JMG Online First, published on January 24, 2017 as 10.1136/jmedgenet-2016-104468

ORIGINAL ARTICLE

STAG1 mutations cause a novel cohesinopathy characterised by unspecific syndromic intellectual disability

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▸ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2016-104468).

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Received 23 November 2016
Revised 26 December 2016
Accepted 27 December 2016

To cite: Lehalle D, Mosca-Boidron A-L, Begtrup A, *et al*. *J Med Genet* Published Online First: [*please include* Day Month Year] doi:10.1136/jmedgenet-2016-104468

ABSTRACT

Background Cohesinopathies are rare neurodevelopmental disorders arising from a dysfunction in the cohesin pathway, which enables chromosome segregation and regulates gene transcription. So far, eight genes from this pathway have been reported in human disease. *STAG1* belongs to the STAG subunit of the core cohesin complex, along with five other subunits. This work aimed to identify the phenotype ascribed to *STAG1* mutations.

Methods Among patients referred for intellectual disability (ID) in genetics departments worldwide, array-comparative genomic hybridisation (CGH), gene panel, whole-exome sequencing or whole-genome sequencing were performed following the local diagnostic standards.

Results A mutation in *STAG1* was identified in 17 individuals from 16 families, 9 males and 8 females aged 2–33 years. Four individuals harboured a small microdeletion encompassing *STAG1*; three individuals from two families had an intragenic *STAG1* deletion. Six deletions were identified by array-CGH, one by whole-exome sequencing. Whole-exome sequencing found de novo heterozygous missense or frameshift *STAG1* variants in eight patients, a panel of genes involved in ID identified a missense and a frameshift variant in two individuals. The 17 patients shared common facial features, with wide mouth and deep-set eyes. Four individuals had mild microcephaly, seven had epilepsy.

Conclusions We report an international series of 17 individuals from 16 families presenting with syndromic unspecific ID that could be attributed to a *STAG1* deletion or point mutation. This first series reporting the phenotype ascribed to mutation in *STAG1* highlights the importance of data sharing in the field of rare disorders.

INTRODUCTION

The evolutionary-conserved cohesin complex plays a crucial role in the control of chromosome segregation during cell division. It is required for the cohesion of sister chromatids, and therefore, ensures the proper distribution of genetic material to daughter cells. Besides this canonical role, recent data demonstrated its involvement and major function in gene transcription and DNA repair and replication.[1–6] The complex is constituted by four subunits, SMC1A, SMC3, RAD21, the HEAT repeat-containing proteins STAG1, 2 or 3 (stromal antigen 1-2-3), and by several regulators involved in the control of the interactions between the complex and the chromatin.[7–9]

Not surprisingly, mutations in the genes coding for the cohesin complex or cofactors have been demonstrated to cause human developmental disorders, known as 'cohesinopathies'. Leading the way in this group of conditions, the most frequent and well-recognised, occurring in 1 in 10 000 newborns, is Cornelia de Lange syndrome (CdLS, OMIM 122470, 300590, 610759, 614701, 300882). CdLS is a multisystemic developmental disorder, characterised by intellectual disability (ID), growth retardation, microcephaly, upper limb reductional defects, visceral malformations and typical facial features. Five genes, all encoding subunits of the cohesion complex or cohesin regulators, account for >70% of CdLS cases. *NIPBL* is responsible for the classical CdLS phenotype.[10] Like *SMC3* and *RAD21*, involved in milder CdLS phenotypes, it follows an autosomal-dominant pattern of inheritance, whereas *SMC1A* and *HDAC8* are X-linked.[11–14] The phenotype is more severe in individuals affected by Roberts syndrome (RS, OMIM 268300), who present with limb defects mimicking phocomelia, growth retardation, dysmorphic features and profound cognitive impairment. RS is due to homozygous or compound heterozygous mutations in the acetyltransferase *ESCO2*.[15] Warsaw breakage syndrome (WBS, OMIM 613398), characterised by ID, severe prenatal and postnatal growth retardation and

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microcephaly, conductive deafness and cutaneous pigmentation anomalies, is due to homozygous or compound heterozygous mutations in *DDX11*.[16–18] In 2015, exome sequencing revealed dominant gain-of-function mutations in *AFF4* as causative in a new CdLS-like syndrome, named CHOPS syndrome (OMIM 616368), standing for Cognitive impairment and Coarse facies, Heart defect, Obesity, Pulmonary involvement and Short stature and Skeletal dysplasia.[19] Interestingly, *SGOL1*, another regulator of the cohesin pathway, has been shown to be involved in a very different autosomal recessive human condition, CAID syndrome (OMIM 616201), characterised by chronic atrial and intestinal dysrhythmia.[20] *STAG2* duplications have been reported in individuals affected by non-syndromic ID, associated in some cases with epilepsy and behavioural issues.[21 22] Finally, two individuals with respectively a *STAG1* deletion and missense *STAG1* variant were previously mentioned in the literature.[23 24] We included them in this series, as patient 3 and patient 7, in order to better characterise their phenotype and compare it with the other individuals with a *STAG1* mutation. Following active data sharing, we now report a series of 17 individuals, all harbouring a deletion or point mutation in the *STAG1* gene, thus allowing to define a novel gene responsible for syndromic unspecific ID. We review the clinical features of this cohort, discuss the putative role of *STAG1* in the phenotype and suggest new insights given by the description of this new syndrome.

PATIENTS AND METHODS

Patients

A cohort of 17 individuals with a deletion or point mutation involving *STAG1* was ascertained through international collaboration (figures 1 and 2, table 1).

Patient 1 is a male, the sixth child of non-related parents. One of his sisters and one brother had minor learning difficulties; the family history is otherwise unremarkable. The pregnancy was marked by intrauterine growth retardation. He was born at 38 WG, with a birth weight of 2370 g (1st centile), length of 48 cm (25th centile) and occipitofrontal circumference (OFC) of 31 cm (1st centile). He presented with severe gastrointestinal reflux in the neonatal period. Developmental delay was noticed from the first months, he walked at 23 months, spoke a few words at 5 years and made sentences at 7 years. He had anxious and quiet behaviour. At the last examination at 8 years old, weight was on −3 standard deviations (SD), height on −2 SD and OFC on −3 SD. Facial dysmorphism is shown in figure 1I, J. Cerebral MRI and tomodensitometry (TDM) were normal.

Patient 2 is a female born at 39 WG after a normal pregnancy. She was small for gestational age, weighted 2450 g (1st centile), measured 47 cm (3rd centile) and OFC was 30.5 cm (<1st centile). She had poor sucking and failure to thrive in the neonatal period. Her development was severely delayed, she walked at 3 years, had no speech at 12 years and showed autistic features. She developed atypical absences at age 5 years. At 12 years old, she was microcephalic (OFC −4 SD), with average growth parameters. The neurological examination demonstrated hypertonia of the lower limbs. Her facial features are shown in figure 1M, N.

