Supplementary Appendix

Supplementary methods:

Variants calling in WES samples:

Bioinformatics analysis of exome sequencing data was performed using our WES pipeline as previously described[1]. Briefly, our pipeline uses Trimmomatic (v.0.35) and BWA (v. 0.5.9) to trim and align sequenced reads to the reference genome (hg19); GATK and Picard (http://broadinstitute.github.io/picard//) to perform local realignment around small insertions and deletions (indels) and to mark read duplicates, respectively.
Next, GATK was applied to assess capture efficiency and coverage for all samples. Mean coverage for all consensus coding sequence (CCDS) in each sample can be found in Supplementary Table 7. Normal and tumor samples were analyzed in parallel. Potential somatic substitutions, single nucleotide variants (SNVs) and indels, were called using Mutect (see https://confluence.broadinstitute.org/display/CGATools/MuTect for method) and IndelLocator (see https://confluence.broadinstitute.org/display/CGATools/Indelocator for methods) on the basis of BWA alignments and were then annotated with ANNOVAR[2].

Interpretation of plausible cancer susceptibility variants:
Those variants which most likely damage the protein (nonsense, canonical splice-site, coding indels and missense) were considered for further analysis. To remove common variants and false positive calls, candidate mutations were subjected to several filtering steps and eliminated if they fulfilled any one of the following criteria: (i) genomic position of variant covered by <3-reads, (ii) <4 reads supported the alternative variant, (iii) variant had allelic ratio <10% for SNVs or <15% for indels, (iv) variant quality < 20; (v) variant had allele frequency > 0.01 in gNOMAD/ExAC databases (release 0.3 2016-01-13) and EVS > 0.01, or seen as homozygote in ExAC database (release 0.3 2016-01-13) (vi) a CADD score < 20[3]. To further interpret missense variants we categorized them using two algorithms in parallel (a) we established a scoring system based of pathogenicity likelihood according to 5 bioinformatic algorithms (SIFT, PolyPhen, MutationTaster, Revel, MCAP) prioritizing variants considered likely pathogenic by 3 or more algorithms and (b) we used the Cancer Genome Interpreter (CGI) that classifies variants according to their potential as drivers (https://www.cancergenomeinterpreter.org)[4–8]. Those variants known to be reported in cancer or predicted tier 1 according to CGI were also prioritized independently of the scoring system. If the variant is predicted pathogenic by the scoring system but CGI considered it driver tier 2, passenger, non-protein affecting or neutral for oncogenesis we investigated its plausible relation with cancer by a search in public databases and literature review, including frequency of the variant in cancer exomes, expression of the gene in thyroid tissue and previously reported data on the variant in particular. Variants localized near the canonical splice sites were further interrogated by splicing predictor algorithms (The Splice Site Prediction by Neural Network from the BDGP and Human Splice Finder)[9,10]. Finally, The Integrative Genomics Viewer was used for the manual examination and visualization of all potential candidate variant[11].
In a first step we focussed on 152 known cancer susceptibility genes from Huang et al 2018[12]. We prioritized those genes mutated in 2 or more samples. For tumor suppressor we selected only those which at least one had a germline and a somatic hit considered likely pathogenic by our algorithm (Supplemental Table 1).

We then extended our analysis to all coding genes. In this case the analysis was performed individually in each normal and tumor pairs of samples (Supplemental Tables 2-7).

Interpretation of plausible somatic driver variants:
For this analysis we focused on the 468 cancer panel gene list elaborated by the MSK-IMPACT™ to compare driver genes involved in adult PDTC with those in our pediatric cases[13]. First all germline variants were filtered out. Then the most likely damaging variants were filtered and classified as described above.

ExomeAI-

Using ExomeAI software and the aforementioned WES data, we searched for genomic CNV events in Chromosome 14 where *DICER1* locus is in these tumors[14]. Briefly, ExomeAI reconstructs genomic allelic imbalance (AI) events by analyzing the exome-wide *B* allele frequency (BAF) profile.

