especially the cerebral cortex,[19] and is involved in myogenesis, which may explain the more severe hypotonia and retardation observed in these two patients. Overexpression of Mef2c in mouse cardiomyocytes has been observed to cause cardiomyopathy.[28] Interestingly, patient 1 presents with concentric myocardial hypertrophy and one copy of *MEF2C* is deleted. The unusual MRI anomalies found in the latter patient might be related to the additional deletion of *NR2F1* (nuclear receptor subfamily 2, group F, member 1), which has been shown to be involved in CNS development[29] and is strongly expressed in the murine cerebral cortex.[19] Patient 2 also displays *RASA1* deletion. Loss of function mutations in this gene, which is also expressed in brain, are related to capillary and arteriovenous malformation[30] and deletion of this gene may underlie her philtral haemangioma (Figure 1g).

Our findings demonstrate a novel genomic disorder characterized by microdeletions encompassing a shortest region of overlap of 1.6 Mb within 5q14.3 and a common clinical phenotype with severe MR, muscular hypotonia, seizures and variable brain and minor anomalies. Haploinsufficiency of one or more of the five genes contained in the commonly deleted region is proposed as the cause of the phenotype. *GPR98/MASS1* and *LYSMD3* in particular are candidate genes for seizures, MR and other CNS symptoms. This report also illustrates the power of DECIPHER as well as of national networks, such as the 'German Mental Retardation Network' (MRNET), in enabling clinical geneticists and molecular cytogeneticists from different departments to share information about rare patients to define critical intervals and evaluate their gene content and thus determine the phenotypic consequences of genomic array findings.

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