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Supplemental Data

RAD21 Mutations Cause a Human Cohesinopathy

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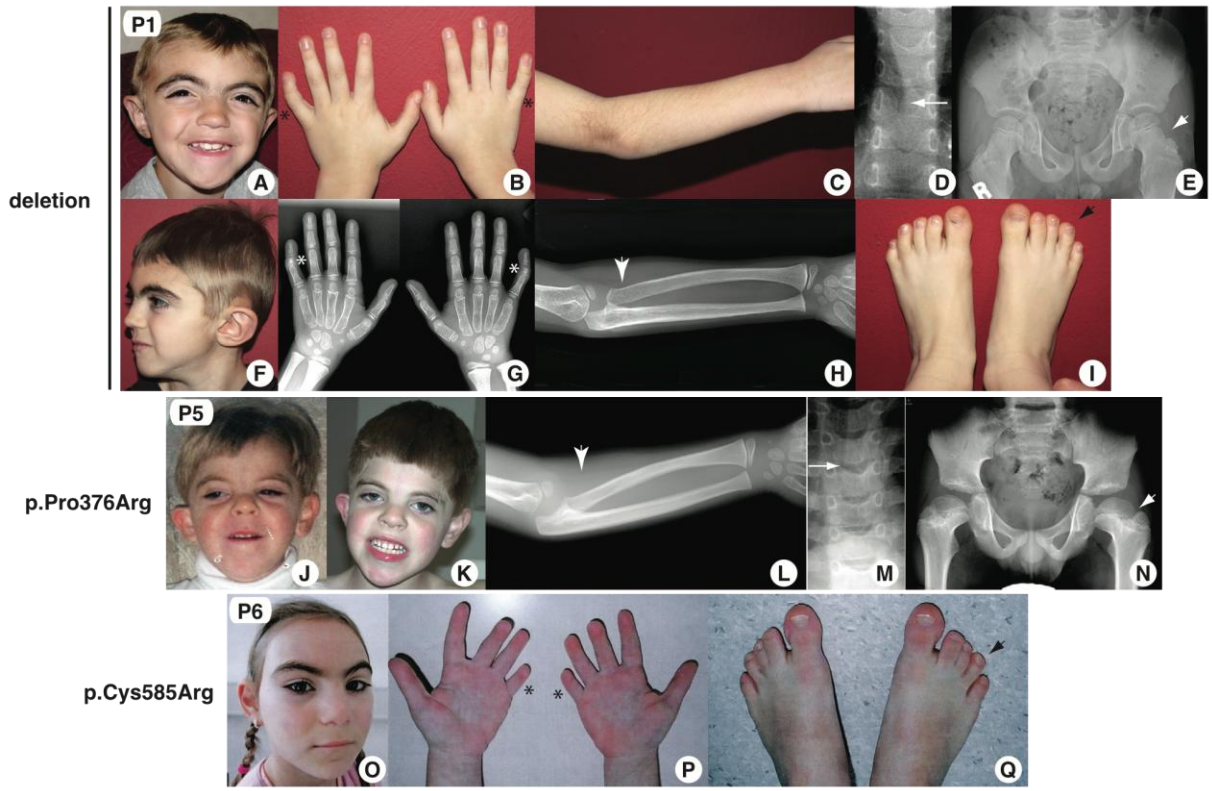


Figure S1. Clinical Features of Children with *RAD21* Mutations.
Facial features seen in individuals with *RAD21* deletions and point mutations include: synophrys (A,J,K,O,V,W), thick eyebrows (all), prominent eyelashes (all), short nose, wide nasal bridge (A,J,K,O,R,S,W), long smooth philtrum (A,J,K,O,V,W). Several consistent mild skeletal features noted included, clinodactyly (* in B,P,T) due to a short middle 5th phalanx (* in G), mild dysplasia of proximal radius (H,L) leading to incomplete extension of elbow (C), mild thoracic vertebral partial (M,U) and full (D) clefts, mild varus deformity and short femoral neck (arrowheads in E,N) with horizontal epiphyses (N). Also noted were shortened 2nd, 3rd and 5th toes, with relative preservation of the 1st and 4th (arrowheads in I and Q).

