SUPPLEMENTARY MATERIALS AND METHODS

A case report of syndromic multinodular goitre in adolescence: Exploring the phenotypic overlap between Cowden and DICER1 syndromes

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***PTEN* germ-line testing:**

RNA analysis: 0.5ml of peripheral blood collected in a heparin collection tube, was mixed with 10ml RPMI 1640 culture medium containing 500U Penicillin/Streptomycin, Fetal Calf Serum, 2mM L-Glutamine, 100U Heparin and 0.2mg Phytohaemagglutin and cells incubated for 4 days in a 37°C incubator. On day 4, 100µl of 10mg/ml Cycloheximide was added to inhibit RNA nonsense mediated decay and the cells incubated for a further 37°C for 2 hours. The contents of the flask were centrifuged for 10 mins at 2000rpm, the supernatant removed and the cell pellet washed twice with 5ml Buffer EL from the Qiagen RNeasy Mini Blood kit. Finally, 350µl of Buffer RLT containing beta mercaptoethanol was added prior to RNA extraction using QIAGEN RNeasy Mini Kit on a QiaCube extraction robot. cDNA was prepared from RNA using the Superscript III Kit by Invitrogen. Two fragments of the *PTEN* gene covering exons 1 to 9 were amplified by PCR in a 25µl reaction volume using 1µM of each primer, 2mM $MgCl_2$, 1x PCR Buffer, 200µM dNTPs, 0.5U Invitrogen Platinum Taq Polymerase and 1µl cDNA. 5µl of PCR product was purified using Ampure (Beckman Coulter) and Sanger sequenced using M13 primers on an Applied BioSystems 3130xl genetic analyser. Results were analysed using ATF Assign sequence analysis software.

DNA analysis: Genomic DNA was prepared from peripheral blood using QIAGEN QIAmp DNA Mini Kit on a QiaSymphony DNA extraction robot. PCR was performed using primers specific for exon 5 of *PTEN* as described above using 50ng genomic DNA. Sanger Sequencing was performed as described above.