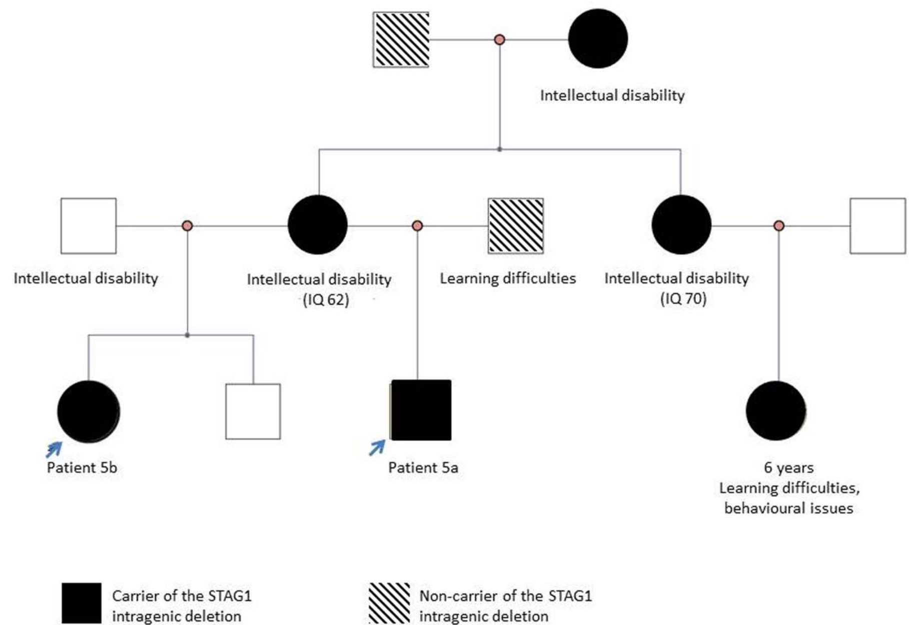
Patient 3 is a female, aged 33 years, who was referred to a clinical geneticist for moderate ID. She was born at 37 WG, with low birth weight (2000 g, 3rd centile), and was initially tube-fed. Poor eye contact was noticed in the first months of life. She showed delayed psychomotor development, walked at 30–36 months and spoke her first words at 3.5 years. She had autistic features. On examination, weight, height and OFC were on average. Her facial features were remarkable with a wide mouth and a high nasal bridge. She had a brain CT scan that showed central atrophy in the right hemisphere.

Patient 4 is the only child of first-cousins parents. He was born full term, with intrauterine growth retardation (birth weight 2600 g, <1st centile). He had a severe developmental delay, head control was acquired at 6 months and he could not sit at 7 years old. He had very few words, almost no non-verbal communication skills and fluctuant eye contact. He developed generalised epilepsy from 15 months. He had myopia. He had a scoliosis that required orthopaedic surgery. Examination at 7 years old showed spastic tetraparesis and bilateral inguinal hernia. Facial features were remarkable by high nasal bridge and wide mouth. He had growth retardation, weight was on −2.5 SD, length on −1 SD and OFC on −1.5 SD. Cerebral MRI revealed global brain atrophy predominant on the cerebellar vermis.

Patient 5a is a 6-year-old boy who was referred to the genetics clinic with a history of familial mild ID (figure 2). His maternal half-sister (patient 5b, figure 1A) presented with global developmental delay, microcephaly (−3 SD), brachycephaly and straight eyebrows with medial flaring. Their mother, maternal aunt, her daughter and the maternal grandmother all also have mild ID.

Figure 1 Facial phenotype of the patients with a STAG1 point mutation or deletion, from childhood to adulthood. Note the deep-set eyes, the wide mouth, that tends to become more obvious with age. (A) Patient 5b, 1 year. (B) Patient 13, 3 years. (C) Patient 11, 8 years. (D) Patient 14, 4 years. (E and F) Patient 9, 5 years. (G and H) Patient 12, 8 years. (I and J) Patient 1, 8 years. (K and L) Patient 5a, 6 years. (M and N) Patient 2, 12 years. (O and P) Patient 8, 29 years.

Figure 2 Family 5 pedigree.



In addition, there is a history of learning difficulties on the paternal sides of the family. He walked at 11–12 months. He had speech delay and started saying sentences at the age of 5 years. He had an average height and weight, but was microcephalic, with an OFC on −2 SD. He was mildly dysmorphic (figure 1K, L).

Patient 6 is a male. The pregnancy was obtained by in vitro fertilisation for paternal infertility. It was complicated by the ultrasound diagnosis of increased nuchal translucency, polyhydramnios, ventriculoseptal defect and single umbilical artery. At 4 weeks, he developed cardiac failure and underwent surgery to cure his ventriculoseptal defect. He had gastroesophageal reflux in the neonatal period. At 6 weeks, he was diagnosed with severe vesicoureteral reflux and had surgery at age 2. He underwent orchidopexy for bilateral cryptorchidism at 2 years old. He had a developmental delay, walked at 24 months and speech delay. He developed partial atonic seizures at 12 months and was treated with levetiracetam. EEG revealed generalised spikes and waves discharges. He benefited from hearing aids for conductive hearing loss. On examination at 6 years old, he had median weight, length on −1 SD, OFC on +1 SD, had hypertelorism, deep-set eyes, wide mouth and thick eyebrows. Brain MRI showed symmetric dilatation of the ventricles and pericerebral space, and possible partial vermis hypoplasia.

Patient 7, a male, was the second child of healthy parents with uneventful family history. He was born at term by caesarean section, following an unremarkable pregnancy. His birth parameters were within the normal range. He had normal early milestones, walked at 16 months and then developed with speech delay and learning difficulties. Aged 9 years and 9 months old, he had an average weight and height and OFC on −1 SD. He went to school for children with special needs, could read and write simple words. He had a sociable and friendly behaviour. He had a temporary mild hearing loss due to recurrent otitis. Facial examination showed deep-set eyes, large central incisors and large ears. He had a normal brain MRI.

Patient 8 is a male, born to healthy non-related parents. He was born at term with normal growth. He had two febrile seizures in infancy, at 4 months and 2 years. He walked with delay at 24 months and had speech delay. He went to a school for special needs children. At 29 years, he could neither write nor read and lived in a home for adults. He had no behavioural issues. On examination, his weight was on −1 SD, height on the medium and OFC on −1 SD. He had a slender build with scoliosis, hyperlaxity and thin hands and feet. He was mildly dysmorphic, as shown in figure 1O, P. Brain MRI showed global brain atrophy especially in the frontal lobes.

Patient 9 is a female, the only child of unrelated parents with no relevant family history. She was born at term with a normal birth weight. She had feeding difficulties. Early milestones were delayed; she rolled at 8 months, sat at 14 months and walked at 2 years. Speech was delayed. At the last referral at 5 years 9 months, she had moderate ID and a short attention span. She had mild behavioural issues, but no autistic features. Height was on −1 SD, weight on average and OFC on −1.5 SD. On examination, she had loose joints in the hands, thick lips and thin eyebrows (figure 1E, F).

