Clinical characterization,
genetic screening
and genotype-phenotype associations in

CEREBELLAR AND BRAINSTEM CONGENITAL DEFECTS (CBCDs)

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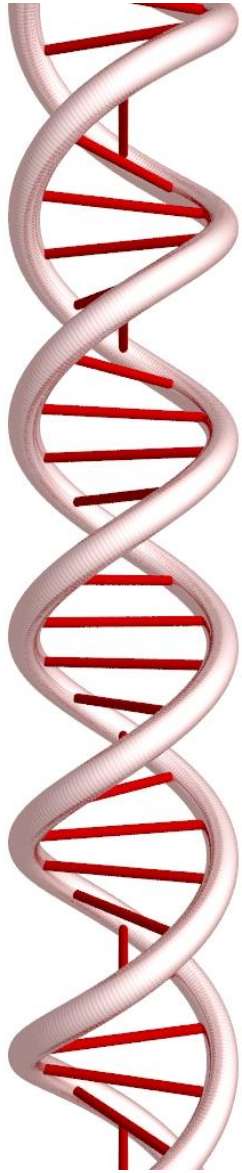
18 Giugno 2020

Coordinatore: Prof. P. Monteleone

Tutor: Prof. P. Vajro



SANTA LUCIA
NEUROSCIENZE
E RIABILITAZIONE

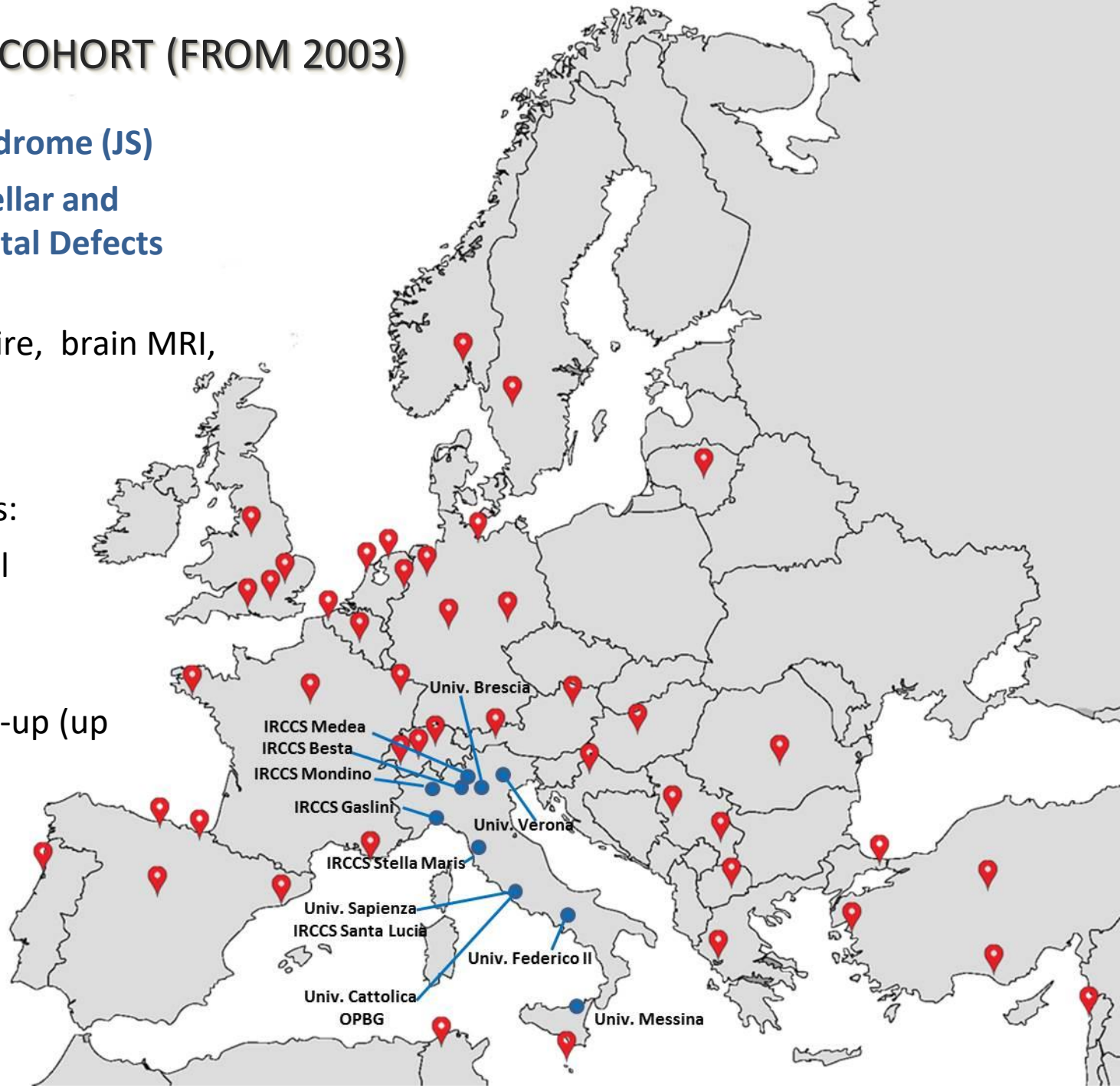
HIPPOCRATICA CIVITAS
STUDIUM SALERNI

OUR RECRUITED COHORT (FROM 2003)

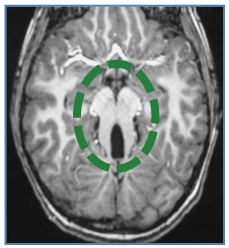
- **~ 600 Joubert Syndrome (JS)**
- **~ 670 other Cerebellar and Brainstem Congenital Defects (CBCDs)**
- Clinical questionnaire, brain MRI, biological samples

In a subset of patients:

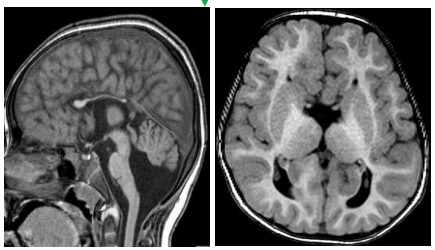
- neuropsychological assessment (++JS)
- DTI-tractography
- ≥18 months follow-up (up to 50%)
- genetic studies



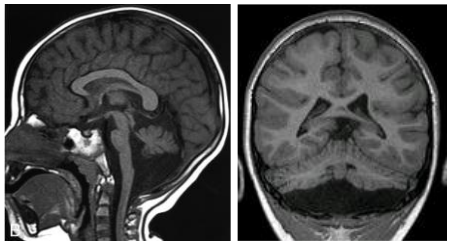
MRI



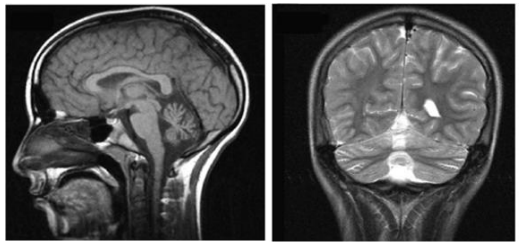
Joubert Syndrome



Tubulinopathy



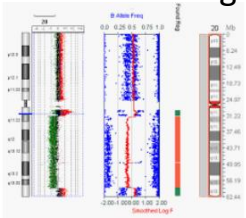
Pontocerebellar hypoplasia



Non-progressive congenital ataxia

Targeted resequencing (JS NGS panel)

if **monoallelic** variant in a recessive JS gene



Custom a-CGH (JS genes)

if negative

Sanger sequencing
Tubulin genes

if negative

Sanger sequencing
founder mutation *TSEN54* gene
MLPA *CASK* gene (qRT-PCR)

Targeted resequencing (PCH NGS panel)

if negative

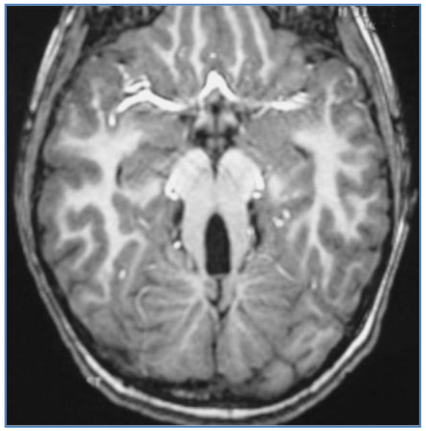
Targeted resequencing (NPCA NGS panel)

if negative

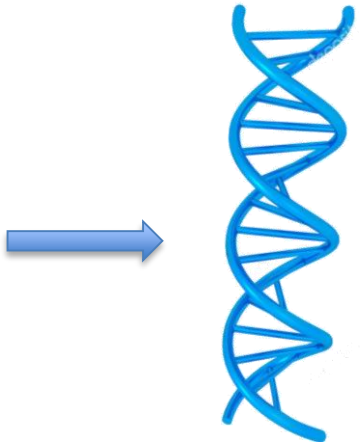
Whole exome sequencing

The Italian CBCD Project

JOUBERT SYNDROME



DIAGNOSIS based on
NEUROIMAGING



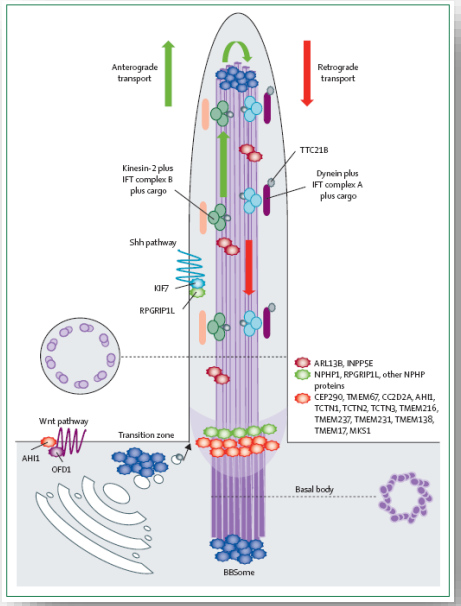
MOLECULAR DIAGNOSIS

- prognostic value
- prenatal diagnosis

>40 known GENES

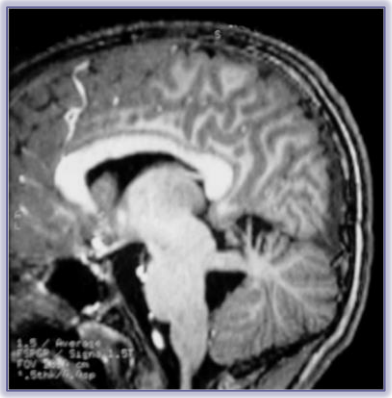
proteins of the primary cilium or its apparatus

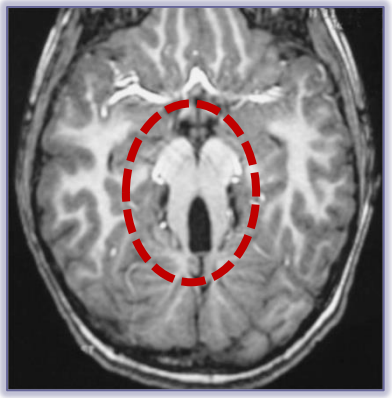
(Shh, Wnt, PDGFα)