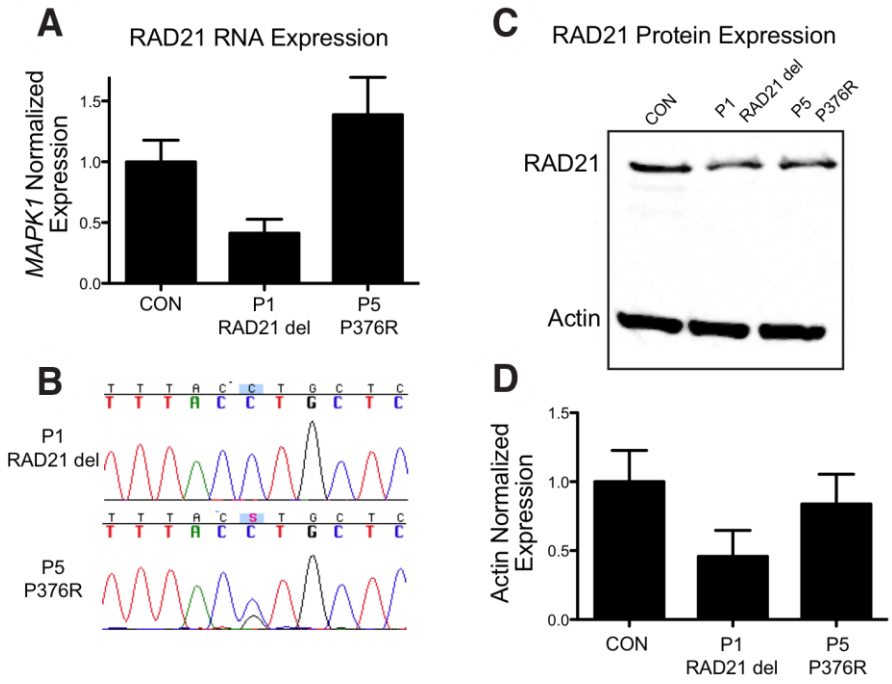


Figure S2. Expression of *RAD21* mutations. A. Quantitative RT-PCR assessment of *RAD21* RNA expression levels from lymphoblastoid cell lines (LCLs). Expression was normalized to *MAPK1* and then to that of a normal control (CON), samples performed in quadruplicate. **B.** Semiquantitative assessment of expression by sequencing of cDNA derived from LCLs (cycloheximide untreated). P1/*RAD21* del shows expression from the retained allele. P5 /P376R (p.Pro376Arg) expression indicates expression of both wild type and mutant alleles. **C**. Western blotting of RAD21 protein from the same LCLs. **D**. Quantitative chemiluminescence of western blots, average of two independent experiments.