Patient 10 is a 2.5-year-old boy who first presented at age 6 months for evaluation of developmental delay. He was an only child. He had a normal birth weight after a 38-week gestation. He was noted to have hypotonia and feeding difficulties with gastro-oesophageal reflux. He walked after age 2. At age 2.5 years, he was not saying words. He had good eye contact and non-verbal communication. He had sleep disturbance, chronic constipation and bilateral inguinal hernia repair. Initial EEG and head MRI studies at 5 months were normal, prompted by odd breathing patterns. He had two episodes of febrile seizures at 17 and 25 months. The second cerebral MRI around age 2 years showed a small area of heterotopia. Examination showed normal growth and OFC. Subtle facial changes included frontal bossing, telecanthus with broad nasal root and simplified ear helix patterns.

Patient 11 is a female. Family history was unremarkable except a brother with attention-deficit/hyperactivity disorder. She was born at term after a normal pregnancy, with normal growth parameters. Neonatal period was normal. She developed delayed milestones, ID and autistic features. Because of staring spells she had a prolonged EEG that was normal. On examination at 8 years old, she had a normal growth and OFC,

Table 1 Comparison of the clinical and cytogenetics or molecular data from the 17 patients of the series

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5a	Patient 5b	Patient 6
Sex	M	F	F	M	M	F	M
Age (years)	8	12	33	7	6	2	6
Family history	–	–	–	First-cousins parents	Family history of intellectual disability, six members carry the same *STAG1* deletion	Maternal half sister of patient 5a	–
Pregnancy	IUGR	IUGR	Normal	IUGR	NA	Normal	Increased nuchal translucency, hydramnios, VSD, single umbilical artery, asymmetric cerebral ventricles and small periventricular cysts
Birth parameters: W/L/OFC (centile)	1st/25th/1st	1st/3rd/<1st	3rd/NA/NA	3rd/NA/NA	25–50th/75th/98th centiles at 2 weeks	9th–25th/NA/NA	70th/80th/80th
Neonatal period	GER	Feeding difficulties	Feeding difficulties	Hypotonia	NA	Early feeding difficulties and suspected GER	GER, vesicoureteral reflux
Intellectual disability	+ (moderate)	+ (severe)	+ (severe)	+ (severe)	+ (mild)	+ (mild)	+
Epilepsy	–	+	–	+	–	–	+
Autistic features	–	+	+	–	–	–	–
Hyperlaxity	–	–	–	+	–	–	–
Brain imaging	Normal	ND	Atrophy right hemisphere	Cerebral atrophy, predominant on the vermis	ND	ND	Dilatation of ventricles and pericerebral space, partial vermis hypoplasia
Growth parameters: W/L/OFC (SD)	−3/−2/−3	0/0/−4	0/0/0	−2.5/−1/−1.5	−1/0/−2	0/NA/−3	0/−1/+1
Facial dysmorphism							
High nasal bridge	+	+	+	–	+	–	+
Deep-set eyes	+	+	+	+	+	+	+
Wide mouth	+	+	+	+	+	–	+
Widely spaced central incisors	+	+	–	–	–	–	–
Thin eyebrows	+	+	+	–	–	+	–
Cytogenetics or molecular result	De novo 3q deletion (*STAG1* and *PCCB*)	De novo 3q deletion (*STAG1* and *PCCB*)	De novo 3q deletion (*STAG1* and *PCCB*)	Intragenic *STAG1* deletion absent in the mother	Intragenic *STAG1* deletion, of exons 2–5/6, maternally inherited	Intragenic *STAG1* deletion, of exons 2–5/6 maternally inherited	3q deletion (*STAG1* and *PCCB*)

	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
Sex	M	M	F	M	F	F	M	F	M	F
Age	9 years	29 years	5 years 9 months	30 months	8 years	8 years	3 years 9 months	4 years	15 years	3 years
Family history	–	–	–		Brother with ADHD	–	–	–	–	–
Pregnancy	Normal	Normal	Normal	Normal	Normal	Normal	Normal karyotype (abnormal genitalia)	Normal	Normal	Normal
Birth parameters: W/L/OFC (centile)	70th/99th/70th	25th/50th/75th	25th/NA/NA	50th/NA/NA	60th/70th/30th	3rd/NA/NA	30th/20th/75th	90th/NA/NA	50th/NA/NA	75th/75th/10th
Neonatal period	Normal	Normal	Feeding difficulties	Hypotonia, feeding difficulties, GER	Normal	Normal	Hypotonia, feeding difficulties	GER, feeding difficulties	Vomiting	Hypotonia
Intellectual disability	+ (moderate)	+ (severe)	+ (moderate)	Mild developmental delay	+	+ (mild)	+ (mild)	Mild developmental delay	+ (severe)	+ (moderate)

Continued

Table 1 Continued

	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
Epilepsy	–	+	–	+	Staring spells	+	–	–	–	+
Autistic features	–	–	–	+	+	+	–	–	+	+
Hyperlaxity	–	+	+	–	–	–	–	+	–	+
Brain imaging	Normal	Global brain atrophy	ND	Small area of heterotopia	Normal	Chiari I	Normal	Normal	NA	Normal
Growth parameters: W/L/OFC (SD)	0/0/–1	–1/0/–1	–1/0/–1.5	+1/–1/–1	0/0/0	0/0/+1	0/–1/–0.5	–1/0/1	NA/0/–1	+1.5/+1.5/–1
Facial dysmorphism										
High nasal bridge	+	+	–	–	–	–	–	–	–	–
Deep-set eyes	+	+	+	–	+	+	+	+	–	–
Wide mouth	–	+	+	–	+	+	+	+	–	+
Widely spaced central incisors	–	–	–	–	–	–	+	+	–	–
Thin eyebrows	+	+	+	–	–	–	–	–	–	+
Cytogenetics or molecular result	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo frameshift *STAG1* variant	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo missense *STAG1* variant	De novo frameshift *STAG1* variant

ADHD, attention-deficit hyperactivity disorder; GER, gastro-oesophageal reflux; IUGR, intrauterine growth retardation; L, length; NA, non-available; ND, not done; OFC, occipitofrontal circumference; VSD, ventriculo-septal defect; W, weight.

deep-set eyes and a wide mouth (figure 1C). Cerebral MRI was normal.

Patient 12 is a female, third child of non-related healthy parents. She was born at 39 WG after an uncomplicated pregnancy, with a birth weight of 2770 g (3rd centile). Developmental milestones were delayed, she sat at 9 months and walked at 21 months of age. At 8 years, she was non-verbal with a good understanding. She had feeding difficulties, constipation, as well as sleep disturbance that required melatonin. She had a happy and friendly behaviour, hyperkinesia and autistic features. She was formally diagnosed with autistic disorder and intellectual deficiency at 5 years old. She developed complex partial seizures from the age of 5 and had a generalised tonic-clonic seizure at age 8; the epilepsy was then controlled by levetiracetam. EEG showed slow background rhythm consistent with encephalopathy. At examination at age 5 years 6 months, she had a normal height, weight and OFC. Subtle facial features are shown in figure 1G, H. Brain MRI revealed a Chiari 1 malformation.

