5q31.3 Microdeletion Syndrome: Clinical and Molecular Characterization of Two Further Cases

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Manuscript Received: 25 February 2013; Manuscript Accepted: 16 May 2013

The 5q31.3 microdeletion syndrome has recently emerged as a distinct clinical entity, and we report two new patients with de novo deletions of this region, bringing the total to seven. Similarly to previously reported cases, the phenotype of our patients is characterized by marked hypotonia, apnea, developmental delay, and feeding difficulties. Both patients had abnormal movements which did not correlate with epileptiform activity on electroencephalogram (EEG). Developmental brain changes on neuroimaging consisted of abnormalities predominantly affecting the white matter and frontal lobes. The 5q31.3 deleted regions overlap those of previously reported cases, and allow further refinement of the shortest region of overlap to 101 kb, including only three genes. Of these, the purine-rich element binding protein A (PURA) gene has an established role in brain development, and we propose that haploinsufficiency for this gene is primarily responsible for the neurodevelopmental features observed. © 2013 Wiley Periodicals, Inc.

Key words: 5q31.3 microdeletion syndrome; neonatal hypotonia; purine-rich element-binding protein A (PURA)

INTRODUCTION

The adoption of array-based technology as the first-tier test for the investigation of multiple congenital abnormalities and developmental disability has resulted in the recognition of many new microdeletion and microduplication syndromes [Li and Andersson, 2009]. The 5q31.3 microdeletion syndrome has recently emerged as a distinct clinical entity, with overlapping deletions in this region having been described in five patients [Shimojima et al., 2011; Hosoki et al., 2012]. We report on two additional patients with microdeletions of the 5q31.3 chromosome region, thus contributing to the clinical and molecular knowledge regarding this syndrome. Refinement of the shortest region of overlap (SRO) to 101 kb highlights PURA as the primary candidate for the neurodevelopmental features observed.

CLINICAL REPORTS

Patient 1 is the first child of nonconsanguineous Caucasian parents, born at term following an uncomplicated pregnancy with a birth weight of 2,740 g (3rd centile). At birth she was transferred to neonatal intensive care with profound central hypotonia, hyporeflexia, central apnea, multifocal myoclonic jerks, and cyclical movements of the limbs. She required ventilation for the first 5 weeks of life, and subsequently a tracheostomy to facilitate airway management. Feeding was via nasogastric tube and subsequent...
gastrostomy due to poor oro-motor function and episodes of aspiration.

Echocardiogram revealed a small ventriculo-septal defect (VSD). Nerve conduction studies were normal. Electroencephalogram (EEG) was consistent with diffuse encephalopathy, showing excessive discontinuity attenuation, asynchrony, and sharp wave activity. However, the seizure-like clinical episodes were not associated with epileptiform discharges on video-EEG monitoring. Cerebral magnetic resonance imaging (MRI) at Day 3 and Day 34 demonstrated diffuse symmetrical white matter T2 hyperintensity without restricted diffusion. Sulcation of both frontal lobes was shallow and immature, with mature sulcation elsewhere.

At 14 months, repeat cerebral MRI showed enlarging cystic spaces within the basal ganglia and white matter of the anterior limbs of the internal capsules. Subsequent cerebral MRI at 2 years of age demonstrated loss of volume of the frontal lobes, generalized white matter volume loss with delayed myelination/dysmyelination and cysts within the periventricular white matter, basal ganglia and subcortical white matter (Fig. 1a–c).

Patient 1 is currently 6 years of age. Her facial features are similar to previously described cases, including hypertelorism, a flat nasal bridge, upslanting palpebral fissures, mild ptosis, prominent cheeks, and micrognathia with a tented upper vermilion border (Fig. 1d,e). She is non-verbal and non-ambulant, consistent with severe intellectual and physical disability. Neurological examination demonstrates generalized central hypotonia with some extensor posturing of the upper limbs, reflexes are mildly increased. Current growth is within the normal range (head circumference 50 cm [25–50th centile], weight 25 kg [75th centile], length not available. She developed central sleep apnea and episodes suggestive of atypical absence seizures at approximately 6 years of age. EEG demonstrated poorly sustained and slow background rhythms for age, without frank epileptiform discharges or focal abnormalities.

Patient 2 is the first child of nonconsanguineous parents, conceived using in vitro fertilization (IVF), and delivered at term following an uncomplicated pregnancy with a birth weight of 3,030 g (50th centile) and head circumference of 34 cm (50th centile). He was admitted to neonatal intensive care at 48 hr of age with severe hypotonia, apneas, and seizure-like episodes consisting of rapid limb jerking, and required ventilation for 10 days. Video EEG monitoring failed to provide evidence of the abnormal movements being seizures on multiple occasions.