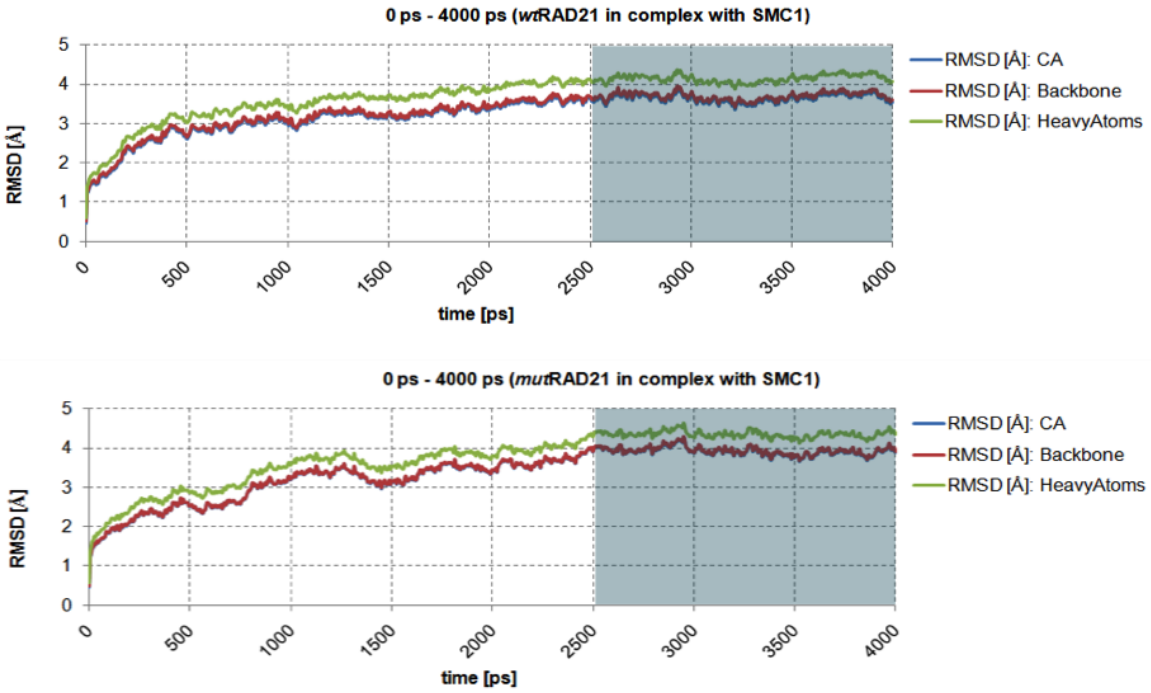


Figure S3. Molecular Dynamic (MD) Simulation by RMSD as a Function of Time. The RMSDBackbone of the two models shows a plateau, representing the stable conformation after around 2.5 ns MD of 3.64 ± 0.08 Å for *wt* model and 3.91 ± 0.08 Å for *mut* model (see diagrams).

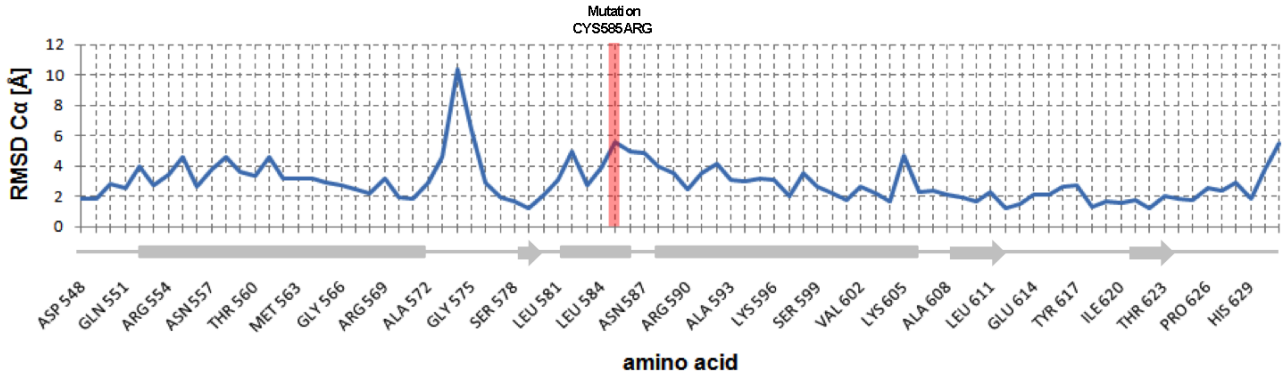


Figure S4. **Altered Loop Region in p.Cys585Arg.** Analyzing the RMSD as a function of RAD21 amino acid residues after generating a *wt* and *mut* average model from 2.5 ns to 4 ns demonstrates a difference in loop regions, which are more flexible than helices or sheets and we detect difference in the region around p.Cys585Arg of RAD21

