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***DICER1* syndrome – clarifying the diagnosis, clinical features and management implications of a pleiotropic tumor predisposition syndrome**

Ingrid Slade,¹ Chiara Bacchelli,¹ Helen Davies,² Anne Murray,¹ Fatemeh Abbaszadeh,¹ Sandra Hanks,¹ Rita Barfoot,¹ Amos Burke,³ Julia Chisholm,⁴ Martin Hewitt,⁵ Helen Jenkinson,⁶ Derek King,⁷ Bruce Morland,⁶ Barry Pizer,⁸ Katrina Prescott,⁹ Anand Saggarr,¹⁰ Lucy Side,¹¹ Heidi Traunecker,¹² Sucheta Vaidya,¹³ Paul Ward,¹⁴ P. Andrew Futreal,² Gordan Vujanic,¹⁵ Andrew G Nicholson,¹⁶ Neil Sebire,¹⁷ John R Priest,¹⁸ Kathryn Pritchard-Jones,^{13*} Richard Houlston,¹ Clare Turnbull,¹ Charles Stiller,¹⁹ Michael R Stratton,^{1,2} Jenny Douglas,¹ Nazneen Rahman¹

¹Section of Cancer Genetics and ¹³Section of Pediatrics, Institute of Cancer Research and Royal Marsden Hospital, Sutton, Surrey, United Kingdom; ²The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom; ³Pediatric Oncology, Addenbrooke's Hospital, Cambridge, United Kingdom; ⁴Department of Pediatric Oncology, ¹¹Department of Clinical Genetics and ¹⁷Department of Histopathology and Pediatric Laboratory Medicine, Great Ormond Street Hospital, London, United Kingdom; ⁵Pediatric Oncology, Nottingham University Hospital, Nottingham, United Kingdom; ⁶Pediatric Oncology, Birmingham Children's Hospital, Birmingham, United Kingdom; ⁷Department of Haematology and Oncology, Royal Aberdeen Children's Hospital, Aberdeen, Scotland, United Kingdom; ⁸Department of Pediatric Oncology, Alder Hey Children's Hospital, Liverpool, United Kingdom; ⁹Department of Clinical Genetics, Chapel Allerton Hospital, Leeds, United Kingdom; ¹⁰Department of Clinical Genetics, St. George's Hospital, London, United Kingdom; ¹²Pediatric Oncology, Children's Hospital for Wales, Cardiff, United Kingdom; ¹⁴Department of Pediatrics, Derriford Hospital, Plymouth, United Kingdom; ¹⁵Department of Histopathology, School of Medicine, Cardiff University, Cardiff, United Kingdom; ¹⁶Department of Histopathology, Royal Brompton Hospital and the National Heart and Lung Institute, Imperial College, London, United Kingdom; ¹⁸International PPB Registry, Children's Hospitals and Clinics of Minnesota, Minneapolis, USA; ¹⁹Childhood Cancer Research Group, Department of Pediatrics, University of Oxford, Oxford, United Kingdom.

*current affiliation Molecular Haematology and Cancer Biology, Institute of Child Health, London, United Kingdom.

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Corresponding author: Professor Nazneen Rahman MD PhD, Professor of Human Genetics, The Institute of Cancer Research; 15 Cotswold Road; Sutton SM2 5NG; United Kingdom. Email: nazneen.rahman@icr.ac.uk

Tel: +44 (0)20 8722 4145 Fax: +44 (0)20 8722 4026

ABSTRACT

Background: Constitutional *DICER1* mutations were recently reported to cause familial pleuropulmonary blastoma (PPB). We sought to investigate the contribution and phenotypic spectrum of constitutional and somatic *DICER1* mutations to cancer.

