

ARTICLE

Nasal speech and hypothyroidism are common hallmarks of 12q15 microdeletions

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

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INTRODUCTION

Array CGH has led to the identification of several microdeletion syndromes by screening individuals with shared phenotypic characteristics, for example, the 12q14 microdeletion syndrome.¹ By combining array CGH data from large cohorts of patients, the screening of overlapping microdeletions is made possible, which in turn can lead to the identification of shared phenotypic characteristics. In this study, we present the clinical and molecular data of three previously unreported patients with submicroscopic overlapping deletions distal to the 12q14 microdeletion syndrome at chromosome bands 12q15q21.1. The deletions presented in this study vary from 2.5 to 2.59 Mb in size, with a 1.34-Mb common deleted region containing six RefSeq genes. To our knowledge, only seven patients have been reported with deletions in this region.^{2–8} The deletion break points in the literature vary from 12q13 to 12q23 and are associated with growth retardation, developmental delay and dysmorphic features. The previously reported cases were detected with standard cytogenetic techniques, except for the patient of Tocyap *et al.*,⁷ in which localization of microsatellite markers was investigated and for the patient from Schluth *et al.*,² in which the deletion was fine mapped using a 1-Mb resolution array. Although a variable clinical phenotype is present in all patients, the three patients in this paper present with developmental delay or learning disability, nasal speech and hypothyroidism. The introduction of array CGH in clinical diagnostics allows the identification of smaller deletions in the genome and simultaneous delineation of the exact break points. This has made the detection of smaller overlapping deletions possible, leading to a better understanding of the genotype–phenotype correlation in these patients.

MATERIALS AND METHODS

Chromosome analysis

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

Array CGH analysis

Patients 1 and 2

Array CGH was performed on patients 1 and 2 using the Agilent SurePrint G3 Human CGH Microarray Kit 4 × 180 K (Agilent Technologies, Santa Clara, CA, USA). The assay was performed according to the manufacturer's instructions with minor modifications. Array analysis on DNA obtained from the patient described by Schluth *et al.*² was also performed, in order to refine the size and break point positions of the deletion in this patient.

Patient 3

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

FISH analysis

Patient 2

Patient 2 was tested with FISH for 22q11 and 17p11.2 (Smith–Magenis region) deletions at the age of 4 years providing normal results. The 12q15q21.1 deletion detected on array was confirmed using FISH with BlueFISH BAC probes RP11-101K2 and RP11-181D11 (BlueGnome, Cambridge, UK) according to the manufacturer's instructions. FISH analysis for this deletion in both parents gave normal results.

Patient 3

Patient 3 was tested with FISH for the 22q11 deletion providing a normal result. FISH analysis for the 12q15q21.1 deletion was performed on metaphase

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slides from patient and parental samples. BAC clones (RP11-90G3, RP11-934P3, RP11-89P15 and RP11-89M22) were selected using the UCSC genome browser (<http://genome.ucsc.edu>) and the March 2006 assembly. The 12q15q21.1 deletion was confirmed in the patient. For both parents, FISH analysis provided normal results.

CLINICAL REPORTS

Patient 1 (Belgium, decipher patient 253774)

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

back remained straight upon anteflexion. Bilateral cubitus valgus was noted. Hands and fingers were long and slender with multiple fine dermatoglyphics in the hand palms. He was not able to fully stretch his fifth fingers. They were held in flexion. In the lower limbs, a mild asymmetry in length was noted. His legs appeared thin with hypoplastic calves. The feet were also slender with hallux valgus. The knee tendon reflexes in the legs were brisk. Radiographs showed mild scoliosis and a S-shaped configuration of the tibiae.