Patient 13 is a male aged 3 years 9 months at evaluation. He had a negative family history. He was born at term with normal growth parameters. He had neonatal hypotonia, walked at 25 months and speech delay. He had feeding difficulties and could eat only soft food. On examination, he had medium weight, height on −1 SD and OFC on −0.5 SD. He had deep-set eyes, wide mouth and widely spaced central incisors, cryptorchidism and a supernumerary nipple (figure 1B). Cerebral MRI was normal.

Patient 14 is a female, referred at 4 years old for developmental delay. The family history was unremarkable. She was born at term after an uneventful pregnancy, with a birth weight on 90th centile. She had feeding difficulties and gastro-oesophageal reflux in the first months. Early milestones were mildly delayed, she walked at 19 months and she had speech delay. On examination, she had normal growth parameters (weight −1 SD, length on the medium range, OFC +1 SD) and subtle dysmorphic features (figure 1D). Brain imaging was normal.

Patient 15 is a male, referred at 15 years old for severe ID. His family history was uncomplicated. He was born after a normal pregnancy, at 40 WG, with a normal birth weight (3300 g). He had feeding difficulties and vomiting from 9 months old. He was late with his development, started to crawl at the age of 14 months. At 15 years old, he could speak 3–4-word sentences, most of the time several words. He had autistic features. On examination, height was medium and OFC 53.7 cm (−1 SD). He did not have specific facial features.

Patient 16 is a female. Family history was unremarkable. She was born at term with a normal birth weight. She had delayed milestones, walked at 23 months and spoke a few words at 3 years old. She developed partial epilepsy that was controlled by lamotrigine. On examination at 3 years, she had behavioural issues with hyperkinesia and autistic features, subtle dysmorphic features with wide mouth and thin eyebrows. Growth was normal. She had a normal brain MRI.

METHODS

Data sharing

Following the local diagnosis of patients 1 and 8 among our cohort of diagnostic array-comparative genomic hybridisation (CGH) and whole exome sequencing (WES), the authors activated data sharing in order to pool together other patients from the diagnostic or research cohort worldwide, and therefore to describe a new disease entity and its causative gene. It required a Decipher search for other micro rearrangements or variants

involving *STAG1*, and the analysis of the list of variants of unknown significance in candidate genes in online supplementary material in publications from large WES and whole genome sequencing (WGS) studies, informal exchanges between collaborators and GeneMatcher data sharing.[25] Patients 2 and 9 were gathered through the Decipher network. The variant of patient 7 was reported as probably disease-causing in a large whole-exome sequencing study.[23] Patient 3 was published in the online supplementary data in ref. 24. Patients 4, 5a and 5b, 6 and 16 were added following network collaboration, after oral and poster communication about the constitution of this series. Patients 10, 11, 12, 13 and 15 were identified through GeneMatcher. Patient 14 was identified through GeneDx.

Array-CGH, WES, gene panel or WGS were performed on DNA extracted from blood for all patients after informed consent following standard methods.

Array-CGH

A diagnostic array-CGH experiment was conducted in six patients. The platform used for patients 1, 2 and 4 was the Human Genome CGH Microarray 180K (patients 1 and 2), or 60K (patient 4) from Agilent according to the manufacturer's protocol (Agilent Technologies, Santa Clara, California, USA). Data were processed with feature extraction (V.9.1) software and the results were analysed with CGH analytics (V.4.0) software (Agilent). The platform used for patients 5a and 5b was the Affymetrix CytoScan 750K SNP genotyping array (Agilent Technologies), and for patient 6 Affymetrix SNPArray (Agilent Technologies), with Human mapping 250K-Nsp1. The result was analysed in the Hg19 genome assembly for all patients. There was no second technique to control these results for patients 5a, 5b and 6. qPCR (SYBR Green from ThermoFisher Scientific) was performed for patients 1, 2 and 4 on a Lightcycler 480.

WES

Trio WES was performed on patients 7–9. Methods can be found in their respective articles.[23 26] Patients 10, 11, 12, 14 and 15 had WES at GeneDx (http://www.genedx.com) whose methods have been reported previously.[27]

Gene panel

A panel of 77 genes involved in ID (DI44 defined by the national system DefiScience) was studied in patient 13, using HaloPlex and Agilent Technologies for hybridation, followed by next generation sequencing (NGS) sequencing on MiSeq Illumina. Alignment was made on the reference human genome GRCh37/hg19 and annotation was performed by three bioinformatics pipelines: PluginBroad 2.7, MiSeqReporter 2.4 (Illumina) and Sequence Pilot (JSI). Data were assembled and filtered in the local database Diagnostic Variants Database.

A panel of 448 genes (DI450) was performed in patient 16, with capture by XT2 (Agilent Technologies), followed by sequencing on HiSeq2500. The lists of the genes whose exonic regions were included in these two panels are available in the online supplementary data.

WGS

Trio WGS was performed in patient 3. Methods can be found in ref. 24.

RESULTS

Clinical data

All the individuals presented with ID, ranging from mild to severe. In addition, four patients had a moderate microcephaly, from −2 to −4 SD; all of them harboured a *STAG1* deletion (p=0.006). Among the seven individuals with a *STAG1* deletion, four had microcephaly below −2 SD, one had an OFC within the lower range on −1.5 SD and only two had a normal OFC. Also, 5 out of 17 had intrauterine growth retardation; however, all but one had a normal postnatal growth. Feeding difficulties and gastro-oesophageal reflux were common in the neonatal period, affecting nine individuals. Seven individuals had behavioural issues, described as autistic features. Seven patients developed epilepsy, ranging from recurrent febrile seizures to epileptic encephalopathy. Five of them had minor cerebral abnormalities: global brain atrophy (two individuals), heterotopia (one individual), brain atrophy with vermis hypoplasia (one individual) and asymptomatic Chiari I malformation (one patient). Hyperlaxity was reported for five patients. Visceral malformations were rare, only one patient had a congenital heart defect, two males had cryptorchidism.

The cytogenetics or molecular results from the 16 families are summarised in table 2.

Array-CGH

Array-CGH in patient 1 showed a deletion encompassing two genes, *STAG1* and *PCCB*, located in 3q22.3 (minimal size 208 kb, maximal size 531 kb, 3:135979743–136510812). The deletion was confirmed by qPCR and parental segregation confirmed a de novo event. *PCCB* is involved in propionic acidemia, in a recessive mode of inheritance. Patient 1 also had a 549 kb 8q11.23 duplication inherited from the healthy father. Array-CGH performed in patient 2 revealed an overlapping 3q22 deletion (chr3: 136035522–136412 948) encompassing *STAG1* and *PCCB*. The deletion was confirmed by FISH analysis and parental segregation confirmed the de novo occurrence. Array-CGH performed on patient 4 revealed a 3q22.3 deletion encompassing 13–18 exons of the *STAG1* gene (minimal size 99 kb, chr3: 136141380–136240231, maximal size 201 kb, chr3: 136109538–136310711), confirmed by qPCR analysis. The deletion was absent in the mother, the father could not be tested. Array CGH performed on patients 5a and 5b identified a 173 bp intragenic *STAG1* deletion within the long arm of chromosome 3, band q22.3, including exons 2–5 or 2–6 within the *STAG1* gene (chr3: 136254742–136427833). The familial segregation was consistent with the pathogenicity of this deletion as it was identified in all the six affected family members and absent from the unaffected father and maternal grandfather. Array-CGH identified in patient 6 a 3q22.3 deletion of minimum 336 kb (chr3: 135969755–136305476) and maximum 486 kb (chr3: 135854035–136340339) encompassing *PCCB* and the 3′ part of *STAG1* from the exon 4 or 5. The parental studies are ongoing.