Methods and Results: We sequenced *DICER1* in constitutional DNA from 823 unrelated individuals with a variety of tumors and in 781 cancer cell lines. We identified constitutional *DICER1* mutations in 19 families including 11/14 with PPB, 2/3 with cystic nephroma, 4/7 with ovarian Sertoli-Leydig type tumors, 1/243 with Wilms tumor (this individual also had Sertoli-Leydig tumor), 1/1 with intra-ocular medulloepithelioma (this individual also had PPB), 1/86 with medulloblastoma/infratentorial PNET and 1/172 with germ cell tumor. The inheritance was investigated in 17 families. *DICER1* mutations were identified in 25 relatives, 17 were unaffected, one mother had ovarian Sertoli-Leydig tumor, one half-sibling had cystic nephroma and six relatives had non-toxic thyroid cysts/goiter. Analysis of eight tumors from *DICER1* mutation-positive individuals demonstrated universal retention of the wild-type allele. We identified *DICER1* truncating mutations in 4/781 cancer cell lines; all were microsatellite unstable lines and hence these are unlikely to be driver mutations.

Conclusion: Constitutional *DICER1* haploinsufficiency predisposes to a broad range of tumors, making a substantial contribution to PPB, cystic nephroma and ovarian Sertoli-Leydig tumors, but a small contribution to other tumors. Most mutation carriers are unaffected indicating that the risk of tumors is modest. We define the clinical contexts in which *DICER1* mutation testing should be considered, the associated tumor risks and the implications for at-risk individuals. We have termed this condition '*DICER1* syndrome'.

INTRODUCTION

DICER1 is an RNase endonuclease important in the production of microRNAs (miRNA) which are non-protein-coding small RNAs that are estimated to regulate the expression of over 30% of protein-coding genes at the post transcriptional level.[1, 2] miRNAs are transcribed as long precursors, known as pri-miRNAs, which are processed in the nucleus to produce pre-miRNAs.[3] The pre-miRNAs are exported to the cytoplasm where DICER1 processing generates a double-strand miRNA duplex.[4] The duplex is unwound to generate the final miRNA which interacts with mRNA to regulate gene expression, typically through translational repression or mRNA degradation. [2, 5, 6]

Over 900 human miRNAs are currently recognised.[7, 8] These have been implicated in a wide range of biological processes including metabolism, morphogenesis, cell fate determination, cell proliferation and apoptosis.[9, 10] There is increasing evidence implicating dysregulation of miRNAs in several human diseases, including cancer.[11, 12] Widespread alteration of miRNA levels is seen in cancers and miRNA profiles characteristic for cancer type and stage are increasingly recognised.[12, 13] Furthermore, global downregulation of miRNAs due to abrogation of miRNA processing has been shown to promote tumorigenesis.[14]

Recently, germline inactivating *DICER1* mutations were shown to cause familial pleuropulmonary blastoma (PPB, OMIM #601200), a rare malignant lung tumor, primarily affecting children before age 6.[15, 16] By linkage analysis Hill *et al* mapped a familial PPB gene to chromosome 14q. They considered *DICER1* to be a promising candidate and identified pathogenic mutations in 11 families.[15] This important finding raises a number of questions. Firstly, what is the contribution of *DICER1* mutations to non-familial, sporadic PPB? Mutations in some cancer predisposition genes contribute appreciably to both familial and sporadic forms of disease, whereas

for others the contribution to non-familial cases is small. Secondly, do constitutional *DICER1* mutations predispose to tumors other than PPB? The International PPB Registry has collected information from over 200 PPB families and a variety of different tumors have been reported in PPB cases and/or their relatives [17, 18] However, it is unknown which tumor types are genuinely associated with PPB, which are related to *DICER1* mutations, and which reflect ascertainment. Thirdly, do somatic *DICER1* mutations contribute to cancer? This is of particular interest as it has been proposed that somatic 14q loss, which has been reported in many cancers, may be targeted at *DICER1*. [19, 20, 21]

To address these questions we have conducted exhaustive *DICER1* sequencing, in >1,600 patient samples, including constitutional DNAs from 823 individuals with a broad range of tumors, but particularly focussing on tumors that have been proposed to be associated with PPB (http://www.ppbregistry.org/pdf/Doc_D.pdf) (Table 1). We also sequenced *DICER1* in DNA from 781 cancer cell lines to assess the impact of somatic *DICER1* mutation on cancer development (Supplementary Table 1).