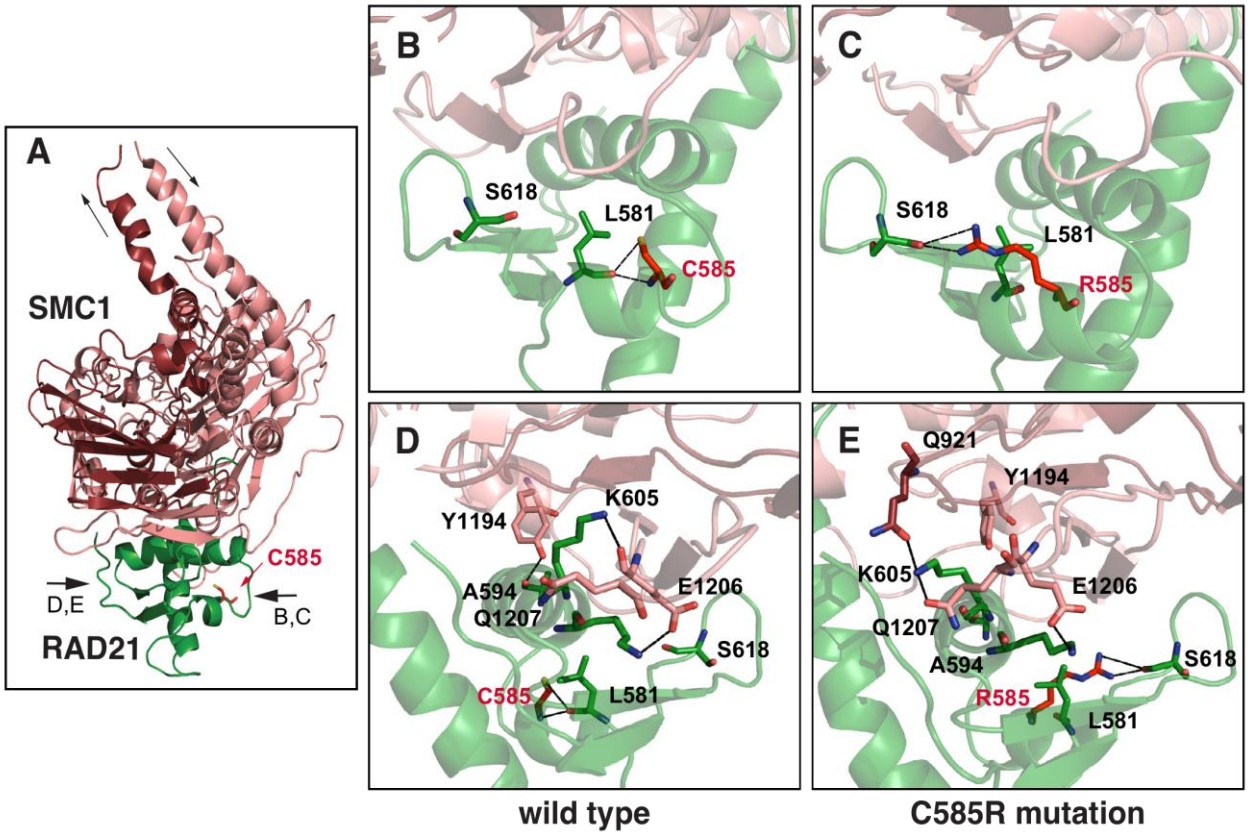


Figure S5. **Structural modeling of wild type and p.Cys585Arg mutant RAD21**. **A**. Modeled Human SMC1-RAD21 interaction. Darker and lighter red indicate the SMC1 N- and C-terminal portions, respectively. RAD21 is colored green. The C585 residue is colored bright red. Arrows indicate the location of the mutation (red) and the views demonstrated in panels B-E. **B, C.** This view illustrates that hydrogen bonds are favored between C585 with L581 in the wild type protein, whereas the R585 model favors hydrogen bonds between R585 and S618. This also suggests a wider distance between SMC1 and RAD21 at the molecular interface. **D,E.** A view of the interface from the opposite side of the complex, illustrates the altered orientation of the helix of RAD21 at the interaction surface with SMC1. This suggests a loss of the hydrogen bonding between wild type RAD21 K591 and SMC1 Y1194. Also of note, the data suggests that the mutant RAD21-SMC1 complex forms stabilizing hydrogen bonds to secure the misfolding as nicely illustrated by the orientation of the helix.