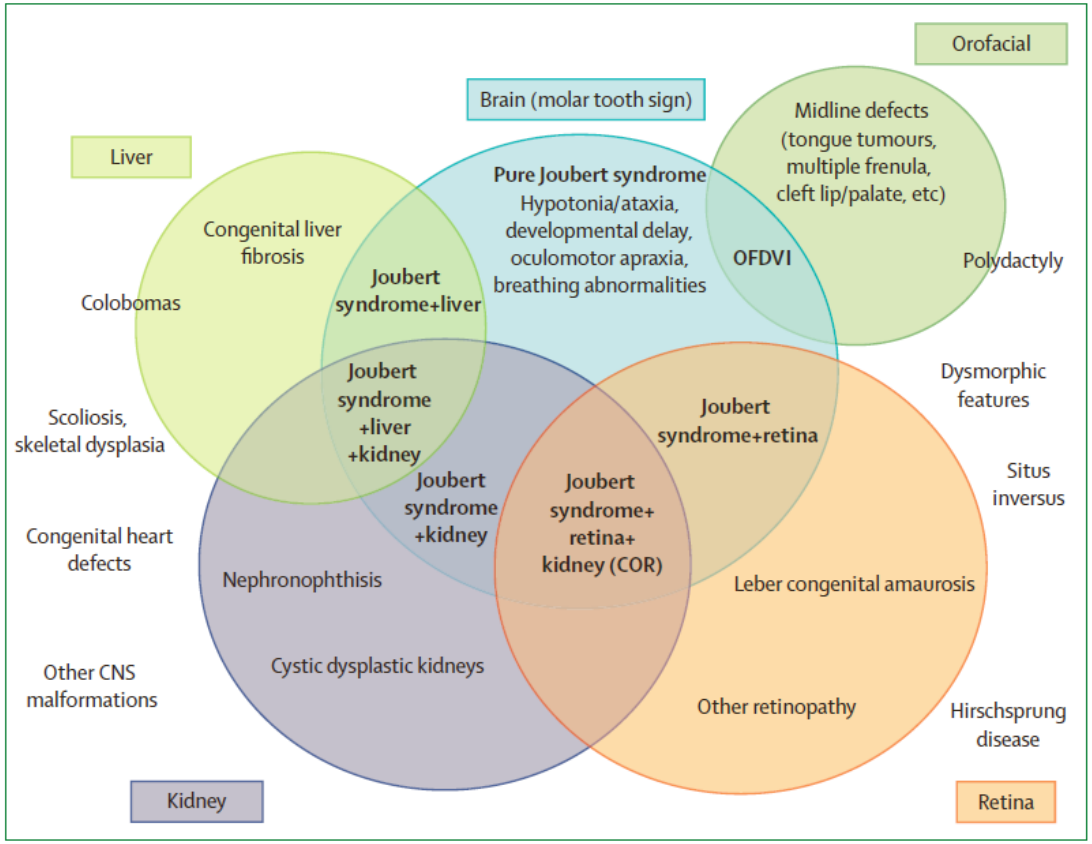


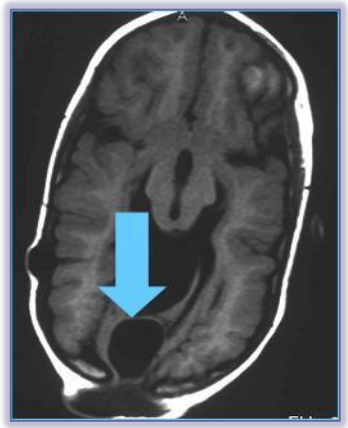
	JBTS	locus	Gene	Prevalent JS phenotypes
2004-2010	JBTS1	9q34	INPP5E	JS±retina; (JS+fegato)
	JBTS2	11q12	TMEM216	JS± rene; (JS+fegato, OFDVI)
	JBTS3	6q23	AHI1	JS±retina (JS+rene)
	JBTS4	2q13	NPHP1	JS+ rene
	JBTS5	12q21	CEP290	JS+retina+ rene
	JBTS6	8q22	TMEM67	JS+ fegato (JS+rene)
	JBTS7	16q12	RPGRIP1L	JS+ rene; (JS+ fegato)
	JBTS8	3q11	ARL13B	JS
	JBTS9	4p15	CC2D2A	JS±retina
	JBTS10	Xp22	OFD1	Fenotipo variabile
	JBTS11*	2q24	TTC21B	--
2011-2014	JBTS12	15q26	KIF7	JS, (OFDVI)
	JBTS13	12q24	TCTN1	JS±retina
	JBTS14	2q33	TMEM237	JS+ rene
	JBTS15	7q32	CEP41	JS
	JBTS16	11q12	TMEM138	Fenotipo variabile
	JBTS17	5p13	C5Orf42	JS±retina±caratteristiche OFD
	JBTS18	10q24	TCTN3	JS± caratteristiche OFD
	JBTS19	16q12	ZNF423	JS+ rene
	JBTS20	16q23	TMEM231	JS+retina+ rene
	JBTS24	12q24	TCTN2	JS
	JBTS21	8q13	CSPP1	JS
	JBTS22	2q37	PDE6D	JS+ rene +retina
	JBTS	1q42	EXOC8	JS
	JBTS28	17q22	MKS1	JS±retina
	JBTS27	17p11	B9D1	JS
2015-2018	JBTS34	19q13	B9D2	JS
	JBTS25	1p36	CEP104	JS
	JBTS29	17p13	TMEM107	JS+retina
	JBTS26	16p12	KIAA0556	JS
	JBTS33	13q21	PIBF1	JS
	JBTS§	11q13	C2CD3	JS+OFD
	JBTS§	1p36	NPHP4	JS+ rene
	JBTS31	5q23	CEP120	JS
	JBTS23	14q23	KIAA0586	JS
	JBTS32	10q24	SUFU	JS+SHH-related disorders
	JBTS§	2p15	TMEM17	JS+OFD
	JBTS§	12q21	POC1B	JS
	JBTS§	17p13	KIAA0753	JS+OFD
	JBTS30	2q37	ARMC9	JS
		1p13.3	CELSR2	JS
	JBTS35	10q24.32	ARL3	JS

