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## Clinical features associated with *CTNNB1* de novo loss of function mutations in ten individuals

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### Abstract

Loss of function mutations in *CTNNB1* have been reported in individuals with intellectual disability [MIM #615075] associated with peripheral spasticity, microcephaly and central hypotonia, suggesting a recognisable phenotype associated with haploinsufficiency for this gene. Trio based whole exome sequencing via the Deciphering Developmental Disorders (DDD) study has identified eleven further individuals with *de novo* loss of function mutations in *CTNNB1*. Here we report detailed phenotypic information on ten of these. We confirm the features that have been previously described and further delineate the skin and hair findings, including fair skin and fair and sparse hair with unusual patterning.

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#### Web resources

This study makes use of data generated by the DECIPHER community. A full list of centres who contributed to the generation of the data is available from <http://decipher.sanger.ac.uk> and via email from [decipher@sanger.ac.uk](mailto:decipher@sanger.ac.uk). Funding for the project was provided by the Wellcome Trust.

#### Accession numbers

Accession numbers on the DECIPHER database are as follows: 267844, 278755, 259002, 259408, 259162, 275999, 264405, 267130, 264159, 259248 and 272034.

## Keywords

*CTNNT1*; intellectual disability; microcephaly

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## Introduction

Whole exome sequencing is increasingly being utilised in the investigation of intellectual disability (ID), with more genes being implicated as a result. A gene, recently linked to a syndromic form of ID, is *CTNNT1*, which encodes for beta 1 catenin. This is a component of the cadherin adhesion complex which mediates cell-cell adhesion, and is also part of the Wnt signalling pathway. This pathway is important in controlling cell growth and differentiation, both in normal and tumour tissue. Beta catenin knockout mice display behaviours consistent with autistic spectrum disorder (Dong et al, 2016), as well as defects in neural development (Brault et al, 2001) and in hair follicle formation (Huelsenken et al, 2001). Somatic mutations in *CTNNT1* have been detected in certain tumours, and more recently germline, loss of function mutations have been linked to intellectual disability [MIM #615075].

Mutations in *CTNNT1* were first linked to intellectual disability when it was identified as a candidate gene during a trio exome sequencing study of 100 patients with intellectual disability (de Ligt et al, 2012). One patient was found to have a frameshift mutation (p.Ser425Thrfs\*11), with a further two patients found to have nonsense mutations (p.Arg515\* and p.Gln309\*) on sequencing of an additional cohort of 765 patients with intellectual disability. The phenotypes of these three patients were investigated in detail, and features included mild to severe intellectual disability, craniofacial anomalies, severe speech delay, microcephaly and childhood hypotonia with progressive peripheral spasticity and delayed motor development (Tucci et al, 2014). A report providing details of a patient with a whole gene deletion of *CTNNT1*, along with a further case series of 16 individuals with loss of function mutations in *CTNNT1*, confirmed these phenotypic features, as well as providing more detail regarding associated dysmorphic features, including thin upper lip vermilion and small alae nasi (Dubruc et al, 2014; Kuechler et al, 2015). One further case of a patient with a *de novo* nonsense mutation in *CTNNT1* has been reported, presenting with characteristic features such as microcephaly, peripheral spasticity and delayed speech, along with atypical features of hyperekplexia and apraxia of upward gaze (Winczewska-Wiktor et al, 2016). This case series aims to further delineate the phenotype associated with loss of function mutations in *CTNNT1*, reviewing ten patients ascertained through the Deciphering Developmental Disorders study.

## Methods

The Deciphering Developmental Disorders (DDD) study is a project which recruited patients through genetics centres in the UK and Ireland, with the aim to utilise genome-wide microarray and whole exome sequencing to increase diagnostic rates for children and adults with severe undiagnosed developmental disorders. Specifically congenital or early onset severe phenotypes were the focus for recruitment, with family trios sampled in the majority

of cases. 13,632 families were recruited, and analysis of 4,293 has been completed so far. DNA samples from patients and their parents were analysed at the Wellcome Trust Sanger Institute with microarray analysis (Agilent 2x1M array CGH [Santa Clara, CA, USA] and Illumina 800K SNP genotyping [San Diego, CA, USA]) to identify copy number variants (CNVs) in the child, and exome sequencing (Agilent SureSelect 55MB Exome Plus with Illumina HiSeq) to investigate single nucleotide variants (SNVs), small insertion-deletions (indels), and CNVs in coding regions of the genome. An automated variant filtering pipeline was used to narrow down the number of putative diagnostic variants, by ruling out common and non-functional variants, and then comparing variants against an in-house database of genes consistently implicated in specific developmental disorders, the Developmental Disorders Genotype-to-Phenotype database (DDG2P). This database includes more than 1000 genes that have been consistently implicated in specific developmental disorders and is updated regularly with newly implicated genes. (Wright et al, 2015)