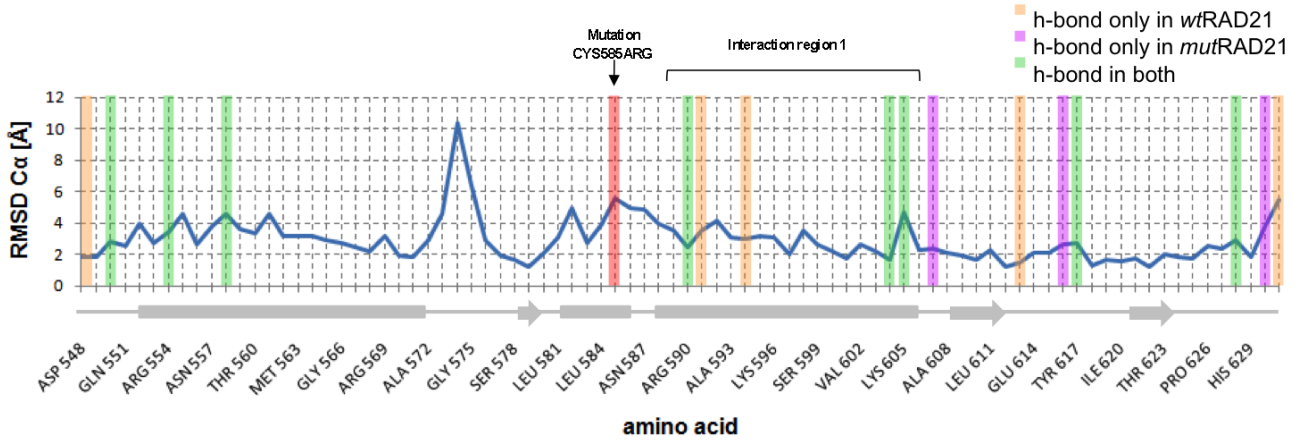


Figure S6. **Alteration of Hydrogen Bonding in the p.Cys585Arg Mutation.** The h-bond interaction of *wt* and *mut*RAD21 to SMC1A during MD simulation (2.5 ns to 4 ns). This diagram shows the $RMSD_{C\alpha}$ as a function of amino acids (*wt*RAD21 versus *mut*RAD21). Amino acids of *wt*/*mut*RAD21 which are involved in h-bond network to SMC1A over 70 % of the time during MD simulation (2.5 ns to 4 ns) are colored.