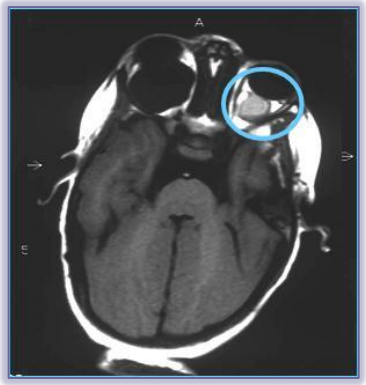
JS AND CILIOPATHIES

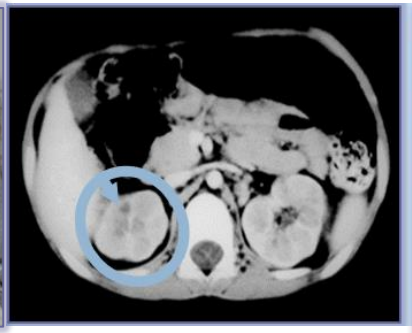


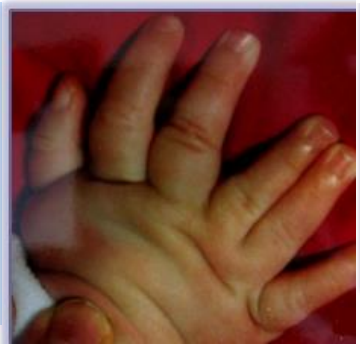


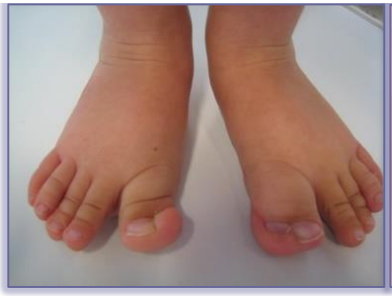


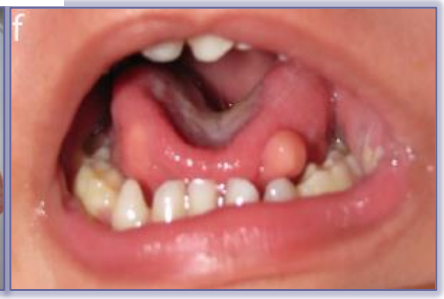








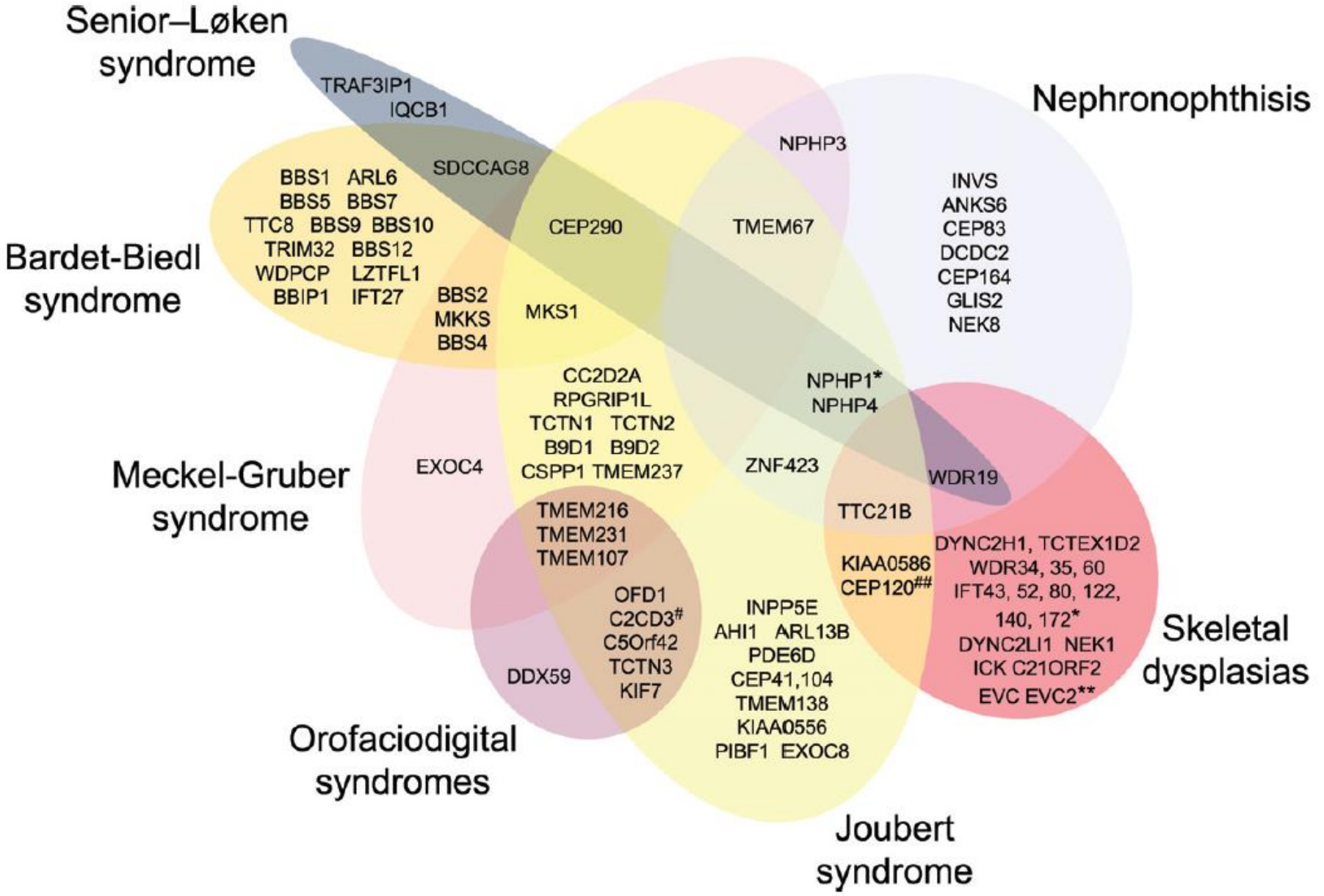




CLINICAL HETEROGENEITY

OVERLAP WITH OTHER CILIOPATHIES

JS AND CILIOPATHIES



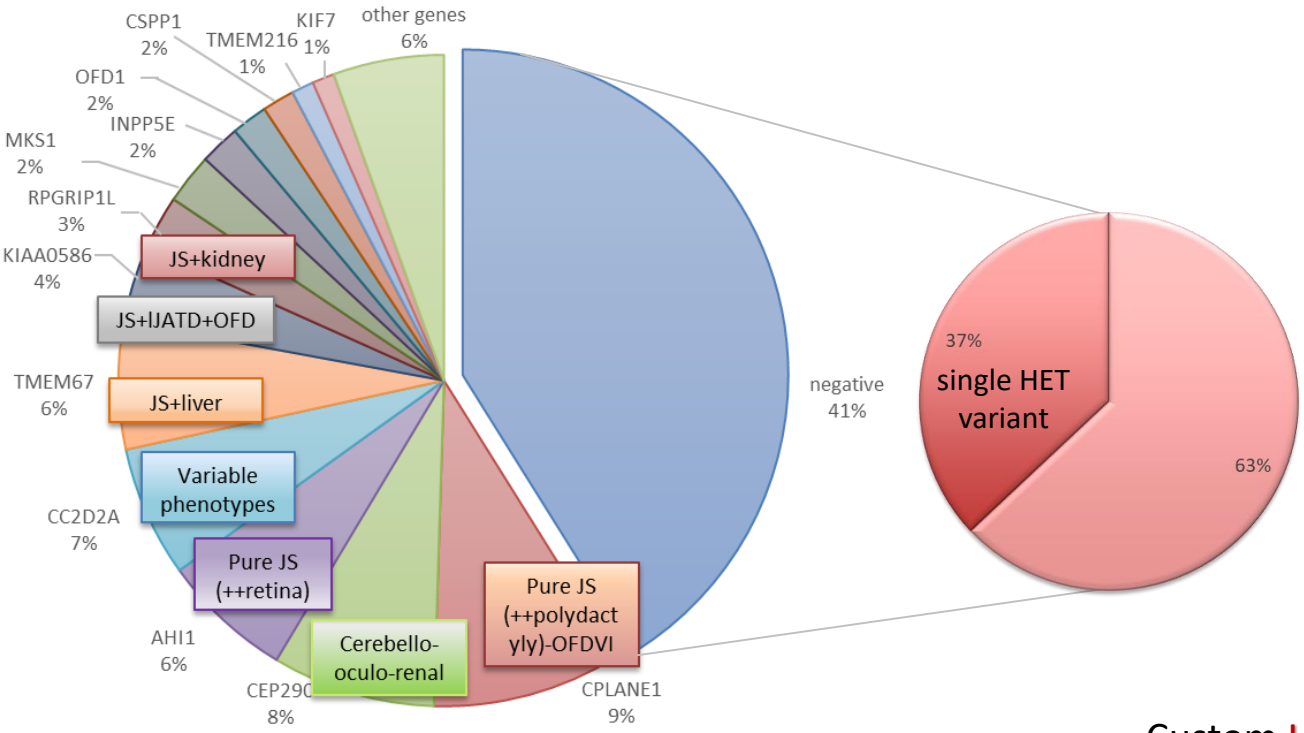
CLINICAL HETEROGENEITY

OVERLAP WITH OTHER CILIOPATHIES

JS GENETIC LANDSCAPE

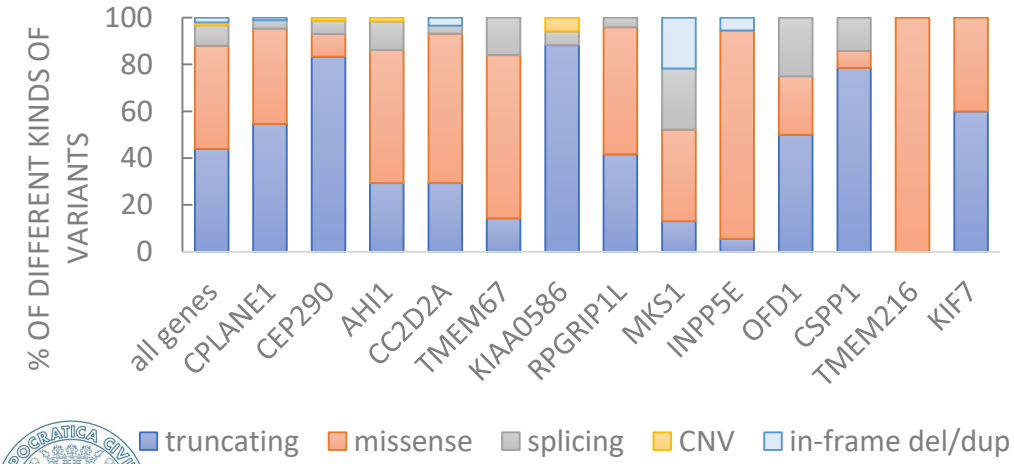
✓ **447** JS screened probands

✓ biallelic or XL variants in 257 (**59%**)



CAUSATIVE VARIANTS

(2nd heterozygous variant missed by sequencing)



Custom HIGH-RESOLUTION aCGH focused on JS genes (CNVs)

- 37 unrelated JS patients
- exon-disruptive CNVs represent the 2nd HET variant in 6 patients **(16%)**
- Genes: *CEP290*, *NPHP1*, *KIAA0586*, *AHI1*, *CPLANE1*

ARTICLE OPEN ACCESS

Age and sex prevalence estimate of Joubert syndrome in Italy

Sara Nuovo, MD, Ilaria Bacigalupo, BSc, Monia Ginevrino, BSc, Roberta Battini, MD, PhD, Enrico Bertini, MD, Renato Borgatti, MD, Antonella Casella, PhD, Alessia Micalizzi, PhD, Marta Nardella, PhD, Romina Romaniello, MD, Valentina Serpieri, BSc, Ginevra Zanni, MD, PhD, Enza Maria Valente, MD, PhD, and Nicola Vanacore, MD, PhD, on behalf of the JS Italian Study Group

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Neurology® 2020;94:1-5. doi:10.1212/WNL.0000000000008996

- 46 Italian centers
- overall, age- and sex-specific prevalence estimates
- JS in Italy on October 8, 2018 (60,483,973 inhabitants, Istat)
- neuroradiologically confirmed diagnosis of JS

Table 1 Population-based prevalence rate of JS in Italy (per 100,000 population, year 2018)

	Males			Females			Total	
Age, y	Cases, n	Population, n	Rate (95% CI)	Cases, n	Population, n	Rate (95% CI)	Cases, n	Rate (95% CI)
0–4	10	1,249,919	0.80 (0.30–1.30)	10	1,181,740	0.85 (0.32–1.37)	20	0.82 (0.46–1.18)
5–9	32	1,432,161	2.23 (1.46–3.01)	31	1,351,543	2.29 (1.49–3.10)	63	2.26 (1.70–2.82)
10–14	33	1,475,522	2.24 (1.47–3.00)	24	1,389,291	1.73 (1.04–2.42)	57	1.99 (1.47–2.51)
15–19	27	1,504,897	1.79 (1.12–2.47)	21	1,393,182	1.51 (0.86–2.15)	48	1.66 (1.19–2.12)
20–24	20	1,557,238	1.28 (0.72–1.85)	14	1,429,282	0.98 (0.47–1.49)	34	1.14 (0.76–1.52)
25–29	11	1,661,411	0.66 (0.27–1.05)	16	1,587,513	1.01 (0.51–1.50)	27	0.83 (0.52–1.14)
30–34	9	1,712,078	0.53 (0.18–0.87)	9	1,682,623	0.53 (0.19–0.88)	18	0.53 (0.29–0.78)
35–39	5	1,911,532	0.26 (0.03–0.49)	3	1,901,851	0.16 (0.00–0.34)	8	0.21 (0.06–0.36)
>40	9	16,922,849	0.05 (0.02–0.09)	0	19,139,341	0.00 (0.00–0.00)	9	0.02 (0.01–0.04)
Total	156	29,427,607	0.53 (0.45–0.61)	128	31,056,366	0.41 (0.34–0.48)	284	0.47 (0.41–0.52)

Abbreviations: CI = confidence interval; JS = Joubert syndrome.

PAEDIATRIC AGE: 1.70 per 100,000

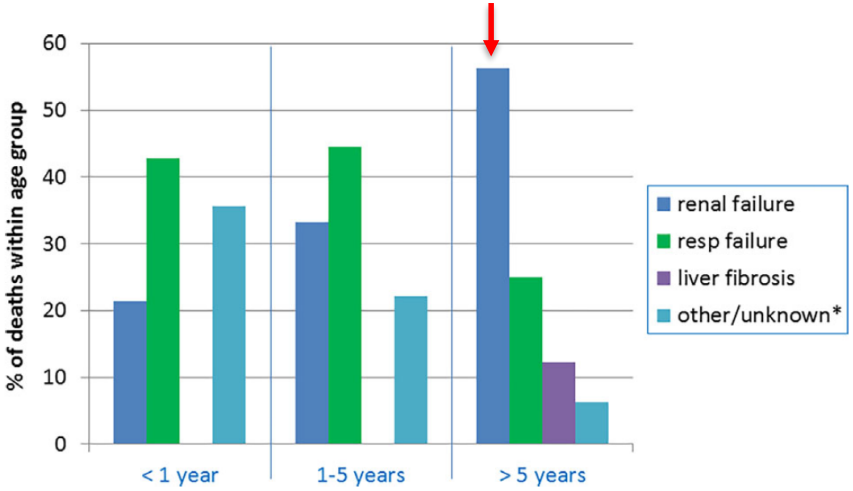
Nuovo S et al., 2020

Nephrol Dial Transplant (2018) 1–8
doi: 10.1093/ndt/gfy333

ndt
Nephrology Dialysis Transplantation

Impaired urinary concentration ability is a sensitive predictor of renal disease progression in Joubert syndrome