WES

Trio exome sequencing performed in patient 7 revealed two heterozygous de novo point mutations: c.641A>G (p.Gln214Arg) in *STAG1* (chr3:136240090G>T) and c.1480dup (p.Met494Asnfs*14) in *SETDB2*, and a maternally inherited missense variant in *PLXNB3* on the X-chromosome: c.1718G>A, p.(Arg573Gln). These findings were previously published in ref. 23. WES in patients 8, 6, 9, 10, 11, 13 and 14 identified de novo heterozygous variant in *STAG1*, respectively, NM_005862.2:c.1433A>C, p.(His478Pro), NM_005862.2:c.646A>G, p.(Arg216Gly), NM_005862.2:c.1118G>A, p.(Arg373Gln), NM_005862.2:c.1460_1464dup, p.(Trp489Valfs*10), NM_005862.2:c.659A>G, p.(His220Arg), NM_005862.2:c.2936A>G, p.(Lys979Arg), and NM_005862.2:c.1052T>G, p.(Leu351Trp). All are absent from the public

Table 2 Summary of the molecular and cytogenetics findings of the 16 families of the series

Patient	Deletion	Deleted genes	Inheritance	Identification
1	chr3: 135979743–136510812	*STAG1*, *PCCB*	De novo	Array-CGH
2	chr3: 136035522–136412948	*STAG1*, *PCCB*	De novo	Array-CGH
3	chr3: 135983184–136383429	*STAG1*, *PCCB*	De novo	WGS
4	chr3: 136109538–136310711	*STAG1* exons 13–18	Absent in the mother (father NA)	Array-CGH
5a	chr3: 136254742–136427833	*STAG1* exons 2–5	Inherited	Array-CGH
5b	chr3: 136254742–136427833	*STAG1* exons 2–5	Inherited	Array-CGH
6	chr3: 135969755–136305476	*STAG1*, *PCCB*	NA	Array-CGH

Patient	Variant	Protein	Exon	Type	Inheritance	Identification
7	NM_005862.2:c.641A>G	p.(Gln214Arg)	7	Missense	De novo	WES
8	NM_005862.2:c.1433A>C	p.(His478Pro)	15	Missense	De novo	WES
9	NM_005862.2:c.646A>G	p.(Arg216Gly)	7	Missense	De novo	WES
10	NM_005862.2:c.1118G>A	p.(Arg373Gln)	11	Missense	De novo	WES
11	NM_005862.2:c.1460_1464dup	p.(Trp489Valfs*10)	15	Frameshift	De novo	WES
12	NM_005862.2:c.659A>G	p.(His220Arg)	7	Missense	De novo	WES
13	NM_005862.2:c.997A>C	p.(Lys333Gln)	10	Missense	De novo	Panel
14	NM_005862.2:c.2936A>G	p.(Lys979Arg)	27	Missense	De novo	WES
15	NM_005862.2: c.1052T>G	p.(Leu351Trp)	11	Missense	De novo	WES
16	NM_005862.2:c.1736dup	p.(Ser580Valfs*21)	17	Frameshift	De novo	Panel

CGH, comparative genomic hybridisation; NA, non-available.

database ExAC, and the missense variants have high predicted pathogenicity scores in PolyPhen, Grantham and CADD, as shown in table 3, which support their pathogenicity.

Gene panel

The DI44 panel studied in patient 13 identified a de novo missense variant in *STAG1* (NM_005862.2:c.997A>C, p.(Lys333Gln), affected a highly conserved among species amino acid, predicted to be deleterious by SIFT, Polyphen and MutationTaster, absent from the ExAC database. The DI450 panel identified a de novo frameshift variant (NM_005862.2: c.1736dup; p.(Ser580Valfs*21) in *STAG1* in patient 16.

WGS

Whole-genome sequencing was performed in patient 3 and his parents and identified a de novo deletion in chromosome 3 involving *STAG1* and *PCCB* (3:135983184 –136383429). These cytogenetics findings were published in ref. 24.

DISCUSSION

The clinical features of the 17 patients of this series with a point mutation or deletion of *STAG1* are summarised in table 1 and their frequency within this series in table 4. Interestingly, 57% (4/7) of the individuals with a *STAG1* deletion had microcephaly, whereas the 10 patients with a *STAG1* single-nucleotide variant had an OFC in the normal range. This result is significant, with a p value <0.05 ($p=0.006$). Half of the microcephalic individuals had an intragenic *STAG1* deletion, half had a larger deletion also encompassing *PCCB*; however, microcephaly has not been reported in individuals with homozygous *PCCB* mutations, which is not in favour of a role of this gene in microcephaly. The observation of one adult with cerebral atrophy but no epilepsy suggests that minor cerebral findings could be directly linked to STAG1 dysfunction rather than being a consequence or cause of epilepsy. Of note, patients without epilepsy are less prone to have a brain imaging performed. The high prevalence of feeding difficulties in infancy is consistent with what is known in other cohesinopathies. The individuals all shared mild but similar dysmorphic features, with a wide mouth and deep-set eyes, which in some cases is reminiscent of the Pitt-Hopkins syndrome gestalt, and which become more obvious with age. Regarding patient 4, who had a phenotype more severe, we cannot rule out a second disorder that would account for this unusual severity. The clinical phenotype

Table 3 Evidences to support the pathogenicity of the eight STAG1 missense variants: frequency in ExAC, prediction scores (Polyphen, Grantham score, CADD score)

Patient	Variant	Frequency (ExAC)	Polyphen score	Grantham score	CADD score
7	NM_005862.2:c.641A>G, p.(Gln214Arg)	0	0.988	43	24.4
8	NM_005862.2:c.1433A>C, p.(His478Pro)	0	0.985	77	24.2
9	NM_005862.2:c.646A>G, p.(Arg216Gly)	0	1	125	22.9
10	NM_005862.2:c.1118G>A, p.(Arg373Gln)	0	0.997	43	36
12	NM_005862.2:c.659A>G, p.(His220Arg)	0	1	29	22.5
13	NM_005862.2:c.997A>C, p.(Lys333Gln)	0	0.989	53	27.5
14	NM_005862.2:c.2936A>G, p.(Lys979Arg)	0	0.998	26	21.9
15	NM_005862.2: c.1052T>G, p.(Leu351Trp)	0	1	61	27.8

Table 4 Frequency of the clinical features observed in the 17 patients

	Number of patients	%
Neurological features		
ID/DD	17/17	100
Epilepsy	7/17	41
Autistic features	7/17	41
Microcephaly	4/17	24
Brain anomaly		
Atrophy	3/17	18
Other unspecific anomaly	3/17	18
Facial features		
Deep-set eyes	14/17	82
Wide mouth	13/17	76
High nasal bridge	7/17	41
Thin eyebrows	8/17	47
Widely spaced central incisors	4/17	24
Growth retardation		
Prenatal	3/17	18
Postnatal	1/17	6
Feeding difficulties/GER	9/17	53
Hyperlaxity	5/17	29
Cryptorchidism	2/9	22
Scoliosis	2/17	12
Congenital heart defect	1/17	6
Supernumerary nipple	1/17	6

DD, developmental delay; GER, gastro-oesophageal reflux; ID, intellectual disability.

associated with *STAG1* aberrations seems to be milder than the phenotype associated with other cohesinopathies, such as CdLS. In particular, the facial features do not resemble any of the CdLS characteristic findings.

