Supplementary Tables

MED20 biallelic pathogenic variants cause a neurodevelopmental disorder altering both transcription activity and Transcription-Coupled Repair pathway

Supplementary Table 1

	Family 1 (France)	Family 2 (Austria)		Family 3 (USA)		Family 4 (Senegal)		
Individual	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Variant, DNA	c.326del c.475G>C	c.341G>C (homozygous)	c.341G>C (homozygous)	c.253C>T (homozygous)	c.253C>T (homozygous)	c.253C>T (homozygous)	c.253C>T (homozygous)	c.253C>T (homozygous)
Variant, protein	p.Lys109Argfs*16 p.Glu159Gln	p.Gly114Ala	p.Gly114Ala	p.Leu85Phe	p.Leu85Phe	p.Leu85Phe	p.Leu85Phe	p.Leu85Phe
Gender	F	F	F	M	M	M	M	M
Age at last report (y)	17	16	14	14	21	29	25	22
Deceased		At age 16						
Presenting symptoms	Developmental delay	Strabismus since birth; Developmental delay	Strabismus since birth; Developmental delay	Developmental delay	Developmental delay	Developmental delay	Developmental delay	Developmental delay
Age at independent walking (y)	No independent walking	Walk with assistance at 2.5 y, never reached independent walking, W wheelchair-bound since the age of 10 y	Walk with assistance at 2.5 y, never reached independen t walking, wheelchair-bound since the age of 14 y	Walked and ran at 2 y, wheelchair bound by age 11 y	2.5 Still able to walk and run at 15 y, decreased ambulatory capacity at 21 y with ataxic gait	3	3	3
Speech	Absence of expression speech	Absence of expression speech, language perception	Absence of expression speech, language perception	Difficulties of speech (1-2 words)	Difficulties of speech (50 words)	No expressive speech; partial receptive language	No expressive speech; partial receptive language	No expressive speech; partial receptive language

		appropriate to the developmental stage of early childhood	appropriate to the developmental stage of early childhood, uses communication devices					
Intellectual disability	Yes, severe	Yes	Yes	Yes, moderate	Yes, moderate	Yes, moderate	Yes, moderate	Yes, moderate
Eyes	Bilateral congenital cataracts	Bilateral cataracts (childhood onset, surgery at 11 y) Convergent strabismus	Bilateral cataracts (childhood onset, surgery at 9 y Convergent strabismus	No cataracts	Bilateral cataracts (childhood onset, surgery at 6 y)	Bilateral cataracts (childhood onset)	Bilateral cataracts (childhood onset)	Bilateral cataracts (childhood onset)
Hearing impairment	No	No	No	No	No	No	No	No
Seizures	Yes	No	Suspected at the age of 14 y	Yes	Yes	No	No	No
Dystonic signs	Yes, progressive and severe	Yes from the age of 3 y, progressive and severe	Yes Progressive, severe	Mild	Mild	Mild	Moderate	Moderate
Pyramidal signs	Infantile onset spasticity	Infantile onset spasticity	Infantile onset spasticity	Moderate to severe, progressive spasticity, moderate dysmetria	Moderate progressive spasticity, hyperreflexia, mild dysmetria	Mild spasticity	Moderate spasticity	Moderate spasticity
Cerebellar signs	Yes	Yes	Yes	Yes	Yes	Mild	No	No
Sensorimotor Neuropathy	ND	Yes (confirmed on EMG/NCS)	ND	ND	ND	ND	ND	ND

