Supporting Information for the article:

Capo-Chichi et al. Identification and biochemical characterization of a novel mutation in *DDX11* causing Warsaw Breakage Syndrome

Supp. Subjects and Methods

We recruited a consanguineous Lebanese family comprised of 2 healthy parents and 3 affected siblings showing intellectual disability and various congenital anomalies. Blood samples were obtained from each of the family members with the informed consent of the parents and the approval of the appropriate institutional ethical review board.

Whole-genome genotyping and homozygosity mapping

Genomic blood DNAs (~2.5µg) from 2 of the affected siblings (IV-1 and IV-3) were used for whole-genome genotyping on the Illumina 660 Quad Chip (Infinium HD). Homozygosity mapping was performed using PLink (v.1.06; http://pngu.mgh.harvard.edu/~purcell/plink/).

Exome capture and massive parallel sequencing

The exome of one affected sibling (patient V-1) was captured from genomic DNA (3ug), using the Agilent SureSelect Human All Exon Capture 50Mb Kit, and sequenced (paired-end, 25x50bp) using the ABI SOLiD4 (Life Technologies; 6 exomes/slide) at the Child Health Genomics Platform at the Sainte-Justine Hospital Research Center (Montreal, Qc, Canada).

Read mapping and variant analysis

Sequence reads were mapped (hg18) using the SOLiD Bioscope. After removing PCR duplicates (Picard's MarkDuplicates; http://picard.sourceforge.net/index.shtml), single-nucleotide variants and small insertions/deletions were called by Bioscope's DiBayes and Small InDel tool, respectively (base coverage >=3x; at least 15% read variant frequency) and annotated using ANNOVAR (Wang, et al., 2010).

Drug-induced chromosomal breakage

Whole-blood cultures (2 dishes/individual) were prepared from 2 affected patients (V-1, V-3) and a healthy control. After incubating for 48 hrs at 37 ºC, the blood cultures (10 ml) were treated with either 30 µl of 1 µg/ml mitomycin C (MMC; Sigma) or the vehicle DPBS (Dulbecco's Phosphate-Buffered Saline) and incubated for additional 24 hrs at 37 ºC, followed by a treatment (2hrs, 37ºC) with 0.1 ml of 10 µg/ml colchicine (Invitrogen), followed by incubating with a hypotonic shock medium (2ml fetal bovine serum + 10ml distilled water) for 7 minutes. The cells were fixed, mounted on a slide, and stained for 12 minutes in a 4% Giemsa (Merck). For each culture, 200 metaphases were analyzed.

DDX11 site-directed mutagenesis

An DDX11-R263Q mutation was introduced into the 6X His-pCDNA3-3XFLAG plasmid DNA using mutagenic primers (sense: 5'-GGT-CTC-CCT-TGG-CTC-CCA-GCA-GAA-CCT-TTG-TG-3' antisense: 5'- CAC-AAA-GGT-TCT-GCT-GGG-AGC-CAA-GGG-AGA-CC-3') and a standard protocol from Quickchange II XL site-directed mutagenesis kit (Stratagene) by Lofstrand labs (Gaithersburg, MD). The open reading frame was sequenced to verify the mutation. The human DDX11 cDNA used herein was provided by the late Dr. Jill M. Lahti (Department of Genetics and Tumor Cell Biology, St. Jude Children's Hospital, Memphis, TN), and requests can be made to Dr. Akira Inoue (St. Jude Children's Hospital).