Patient 2 (United Kingdom, decipher patient 251427)

The second patient is an 11-year-old girl born to non-consanguineous healthy parents. At birth, she weighed 2.85 kg (P10) and presented with Down syndrome-like features and feeding problems. At the age of 3 years, she was diagnosed with acute lymphoblastoid leukemia, but was in complete remission at the age of 6 years. At 9 years of age, she started thyroxine treatment because of a persistently high TSH. The patient is now 11-years old, her height is on the ninth percentile and her head circumference is 56.5 cm (> P98). Her facial features include mid-facial hypoplasia, straight eyebrows, high forehead, low-set ears and dorsally rotated auricles (Figures 1c and d). She also has hammertoes and hypoplastic fifth toenails. Her speech has a nasal quality and she requires learning support in mainstream school because of dyslexia and dyspraxia. Recent psychological assessment places her full-scale IQ on the seventh centile (IQ=69) and her literacy skills as 3-years behind her chronological age, whereas her numeric skills have shown no progress in 2 years.

Patient 3 (Sweden, decipher patient 248767)

The third patient is a 21-year-old woman, the second child of four, born to healthy, unrelated parents. The siblings, two brothers and one sister, are all healthy. She was born after an uneventful pregnancy with a birth weight of 3.5 kg (P50). She had a history of recurrent upper airway infections and middle ear infections. Since preschool age, she has had nasal speech and she has undergone several operations for this without success, as her speech is still very nasal. She was tested with FISH for the 22q11 deletion, providing a normal result. Growth has been normal. She has a slender build, small low-set ears and straight



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

eyebrows (Figures 1e–h). There are no known malformations. She wears glasses because of a refraction error and her hearing is normal. Up to seventh grade she followed regular school but after that, she has received special schooling. Her IQ is within the low normal range (total IQ 74, verbal scores higher than performance). In her late teens, she developed anorexia nervosa. She recently gave birth to a healthy baby girl, who has been tested negative for the 12q15q21.1 deletion present in the mother. During the pregnancy, she developed hypothyroidism.

RESULTS

Conventional chromosome analysis did not reveal any structural rearrangements in any of the patients. Using oligonucleotide arrays (Agilent), a deletion involving chromosome bands 12q15q21.1 was detected in all patients. The larger deletion in the patient from Schluth *et al*² was also finemapped on a 180-k oligonucleotide array. In patient

2, the deletion was confirmed with FISH using BAC probes RP11-101K2 and RP11-181D11. For patient 3, the 12q15q21.1 deletion was confirmed with FISH using BAC probes RP11-90G3, RP11-934P3, RP11-89P15 and RP11-89M22. Figure 2 depicts the deletions found in all three patients and the fine mapped deletion in the patient from Schluth *et al*.² The deletions in patients 2 and 3 are similar in size and in break-point location, whereas the deletion in patient 1 is slightly centromeric shifted. Analysis of the parents revealed that all the deletions occurred *de novo*. The data of these three patients were submitted to the DECIPHER database (DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources, <https://decipher.sanger.ac.uk/>).

DISCUSSION

To date, only seven patients with larger deletions containing this region have been reported in the literature.^{2–8} The deletion