The cytogenetics and molecular findings of all 16 patients or families are summarised in table 2. Four unrelated patients had a small microdeletion of similar size encompassing both *STAG1* and *PCCB*, the latter being involved in a recessive metabolic disease, propionic academia. Classically, the heterozygous parents of the homozygous individuals affected by propionic academia are asymptomatic, suggesting that haploinsufficiency of *PCCB* is not pathogenic. Despite this deletion being in the same locus in four individuals, there is no evidence for a recurrent event as there is no low copy repeat or segmental duplication at the distal or proximal breakpoints. Three patients including two siblings had a *STAG1* intragenic deletion. Among the 10 de novo heterozygous *STAG1* variants, 8 were missense and 2 frameshift. Three were localised in exon 7, two in exon 11 and two in exon 15; one each was in exons 10, 17 and 27. There is no recurrent mutation in the series. Apart from microcephaly, there was no striking difference in this series among patients with a deletion or a point mutation in *STAG1*.

The canonical role of the cohesin complex in chromosomal segregation and cohesion of sister chromatids has long been studied. It was demonstrated that *STAG2* was required for centromeric cohesion, whereas *STAG3* was required to stabilise cohesin on chromosome axes and *STAG1* had a specific role in telomere cohesion.[9 28] Besides its canonical role in chromosome segregation, the cohesin complex indeed directly influences transcription via an essential role in transcriptional enhancer function.[2] Cohesin promotes the transition of promoter-proximal paused RNA polymerase II (Pol II) to elongation at the genes that it binds by facilitating enhancer-promoter contact. Moreover, cohesin depletion reduces the level of transcriptionally engaged Pol II at the promoter of most genes that do not bind cohesin, suggesting a wider role for the cohesin complex in the expression of broadly acting transcription factors that regulates many or most genes. Cohesin subunits participate in all cohesin-related processes, including chromatid cohesion during the S phase, gene transcription regulation during the interphase and DNA damage repair. Therefore, mutations in genes encoding these proteins have the potential to disrupt all these processes.[29] The importance of gene dosage is illustrated by the observations of the effect on gene transcription but not on chromosome segregation upon the reduction of cohesin levels by 80% in *Drosophila* cells.[30] In human cells, complete lack of a cohesin subunit is associated with cohesion defects, a classical feature of recessive cohesinopathies, whereas dominant cohesinopathies arise through transcriptional dysregulation. As examples, chromosomes of RS cells exhibit premature centromere separation,[31] karyotypes of fibroblasts from individuals with homozygous *SGOL1* mutations harbour the railroad appearance marker of centromeric cohesion defects[20] and the cellular features of WBS are both increased breakage in the presence of mitomycin C and cohesion defects, suggesting an underlying DNA repair dysfunction.[18 32] On the other hand, CdLS arises through transcriptional dysregulation, as demonstrated by genome-wide transcriptional microarrays and proteomics approach in cells mutated in *NIPBL*, *SMC1A*, *SMC3* and *RAD21*.[33 34] Along this line, AFF4 is a component of the super elongation complex that plays a crucial role in transcriptional regulation. Transcriptome analyses revealed a similar expression pattern of dysregulated genes in CHOPS and CdLS syndromes.[19] Given the phenotypic homogeneity in this series, it is likely that the underlying physiopathology is common in *STAG1* deletions, frameshift variants and missense variants we report, strongly suggesting that this phenotype is caused by *STAG1* haploinsufficiency. Therefore, transcriptional dysregulation is likely to be the causative mechanism underlying the associated phenotype.

Essential data about the function of STAG1 indeed have been provided by the observations and studies made on a mouse model deficient for *STAG1*.[35–37] Homozygous Stag1-null mice did not survive the embryonic stage, demonstrating the essential function of *STAG1* in viability, and the non-redundant role of *STAG1* and *STAG2*. Stag1−/− embryos showed severe developmental anomalies. Stag1+/− mice had an increased incidence of early-onset tumours, features of premature ageing and a shorter lifespan. It was shown that STAG1 contributes to the chromatin architecture in the regenerative islet-derived gene clusters of the pancreas of the *STAG1* heterozygous mice.[37] Moreover, the studies of the transcriptomes of STAG1-null and wildtype mouse embryonic fibroblasts revealed transcriptional changes in the STAG1-null cells. These data suggested a specific and role of STAG1 in gene regulation, essential for the embryonic development.[38]

In conclusion, this series reports 17 patients with *STAG1* deletions or single-nucleotide variants, all with a presentation of ID, similar facial gestalt and variable associated features. The spread of deep-sequencing techniques, such as whole-exome and whole-genome sequencing, in patients with ID, will probably add more reports and allow a better characterisation of this syndrome in the near future. Moreover, the constitution of this series adds another building block to the power of data sharing in order to cluster and delineate new entities based on molecular data.

URLs

ExAC Browser, exac.broadinstitute.org
Decipher, https://decipher.sanger.ac.uk/
GeneDx, http://www.genedx.com
Polyphen 2, http://genetics.bwh.harvard.edu/pph2/
CADD score: http://cadd.gs.washington.edu/

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Acknowledgements The authors wish to thank the patients and their families involved in the study, in particular the parents of patient 12. The Regional Council of Burgundy and the Centre Hospitalo-Universitaire de Dijon supported this work (PARI 2015). The authors acknowledge the GeneMatcher and Decipher initiatives in promoting data sharing. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003], a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute [grant number WT098051]. The views expressed in this publication are those of the author(s) and not necessarily those of the Wellcome Trust or the Department of Health. The study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network.

Contributors DL wrote the paper. A-LM-B/PCa and AC/IS analysed the array CGH (patients 1 and 5), AB/MTC, BG, PK/J-BR/JT/LD-J, AR, TS and KVG analysed the WES data (patients 10–12 and 14–15, 3, 16, 8, 7, 13 and 15), OB-B, PCh/AJ, OD, AM-P, CMD, SM, HO, S-MP/ALTT, NR, DJS, PT, CV-D, MHW, GNW and CZ are the referring clinician (patients 16, 8, 11, 1, 14, 4, 2, 5, 6, 9, 15, 13, 3, 10 and 7), CT-R and LF supervised this work.