Progressive neurological features /loss of acquired skills	Yes	Yes, from the age of 3 y, loss of assistant walking at 6 y	Yes from the age of 3 y, loss of assisted walking at 10 y, loss of crawling at age 14 y, loss of nonverbal communication skills since the age of 10 y	Yes, loss of ambulation and speech at 11 y	Stable	Stable	Stable	Stable
Behavior : cheerful disposition	Yes (initially, before cognitive decline)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dysmorphic features (see supplemental data for details)	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Growth	Poor weight gain	Normal	Normal	Normal height, poor weight gain beginning at age 10 y	Normal	Normal	Normal	Normal
Head circumference	Progressive microcephaly -1.5SD at birth, -4SD at 4 y	80^{th} centile at 13 months then microcephaly <- 3SD from age 4.5 y	90^{th} centile at 2.5 y	50th centile at birth and (53 cm) 30th centile at age 12 y	(55.2 cm) 62^{nd} centile at 13 y	ND	ND	ND
Brain imaging	Cerebral and cerebellar atrophy, brainstem	Progressive cerebral atrophy and cerebellar	Cerebral and cerebellar atrophy, enlarged	Cerebral and cerebellar atrophy,	Mild inferior vermian hypoplasia	Mild cerebral atrophy	ND	ND

	hypoplasia, calcification in the falx cerebri	atrophy, with progressive alterations of the basal ganglia, thinning of the isthmus of the corpus callosum	ventricles, and atrophy of the basal ganglia	enlarged ventricles				
Other manifestations	Choanal atresia, scoliosis (surgery at 13 y)	Scoliosis (surgery at 16 y)		Hirschsprung disease, scoliosis	Scoliosis, genu valgum, unilateral cryptorchidism	No	No	No
Treatment response	Improvement with levodopa		Improvement with levodopa and gabapentin					
Other Variants				*SCN1B* c.253C>T;p.(L385F) pathogenic, presumed to be cause of seizures; RET c.1009G>A;p.(E337K) VUS; likely the cause of Hirschsprung disease in this family	*SCN1B* c.253C>T;p.(L385F) pathogenic, presumed to be cause of seizures; RET c.1009G>A;p.(E337K) VUS; likely the cause of Hirschsprung disease in this family			

Supplementary Table 2

cDNA (c.)	Protein (p.)	CADD	PolyPhen-2	SIFT	AlphaMissense	SPiP	SpliceAI
c.253C>T	p.(Leu85Phe)	24.3	Probably damaging (0.999/0.961)	Tolerated (0.09)	Likely pathogenic (0.6886)	NC	NC
c.341G>C	p.(Gly114Ala)	27.4	Probably damaging (0.997/0.97)	Deleterious (0.00)	Likely pathogenic (0.9856)	NC	NC
c.475G>C	p.(Glu159Gln)	26.5	Probably damaging (0.993/0.957)	Deleterious (0.00)	Likely pathogenic (0.916)	NC	NC

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Supplementary Table 3

Patient	Family	Chr	Start	End	Length (Mb)	#SNP	Total Homozygosity (Mb)
P6	4	6	37,116,078	42,282,393	5.17	1,494	30.1
P7	4	6	37,116,078	42,282,393	5.17	1,494	123.75
P8	4	6	37,116,078	42,282,393	5.17	1,494	52.16
P4	3	6	36,974,212	45,743,286	8.77	2,436	159.06

Supplementary Table 4

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Alignment 1
Hs MED20 1   MGVTCVSQMPVAEGKSVQQTVELLTRKLEMLGAEKQGTFCVDCETYHTAASTLGSQGQTGKLMYVMHNSEYPLSCFALFE  80
Sc Med20 1   MGKSAVIFVERATPATLTELKDALSNSI----LSVRDPWSIDFRTYRCSIKNL--PADVSKLMYSITFHHHGRQTVLIKD  74

Hs MED20 81  NGP-----QL---------------IADTNFDVLMVKLKGFFQSAKASKIETRGTRYQYCDFLVKVGTVTMGPSARGISV  140
Sc Med20 75  NSAMVTTAAAADIPPALVFNGSSTGVPESIDTILSSKLSNIWMQRQLIKGDA-GETLILDGLTVRLVNLFSSTGFKGLLI  153