Sara Nuovo [1,2], Laura Fuiano[3], Alessia Micalizzi[1], Roberta Battini[4,5], Enrico Bertini[6], Renato Borgatti[7], Gianluca Caridi [8], Stefano D'Arrigo[9], Elisa Fazzi[10,11], Rita Fischetto[12], Gian Marco Ghiggeri[8], Lucio Giordano[10], Vincenzo Leuzzi[13], Romina Romaniello[7], Sabrina Signorini[14], Gilda Stringini[3], Ginevra Zanni[6], Marta Romani[15], Enza Maria Valente[1,16] and Francesco Emma[3]



Dempsey JC S et al., 2017

93 patients (76 families) JS

25-30 % of JS patients

RENAL

- serum/urine electrolytes
- polyuria yes/no
- kidney US
- basal urine osmolality
  < 800 mOsm/Kg H_2O
- DDAVP test (MINIRIN)

NEPHRONOPHTHISIS

- progressive tubular atrophy, interstitial fibrosis
- small corticomedullary cysts
- progression in CKD

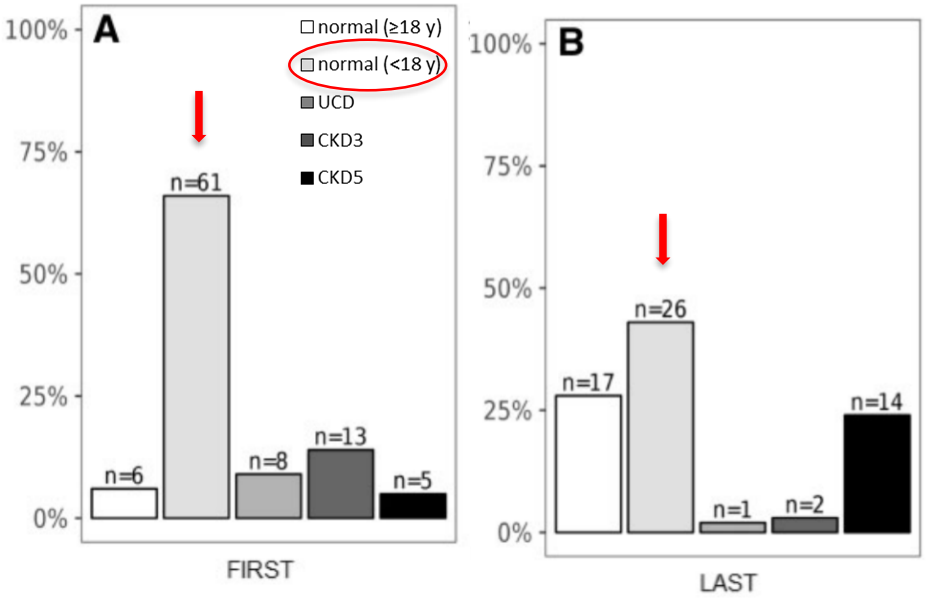
FOLLOW-UP (60 subjects)

- initial normal eGFR
- DURATION (median): 9 [1-14] years

CYSTIC DYSPLASTIC KIDNEYS

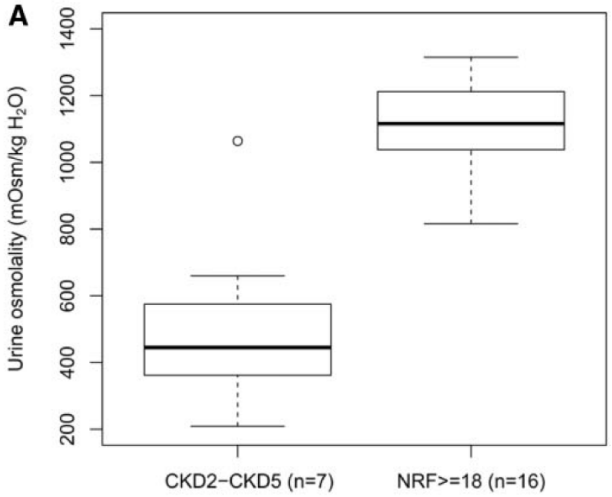
asymptomatic for years, lack of BIOMARKER

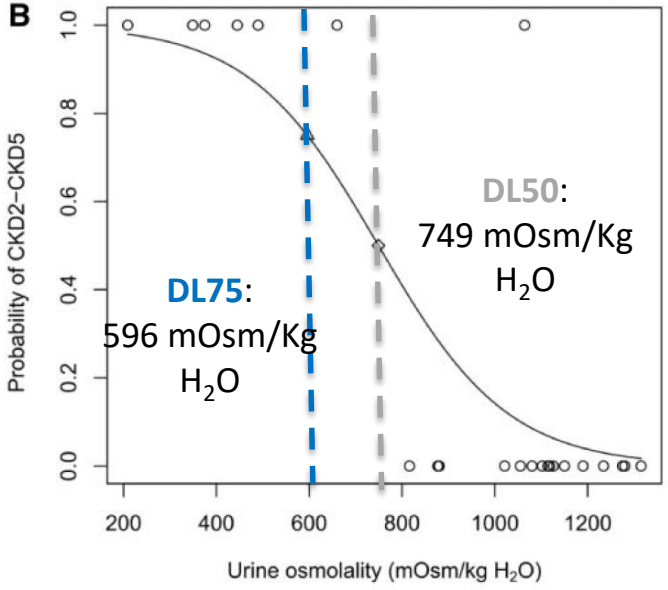
RENAL FUNCTION: FOLLOW-UP



CHRONIC KIDNEY DISEASE IN JS PATIENTS: 29%

CANDIDATE BIOMARKER (URINE OSMOLALITY) FOR EARLY RECOGNITION OF RENAL DISEASE



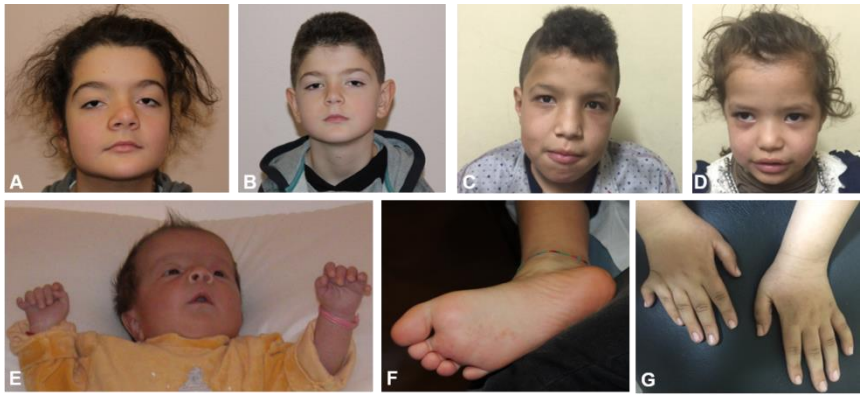


DL50: probability of adverse renal outcome >50%

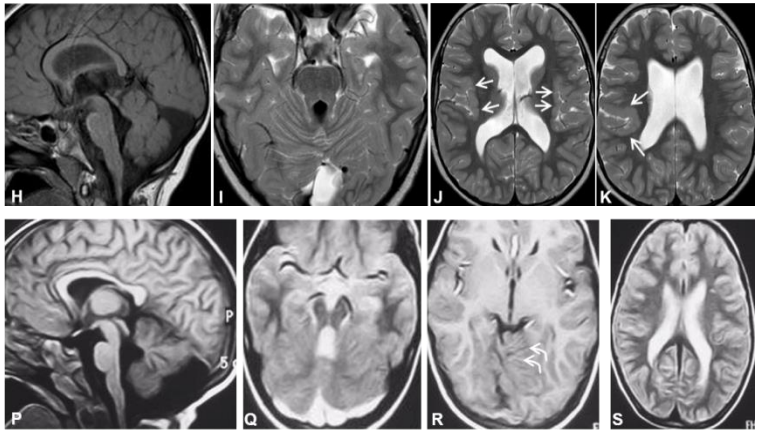
DL75: probability of adverse renal outcome >75%

Nuovo S et al., 2018

JS-WES APPROACH



macrocephaly/macrosomy/hypertelorism/polydactyly/
frontal bossing/depressed nasal bridge/ PMD/ID

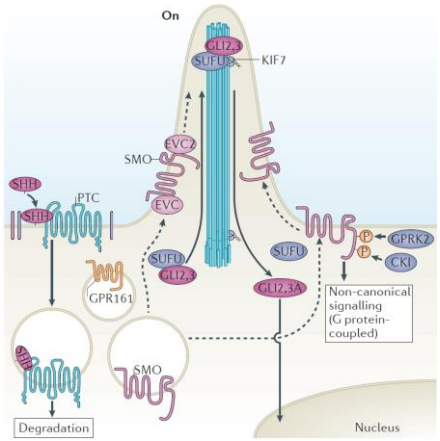


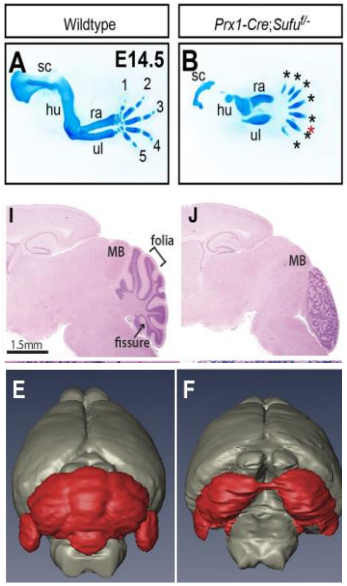
mild molar tooth ± polymicrogyria

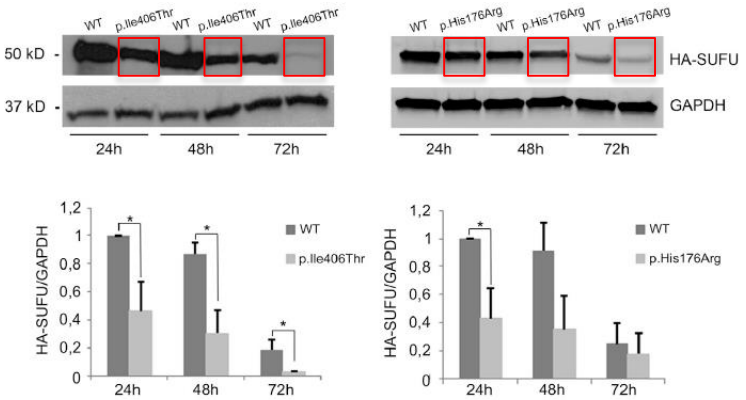
REPRESSOR OF SHH SIGNALING

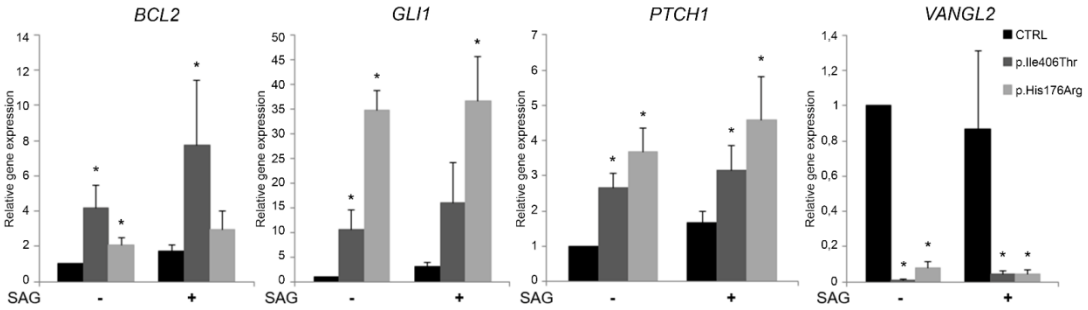
WES *SUFU* p.Ile406Thr; p.His176Arg

Conditional KO: polydactyly, cerebellar hypoplasia









De Mori et al., 2017

JS-WES APPROACH

ARTICLE

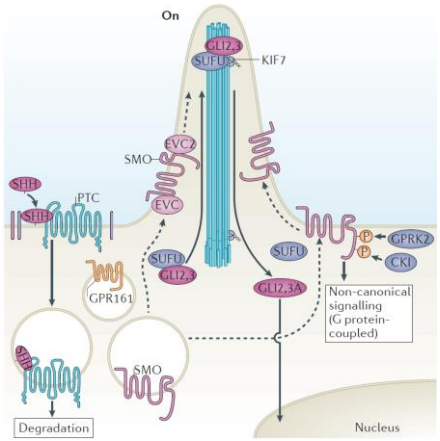
Hypomorphic Recessive Variants in *SUFU* Impair the Sonic Hedgehog Pathway and Cause Joubert Syndrome with Cranio-facial and Skeletal Defects