**FIG. 1.** a: Axial T2 image of Patient 1 on Day 3 of life demonstrating shallow frontal lobe sulcation [small arrow] and increased white matter signal intensity [long arrows]. b: Axial T2 image at 1 month of age demonstrates similar findings with development of cysts in both internal capsules and basal ganglia [black arrows]. c: Axial T2 image at 2 years of age demonstrates white matter volume loss with ex vacuo dilatation of the ventricles and severely delayed myelination. Note the disproportionate shrinkage of both frontal lobes [stars] with atrophy of the anterior corpus callosum [white arrow]. Cysts are in the anterior limbs of the internal capsules [black arrow] and in the higher subcortical white matter [not shown]. d: Patient 1 at age 7 months and e: 6 years demonstrating low set ears, depressed nasal bridge, short nose with anteverted nares, sparse eyebrows, mild ptosis, prominent cheeks, and micrognathia with tented upper lip. f: Axial T2 weighted image in patient 2 on Day 9 of life demonstrates shallow frontal lobe sulcation [short arrow] and increased signal intensity in the white matter especially in both frontal lobes [long arrow].
Cerebral MRI on Day 9 of life showed diffuse symmetrical white matter T2 hyperintensity without restricted diffusion. This was most marked in the frontal lobes which also demonstrated shallow, immature sulcation relative to the rest of the brain. There were enlarged extra-axial spaces and a small but fully formed cerebellum (Fig. 1f).

Patient 2 is currently 2.5 years of age and has not had any abnormal movements for the past year. His tone is improving, and although his facial appearance was initially myopathic, he is not dysmorphic. Power and reflexes are normal, and cranial nerves are intact. He has hypermetropia, and a minor alternating esotropia but otherwise structurally normal eyes. Audiology assessment shows moderate conductive hearing loss. His current growth parameters are height 87 cm, weight 13 kg, and head circumference 50 cm (all 50th centile). He smiled responsively at 4 months of age, batted objects at 6 months, sat unsupported at 2 years, and is now standing with support, has pincer grasp, and is able to indicate needs with gestures.

**MOLECULAR CYTOGENETIC ANALYSIS AND RESULTS**

Copy number analysis was conducted on DNA extracted from peripheral blood lymphocytes, using the Affymetrix Genome-Wide SNP Array 6.0 (Patient 1) and the Affymetrix Whole-Genome Wide 2.7 M Array (Patient 2), with resolution reported at 0.2 Mb for both platforms.

Patient 1 has an interstitial deletion of approximately 3.2 Mb at 5q31.2–31.3 (137,069,214–140,430,729), and Patient 2 has a smaller interstitial deletion of approximately 1.9 Mb at the region 5q31.3 (139,422,959–141,309,459) (Fig. 2). In addition, there appeared to be a duplication distal to the deletion in Patient 1. Interpretation was made using the UCSC Genome Browser March 2006 build hg18.

Both deletions were confirmed; Patient 1 using the Illumina HumanCytoSNP-12 v2.1 and Patient 2 using the Affymetrix Cytoscan 750 K, the latter demonstrating that the NRG2 gene is not deleted and is centromeric to the proximal break point. The distal

![Image](image-url)
duplication in Patient 1 was not confirmed, with both the copy number (log R) and the genotyping data being normal (Fig. S1), and was reinterpreted as an artifact.

Parental analysis has confirmed these microdeletions to be de novo in both cases. Genotype analysis (using SNP trio) (Ting et al., 2007) on DNA samples from Patient 2 and his parents showed this deletion to be on the paternally inherited chromosome 5. Determination of parental origin of the deleted chromosome for Patient 1 was not possible, since the original studies used MLPA and additional DNA samples were not available.

DISCUSSION

We have described two new patients with microdeletions of the 5q31.3 region, bringing the total number of reported cases to seven. The clinical features shared by all seven individuals include profound hypotonia, developmental delay and feeding difficulty, “Seizures” (5/7) and respiratory distress/apnea (5/7) were also common features. Both patients described here demonstrated abnormal seizure-like movements in the neonatal period, which in both cases prompted treatment with anti-epileptic medication. However, video-EEG monitoring failed to provide correlation that the abnormal movements were seizures. Of note, one previously published patient (Patient 3) [Hosoki et al., 2012] similarly had clinical seizure-like events which did not show correlation on video-EEG. Given that the seizure-like events observed in three of the 5q31.3 patients appear to be non-epileptic, we suggest that video-EEG monitoring should form an integral part of evaluation of abnormal movements in patients with this disorder.