Hs MED20 141 EVEYGPCVVASDCWSLLLEFLQSFLGSHTPGAPAVFGNRHDAVYGP---------ADTMVQYMELFNKIRKQQQVPVAGIR 212
Sc Med20 154 ELQADEA---GEF-ETKIAGIEGHLAEI-------RAKEYKTSSDSLGPDTSNEICDLAYQYVRALEL              210
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Alignment 2
Hs MED20 1   MGVTCVSQMPVAEGKSVQQTVELLTRKLEMLGAEKQGTFCVDCETYHTAASTLGSQGQTGKLMYVMHNSEYPLSCFALFE  80
Sc Med20 1   MGKSAVIFVERATPATLTELKDALSNSILSV----RDPWSIDFRTYRCSIK--NLPADVSKLMYSITFHHHGRQTVLIKD  74

Hs MED20 81  NGP--------------------QLIADTNFDVLMVKLKGFFQSAKASKIETRGTRYQYCDFLVKVGTVTMGPSARGISV  140
Sc Med20 75  NSAMVTTAAAADIPPALVFNGSSTGVPESIDTILSSKLSNIWMQRQLIKGDA-GETLILDGLTVRLVNLFSSTGFKGLLI  153

Hs MED20 141 EVEYGPCVVASDCWSLLLEFLQSFLGSHT-PGAPAVFGNRHDAVYGPADTMVQYMELFNKIRKQQQVPVAGIR  212
Sc Med20 154 ELQA---DEAGEFETKIAGIEGHLAEIRAKEYKTSSDSLGPDTSNEICDLAYQYVRALEL              210
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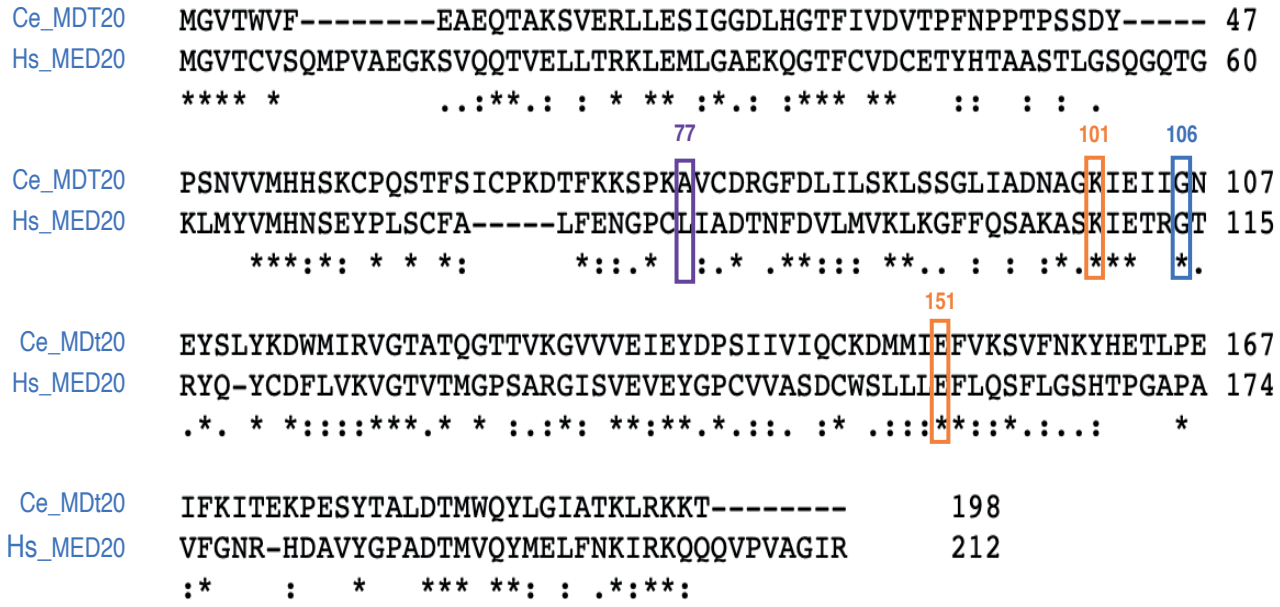
Hs MED20		Sc Med20
Leu85Phe	→	Ala84Phe and Gly99Phe
Lys109Argfs*16	→	Δ123
Gly114Ala	→	Gly127Ala
Glu159Gln	→	Ala168Gln and Gly169Gln