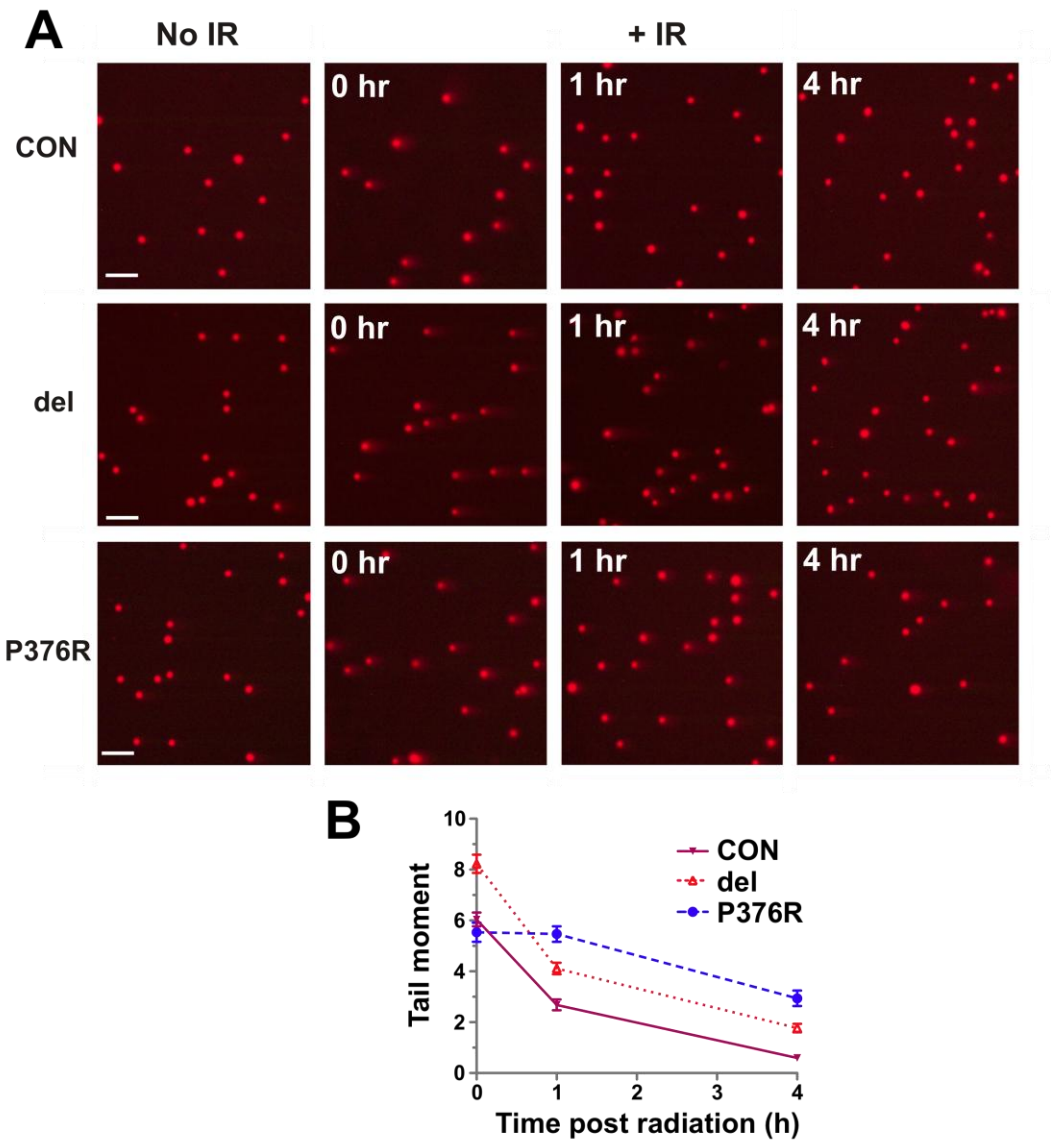


Figure S7. The Basal Level of Radiation-induced DNA Damage in the Control (CON) and *RAD21*-mutant Cell Lines. A. Representative images used for the quantitation of the tail moment in the comet assay. All cells in each image were measured for the tail moment using CASP image analysis software. Eight to ten images with a minimum of 50 cells were measured for each data point. Bar = 10 µm. B. The tail moment of the control (CON) and *RAD21* mutant cells at 0 hr, 1 hr and 4 hr following IR at 8 Gy. The values represent the mean of the tail moment measured from a minimum 50 cells. Del=*RAD21* deletion, P376R=p.Pro376Arg. Error bars represent standard deviations.

SUPPLEMENTAL TABLES

Individual	P1	P2	P3	P4	P5	P6
Reference	this report	McBrien 2008[1]	Wuyts 2002[2]	this report	this report	this report
Mutation	Heterozygous deletion chr8:117,708,713-121,024,193 (hg18)	Heterozygous deletion chr8:117,640,909-119,330,085 (hg18)	Heterozygous deletion chr8:117237890-122631628 (hg18)	Heterozygous deletion chr8: 116,950,003-118,944,486 (hg18)	c.1127C>G p.Pro376Arg	c.1753T>C p.Cys585Arg
SIFT/PolyPhen score	N/A	N/A	N/A	N/A	0.02	0.12
Weight	5%	BW; 2% 19 m; 2%	12y; 50%		5%	
Height	<5%	BL; 2% 19 m; 10%	12y; 25%	<3%	5%	
Head circumference	<<5%	BHC 2% 19 m; <2%	12y; 3%	3%	microcephaly	microcephaly
Skull	Mild brachycephaly, thin temporal hair and over ears	19m CdLS: 95% sparse fine scalp hair, prominent metopic ridge			asymmetric	
Eyebrows	Full arched eyebrows, lateral disorganization	thick and bushy	thick and bushy	thick and bushy	Thick, Lateral disorganization	thick and bushy, arched
Synophrys	+	-	+	-	+	+
Lashes	prominent	long	long	long	Prominent	long
Lids	nl		telecanthus		Bilateral ptosis	upslanted
Nose	Wide nasal bridge	Wide nasal bridge	broad	Wide nasal bridge	Upturned tip, wide bridge	short, broad bridge
Philtrum		long	long	long	smooth	long
Upper lip		thin	thin	thin	Bowed, thin	thin
Palate	Cleft palate	-		normal	Velopharyngeal insufficiency; submucous cleft	
Micrognathia	++ requiring surgery as infant	-	mild	-		
Teeth	caries	normal		normal	Lack of enamel	
Hearing/ears	normal	normal	normal	normal	Stapes fixation	
Small hands	L bridged crease	mild PIP flexion deformity of 4th right		-	transverse palmar crease, MF 4.8, PL 7.1@6y	
Proximal thumb	+			+		
5th Clinodactyly	++			-		+
Fingers	Mild hyperextensible		clinodactyly 1st finger	normal	thin	short
Elbow limitation	+	-		-	L radioulnar synostosis	
Skeletal age		normal			delayed	
Vertebrae	Thoracic vertebral cleft	hemivertebrae at T10 and T11			Vertebral cleft L1	