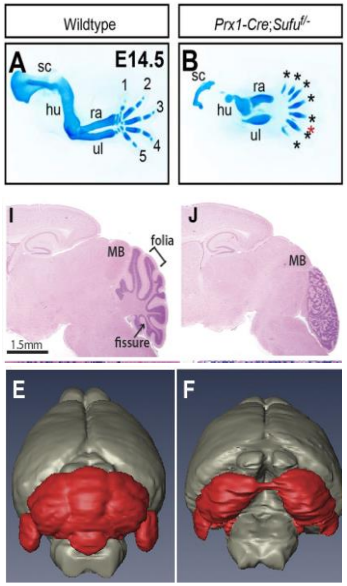
Roberta De Mori,[1,2,20] Marta Romani,[3,20] Stefano D'Arrigo,[4] Maha S. Zaki,[5] Elisa Lorefice,[1] Silvia Tardivo,[1] Tommaso Biagini,[6] Valentina Stanley,[7] Damir Musaev,[7] Joel Fluss,[8] Alessia Micalizzi,[1,2] Sara Nuovo,[1,9] Barbara Illi,[10] Luisa Chiapparini,[11] Lucia Di Marcotullio,[12] Mahmoud Y. Issa,[5] Danila Anello,[1] Antonella Casella,[1] Monia Ginevrino,[1,13] Autumn Sa'na Leggins,[7] Susanne Roosing,[14] Romina Alfonsi,[12] Jessica Rosati,[15] Rachel Schot,[16] Grazia Maria Simonetta Mancini,[16] Enrico Bertini,[17] William B. Dobyns,[18,19] Tommaso Mazza,[6] Joseph G. Gleeson,[7] and Enza Maria Valente[1,13,*]

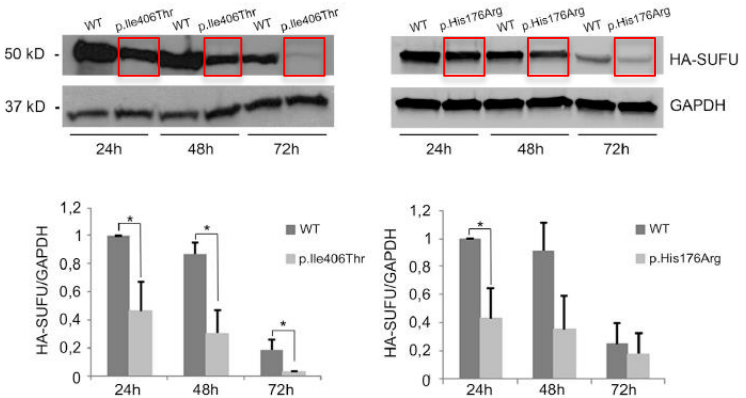
REPRESSOR OF SHH SIGNALING

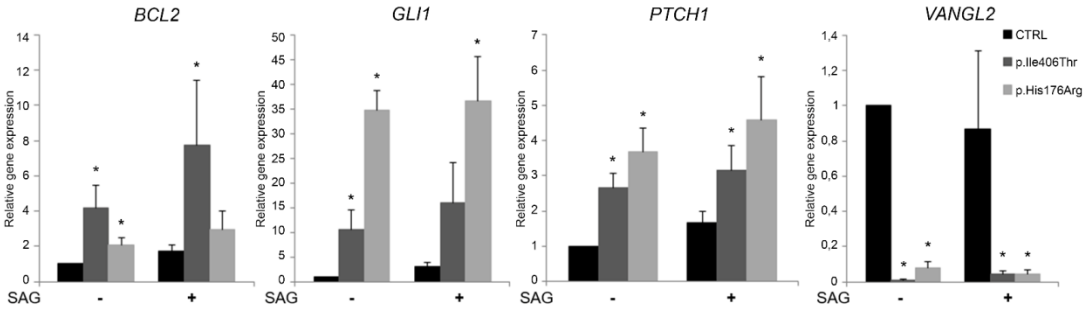
WES *SUFU* p.Ile406Thr; p.His176Arg

Conditional KO: polydactyly, cerebellar hypoplasia







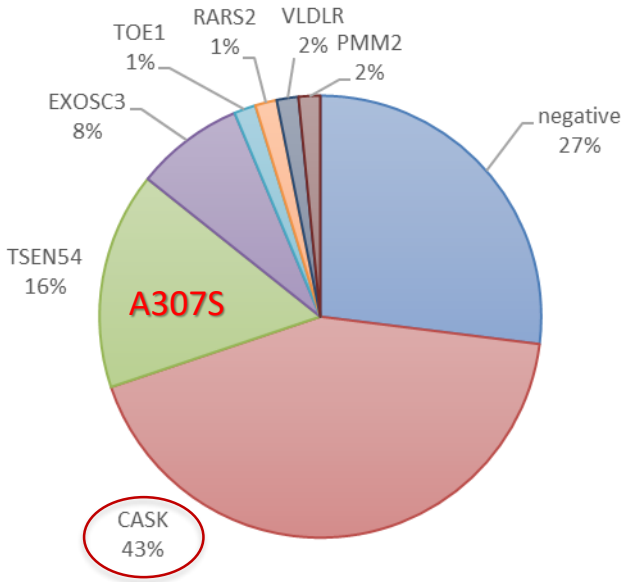


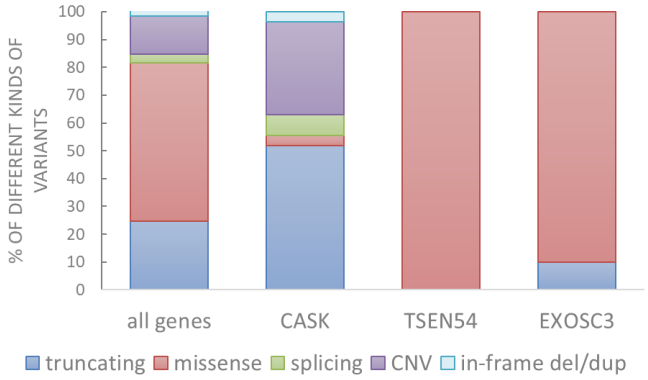
De Mori et al., 2017

PONTOCEREBELLAR HYPOPLASIA

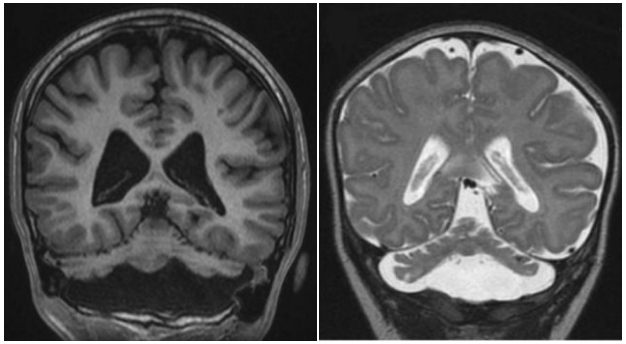
13 SUBTYPES, over 21 GENES

- ✓ **63** screened probands
- ✓ biallelic or XL variants in 46 (**73%**)

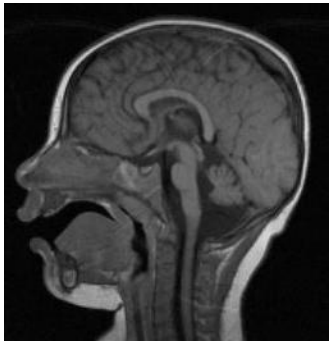




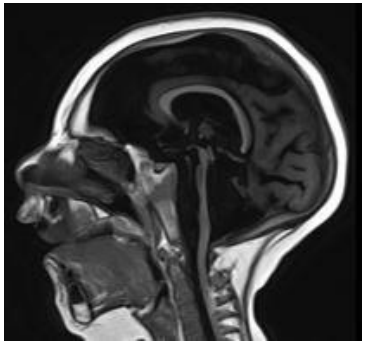
DRAGONFLY BUTTERFLY



CASK PCH males → **4/25 (16%)**



Arg28* (mosaic)