Table 1. Tumors in individuals screened for constitutional *DICER1* mutations

Tumor Type	Number of cases	Number with <i>DICER1</i> mutation
<u>Lung tumors</u>		
Pleuropulmonary blastoma	14	11
Congenital lung cyst	1	
<u>Sex-cord stromal tumors</u>		
Testicular Sertoli-Leydig/Sertoli/Leydig tumor	11	
Ovarian Sertoli-Leydig tumor	6	3
Ovarian granulosa cell tumor	6	
Gonadal stromal cell tumor unclassified	5	
Mixed stromal cell tumor	1	
Stromal sex-cord tumor unclassified	1	1
<u>Renal tumors</u>		
Wilms tumor	243	1 ^a
Cystic nephroma	3	2
Clear cell sarcoma	3	
<u>CNS tumors</u>		
Medulloblastoma/Infratentorial PNET	84	1
<u>Eye tumors</u>		
Medulloepithelioma (dictyoma)	1	1 ^b
<u>Germ cell tumors</u>		
Seminoma	71	1
Non-seminoma (testis)	52	
Mixed testicular cancer	14	
Embryonal carcinoma	13	
Testicular cancer unspecified	12	
Teratoma	10	
Dysgerminoma/Germinoma	8	
Yolk-sac tumor	4	
Gonadoblastoma	1	
<u>Neuroblastoma</u>	73	
<u>Thyroid carcinoma</u>		
Papillary thyroid carcinoma	51	
Follicular thyroid carcinoma	18	
Thyroid cancer unspecified	15	
Anaplastic thyroid carcinoma	4	
<u>Soft tissue and other extraosseous sarcomas</u>		
Rhabdomyosarcoma	19	
Fibrosarcoma	7	
Other soft tissue sarcomas	49	
<u>Malignant bone tumors</u>		
Ewings sarcoma	8	
Osteosarcoma	7	
Chondrosarcoma	4	
<u>Hepatic tumors</u>		
Hepatoblastoma	2	
Hepatocellular carcinoma	2	

^athis patient also had ovarian Sertoli-Leydig cell tumor

^bthis patient also had pleuropulmonary blastoma

METHODS

Samples

Constitutional DNA was extracted from EDTA-venous blood samples and collected through the Factors Associated with Childhood Tumors (FACT) study, the Royal Marsden Hospital (RMH) cancer collections, and the Institute of Cancer Research UK-wide testicular germ cell tumor collections, all of which have been approved by an appropriate Ethics Board. All samples were obtained with full informed consent. The research was undertaken as part of the Factors Associated with Childhood Tumors Study (FACT study) which was approved by the London Multicentre Research Ethics Committee (05/MRE02/17). The FACT study aims to identify genetic factors that predispose to the development of childhood tumors (www.icr.ac.uk/fact). The National Registry of Childhood Tumors (NRCT) was utilised to identify the total number (indicated in brackets) of cases nationally for PPB (20, 5 of whom were deceased), cystic nephroma (15, none deceased) and Sertoli-Leydig cell tumors (7, none deceased) that had been registered since its inception in 1962. The clinicians were contacted to request that they recruit these patients to the FACT study. In addition, a small minority of patients referred to our Clinical Genetics service with these tumors were recruited directly. Tumor DNA from *DICER1* mutation-positive paraffin-embedded tissues was extracted using QIAamp DNA FFPE Tissue kit (QIAGEN) according to manufacturer instructions. We analysed whole genome amplified DNA from 781 cancer cell lines as part of the Cancer Genome Project, Cell Line Project (Supplementary Table 1).

***DICER1* Sequencing**

For analysis of the constitutional DNA we designed PCR primers to amplify the 26 coding exons and intron-exon boundaries of *DICER1* in a multiplex PCR reaction (Supplementary Table 2). Products were sequenced by capillary sequencing using the BigDye Terminator Cycle Sequencing Kit and an ABI3730 Genetic Analyzer

(Applied Biosystems, Foster City CA). Sequences were analysed using Mutation Surveyor software v3.20 (SoftGenetics, USA). We only included samples in which at least 90% or more of the coding sequence was successfully screened in subsequent analyses. For the cancer cell lines, PCR primers that amplify 500bp PCR products encompassing the 26 coding *DICER1* exons and intron-exon boundaries were designed and sequenced as described above. Sequence traces were analyzed using AutoCSA software[22], followed by manual inspection of putative variants. All putative variants were confirmed by bidirectional sequencing of a second independently amplified PCR product. Matched normal cell lines were available for forty cell lines. The somatic status of variants identified in these forty cell lines was determined by sequencing DNA from the corresponding normal. In the remaining cell lines we assumed that cell line variants that were also identified in the constitutional DNA screen were not somatic. We evaluated the likely pathogenicity of sequence variants using Polyphen, SIFT and NNSplice software.