By interrogating the DECIPHER database, where DDD results can be scrutinised, we identified eleven patients with an inactivating mutation of *CTNNB1*. We then contacted the responsible clinicians to obtain detailed phenotypic data on these patients, and received responses for ten patients. Consent was obtained for publication of photographs.

### Clinical Features

The patients' ages range from 3-27 years, with three males and seven females. In all patients symptoms occurred within the first year of life. Table 1 shows the pertinent clinical features for each patient.

### Craniofacial

Mild dysmorphic features have been previously reported. Our patients displayed a range of features, with a thin upper lip being the most common (7/10 patients). Other features seen include low set ears, wide spaced teeth, prominent columella (previously noted in older patients) and a prominent nose (Fig 1). Dubruc et al's case report noted that a patient with a whole gene deletion of *CTNNB1* had fair, sparse hair and fair skin, and so we specifically asked about these features when collecting clinical details. 6/10 patients were reported to have fair skin, and 7/10 patients were reported to have fair and/or fine hair compared to other family members, with two of those having an unusual hair pattern such as a cowlick or unusual hair whorl.

### Growth

3/10 patients were noted to have intrauterine growth retardation on antenatal scanning. Postnatal microcephaly was a common finding, with 7/10 patients having a head circumference of -3 standard deviations or smaller. Figure 2 visualises the growth parameters and developmental progress of the 11 patients ascertained through the DDD study.

### Development

All of the patients have significant motor delay, with the earliest age of walking at 2.5 years. 3/10 patients are unable to walk independently, the oldest being 6 years 2 months of age. All

of the patients have significant speech delay, as has previously been reported. Behavioural problems are a common feature, with 9/10 patients reported to have issues such as aggressive outbursts and poor sleep. Visual problems have previously been reported, and 4/10 patients have strabismus, with 2/10 also affected by hypermetropia.

## Neurology

As noted in previous reports, 9/10 patients were noted to have truncal hypotonia, usually presenting within the first year of life. 2/10 patients had problems feeding in the neonatal period. Peripheral spasticity is a common feature which seems to present after initial hypotonia, with 7/10 patients developing this feature. 2/10 patients were noted to have broad based or ataxic gait, which became evident at 3 to 4 years of age. Dystonic posturing was also noted in 2/10 patients, presenting at 18 months to 2 years of age. None of our patients were reported to have seizures; there was a query regarding possible absence seizures in 1 patient, but EEG was normal. All 10 patients have had MRI brain scans, which were normal except for 1 patient, who was noted to have atrophy of the left temporal lobe.

## Mutations

In our patients, 8 nonsense and 2 frameshift *de novo* mutations were detected in the *CTNNB1* gene by whole exome sequencing of family trios (Table 1). These are all predicted to lead to premature truncation of the protein. This concurs with previously reported mutations and deletions, which were all *de novo* and caused loss of function of the gene.

## Discussion

Our case series reinforces the phenotypic features previously reported in conjunction with inactivating *CTNNB1* mutations, including intellectual disability, postnatal microcephaly, truncal hypotonia and peripheral spasticity, mild dysmorphic features and behavioural problems. An additional patient with *CTNNB1* loss of function mutations identified by the DDD study also displays typical phenotypic features when looking at the DECIPHER database; we have not included this patient in our clinical report as we were not able to collect further updated phenotypic data for them. This patient (DECIPHER ID 264159), who has a *de novo* frameshift variant (c.1686\_1690delGGGGGinsGGGG; p.Val564SerfsTer6), has clinical features listed on DECIPHER including delayed speech and language development, truncal hypotonia and abnormal hair pattern. Our case series appears to show an excess of female patients (7 females as opposed to 3 males). On looking at the previously described 22 cases, 12 were female and 10 male, so there doesn't appear to be a significant gender bias in those affected by inactivating *CTNNB1* mutations, although these are small numbers from which to infer such a finding and this may become more obvious as more cases are identified. In the DDD cohort, 11 cases were found from the 4,293 families where analysis has been completed, giving a frequency of 0.0026.