Supplementary References

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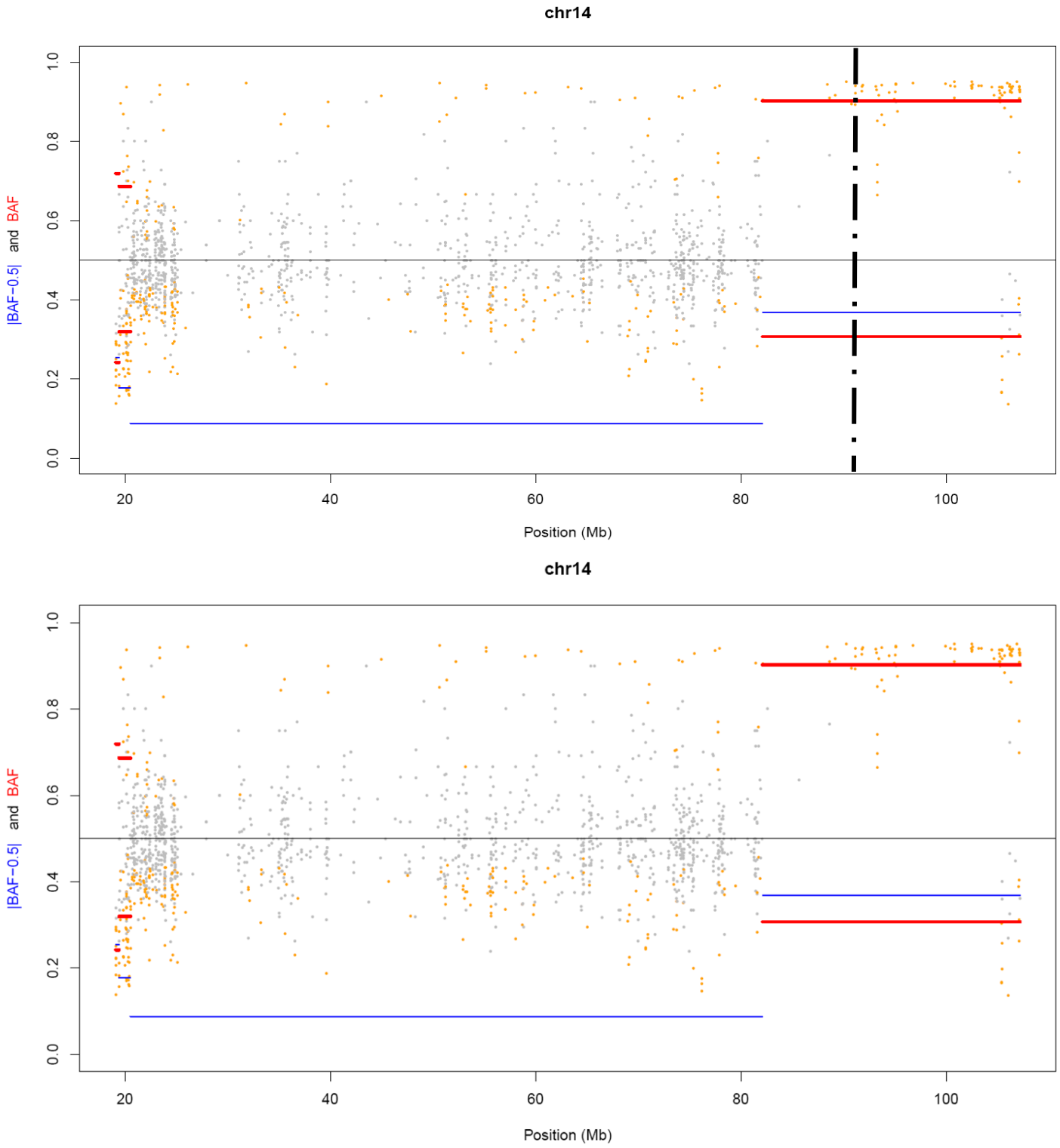

eFigure 1: Tumor sample from individual case 4. ExomeAI shows allelic imbalances affecting a large segments of chromosome 14q where DICER1 is localized (marked by a vertical doted line) . In this case, the mutant allele at c.G5437A;p.E1813Q showed a variant allele frequency of 95.7%.

Supplemental Table 8. Literature review of pediatric poorly differentiated thyroid carcinoma

Publication	Turin criteria	Patient age	Sex	Stage	Patient Status	Length of follow up (mo)
1. Sironi et al (1992)	NA	16	F	T4bN1MX	AWD	78
2. Kotiloglu et al (1995)	NA	14	F	T3N0M0	AWOD	60
3. Hassoun et al (1997)	NA	15	F	T3N1M0	AWOD	22
4. Hassoun et al (1997)	NA	16	F	NA	DOD	31
5. Pilotti et al (1997)	NA	17	F	T4N1M0	Alive[b]	114
6. Rodriguez et al (1998)	NA	14	F	T1N0M0	AWOD	68
7. Rodriguez et al (1998)	NA	11	M	T2N0M0	AWOD	42
8. Takeuchi et al (1999)	NA	20	M	NA	AWOD	130
9. Takeuchi et al (1999)	NA	9	F	NA	AWOD	122
10. Lam et al (2000)	NA	15	F	NA	Alive[b]	336
11. Lo et al (2000)	NA	15	F	NA	AWOD	312
12. Lo et al (2000)	Yes	15	M	At least T3	DOD	3
13. Zettinig et al (2000)	NA	14	F	TxNXM1	AWOD	288
14. Rijhwani and Satish (2003)	NA	10	F	T3N1MX	NA	NA
15. Yusuf et al (2003)	NA	15	F	T3	AWOD	24
16. Kumagai et al (2006)	NA	12	F	T3NXMX	NA	NA
17. Prommegger et al (2006)	NA	7	F	T3N1MX	AWOD	48
18. Donnellan et al (2009)	NA[a]	4	F	T3N1M1	Alive[b]	12
19. Wu et al (2011)	Yes	9	M	T2N0M0	AWOD	5
20. Hod et al (2013)	Yes	16	NA	NA	NA	NA
21. Liu et al (2015)	NA	16	M	TXN1M1	DOD	20
22. Mitsutake et al (2015)	NA	NA	NA	NA	NA	NA
23. Norlen et al (2015)	NA	NA	NA	NA	NA	NA
24. Win et al (2017)	Yes	20	F	NA	AWOD	>60
25. Mao et al (2017)	NA	>12	NA	NA	Alive[b]	60

NA = Not assessable; Mo = Months; AWD= alive with disease; AWOD = alive without disease

[a]Histopathology images provided in case report are most consistent with papillary rather than poorly differentiated thyroid carcinoma

[b]Disease status uncertain