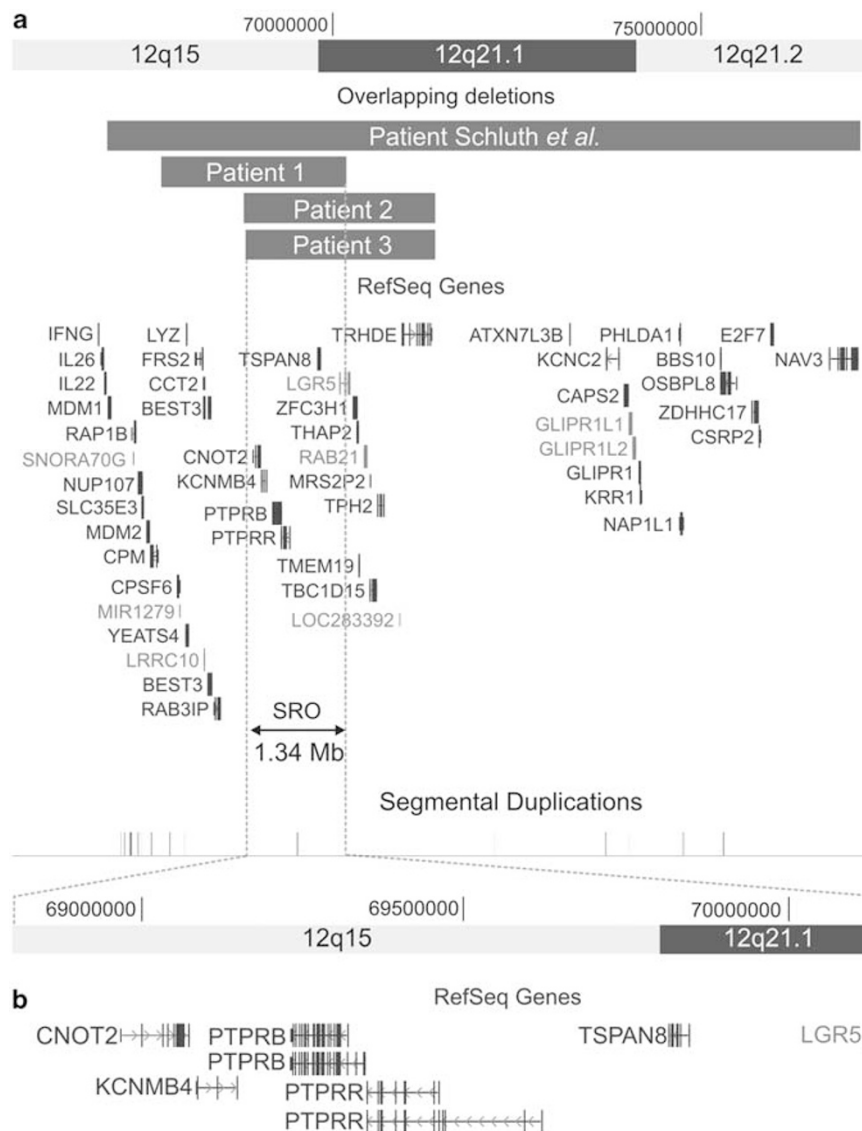


Figure 2 (a) Overview of the 12q15q21.1 microdeletions in patients 1–3 and the finemapped deletion in the patient from Schluth *et al.*² Break point locations were uploaded into the UCSC Genome Browser (March 2006 assembly). Patient 1: arr 12q15q21.1(67640963-70139285)×1 dn; Patient 2: arr 12q15q21.1(68782918-71372314)×1 dn; Patient 3: arr 12q15q21.1(68802240-71372314)×1 dn; Patient from Schluth *et al*²: arr 12q15q21.2(66869019-77078118)×1 dn. The smallest region of overlap (SRO) indicated by the dashed lines, is 1.34 Mb in size and contains six RefSeq genes. (b) Overview of the SRO.

Table 1 Array CGH analysis results and phenotypic features of patients 1–3 and the patient obtained from Schluth *et al*²

	Schluth <i>et al</i> ²	Patient 1 (Belgium)	Patient 2 (UK)	Patient 3 (Sweden)
<i>Array CGH analysis</i>				
Proximal breakpoint (bp)	66 869 019	67 640 963	68 782 918	68 802 240
Distal breakpoint (bp)	77 078 118	70 139 285	71 372 314	71 372 314
Location of deletion	12q15q21.2	12q15q21.1	12q15q21.1	12q15q21.1
Size of deletion	10.21 Mb	2.50 Mb	2.59 Mb	2.57 Mb
Heredity	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>	<i>De novo</i>
<i>Characteristics</i>				
Gender	F	M	F	F
<i>General</i>				
Birth weight (kg)	2.1 (P3)	2.63 (P25–50)	2.85 (P10)	3.5 (P50)
Head circumference (cm)	32	33		
Developmental delay	+	+	+	+
Feeding problems	–	+	+	–
Hypotonia	+	+	–	–
Hypothyroidism	NI	+	+	During pregnancy
<i>Facial</i>				
Flat face/midfacial hypoplasia	–	+	+	+
Large anterior fontanel	+	+	–	NI
High forehead	+	–	+	+
Hyper/hypotelorism	Hyper	Hypo	–	–
Straight eyebrows	–	+	+	+
Low-set ears	+	–	+	+
Micrognathia	+	+	–	–
Long philtrum	+	+	–	–
<i>Hands and feet</i>				
Hammer toes	–	+	+	–
<i>Stature</i>				
Slender	+	+	–	+
<i>Speech</i>				
Delayed speech	+	+	–	–
Nasal speech	–	+	+	+
<i>Developmental</i>				
<i>Psychomotor</i>				
Development delay	+	+	+	+
Walking delay	+	+	–	–
<i>Others</i>				
Refraction error	–	–	+	+
Otitis media	–	+	–	+

Abbreviation: NI, not investigated.