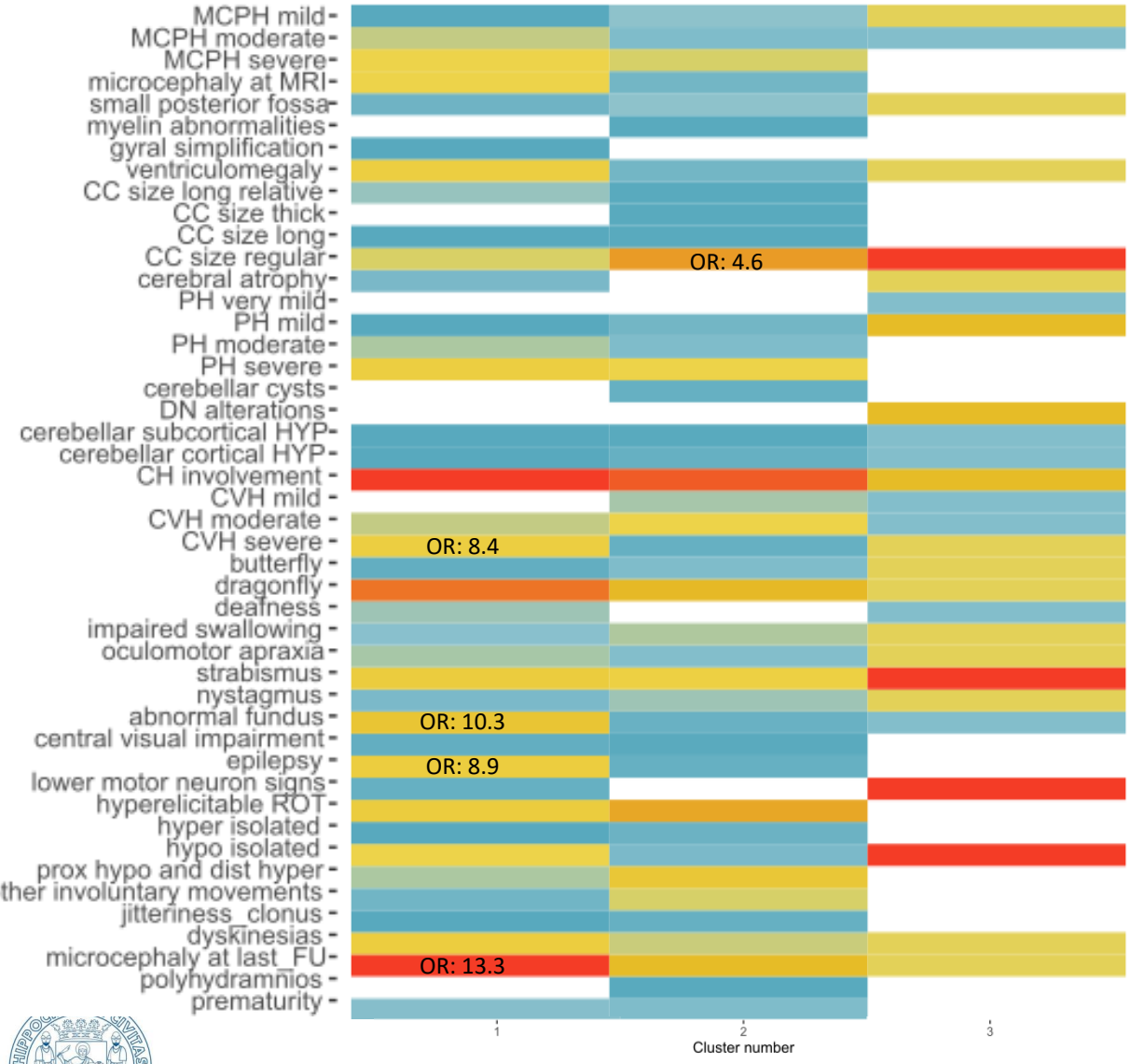
ex9_ex10 del

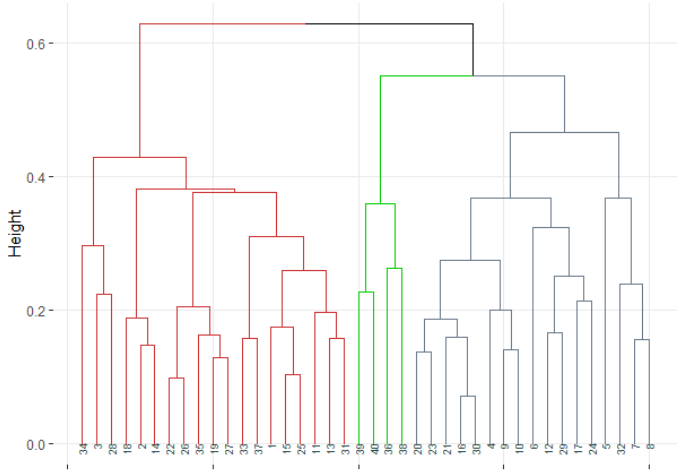
mosaic LoF, severe miss → hemiz LoF

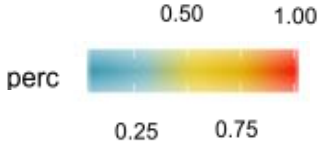
- microcephaly
- PCH
- ID
- variable seizures
- deafness
- butterfly/dragonfly

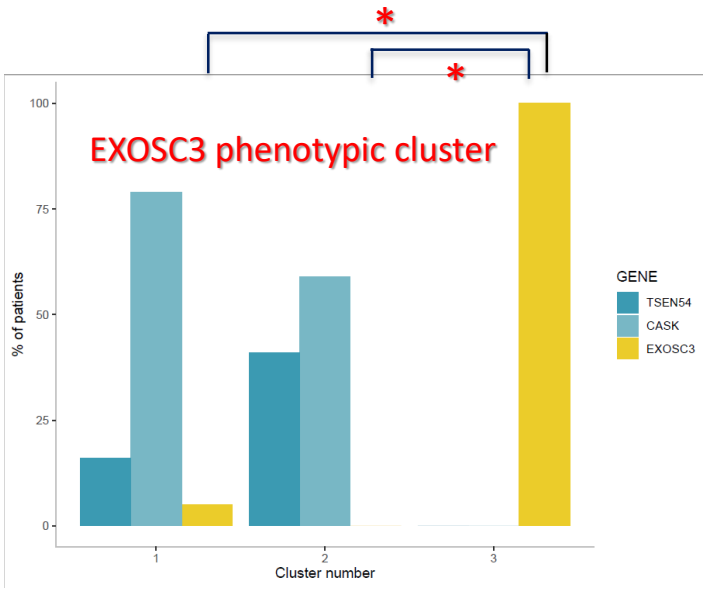
- **often lethal**
- PCH
- psychomotor delay
- microcephaly
- cortical atrophy
- seizures

CLUSTER ANALYSIS









TUB-CEREBELLUM

Eur Radiol
DOI 10.1007/s00330-017-4945-2
NEURO

Tubulin-related cerebellar dysplasia: definition of a distinct pattern of cerebellar malformation

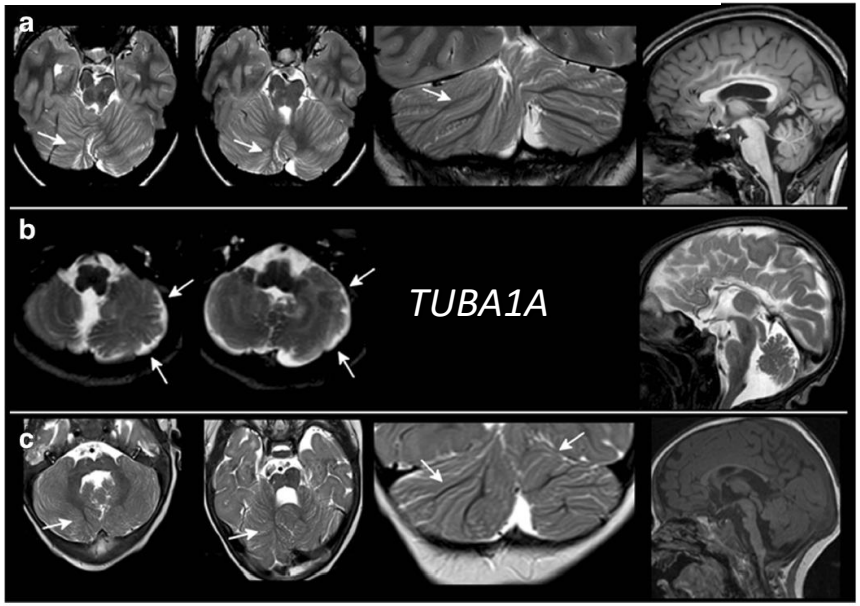
Romina Romaniello[1] · Filippo Arrigoni[2] · Elena Panzeri[3] · Andrea Poretti[4,5] · Alessia Micalizzi[6,7] · Andrea Citterio[3] · Maria Francesca Bedeschi[8] · Angela Berardinelli[9] · Margherita Cusmai[10] · Stefano D'Arrigo[11] · Alessandro Ferraris[12] · Annette Hackenberg[13] · Alma Kuechler[14] · Margherita Mancardi[15] · Sara Nuovo[6,16] · Barbara Oehl-Jaschkowitz[17] · Andrea Rossi[18] · Sabrina Signorini[9] · Frank Tüttelmann[19] · Dagmar Wahl[20] · Ute Hehr[21] · Eugen Boltshauser[22] · Maria Teresa Bassi[3] · Enza Maria Valente[6,23] · Renato Borgatti[1]

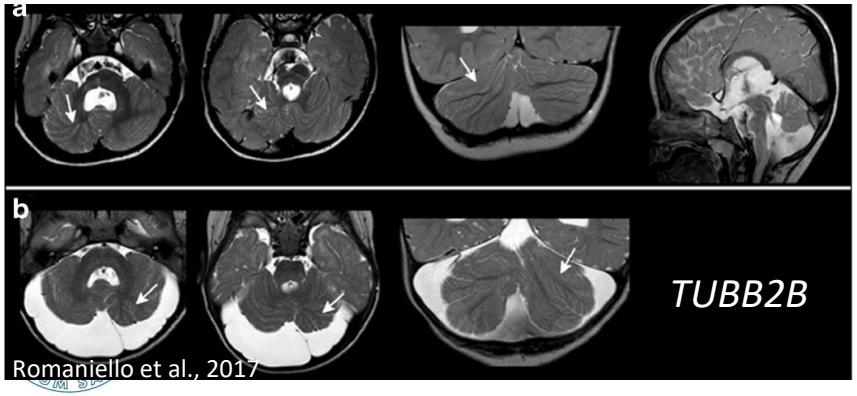
- 28 patients mutated in *TUBA1A*, *TUBB2B* or *TUBB3*
- 24 children (age 5m-11y)
- 4 adults (age 22-25y)
- focus on CEREBELLUM (MRI)

CEREBELLAR anomalies in 24/28 (86%)

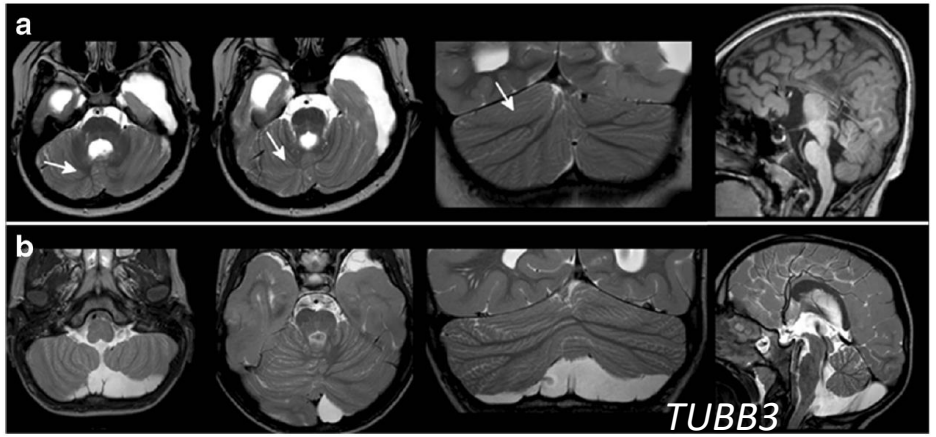
↓

DYSPLASIA in 19/28 (68%)





Romaniello et al., 2017



"TUBULIN-RELATED CEREBELLAR DYSPLASIA"