Individual	P1	P2	P3	P4	P5	P6
Feet	Long 4th metacarpal	bilateral talipes calcaneo-valgus, prominent 1st toe, fetal toe pads		normal	Mild 2-3 syndactyly	small, prominent first toe, mild 2-3 syndactyly, long 4th metacarpal
Other skeletal	coxa vara, short femoral neck, exotoses	exostoses	exostoses	exostoses	Pectus carinatum, coxa vara, short femoral neck	
Hirsutism	-	-	-	+		-
Cutis marmorata	Cutis marmorata, hyperpigmentation behind hears	generalized, fading with time		-		+
Cardiac	normal	PFO		-	Tetralogy of Fallot	
Gastrointestinal/ Genitourinary	Mild GER	bifid scrotum		-	GER, malrotation	
Neurologic			generalized seizures at 12y. partial complex vs. atypical absence			
Cognitive	Pleasant, Social, Normal at 7 yrs	pleasant,borderline delay (walk at 19 mo, 30 words at 26 m.	language delay first noted at 5y. IQ of 55-60. Works as nurse assistant	delayed	ADHD, severe delay, verbal>motor	
Imaging			Brain MRI with T2 hyperintensity in tuber cinereum.			
Notes	Pneumothorax at birth	Upper body strength weak in shoulders/arms healthy child now did have repeated chest infections in first two years	Parental history of infertility, clomifen-induced			

Table S1. Clinical Features in Children with de novo *RAD21* Mutations Numbering is per den Dunnen[3]. SIFT scores are felt to be significant for values <0.10[4]. Growth Percentages estimated from CDC Growth charts[5].

Variant	Protein effect	Location	SIFT	Allele Frequency	#/# in cases	#/# in controls	Comments
Rare Variants							
c.805A>G	p.Asn269Asp	Exon 7	0.56	<0.01	1/255	1/192	INP
c.1242T>G	p.Asp414Glu	Exon 10	0.62	<0.01	1/255	0/192	parents unavailable
c.1352T>G	p.Leu451Arg	Exon 11	0.03	<0.01	1/255	0/192	INP
c.1711C>T	p.Leu571Phe	Exon 14	0.21	<0.01	1/255	0/192	INP
c.1811A>G	p.Lys604Arg	Exon 14	0.26	<0.01	1/255	0/192	INP
Polymorphisms							
c.144+94G>A		Intron 2	n/a	0.17	40/115		
c.144+145A>T		Intron 2	n/a	0.03	8/147		
c.274+37_38insGTT		Intron 3	n/a	0.02	4/96		
c.275-82G>A		Intron 3	n/a	0.01	2/96		
c.688+8G>A		Intron 6	n/a	0.06	28/227		
c.688+46T>C		Intron 6	n/a	0.21	41/96		
c.938-27delT		Intron 7	n/a	0.01	1/96		
c.938-11T>C		Intron 8	n/a	0.18	90/249		
c.1161+60G>A		Intron 9	n/a	0.48	62/96		
c.1440T>C	p.Ala480Ala	Exon 11	0.84	0.25	53/198		

Table S2. Rare inherited variants and polymorphisms in *RAD21.* Numbering is per den Dunnen[3]. SIFT[4] scores are felt to be significant for values <0.10. INP=inherited from normal parent

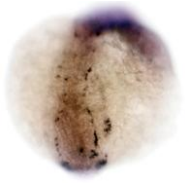
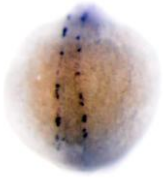
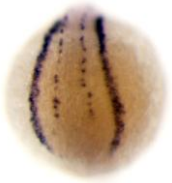
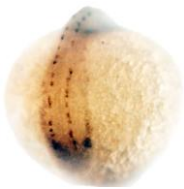
RAD21 variant injected (400pg)	Total embryos (n)	 Wild type embryos % (n)	 Extra activity in WT[a] % (n)	 Total mutants % (n)	 Full rescued mutants[b] % (n)	 Partial rescued mutants[b] % (n)	Total rescued mutants %
Uninjected	163	82 (134)	10 (13)	18 (16)	3 (1)[c]	0	3
rad21^{nz171} mRNA	50	64 (32)	0 (0)	36 (18)	17 (3) [c]	0	17
WT *rad21* (zebrafish)	83	84 (70)	34 (25)	16 (13)	31 (4)	69 (9)	100
P377R (zebrafish)	124	84 (104)	65 (68)	16 (20)	35 (7)	25 (5)	65
C597R (zebrafish)	122	89 (108)	20 (22)	11 (14)	14 (2)	0	14

Table S3. Rescue of *runx1* expression at 12 somites, following injection of mRNAs from zebrafish Rad21 variant constructs. [a] Extra activity refers to genotypically WT embryos with reduction or disruption of wild type staining, or extra regions of staining. [b] Rescued mutants include genotyped homozygous mutants called as either some stripes (partial rescue) or full stripes (full rescue). [c] Mistyping has called an occasional phenotypic wild type embryo as mutant in the controls.