Breakpoint positions are given in hg18 build (March 2006 assembly) coordinates.

break-points vary from 12q13 to 12q23, and the phenotype differs from patient to patient depending on the size of the deletion. Phenotypic features include intrauterine growth retardation (6/7), developmental delay (5/7), delayed walking (4/7) and dysmorphic features. Low-set ears and a high-arched palate were present in 6/7 and 3/7 patients, respectively.^{2–8} In this study, we report three new cases with an overlapping submicroscopic deletion of 12q15q21.1, with a common deleted region of 1.34 Mb. These patients present with developmental delay (3/3), nasal speech (3/3) and hypothyroidism (3/3), in addition to other congenital anomalies (Tables 1 and 2).

Patients 2 and 3 appear to share the same proximal and distal break-point (Figure 2). The analysis of the genomic structure of that region did not reveal the presence of any segmental duplications or low-copy repeats. However, a common HERVH element is present at both sides of the deletion for both patients. Recently Visser *et al*⁹ indicated that rare pathogenic CNVs do not seem to occur in random genomic sequences, but may favor locations with a high content of

specific architectural features. The presence of this microhomology at the break-point junctions in patients 2 and 3 is suggestive for a microhomology-mediated rearrangement.

In the smallest region of overlap (SRO), six RefSeq genes are located: *CNOT2*, *KCNMB4*, *PTPRB*, *PTPRR*, *SPAN8* and *LGR5*. Little is known about these genes, and none of them have been implicated in palatal development.

CNOT2, a homeobox gene, is part of the CCR4–NOT complex.^{10,11} This complex functions as a general transcription regulation complex and is ubiquitously expressed. Abdelkhalik *et al*¹² showed that in mouse embryos loss of the ortholog *Not* results in abnormal notochord formation in and caudal to the posterior trunk.

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

Both *PTPRB* and *PTPRR* belong to the protein tyrosine phosphatase (PTP) family. This family of enzymes consists of key factors in a variety of cellular processes including cell growth, differentiation,

Table 2 Additional phenotypic characteristics seen in patients 1–3 and the patient from Schluth *et al*²

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

^aIntra-uterine growth retardation.

metabolism, cell cycle, cell–cell communications, cell migration, gene transcription, ion channels, immune response and survival.¹⁵ Malfunction of PTP activity is associated with a number of human disorders, including cancers, diabetes, rheumatoid arthritis and hypertension.¹⁶ Studies of PTPRR-deficient mice showed that PTPRR isoforms¹⁷ are physiological regulators of MAPK phosphorylation. These mice displayed impaired motor coordination and defects in their balance skills, reminiscent of a mild ataxia because of altered MAPK signaling.¹⁸