Recombinant DDX11 protein purification

DDX11-WT and DDX11-R263Q proteins were purified using a protocol previously described (Wu, et al., 2012). Briefly, 6X His-pCDNA3-3XFLAG plasmid containing human DDX11-WT and DDX11-R263Q cDNA was transfected into 293T cells using Lipofectamine 2000 (Invitrogen) as recommended by the manufacturer. Ten to twenty 10-cm plates were grown to ~80% confluence for transfection. Cells were harvested 54 h after transfection by trypsinization and centrifugation. Pellets were washed with cold PBS and cold PBS with proteinase inhibitors (Roche Applied Science) sequentially, resuspended in 5 ml of hypotonic buffer (10 mM Tris HCl pH 7.4, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.5 mM PMSF, proteinase inhibitors) and incubated on ice for 15 min. Cells were lysed by Dounce homogenization (30 strokes) and centrifuged at 4 °C for 30 min at 2500 x *g* to separate nuclear pellets from cytosolic

fraction. The cytosolic fraction was collected and kept on ice. The nuclear pellets were suspended in 5 ml of nuclear buffer (20 mM Tris HCl, pH 7.4, 0.15 M NaCl, 1.5 mM $MgCl_2$, 10 % glycerol, 0.2 mM EDTA, 1 mM DTT, 0.5 mM PMSF and proteinase inhibitors) and incubated in rocking at 4 °C for 30 min. The cytosolic and nuclear fractions were pooled and centrifuged at 43,500 x *g* for 1 h at 4 °C. The supernatant (10 mL) was incubated with 0.4 ml of FLAG resin (Sigma) in FLAG buffer (20 mM Tris HCl, pH 7.4, 0.15 M NaCl, 1.5 mM $MgCl_2$, 10 % glycerol, 0.05 % Nonidet P 40, 0.2 mM EDTA, 1 mM DTT, 0.5 mM PMSF and proteinase inhibitors) at 4 °C for 2 h. The resin was then washed twice with high salt containing FLAG buffer buffer (20 mM Tris HCl, pH 7.4, 0.5 M NaCl, 1.5 mM $MgCl_2$, 10 % glycerol, 0.05 % Nonidet P 40, 0.2 mM EDTA, 1 mM DTT, 0.5 mM PMSF and proteinase inhibitors) followed by one washing with FLAG buffer. DDX11 protein was eluted with 4 µg/ml 3X FLAG petide (Sigma) in elution buffer (25 mM TrisHCl, pH 7.4, 1 mM EDTA, 0.15 M NaCl, 1 mM DTT, 0.01% nonidet P-40, protein inhibitors). The FLAG tagged DDX11 protein was dialysed at 4 °C for 2 h in elution buffer. Aliquots were frozen in liquid nitrogen and stored at -80 °C. Purified recombinant DDX11 proteins were analyzed for purity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie staining. Protein concentration was determined by the Bradford assay using bovine serum albumin (BSA) as a standard. A total of 3 independent purifications were prepared for each wild type and mutant DDX11, each of which was used in the different assays described herein.

Radiometric helicase assays

The helicase assays were performed as described previously (Wu et al., 2012). Briefly, reaction mixtures (20 µl) contained 25 mM Hepes-NaOH, pH 7.5, 25 mM potassium acetate, 1 mM magnesium acetate, 1 mM DTT, 100 µg/ml BSA, 1 mM ATP, 10 fmol of the fork duplex substrate or 2 fmol of OX-1 G4 DNA substrate and the indicated concentrations of the specific DDX11 protein. Helicase reactions were initiated by addition of DDX11 protein and then incubated at 37 °C for 15 minutes. The reactions were stopped by stop buffer (75.5 mM EDTA, 0.3% SDS, 12.5 % glycerol, 0.02% bromophenol blue, 0.02% xylene cyanol blue). For standard duplex substrate, a 10-fold excess of unlabeled oligonucleotide with the same sequence as the labeled strand was included in the stop buffer to prevent reannealing. Reactions were run in 12 % native PAGE for duplex substrate, and 12% native PAGE containing 10 mM KCl for G4

substrate. The reaction product was visualized in phosphoimager and quantified by Image quant software. The percentage of helicase substrate unbound was calculated by 100X (P/(S+P) where P is the product and S is the substrate.