Competing interests MTC and AB are employees of GeneDx.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Ethics approval Obtained.

REFERENCES

1 Horsfield JA, Print CG, Mönnich M. Diverse developmental disorders from the one ring: distinct molecular pathways underlie the cohesinopathies. *Front Genet* 2012;3:171.
2 Schaaf CA, Kwak H, Koenig A, Misulovin Z, Gohara DW, Watson A, Zhou Y, Lis JT, Dorsett D. Genome-wide control of RNA polymerase II activity by cohesin. *PLoS Genet* 2013;9:e1003382.
3 Zakari M, Yuen K, Gerton JL. Etiology and pathogenesis of the cohesinopathies. *Wiley Interdiscip Rev Dev Biol* 2015;4:489–504.
4 Ball ARJ, Chen YY, Yokomori K. Mechanisms of cohesin-mediated gene regulation and lessons learned from cohesinopathies. *Biochim Biophys Acta* 2014;1839:191–202.
5 Kong X, Ball ARJ, Pham HX, Zeng W, Chen HY, Schmiesing JA, Kim JS, Berns M, Yokomori K. Distinct functions of human cohesin-SA1 and cohesin-SA2 in double-strand break repair. *Mol Cell Biol* 2014;34:685–98.
6 Dorsett D, Ström L. The ancient and evolving roles of cohesin in gene expression and DNA repair. *Curr Biol* 2012;22:R240–50.
7 Barbero JL. Genetic basis of cohesinopathies. *Appl Clin Genet* 2013;6:15–23.
8 Haering CH, Jessberger R. Cohesin in determining chromosome architecture. *Exp Cell Res* 2012;318:1386–93.
9 Ward A, Hopkins J, Mckay M, Murray S, Jordan PW. Genetic interactions between the Meiosis-specific cohesin components, STAG3, REC8, and RAD21L. *G3 (Bethesda)* 2016;6:1713–24.
10 Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, Nowaczyk MJM, Toriello H, Bamshad MJ, Carey JC, Rappaport E, Kawauchi S, Lander AD, Calof AL, Li H-H, Devoto M, Jackson LG. Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B. *Nat Genet* 2004;36:631–5.
11 Ansari M, Poke G, Ferry Q, Williamson K, Aldridge R, Meynert AM, Bengani H, Chan CY, Kayserili H, Avci S, Hennekam RCM, Lampe AK, Redeker E, Homfray T, Ross A, Falkenberg Smeland M, Mansour S, Parker MJ, Cook JA, Splitt M, Fisher RB, Fryer A, Magee AC, Wilkie A, Barnicoat A, Brady AF, Cooper NS, Mercer C, Deshpande C, Bennett CP, Pilz DT, Ruddy D, Cilliers D, Johnson DS, Josifova D, Rosser E, Thompson EM, Wakeling E, Kinning E, Stewart F, Flinter F, Girisha KM, Cox H, Firth HV, Kingston H, Wee JS, Hurst JA, Clayton-Smith J, Tolmie J, Vogt J, Tatton-Brown K, Chandler K, Prescott K, Wilson L, Behnam M, McEntagart M, Davidson R, Lynch SA, Sisodiya S, Mehta SG, McKee SA, Mohammed S, Holden S, Park SM, Holder SE, Harrison V, McConnell V, Lam WK, Green AJ, Donnai D, Bitner-Glindzicz M, Donnelly DE, Nellåker C, Taylor MS, FitzPatrick DR. Genetic heterogeneity in Cornelia de Lange syndrome (CdLS) and CdLS-like phenotypes with observed and predicted levels of mosaicism. *J Med Genet* 2014;51:659–68.
12 Deardorff MA, Wilde JJ, Albrecht M, Dickinson E, Tennstedt S, Braunholz D, Mönnich M, Yan Y, Xu W, Gil-Rodríguez MC, Clark D, Hakonarson H, Halbach S, Michelis LD, Rampuria A, Rossier E, Spranger S, Van Maldergem L, Lynch SA, Gillessen-Kaesbach G, Ludecke HJ, Ramsay RG, McKay MJ, Krantz ID, Xu H, Horsfield JA, Kaiser FJ. RAD21 mutations cause a human cohesinopathy. *Am J Hum Genet* 2012;90:1014–27.
13 Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodríguez C, Arnedo M, Loeys B, Kline AD, Wilson M, Lillquist K, Siu V, Ramos FJ, Musio A, Jackson LS, Dorsett D, Krantz ID. Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation. *Am J Hum Genet* 2007;80:485–94.
14 Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, Saitoh K, Komata M, Katou Y, Clark D, Cole KE, De Baere E, Decroos C, Di Donato N, Ernst S, Francey LJ, Gyftodimou Y, Hirashima K, Hullings M, Ishikawa Y, Jaulin C, Kaur M, Kiyono T, Lombardi PM, Magnaghi-Jaulin L, Mortier GR, Nozaki N, Petersen MB, Seimiya H, Siu VM, Suzuki Y, Takagaki K, Wilde JJ, Willems PJ, Prigent C, Gillessen-Kaesbach G, Christianson DW, Kaiser FJ, Jackson LG, Hirota T, Krantz ID, Shirahige K. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. *Nature* 2012;489:313–17.
15 Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, van Gosliga D, Kayserili H, Xu C, Ozono K, Jabs EW, Inui K, Joenje H. Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. *Nat Genet* 2005;37:468–70.
16 Bailey C, Fryer AE, Greenslade M. Warsaw breakage syndrome—a further report, emphasising cutaneous findings. *Eur J Med Genet* 2015;58:235–7.
17 Capo-Chichi JM, Bharti SK, Sommers JA, Yammine T, Chouery E, Patry L, Rouleau GA, Samuels ME, Hamdan FF, Michaud JL, Brosh RM Jr, Mégarbane A, Kibar Z. Identification and biochemical characterization of a novel mutation in DDX11 causing Warsaw breakage syndrome. *Hum Mutat* 2013;34:103–7.
18 van der Lelij P, Chrzanowska KH, Godthelp BC, Rooimans MA, Oostra AB, Stumm M, Zdzienicka MZ, Joenje H, de Winter JP. Warsaw breakage syndrome, a cohesinopathy associated with mutations in the XPD helicase family member DDX11/ChlR1. *Am J Hum Genet* 2010;86:262–6.
19 Izumi K, Nakato R, Zhang Z, Edmondson AC, Noon S, Dulik MC, Rajagopalan R, Venditti CP, Gripp K, Samanich J, Zackai EH, Deardorff MA, Clark D, Allen JL, Dorsett D, Misulovin Z, Komata M, Bando M, Kaur M, Katou Y, Shirahige K, Krantz ID. Germline gain-of-function mutations in AFF4 cause a developmental syndrome functionally linking the super elongation complex and cohesin. *Nat Genet* 2015;47:338–44.
20 Chetaille P, Preuss C, Burkhard S, Côté JM, Houde C, Castilloux J, Piché J, Gosset N, Leclerc S, Wunnemann F, Thibeault M, Gagnon C, Galli A, Tuck E, Hickson GR,

El Amine N, Boufaied I, Lemyre E, de Santa Barbara P, Faure S, Jonzon A, Cameron M, Dietz HC, Gallo-McFarlane E, Benson DW, Moreau C, Labuda D, FORGE Canada Consortium, Zhan SH, Shen Y, Jomphe M, Jones SJM, Bakkers J, Andelfinger G. Mutations in SGOL1 cause a novel cohesinopathy affecting heart and gut rhythm. *Nat Genet* 2014;46:1245–9.