- CORTICAL ± VERMIS DYSPLASIA
- PREVALENT UNILATERAL PATTERN (RIGHT > > LEFT)
- OFTEN IN THE POSTERO-SUPERIOR HEMISPHERIC REGION
- REGULAR ASPECT OF THE CEREBELLAR CORTEX (NO CYSTS, THICKENING OF THE CEREBELLAR FOLIA OR SIGNAL ALTERATIONS)

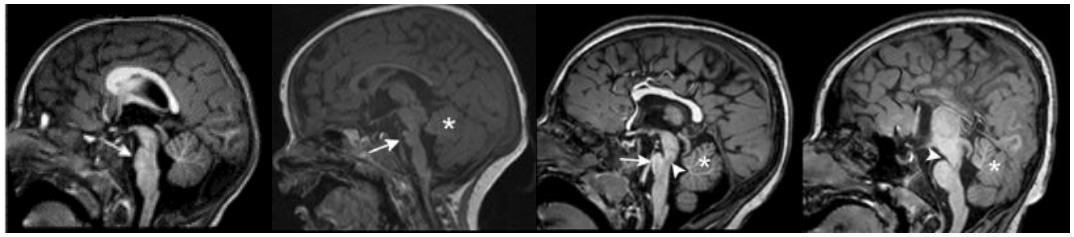
TUB-BRAINSTEM

European Radiology
https://doi.org/10.1007/s00330-018-5610-0

NEURO

The spectrum of brainstem malformations associated to mutations of the tubulin genes family: MRI and DTI analysis

Filippo Arrigoni[1] · Romina Romaniello[2] · Denis Peruzzo[1] · Andrea Poretti[3] · Maria Teresa Bassi[4] · Carlo Pierpaoli[5] · Enza Maria Valente[6,7] · Sara Nuovo[7,8] · Eugen Boltshauser[9] · Thierry André Gerard Marie Huisman[3] · Fabio Triulzi[10] · Renato Borgatti[2]



- 15 patients mutated in *TUBA1A-TUBB2B-TUBB3*
- median age 1.25y (1m-31y)
- focus on BRAINSTEM (MRI, DTI)

BRAINSTEM malformation in 14/15 (93%)

ASYMMETRY (80%)
- pons
- pons + medulla oblongata and/or mesencephalon
- crossed between medulla and pons

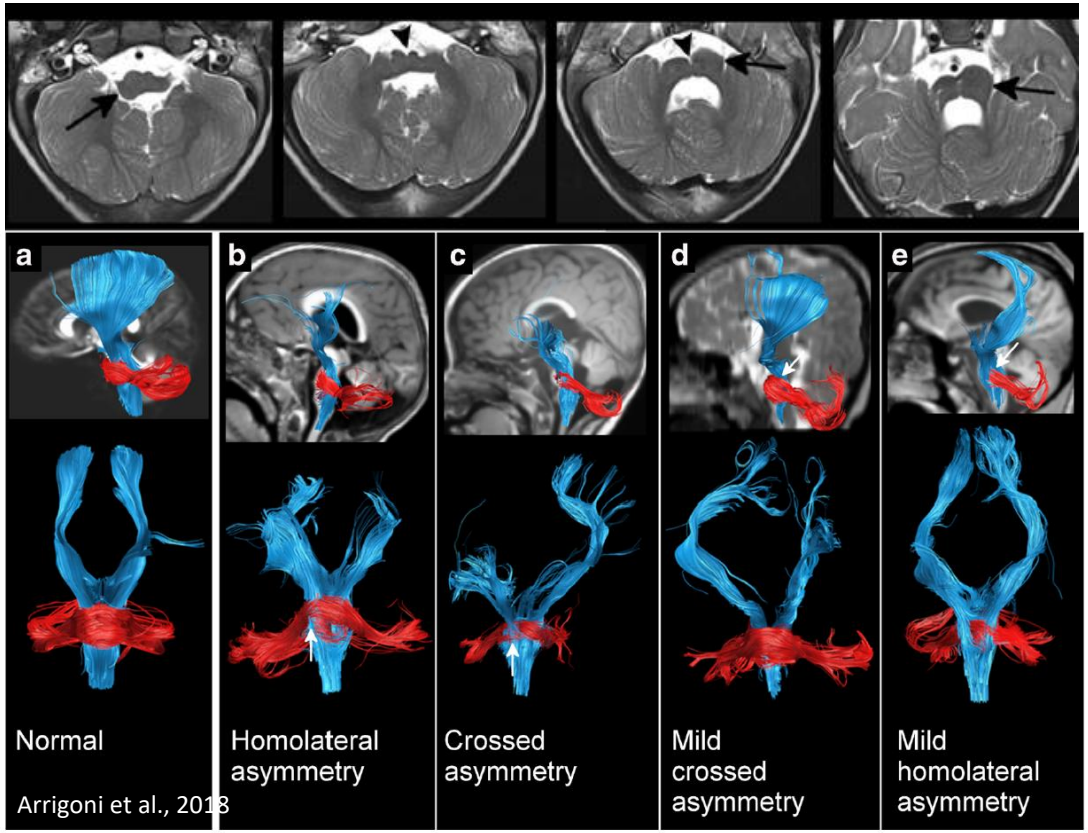
ANTERIOR CLEFT (40%)

SHORT/SMALL PONS (67%)

IRREGULAR PONTO-MESENCEPHALIC JUNCTION (33%)

SHORT/ASYMMETRIC MCP_S (47%)

"CROSSED ASYMMETRY" PATTERN

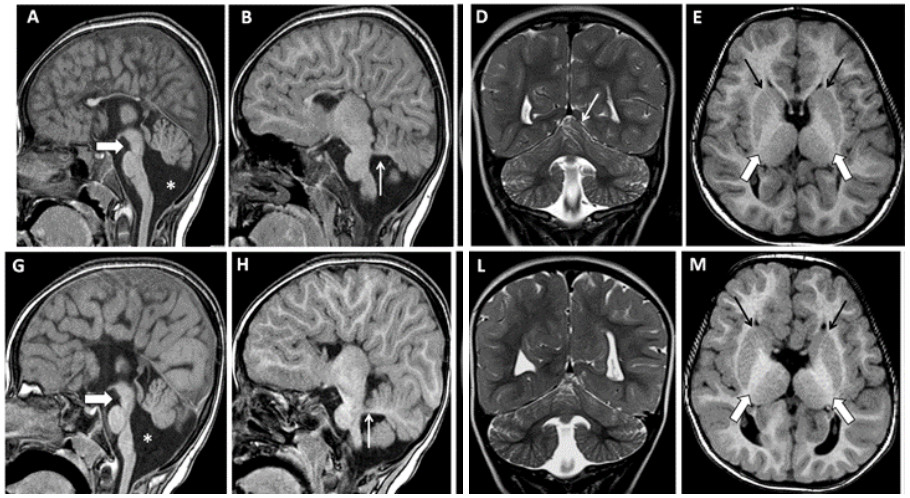


altered organisation of WHITE MATTER TRACTS

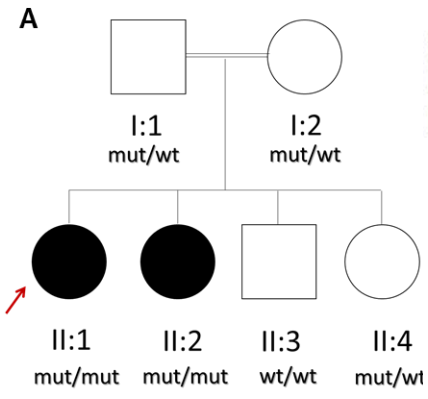
Cortico-spinal tract (CST)

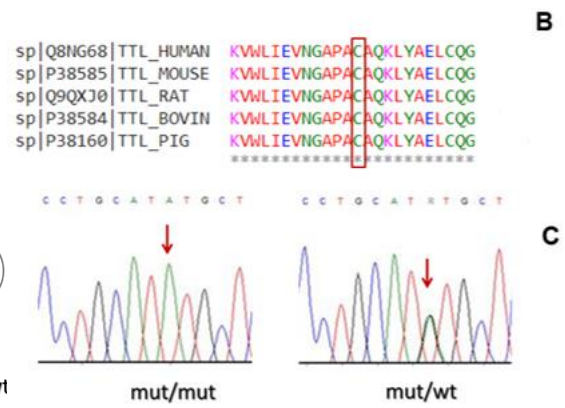
Transverse Pontine Fibers (TPF)

(1) THINNING/ATROPHY OF CST
(2) ABNORMAL COURSE OF CST
(3) THINNING/THICKENING OF TPF
(4) IRREGULAR COURSE OF TPF
(5) COMBINED



TUB-WES APPROACH

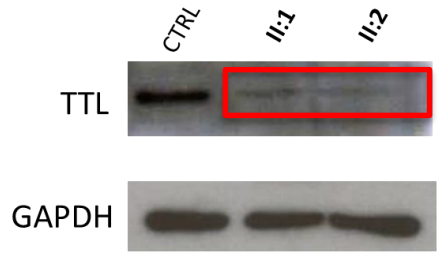


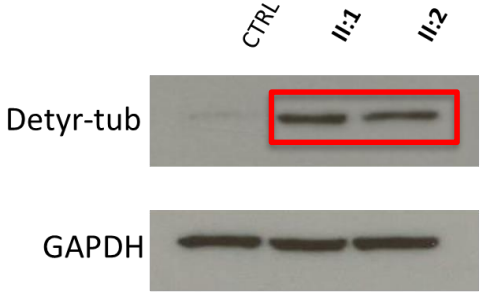


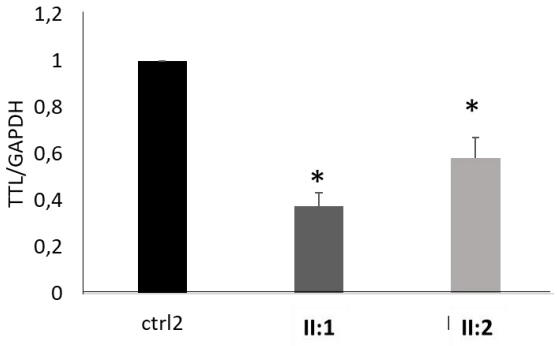
- commissure agenesis/hypoplasia
- hypodysplastic-counterclockwise rotated cerebellar vermis
- horizontalized SCPs
- enlarged 4th ventricle
- dysplastic brainstem
- dysplastic internal capsule

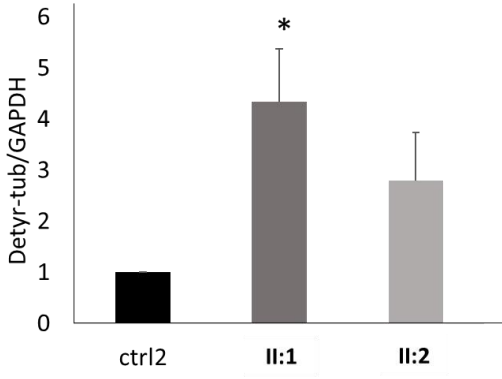
DECREASED LEVELS OF TTL mutant protein and **HIGHER DETYROSINATED TUBULIN EXPRESSION**