Patients with 5q31.3 microdeletions have also consistently demonstrated developmental brain changes on neuroimaging, primarily affecting the white matter and the frontal lobes. Abnormal white matter signal is evident in the neonatal period, and this is followed by progressive volume loss and delayed myelination/dysmyelination. Serial imaging in our Patient 1 has revealed a progressive leukoencephalopathy, and it remains to be seen whether this observation will be confirmed in longitudinal follow-up of other patients with 5q31.3 microdeletions. In addition, there is simplification of frontal lobe sulcation and disproportionate volume loss of the frontal lobes over time. This pattern of frontal lobe involvement has previously been noted [Hosoki et al., 2012], and is also evident in the imaging provided by Shimojima et al. [2011].

The 5q31.3 region contains several genes known to be involved in neuron development and function, notably the PCDH cluster, NRG2, and PURA. These have all been put forward as possible candidates for the observed neurodevelopmental phenotype [Shimojima et al., 2011; Hosoki et al., 2012]. Patient 1 demonstrates a large deletion of 3.2 Mb, with loss of 89 genes including all previously proposed candidate genes and a severe phenotype. However, the smaller deletion of 1.9 Mb identified in Patient 2 allows refinement of the proximal breakpoint of the 5q31.3 microdeletion syndrome and reduces the previously suggested SRO [Hosoki et al., 2012] to 101 kb (Fig. 2). Within this refined SRO are only three genes, PURA, C5orf53, and C5orf32. Of these, PURA is the best candidate for the observed phenotype. The function of C5orf53 and C5orf32 remains to be elucidated, and therefore their contribution to the phenotype, if any, is unknown.

PURA encodes a single-stranded DNA binding protein, pur-alpha (PURA), that has a variety of roles including regulation of transcription initiation and RNA translation. PURA may play a role in CGG-repeat mediated neurodegeneration in Fragile X Tremor/Ataxia Syndrome [Jin et al., 2007], and over-expression of PURA has a dose-dependent inhibitory effect on neurodegeneration in animal models. PURA knock-out mice exhibit seizures, tremor, and early death with abnormalities of the white matter, reduced neuronal numbers, and disorganized synapse formation on histopathological studies [Khalili et al., 2003]. PURA demonstrates high evolutionary conservation of the DNA binding region and appears to be critical for the regulation and timing of the expression of neurodevelopmental genes. PURA has previously been proposed as a potential candidate for the key features of 5q31.3 microdeletion phenotype [Hosoki et al., 2012], and our case 2 provides strong support for this hypothesis. Although none of the 5q31.3 microdeletion patients described so far have had tremor, it is of interest that three patients including the two described in this report have had significant abnormal movements not correlated with seizure activity on EEG.

Of note, our Patient 2 demonstrates a milder neurodevelopmental phenotype than the other six cases. Unlike the other six cases, his deletion does not include loss of NRG2. The neuroregulins play a role in neuronal and glial growth and differentiation. NRG2 knock-out mice do not display neuroanatomical differences when compared to wild-type, however restricted growth and early morbidity have been observed [Britto et al., 2004]. We propose that when NRG2 is deleted in combination with PURA, this results in a more severe phenotype. In addition, our Patient 2 is not dysmorphic. It may be that at least some of the dysmorphic features described in the other patients are secondary to the severe hypotonia as already suggested [Shimojima et al., 2011] or that the dysmorphic features are due to haploinsufficiency of gene(s) not involved in this patient's microdeletion.

In conclusion, we have provided additional clinical and molecular evidence supporting the existence of the 5q31.3 microdeletion syndrome, bringing the number of described cases to seven. Cases demonstrate core clinical features of profound hypotonia, developmental delay and a range of abnormalities on cerebral imaging primarily affecting the white matter and the frontal lobes. Narrowing the SRO to include only three genes lends further support for PURA as the likely primary candidate gene for the core neurodevelopmental features of this syndrome. Identification of point mutations within PURA in individuals with similar phenotypes will further validate this hypothesis.

ACKNOWLEDGMENT

We thank the families for their participation in this study.

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher’s web-site.

FIG. S1. Expanded view of the 5q31.3 deleted region in Patient 1 obtained using the Illumina Human CytoSNP-12 v2.1 platform, demonstrating no evidence of the distal duplication originally observed using Affymetrix Genome-Wide SNP Array 6.0, with both the copy number (logR) and the genotyping data being normal in the highlighted region.