Electrophoretic Mobility Shift Assays (EMSA)

DNA binding of DDX11 proteins were performed in a reaction mixture (20 µl) that contained the indicated concentrations of DDX11 and a 0.5 nM concentration of the specified ^{32}P-end-labeled DNA substrate in the same reaction buffer as that used for helicase assays except ATP was omitted. The binding mixtures were incubated on ice for 30 min after the addition of DDX11. After incubation, 3 µl of loading dye (74% glycerol, 0.01% xylene cyanol, 0.01%bromphenol blue) was added to each mixture, and samples were resolved on native 5% (19:1 acrylamide:bisacrylamide) polyacrylamide gels at 200 V for 1 h and 30 min at 4 °C. The radiolabeled species were visualized using a PhosphorImager and analyzed with ImageQuant software. The specific activity of ^{32}P-gamma ATP used for radiolabeling oligonucleotide to prepare 19 bp forked duplex DNA substrate was 3000 Ci (111TBq)/mmol (Perkin Elmer).

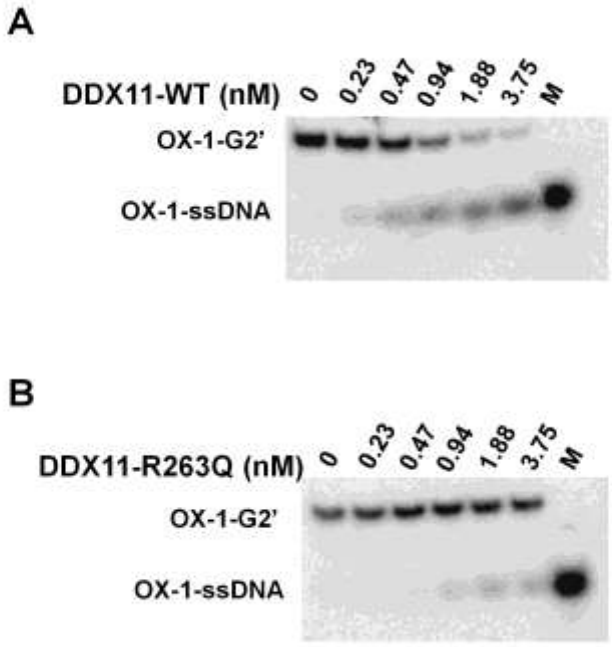
ATP Hydrolysis Assays

ATP hydrolysis was measured using [γ-^{32}P] ATP (PerkinElmer Life Sciences) and analysis by thin layer chromatography (TLC) on polyethyleneimine-cellulose plates (Mallinckrodt Baker). The standard reaction mixture (20 µl total volume) contained 25 mM Hepes-NaOH, pH 7.5, 25 mM potassium acetate, 1 mM magnesium acetate, 1 mM DTT, 100 µg/ml BSA, 250 µM [γ-^{32}P] ATP, and 30 nM DDX11 protein and was incubated at 37 °C. Reactions were quenched with 50 mM EDTA (final concentration). The reaction mixture was spotted onto a polyethyleneimine-cellulose TLC plate and resolved using 0.5 M LiCl, 1 M formic acid as the carrier solvent. The TLC plate was exposed to a phosphorimaging cassette for 1 h, visualized using a PhosphorImager, and analyzed with ImageQuant software. For experiments to determine K_m (ATP) and V_{max}, M13mp18 ssDNA was 2.1 nM, the concentration of ATP ranged from 16 to 2000 µM, and the reaction was incubated for 30 min. For determination of k_{cat}, the concentration of ATP was 4.4 mM. Five-microliter aliquots were removed and quenched with 5 µl of 0.1 M EDTA at 0, 7.5, 15, 30 and 45 min, respectively.

Supp. References

Wang K, Li M, Hakonarson H. 2010. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38(16):e164.

Wu Y, Sommers JA, Khan I, de Winter JP, Brosh RM, Jr. 2012. Biochemical characterization of Warsaw breakage syndrome helicase. J Biol Chem 287(2):1007-21.