$\beta$ -catenin has been shown to play a role in skin development and the formation of hair follicles in mice (Huelsen et al, 2001). Changes in hair and skin have only been, so far, described in detail by Dubruc et al (2014) in a patient who has a whole gene deletion of *CTNNB1*. Winczewska-Wiktor et al (2016) also mention that their patient has a right frontal

hair upsweep, which would further support our findings. We have demonstrated in our patients that hair and skin anomalies are a common feature associated with *CTNNB1* inactivating mutations, specifically fair skin, and fair, fine hair, with unusual hair patterning, which is out of keeping with the familial background.

In summary, our case series confirms findings previously described in association with *CTNNB1* inactivating mutations, and further delineates specific skin and hair findings, which may be helpful in identifying likely affected patients.

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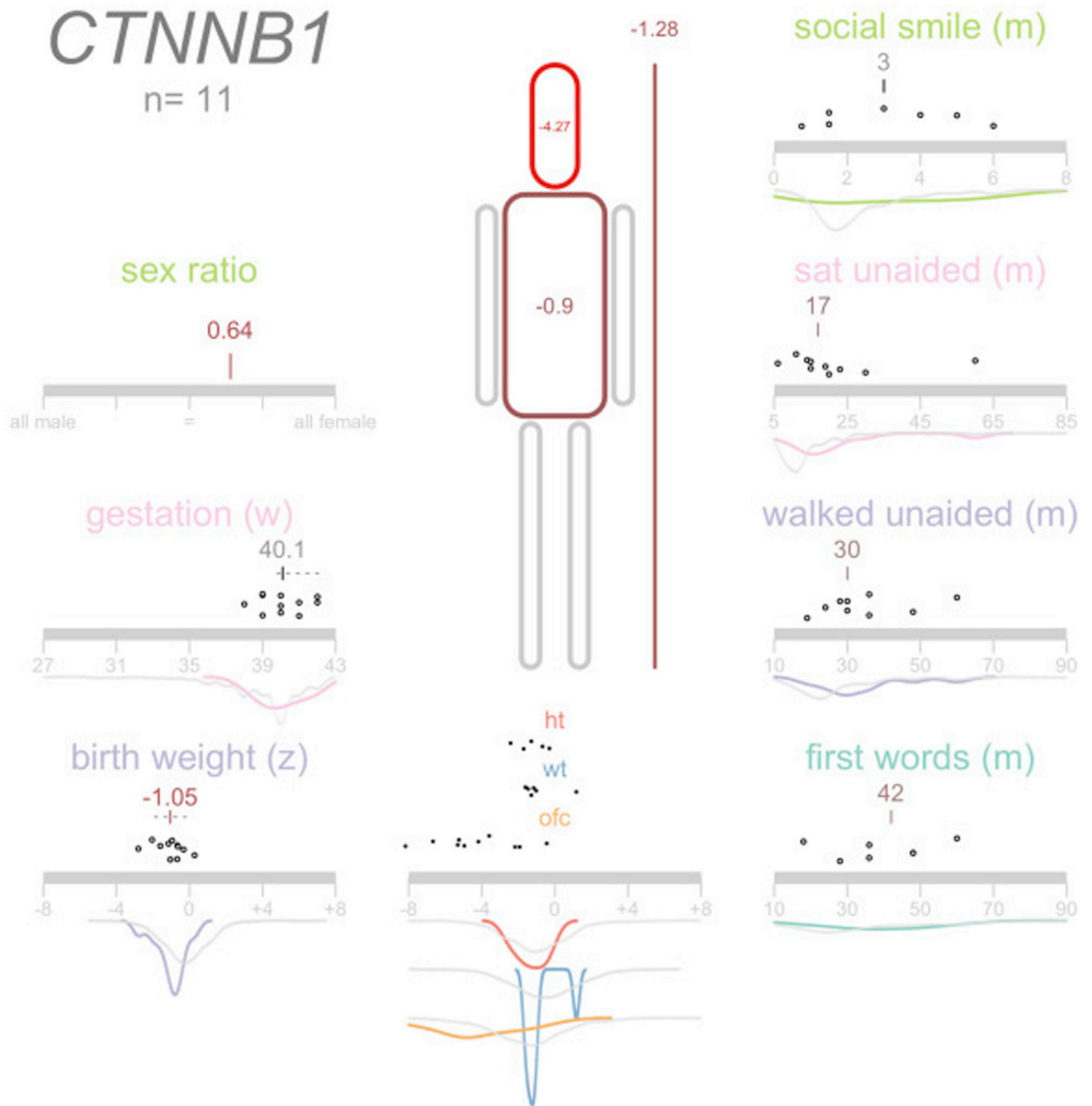
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**Figure 1.**

Photographs of 4 patients with *CTNNT1* mutations; from left to right, patient 2 (age 20 months), patient 5 (age 8 years), patient 6 (age 27 years) and patient 8 (age 7 months). Note fair and fine hair, and thin upper lip.