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

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

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

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

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

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

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

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

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

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- Menten B, Buysse K, Zahir F *et al*: Osteopoikilosis, short stature and mental retardation as key features of a new microdeletion syndrome on 12q14. *J Med Genet* 2007; **44**: 264–268.
- Schluth C, Gesny R, Borck G *et al*: New case of interstitial deletion 12(q15-q21.2) in a girl with facial dysmorphism and mental retardation. *Am J Med Genet A* 2008; **146A**: 93–96.
- Meinecke P, Meinecke R: Multiple malformation syndrome including cleft lip and palate and cardiac abnormalities due to an interstitial deletion of chromosome 12q. *J Med Genet* 1987; **24**: 187.
- Watson MS, McAllister-Barton L, Mahoney MJ, Breg WR: Deletion (12)(q15q21.2). *J Med Genet* 1989; **26**: 343–344.
- Perez Sanchez C, Ayensa F, Lloveras E *et al*: Prenatal diagnosis of an interstitial 12q chromosome deletion. *Ann Genet* 2004; **47**: 177–179.
- James PA, Oei P, Ng D, Kannu P, Aftimos S: Another case of interstitial del(12) involving the proposed cardio-facio-cutaneous candidate region. *Am J Med Genet A* 2005; **136**: 12–16.
- Tocay ML, Azar N, Chen T, Wiggs J: Clinical and molecular characterization of a patient with an interstitial deletion of chromosome 12q15-q23 and peripheral corneal abnormalities. *Am J Ophthalmol* 2006; **141**: 566–567.
- Yamanishi T, Nishio J, Miya S *et al*: 12q interstitial deletion with bilateral cleft lip and palate: case report and literature review. *Cleft Palate Craniofac J* 2008; **45**: 325–328.
- Visser LE, Bhatt SS, Janssen IM *et al*: Rare pathogenic microdeletions and tandem duplications are microhomology-mediated and stimulated by local genomic architecture. *Hum Mol Genet* 2009; **18**: 3579–3593.

- 10 Collart MA: Global control of gene expression in yeast by the Ccr4-Not complex. *Gene* 2003; **313**: 1–16.
- 11 Denis CL, Chen J: The CCR4-NOT complex plays diverse roles in mRNA metabolism. *Prog Nucleic Acid Res Mol Biol* 2003; **73**: 221–250.
- 12 Abdelkhalek HB, Beckers A, Schuster-Gossler K *et al*: The mouse homeobox gene Not is required for caudal notochord development and affected by the truncate mutation. *Genes Dev* 2004; **18**: 1725–1736.
- 13 Behrens R, Nolting A, Reimann F, Schwarz M, Waldschutz R, Pongs O: hKCNMB3 and hKCNMB4, cloning and characterization of two members of the large-conductance calcium-activated potassium channel beta subunit family. *FEBS Lett* 2000; **474**: 99–106.
- 14 Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW: Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J Biol Chem* 2000; **275**: 6453–6461.
- 15 Hunter T: Signaling—2000 and beyond. *Cell* 2000; **100**: 113–127.
- 16 Zhang ZY: Protein tyrosine phosphatases: prospects for therapeutics. *Curr Opin Chem Biol* 2001; **5**: 416–423.
- 17 Hendriks WJ, Dilaver G, Noordman YE, Kremer B, Fransen JA: PTPRR protein tyrosine phosphatase isoforms and locomotion of vesicles and mice. *Cerebellum* 2009; **8**: 80–88.
- 18 Chirivi RG, Noordman YE, Van der Zee CE, Hendriks WJ: Altered MAP kinase phosphorylation and impaired motor coordination in PTPRR deficient mice. *J Neurochem* 2007; **101**: 829–840.
- 19 Kuhn S, Koch M, Nubel T *et al*: A complex of EpCAM, claudin-7, CD44 variant isoforms, and tetraspanins promotes colorectal cancer progression. *Mol Cancer Res* 2007; **5**: 553–567.
- 20 Scholz CJ, Jacob CP, Buttenschon HN *et al*: Functional variants of TSPAN8 are associated with bipolar disorder and schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 967–972.
- 21 Hsu SY, Liang SG, Hsueh AJ: Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region. *Mol Endocrinol* 1998; **12**: 1830–1845.
- 22 Hsu SY, Kudo M, Chen T *et al*: The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol Endocrinol* 2000; **14**: 1257–1271.
- 23 Morita H, Mazerbourg S, Bouley DM *et al*: Neonatal lethality of LGR5 null mice is associated with ankyloglossia and gastrointestinal distension. *Mol Cell Biol* 2004; **24**: 9736–9743.
- 24 Curry CJ, Mao R, Aston E *et al*: Homozygous deletions of a copy number change detected by array CGH: a new cause for mental retardation? *Am J Med Genet A* 2008; **146A**: 1903–1910.