Supp. Figure S1. Helicase activity of DDX11-WT and DDX11-R263Q on antiparallel G2' G-quadruplex DNA substrate. Helicase reactions (20 µl) were performed by incubating DDX11-WT or DDX11-R263Q with 0.1 nM OX-1-G2' DNA substrate at 37 °C for 15 min under standard helicase assay conditions as described under "Supp. Subjects and Methods". Products were resolved on native 10% polyacrylamide gels and representative phosphorimages of typical gels are shown. *M*, radio-labeled oligonucleotide marker.

Supp. Table S1. Shared homozygosity regions > 1Mb between patient V-1 and patient V-3

Chr	Marker start	Marker end	Position start	Position end	Size (Mb)
4	rs13114034	cnvi0013790	6 575 335	18 621 527	12.0
7	rs4074750	rs17413316	7 006 834	8 767 495	1.8
9	rs715666	rs1060501	114 570 514	116 030 088	1.5
11	rs590586	rs1790157	71 544 815	74 827 791	3.3
	cnvi0005376	rs4937823	130 640 250	133 110 660	2.5
12	rs12313656	rs11062260	1 305 458	2 517 044	1.2
	rs7134402	rs3819545	12 988 594	46 551 273	33.6
14	rs17127035	rs7151711	53 427 887	78 734 800	25.3
17	rs9910227	rs9652853	51 338 491	52 636 590	1.3
22	rs5996657	rs5998853	22 715 105	32 022 122	9.3

Chr, chromosome. Markers start and end delimit the regions of shared homozygosity at their corresponding positions (position start and end). Markers positions are relative to the hg18 reference.

Supp. Table S2. Prioritization of the variants detected in the exome of patient V-1

Variant filters applied sequentially	**Variant count**
Non-synonymous, nonsense, splicing, and coding/splicing Indels	8 087
Homozygous	2 819
In HR	148
Not in 198 in-house control exomes	5
Not in dbSNP135 or EVS or 1000 Genomes[(a)]	2
Confirmed homozygous in the 3 affected siblings and heterozygous in the parents [(b)]	1 (*DDX11* p.R263Q)

HR, Shared homozygosity regions > 1M. EVS, exome variant server (http://evs.gs.washington.edu/EVS/); (a) only variants that were found in these databases at minor allele frequencies > 0.5% were excluded; (b) based on Sanger sequencing of both DNA strands..

Supp. Table S3. ATPase activity of DDX11-WT and DDX11-R263Q

Protein	K_m (mM)[a,b]	V_{max} (nmol min^{-1})[a,b]	k_{cat} (min^{-1})[a,b,c]
DDX11-WT	0.66 ± 0.04	0.56 ± 0.05	220 ± 40
DDX11-R263Q	0.19 ± 0.05	0.066 ± 0.008	12 ± 4

[a] DDX11 proteins were 30 nM final concentration.[b] M13mp18 ssDNA concentration was 2.1 nM. [c]ATP concentration was 4.4 mM.

Supp. Table S4. Clinical features of the patients with DDX11 mutations

Description	Patient V-1	Patient V-2*	Patient V-3	van der Lelij, et al., 2010a
DDX11 mutation	p.R263Q/ p.R263Q	p.R263Q/ p.R263Q	p.R263Q/ p.R263Q	c.2271+2T>C / c.2689_2691del
Gender	F	M	F	M
Growth retardation	severe	severe	severe	severe
ID	severe	severe	severe	mild
Head circumference, centile	<3rd centile	< 3rd centile	< 3rd centile	< 3rd centile
Facial dysmorphy	+	+	+	+
Cardiac malformations	-	+(b)	-	+(c)
Deafness(a)	+	+	+	+
Abnormal skin pigmentation	-	-	-	+
Clinodactyly /Syndactyly	+/-	-/-	-/-	+/+

a) deafness due to cochlear structural abnormalities; b) tetralogy of Fallot; c) small ventricular septal defects; ID, intellectual disability. * Patient V-2 died at the age of 4 years because of heart failure.