21 Leroy C, Jacquemont ML, Doray B, Lamblin D, Cormier-Daire V, Philippe A, Nusbaum S, Patrat C, Steffann J, Colleaux L, Vekemans M, Romana S, Turleau C, Malan V. Xq25 duplication: the crucial role of the STAG2 gene in this novel human cohesinopathy. *Clin Genet* 2016;89:68–73.

22 Kumar R, Corbett MA, Van Bon BW, Gardner A, Woenig JA, Jolly LA, Douglas E, Friend K, Tan C, Van Esch H, Holvoet M, Raynaud M, Field M, Leffler M, Budny B, Wisniewska M, Badura-Stronka M, Latos-Bielenska A, Batanian J, Rosenfeld JA, Basel-Vanagaite L, Jensen C, Bienek M, Froyen G, Ullmann R, Hu H, Love MI, Haas SA, Stankiewicz P, Cheung SW, Baxendale A, Nicholl J, Thompson EM, Haan E, Kalscheuer VM, Gecz J. Increased STAG2 dosage defines a novel cohesinopathy with intellectual disability and behavioral problems. *Hum Mol Genet* 2015;24:7171–81.

23 Rauch A, Wieczorek D, Graf E, Wieland T, Endele S, Schwarzmayr T, Albrecht B, Bartholdi D, Beygo J, Di Donato N, Dufke A, Cremer K, Hempel M, Horn D, Hoyer J, Joset P, Röpke A, Moog U, Riess A, Thiel CT, Tzschach A, Wiesener A, Wohlleber E, Zweier C, Ekici AB, Zink AM, Rump A, Meisinger C, Grallert H, Sticht H, Schenck A, Engels H, Rappold G, Schröck E, Wieacker P, Riess O, Meitinger T, Reis A, Strom TM. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012;380:1674–82.

24 Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BWM, Willemsen MH, Kwint M, Janssen IM, Hoischen A, Schenck A, Leach R, Klein R, Tearle R, Bo T, Pfundt R, Yntema HG, de Vries BBA, Kleefstra T, Brunner HG, Vissers LELM, Veltman JA. Genome sequencing identifies major causes of severe intellectual disability. *Nature* 2014;511:344–7.

25 Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: A Matching Tool for Connecting Investigators with an Interest in the Same Gene. *Hum Mutat* 2015;36:928–30.

26 Thevenon J, Milh M, Feillet F, St-Onge J, Duffourd Y, Jugé C, Roubertie A, Héron D, Mignot C, Raffo E, Isidor B, Wahlen S, Sanlaville D, Villeneuve N, Darmency-Stamboul V, Toutain A, Lefebvre M, Chouchane M, Huet F, Lafon A, de Saint Martin A, Lesca G, El Chehadeh S, Thauvin-Robinet C, Masurel-Paulet A, Odent S, Villard L, Philippe C, Faivre L, Rivière JB. Mutations in SLC13A5 cause autosomal-recessive epileptic encephalopathy with seizure onset in the first days of life. *Am J Hum Genet* 2014;95:113–20.

27 Tanaka AJ, Cho MT, Millan F, Juusola J, Retterer K, Joshi C, Niyazov D, Garnica A, Gratz E, Deardorff M, Wilkins A, Ortiz-Gonzalez X, Mathews K, Panzer K, Brilstra E, van Gassen KLI, Volker-Touw CM, van Binsbergen E, Sobreira N, Hamosh A, McKnight D, Monaghan KG, Chung WK. Mutations in SPATA5 Are Associated with Microcephaly, Intellectual Disability, Seizures, and Hearing Loss. *Am J Hum Genet* 2015;97:457–64.

28 Canudas S, Smith S. Differential regulation of telomere and centromere cohesion by the Scc3 homologues SA1 and SA2, respectively, in human cells. *J Cell Biol* 2009;187:165–73.

29 Skibbens RV, Colquhoun JM, Green MJ, Molnar CA, Sin DN, Sullivan BJ, Tanzosh EE. Cohesinopathies of a feather flock together. *PLoS Genet* 2013;9:e1004036.

30 Schaaf CA, Misulovin Z, Sahota G, Siddiqui AM, Schwartz YB, Kahn TG, Pirrotta V, Gause M, Dorsett D. Regulation of the Drosophila Enhancer of split and invected-engrailed gene complexes by sister chromatid cohesion proteins. *PLoS ONE* 2009;4:e6202.

31 Gerkes EH, van der Kevie-Kersemaekers AM, Yakin M, Smeets DFCM, van Ravenswaaij-Arts CMA. The importance of chromosome studies in Roberts syndrome/SC phocomelia and other cohesinopathies. *Eur J Med Genet* 2010;53:40–4.

32 van der Lelij P, Oostra AB, Rooimans MA, Joenje H, de Winter JP. Diagnostic Overlap between Fanconi Anemia and the Cohesinopathies: Roberts Syndrome and Warsaw Breakage Syndrome. *Anemia* 2010;2010:565268.

33 Gimigliano A, Mannini L, Bianchi L, Puglia M, Deardorff MA, Menga S, Krantz ID, Musio A, Bini L. Proteomic profile identifies dysregulated pathways in Cornelia de Lange syndrome cells with distinct mutations in SMC1A and SMC3 genes. *J Proteome Res* 2012;11:6111–23.

34 Liu J, Zhang Z, Bando M, Itoh T, Deardorff MA, Clark D, Kaur M, Tandy S, Kondoh T, Rappaport E, Spinner NB, Vega H, Jackson LG, Shirahige K, Krantz ID. Transcriptional dysregulation in NIPBL and cohesin mutant human cells. *PLoS Biol* 2009;7:e1000119.

35 Remeseiro S, Cuadrado A, Gómez-López G, Pisano DG, Losada A. A unique role of cohesin-SA1 in gene regulation and development. *EMBO J* 2012;31:2090–102.

36 Remeseiro S, Cuadrado A, Carretero M, Martínez P, Drosopoulos WC, Cañamero M, Schildkraut CL, Blasco MA, Losada A. Cohesin-SA1 deficiency drives aneuploidy and tumourigenesis in mice due to impaired replication of telomeres. *EMBO J* 2012;31:2076–89.

37 Cuadrado A, Remeseiro S, Graña O, Pisano DG, Losada A. The contribution of cohesin-SA1 to gene expression and chromatin architecture in two murine tissues. *Nucleic Acids Res* 2015;43:3056–67.

38 Cuadrado A, Remeseiro S, Gómez-López G, Pisano DG, Losada A. The specific contributions of cohesin-SA1 to cohesion and gene expression: implications for cancer and development. *Cell Cycle* 2012;11:2233–8.