**Figure 2.** Phenicon demonstrating data collected for DDD patients with CTNNB1 mutations.

Table 1

Representation of the phenotypic data collected for each of the 10 patients with CTNBN1 mutations.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	
DECIPHER ID	267,844	278,755	259,002	259,408	259,162	275,999	264,405	272,034	267,130	259,248	
Mutation	c.1801C>T p.Gln601Ter	c.1603C>T p.Arg535Ter	c.1981C>T p.Arg661Ter	c.1603C>T p.Arg535Ter	c.1038_1044delGCTATCTinsGCT p.Val349AlafsTer9	c.999C>G p.Tyr333Ter	c.1420C>T p.Arg474Ter	c.799_809 delGAAGGAGCTAA insGAA p.Gly268TrpfsTer5	c.1612C>T p.Gln538Ter	c.998dupA p.Tyr333Ter	
Mutation type	Nonsense	Nonsense	Nonsense	Nonsense	Frameshift	Nonsense	Nonsense	Frameshift	Nonsense	Nonsense	
Sex	Male	Male	Female	Male	Female	Female	Female	Female	Female	Female	
Age of onset of symptoms	Birth	3 months	Birth	Birth	Within 1st year	Birth	7 months	3 months	Within 1st year	9 months	
Age at diagnosis	6 years 2 months	3 years 3 months	9 years 2 months	14 years	11 years	27 years	13 years	7 years	4 years 6 months	9 years	
Maternal Age	27	39	27	28	32	21	29	29	32	32	
Paternal Age	27	43	30	38	28	22	34	34	33	41	
Prenatal issues	Intrauterine growth retardation	None	Microcephaly and intrauterine growth retardation	None	Intrauterine growth retardation	None	None	None	Gestational diabetes; Exposure to anticonvulsant drugs	None	
Gestation (weeks)	39	40	38	41	40	39	41	42	41	40	
Birthweight (SD)	2.098 kg (2.8SD)	3.05 kg (1.05SD)	2.53 kg (1.15SD)	3.4 kg (0.32SD)	2.7 kg (1.6SD)	2.92 kg (0.66SD)	3.118 kg (0.63SD)	3.1 kg (0.67SD)	2.345 kg (2.05SD)	2.98 kg (0.95SD)	
Neonatal issues	Floppy	Low oxygen saturations	Poor feeding, reflux	Persistent Pulmonary Hypertension	Strabismus	Difficulty feeding, floppy	None	None	In SCBU for 21 days	Small anterior fontanelle, irritable	
Growth Parameters (SD)	At 3 years: OFC 46.6 cm (3.6SD) Weight 11.4 kg (2.74SD) Height 87.2 cm (2.82SD)	At 21 months: OFC 43 cm (5.25SD) Weight 10.45 kg (1.29SD) Height 83.5 cm (0.31SD)	At 5 years: OFC 42 cm (8.18SD) Weight 15.2 kg (1.62SD) Height 99 cm (2.43SD)	At 6 years: OFC 50.4 cm (1.93SD) Weight 26 kg (1.17SD)	At 4 years: OFC 48.9 cm (2.18SD) At 11 years: OFC 51.1 cm (2.6SD) Height 0SD Weight 0SD	At 26 years: OFC 52.7 cm (2.03SD) Weight 118 kg (3.25SD) Height 152 cm (1.96SD)	At 2 years: OFC 45 cm (3.35SD) Weight 12.15 kg (0.14SD) Height 86.3 cm (0.11SD) At 9 years: OFC 47 cm (5.32SD)	At 2 years: OFC 45 cm (3.35SD) Weight 12.15 kg (0.14SD) Height 86.3 cm (0.11SD) At 9 years: OFC 47 cm (5.32SD)	At 3 years: OFC 42.6 cm (6.67SD) Weight 13 kg (1.16SD) Height 92.1 cm (1.29SD)	At 3 years: OFC (4.96SD) Weight 12.84 kg (1.46SD) Height 95.5 cm (0.68SD)	At 4 years: OFC 46.9 cm (4.19SD) Weight 15.45 kg (1.03SD) Height 99 cm (1.71SD)
Developmental progress	Smiled e4 months; Head control - 9 months; Rolling back to front from 2 years;	Smiled e4 months; Sat supported - 8 months; Rolling - 9 months; not walking at 3	Smiled e6 weeks; Rolling - 6 months; Sat - 11 months; Walked - 30 months	Sat e15 months Walked with rollator - age 4; Can't walk unaided at age 14 years	Late sitting; Crawled - 13.5 months; Walked - 42 months, ataxic	Sat independently - 30 months; Walked - 4e5 years, ataxic	Sat independently 13 months; Walked - 42 months	Sat independently 18 months; Walked - 36 months, broad based gait	Social smile e24 weeks; Sat independently - 23 months; Walked - 2.5e3 years, ataxic	Social smile - 3 weeks; Sat independently - 14 months;	



