SUPPLEMENTARY MATERIALS AND METHODS

Functional characterization of multiple *DICER1* mutations in an adolescent

Exon skipping

In order to evaluate the EX-SKIP predictions for missense mutations, we employed an exon cassette assay. Briefly, we compared the spliced products of a wild-type exon 25 flanked by its 5' and 3' introns to those of an exon 25 bearing the missense mutations in question. The c.5441C>T mutation was introduced into the pSPL3-EX25 plasmid (Wu, et al. 2013) via site directed mutagenesis using the primers 5'-GGATATTTTTGAGTTGCTTGCTGGTGCC-3' and 5'-GGCACCAGCAAGCAACTCAAAAATATCC-3'to make pSPL3-S1814L. The c.5428G>T mutation was introduced using primers 5'-CTTAGAATTCCTGGGATATGCGATTTTGGAACACC-3' and 5'-GGTAGTCCAAAATCGCATATCCCAGGAATTCTAAG-3'. The plasmids pSPL3, pSPL3-EX25, pSPL3-S1814L (in triplicate), pSPL3-D1810Y, and mock control were each transfected into either HEK 293 or COS-1 cells using lipofectamine 2000 (Life Technologies) according to manufacturer's instructions. RNA was extracted using the Allprep DNA/RNA kit (Qiagen) using the manufacturer's protocol. cDNA was made using the Quantitect Reverse Transcription Kit (Qiagen). PCR amplification and analysis of the exon cassette was performed as described previously (Wu et al. 2013).

In vitro cleavage assay

FLAG-tagged DICER1 was subcloned from pCAGGS-FLAGDICER1 (Gurtan, et al. 2012) into the pQCXIB retroviral vector. Missense mutations were introduced using site directed mutagenesis (S1814L and D1810Y were made using the primers described in the exon skipping experiment). Retroviruses were packaged by transfecting HEK 293T cells with pQCXIB constructs, as well as

pUMVC and pVSVG plasmids. Viral supernatant was collected 48 hours and 72 hours post transfection, filtered and used to transduce HEK 293 cells. Stably transduced cells were selected using blasticidin (Wisent). FLAG-DICER1 expression was assessed by Western blot analysis. FLAG-DICER1 expressing cell lines were grown to near confluence on 15 cm cell culture dishes, rinsed with PBS, and collected by scraping. Cell pellets were lysed in 500 ul NP-40 lysis buffer (50 mM HEPES, pH 7.5; 150 mM KCl; 2 mM EDTA; 1 mM NaF; 0.5% (v/v) NP-40; 0.5 mM DTT; complete protease inhibitors), pre-cleared with protein G sepharose beads for 1 hour, immunoprecipitated with 50 ul packed FLAG-M2 resin (Sigma) for 4 hours, washed 3X with IP wash buffer(50 mM HEPES, pH 7.5; 300 mM KCl; 0.05% (v/v) NP-40; 0.5 mM DTT; complete protease inhibitors), 1X with high salt buffer (50 mM HEPES, pH 7.5; 500 mM KCl; 0.05% (v/v) NP-40; 0.5 mM DTT; complete protease inhibitors), 1X TBS, and then stored in 50 μl DICER1 storage buffer (25 mM Tris HCl, pH 7.0; 50mM NaCl; 1mM $MgCl_2$; 50% (v/v) glycerol) with 1 μl RNAsin (Promega) and stored at -20^{o}C. The amount of slurry used per *in vitro* cleavage was determined by Western blot of immunoprecipitated material. A template for in vitro transcribing pre-miR122 RNA was generated by 3-primer PCR using 5'-ATAGTTTAGACACAAACACCATTGTCACACTCCACTATAGTGAGTCGTATTA-3' as a template as well as Forward (5'-TATTTAGTGTGATAATGGCGTTTGATAGTTTAGAC-3') and Reverse (5'-TAATACGACTCACTATAG-3') primers. The PCR product, which contains a T7 promotor, was gel purified used to perform *in vitro* transcription (Ambion MAXIScript kit) with 6 ul gamma-^{32}P-UTP (Perkin Elmer). Radioactive pre-miR122 was column purified (Roche), heat denatured, slow cooled and 2 μl of probe was used per *in vitro* cleavage reaction. Samples were incubated with an appropriate amount of slurry (normalized to FLAG-DICER levels as assessed by Western Blot) at 37^{o}C in 40 μl reaction volume in reaction buffer (500 mM Tris, pH 7.0; 500 mM NaCl; 25 mM $MgCl_2$; 20mM DTT; 0.5% triton X-100). The reaction was stopped by adding an equal volume of 2X loading dye (Ambion)

at defined time points. Products were resolved on a 15% denaturing acrylamide gel, along with a end-labelled small RNA ladder (NEB) and analyzed via phosphorimager. Controls are shown in Supplementary Figure 6.

Cycloheximide treatment

In order to determine if the paternally-inherited *DICER1* mutation c.5441C>T could cause exon 25 skipping, immortalized lymphocytes for the proband's parents were generated and treated with and without cycloheximide to prevent nonsense mediated decay as previously described (Rio Frio, et al. 2011). RNA was extracted, reverse transcribed to cDNA and then amplified over the exon 24-26 region to determine presence/absence of exon 25 (see Supplemental Figure 5).

References

Gurtan AM, Lu V, Bhutkar A & Sharp PA 2012 In vivo structure-function analysis of human Dicer reveals directional processing of precursor miRNAs. *RNA* **18** 1116-1122.
Rio Frio T, Bahubeshi A, Kanellopoulou C, Hamel N, Niedziela M, Sabbaghian N, Pouchet C, Gilbert L, O'Brien PK, Serfas K, et al. 2011 DICER1 mutations in familial multinodular goiter with and without ovarian Sertoli-Leydig cell tumors. *JAMA* **305** 68-77.
Wu MK, Sabbaghian N, Xu B, Addidou-Kalucki S, Bernard C, Zou D, Reeve AE, Eccles MR, Cole C, Choong CS, et al. 2013 Biallelic DICER1 mutations occur in Wilms tumours. *J Pathol* **230** 154-164.