WES analysis: c. 1013G>A (p.Cys338Tyr) in *TTL*

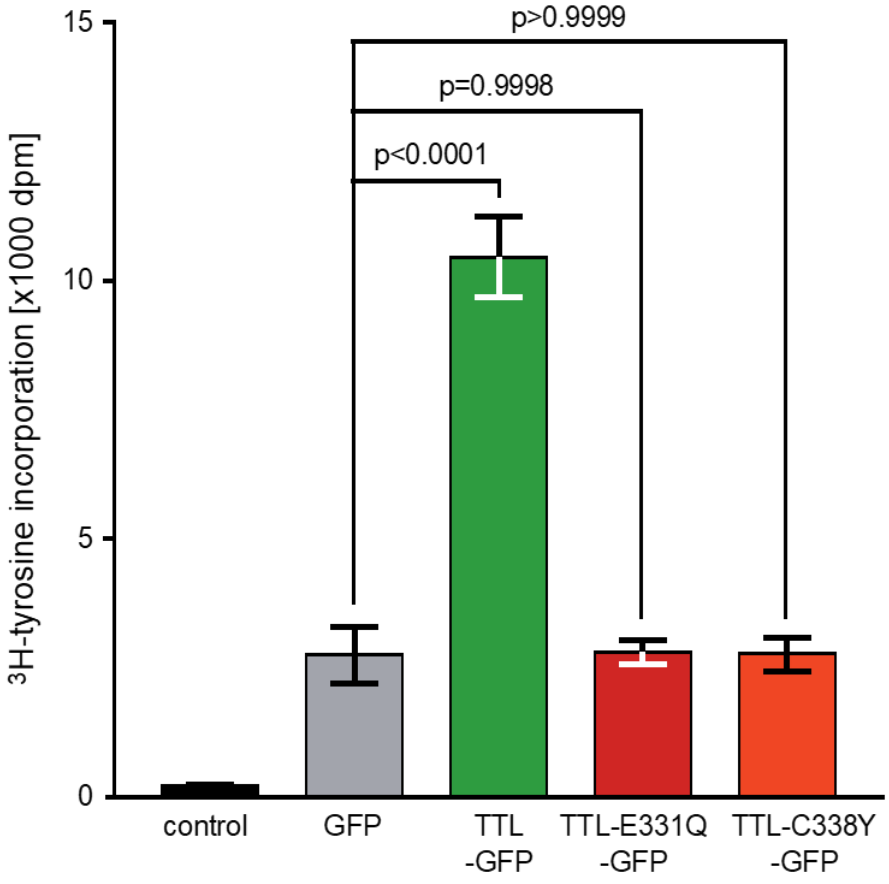




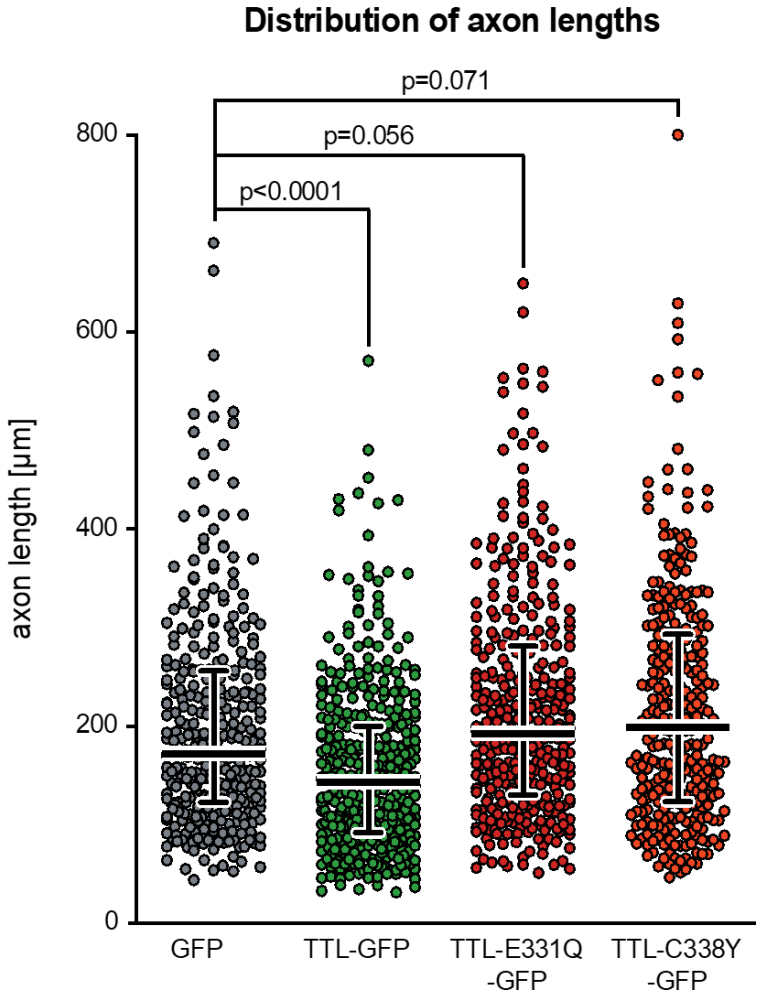




TUB-WES APPROACH



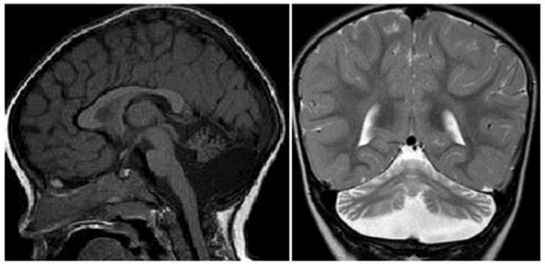
BOTH TTL MUTANTS ARE **ENZYMATICALLY INACTIVE**



INCREASED **AXONAL LENGTH**

RECESSIVELY INHERITED TUBULINOPATHY-LIKE DISORDER, CAUSED BY BIALLELIC LOSS OF FUNCTION MUTATIONS IN THE *TTL* GENE

NPCA-*SPTBN2*



SPTBN2 c.I1438C>T; p.Arg480Trp

- 2-YEAR-OLD GIRL
- GENERALIZED HYPOTONIA, GLOBAL DEVELOPMENTAL DELAY, ALTERNATING ESOTROPIA
- CEREBELLAR SYNDROME WITH GAIT ATAXIA AND DYSARTHRIA
- MRI: GLOBAL CEREBELLAR HYPOPLASIA WITH ENLARGED INTERFOLIAL SPACES

Nuovo S et al., 2018

	SCA5					"R480W-phenotype"	SCAR14			
Reference	2	2	2	3	4	3, 9, present study	6	1	5	7
Genetic variant	c.1592_1630del p.E532_M544del	c.1886_1900del p.L629_R634 delinsW	c.758T>C p.L253P	c.1415 C>T p.T472M	c.2608_2610del p.E870del	c.1438C>T p.R480W	c.6375-1G>C	c.2864_2868del p.T955Sfs*120	c.1881C>A p.C627X	c.1572C>T p.R414C
Inheritance			het AD			het de novo		hom AR		
Exon	12	14	7	12	14	12	splice site	16	14	2
Domain	3rd SPEC	3rd SPEC	CH	2nd SPEC	6th SPEC	2nd SPEC	PH	6th SPEC	3rd SPEC	1st SPEC
N. of affected	90	6	12	3	5	3	9	3	3	2
Mean age of onset (years)	45	27	33	45	53	congenital		congenital		
Cerebellar syndrome	+	+	+	+	+	+	+	+	+	+
Abnormal ocular movements	-	+	+	-	-	+	-	-	+	+
Hypotonia	-	-	-	-	-	2/3	+	-	1/3	+
Pyramidal signs	4/90	5/6	-	-	1/5	1/3	-	+	-	-
Tremor	-	-	7/12	-	-	1/3	3/9	-	-	-
Focal dystonia	-	1/6	-	-	-	-	-	-	-	-
Facial myokymia	-	4/6	-	-	-	1/3	-	-	-	-
Bulbar dysfunction	2/90	-	-	-	1/5	-	-	-	-	-
DD/ID	-	-	-	-	-	+	+	+	+	+
Behavioral problems	-	-	-	-	-	-	8/8[c]	-	-	-
Cerebellar atrophy	+	+	+	+	+	+[a]	+	NA	2/3[e]	+
Clinical progression	+	+	+	+	+	1/2[b]	+[d]	no	no	no

European Journal of Human Genetics
https://doi.org/10.1038/s41431-018-0158-7

ESHG

CORRESPONDENCE

Check for updates

Between SCA5 and SCAR14: delineation of the *SPTBN2* p.R480W-associated phenotype

Sara Nuovo[1,2] · Alessia Micalizzi[1] · Stefano D'Arrigo[3] · Monia Ginevrino[1,4] · Tommaso Biagini[5] · Tommaso Mazza[5] · Enza Maria Valente[1,4]

Inheritance			het AD			het de novo		hom AR		
Exon	12	14	7	12	14	12	splice site	16	14	2
Domain	3rd SPEC	3rd SPEC	CH	2nd SPEC	6th SPEC	2nd SPEC	PH	6th SPEC	3rd SPEC	1st SPEC
N. of affected	90	6	12	3	5	3	9	3	3	2
Mean age of onset (years)	45	27	33	45	53	congenital		congenital		
Cerebellar syndrome	+	+	+	+	+	+	+	+	+	+
Abnormal ocular movements	-	+	+	-	-	+	-	-	+	+
Hypotonia	-	-	-	-	-	2/3	+	-	1/3	+
Pyramidal signs	4/90	5/6	-	-	1/5	1/3	-	+	-	-
Tremor	-	-	7/12	-	-	1/3	3/9	-	-	-
Focal dystonia	-	1/6	-	-	-	-	-	-	-	-
Facial myokymia	-	4/6	-	-	-	1/3	-	-	-	-
Bulbar dysfunction	2/90	-	-	-	1/5	-	-	-	-	-
DD/ID	-	-	-	-	-	+	+	+	+	+
Behavioral problems	-	-	-	-	-	-	8/8[c]	-	-	-
Cerebellar atrophy	+	+	+	+	+	+[a]	+	NA	2/3[e]	+
Clinical progression	+	+	+	+	+	1/2[b]	+[d]	no	no	no

ACKNOWLEDGEMENTS

❖ **ASSOCIAZIONE AISJAC**

❖ **Prof.ssa Enza Maria Valente**

- Alessia Micalizzi, Monia Ginevrino, Roberta De Mori, Simona Petrucci, Silvia Tardivo, Antonella Casella, Elisa Lorefice, Danila Anello, Romina Cutigni
- Ivan Limongelli (enGenome)

Agilent Technologies

illumina

❖ **Collaborazioni Internazionali**

- Joe Gleeson (San Diego)
- Eugen Boltshauser, (Zurich, Baltimore)
- Tania Attie-Bitach (Paris)
- The International JSRD Study Group

Andrea Poretti, M.D.

Assistant Professor of Radiology and Radiological Science

Expertise: Pediatric Imaging, Pediatric Neurology

Aprile 1977-Marzo 2017

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European Research Council
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The Italian CBCD Project

Ad Andrea

SANTA LUCIA
NEUROSCIENZE
E RIABILITAZIONE