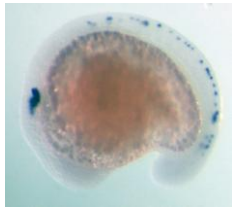
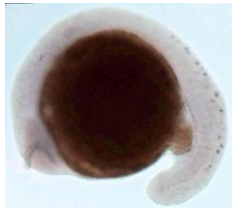
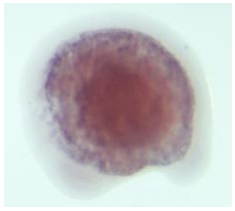
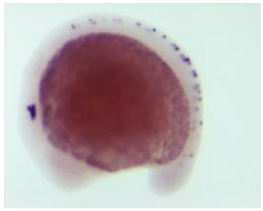
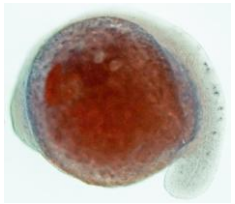
rad21 variant injected (400pg)	Total embryos (n)	 Wild type embryos % (n)	 Extra activity in WT[a] % (n)	 Total mutants % (n)	 Full rescued mutants [b] % (n)	 Partial rescued mutants [b] % (n)	Total rescued mutants %
Uninjected	100	79 (79)	3 (2)	21 (21)	14 (3) [c]	0	14
rad21^{nz171} mRNA	59	88 (52)	12 (6)	12 (7)	8 (1) [c]	0	8
WT *rad21* (zebrafish)	100	83 (83)	47 (39)	17 (17)	24 (4)	76 (13)	100
P377R (zebrafish)	147	85 (125)	50 (62)	15 (22)	36 (8)	64 (14)	100
C597R (zebrafish)	99	89 (88)	43 (38)	11 (11)	0	18 (2)	18

Table S4. Rescue of *runx3* expression at 17 somites, following injection of mRNAs from zebrafish Rad21 variant constructs. [a] Extra activity refers to genotypically WT embryos with reduction or disruption of wild type staining, or extra regions of staining. [b] Full rescued mutants have pattern and staining of a WT. To be counted as partially rescued, the number of cells to show staining had to be at least 4 per embryo. [c] Mistyping has called an occasional phenotypic wild type embryo as mutant in the controls.

Primer Name	Primer Sequence
Human	
RAD21P376Rf	CTGTTTTCTTTACGTGCTCAGCCTTTGTGG
RAD21P376Rr	CCACAAAGGCTGAGCACGTAAAGAAAACAG
RAD21C585Rf	CAGTTTGCTTGAGTTACGTCGAAATACGAACAG
RAD21C585Rr	CTGTTCGTATTTCGACGTAACTCAAGCAAACTG
Zebrafish	
zfRAD21_P377R_A	GGAGAAGCTCTTCTCATTGCGTGCTCAGCCTCTCTGG
zfRAD21_P377R_B	CCAGAGAGGCTGAGCACGCAATGAGAAGAGCTTCTCC
zfRAD21_C597R_A	GCCTGCTGGAGCTGCGCAGAAACAACAAC
zfRAD21_C597R_B	GTTGTTGTTTCTGCGCAGCTCCAGCAGGC

Table S5. Mutagenesis primers.

SUPPLEMENTAL DATA REFERENCES

1. McBrien, J., Crolla, J.A., Huang, S., Kelleher, J., Gleeson, J., and Lynch, S.A. (2008). Further case of microdeletion of 8q24 with phenotype overlapping Langer-Giedion without TRPS1 deletion. Am J Med Genet A *146A*, 1587-1592.
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3. den Dunnen, J.T., and Antonarakis, S.E. (2000). Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. Hum Mutat *15*, 7-12.
4. Ng, P.C., and Henikoff, S. (2003). SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res *31*, 3812-3814.
5. Kuczmarski, R.J., Ogden, C.L., Grummer-Strawn, L.M., Flegal, K.M., Guo, S.S., Wei, R., Mei, Z., Curtin, L.R., Roche, A.F., and Johnson, C.L. (2000). CDC growth charts: United States. Adv Data *314*, 1-27.