Supplementary Table 5



Hs MED20		Ce MDT-20
Leu85Phe	→	Not conserved
Lys109Argfs*16	→	Lys101Argfs*2 (conserved aa)
Gly114Ala	→	Gly106Ala (conserved aa)
Glu159Gln	→	Glu152Gln (conserved aa)

Supplementary Table 6

Name	Genotype	Collection
YPH499	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Y8270
med20-A84F	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-A84F::kanMX*	Y8064
med20-G99F	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G99F::kanMX*	Y8065
med20-G127A	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G127A::kanMX*	Y8066
med20-A168Q	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-A168Q::kanMX*	Y8067
med20-G169Q	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G169Q::kanMX*	Y8063
med20Δ-123	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20Δ123::kanMX*	Y8055
med20Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20::kanMX*	Y8059
YPH499 *rad7Δ*	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad7::HIS3*	Y8275
med20-A84F rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-A84F::kanMX rad7::HIS3*	Y8070
med20-G99F rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G99F::kanMX rad7::HIS3*	Y8071
med20-G127A rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G127A::kanMX rad7::HIS3*	Y8072
med20-A168Q rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-A168Q::kanMX rad7::HIS3*	Y8073
med20-G169Q rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20-G169Q::kanMX rad7::HIS3*	Y8196
med20Δ-123 rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20Δ123::kanMX rad7::HIS3*	Y8166
med20Δ rad7Δ	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 med20::kanMX rad7::HIS3*	Y8076

Supplementary Table 7

Genes	Forward	Reverse
GAPDH	Hs_GAPDH_1_SG QuantiTect Primer Assay QT00079247	
MED20	Hs_MED20_1_SG QuantiTect Primer Assay QT00043505	
GAPDH	gagtccactggctcttcac	ggactgtggtcatgagtccttc
CDK5RAP2	acagacaaccagcacctgaaga	tccttgctagccagaagctctgtt
NIPBL	tctggtccgttgtctggcaattca	ttagcctgtgcaccatgtgtaggt
	aggacgtcttaatggcacagga	atgaggagagtctcggtaggaa

Supplementary Table 8

Antibodies	Host	References	Manufacturer
ATF3	Rabbit	Ab207434	Abcam
B-tubulin	Mouse	AG-tub-2012	Euromedex
GAPDH	Mouse	GT239	Genetex
CPD	Mouse	CAC-NM-DND-001	Cosmo-bio
6-4PP	Mouse	CAC-NM-DND-002	Cosmo-bio
RNAPII	Rat	#61081	Active Motif
RNAPII	Mouse	#39097	Active Motif
RNAPII S5P	Mouse	#91119	Active Motif
RNAPII S2P	mouse	#91115	Active Motif
TFIIB	Rabbit	GTX107327	Genetex
XPD	Rabbit	#NBP3-15112	Bio-Techne
MAT-1	Rabbit	H719-1-AP	ProteinTech
CDK7	Rabbit	BE-A300-405A	Bethyl
CDK9	Rabbit	sc8338	Santa Cruz Biotechnology
TFIIEα	Mouse	#2A1	IGBMC
XPC	Rabbit	A301-122A	Bethyl
Flag	Mouse	MA1-91878	Thermofischer
MED4	Rabbit	ab129170	Abcam
MED8	Rabbit	PA5-50053	Thermofischer
MED17	Mouse	#00009440-MA2	Abnova
MED18	Rabbit	15352-1AP	ProteinTech
MED20	Rabbit	17598-1	ProteinTech
MED27	Mouse	sc-390295	Santa Cruz Biotechnology