	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Speech and Language development	Bottom shuffles but unable to walk at age 6 years 2 months.	Makes lots of noises but no words.	First words 3e4 years	Single words at age 14 years	Single words at age 5 years, talks in sentences at age 11 years	First words 4e5 years; can speak in sentences as adult but speech very unclear	First words 4e5 years	No speech	First words 3e4 years	Significant speech delay e first words age 4; more fluent speech age 6; said to be 3 years behind with verbal skills
Fair skin (ethnic origin)	Yes e but parents reported being as fair as children. (White British)	Yes (Scottish Caucasian)	Yes (Middle Eastern e Iran/Lebanon)	Yes (White British)	No (Egyptian)	Yes (White British)	Described as clear, has pigmented patch right knee (Caucasian)	Yes (Caucasian)	Normal for family (Caucasian)	No (Moroccan Sephardic Jewish)
Hair	Very blonde	Very blonde	Very slow growth and fine	Fair, not sparse, has cowlick	Fair and sparse, especially over temples	Fair as child, unusual hair whorl pattern, has fine hair as adult	Normal	Fine, blonde hair	Normal	Dark
Dysmorphic features	Slightly prominent nose in earlier childhood; thin upper lip.	Thin upper lip and prominent lower lip; long smooth philtrum; small ears; brachycephaly.	Wide spaced teeth; sacral dimple; tented and thin upper lip; 5th finger clinodactyly; low set ears	Low set ears; short philtrum; thin upper lip; high palate; prominent chin	Periorbital fullness; slightly low set ears with fleshy lobes; full tip to nose	Thin lower lip; downslanting palpebral fissures; prominent columella; large chin; wide spaced teeth	Deep set eyes with darker periorbital skin; epicanthic folds; short prominent nose with bulbous tip; smooth philtrum; thin upper lip; high palate; micrognathia with pointed chin; gum hypertrophy	Thin upper lip; slightly low columella	Prominent eyes	Low set ears
Peripheral spasticity	Unsure	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes
Truncal hypotonia	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Autistic features/behavioural problems	Occasional temper; frustration. Can bite others and himself; large appetite; constantly active e head rocking movements.	No	Some autistic traits - obsessional behaviour, global delay	Aggressive outbursts; self harm; poor sleep	Stereotypes e.g. clapping repeatedly, temper tantrums, aggressive to family members	Aggressive, temper tantrums, self injurious (biting, picking)	Severe behavioural problems/ADHD; aggressive; poor sleep; teeth grinding; mouths objects	Yes	Yes	No ASD but violent outbursts associated with difficulty expressing emotions
Seizures	No	No	No	No	No	No	No	No	No	?possible absence seizures in early childhood; EEG normal

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Additional clinical features	Single supernumerary upper incisor; bilateral orchidopexies	Strabismus and hypermetropia, dystonic posturing	Poor balance; oromotor dyspraxia; slightly delayed bone age; hypermobile joints; glue ear	Truncal obesity; absent left testis; brachydactyly; Achilles tendon contracture; high arched palate	Strabismus and hypermetropia acquired teeth early, from 3 months	Asthma; left clubfoot; increased dermatoglyphic whorls	Bilateral strabismus	Intermittent loss of skills	Strabismus; congenital ichthyosiform erythroderma (probably paternally inherited)	Dystonic posturing