RESULTS

Germline *DICER1* mutation analysis

We identified pathogenic mutations in 19/823 index individuals (Table 2 and Supplementary Figure 1). Seventeen mutations lead to premature protein truncation as a result of frameshift, nonsense or consensus splice-site mutations. Two mutations are missense alterations for which there is substantial evidence of pathogenicity. Firstly, they are the only two missense alterations in the 3214 chromosomes screened that are predicted to be pathogenic by SIFT and Polyphen. Secondly, both target highly conserved residues in the RNase III domain. Thirdly, they are in the vicinity of a missense variant identified by Hill *et al*, which resulted in a similar *DICER1* histochemical profile to truncating mutations.[15] We also identified several non-pathogenic variants including nine missense variants, 23 synonymous variants and five intronic variants (Supplementary Table 3).

Pleuropulmonary blastoma

We identified *DICER1* mutations in 10 individuals with PPB and in the mother of a child that had died from PPB but from whom no sample was available. One child developed an intra-ocular medulloepithelioma four years after PPB. Three-generational pedigrees were available for most cases and no relative had PPB. One sibling died from neuroblastoma; her *DICER1* status is not known, but a mutation was present in her mother. There were three children with PPB in whom we did not identify a *DICER1* mutation.

Cystic nephroma

The commonest reported association of PPB is cystic nephroma[17], a rare benign renal tumor that typically presents as a multicystic renal mass without solid nodules. It has a bimodal incidence with 50% occurring in children less than 4 years and 30% in the 5th and 6th decades.[23] We had DNA from three unilateral childhood cases

and in two we identified truncating *DICER1* mutations. One of the children had a half-sibling with cystic nephroma who also has the mutation. The second *DICER1* mutation-positive case was recently found to have a small lung cyst which is being monitored, but has not had histological evaluation.

Ovarian Sertoli-Leydig tumors

We analysed DNA from 30 individuals with sex cord tumors of which six were ovarian Sertoli-Leydig tumors, which are sex cord-tumors that exhibit testicular differentiation.[24] The age range of diagnosis is between 2-75 years but ~75% present in the second or third decades.[24] We identified truncating *DICER1* mutations in four individuals; three had young-onset bilateral ovarian Sertoli-Leydig tumors, and one had a unilateral ovarian sex cord tumor that could not be further classified because of necrosis. One of the bilateral cases previously had Wilms tumor (see below). The mother of one case had also developed a Sertoli-Leydig tumor at 21 years and carried the *DICER1* mutation. She has subsequently had melanoma at 50 years, endometrial cancer at 62 years and breast cancer at 68 years.

Wilms tumor

Wilms tumor is an embryonal cancer of the kidney that affects ~1 in 10,000 children, usually before the age of 6 years.[25, 26] We analysed DNA from 243 individuals with Wilms tumor. We identified one truncating *DICER1* mutation, in a child who developed Wilms tumor of atypical histology at the unusually late age of 8 years. Four years after treatment the child developed bilateral ovarian Sertoli-Leydig cell tumors.

Medulloblastoma/Infratentorial PNET

Medulloblastoma is a primitive neuro-ectodermal tumor that arises in the posterior fossa.[27] We analysed 84 childhood medulloblastoma/infratentorial PNET cases and

identified one truncating mutation in a child of 13 years. No other information or samples were available.

Seminoma

We analysed DNA from 185 individuals with germ cell tumor, of which 128 had a family history of testicular cancer. We identified one missense *DICER1* mutation, Q1580H. A maternal first cousin once removed of this proband developed testicular cancer at 27 years but the proband's mother does not carry the *DICER1* mutation. It is not possible to conclusively establish whether this mutation is pathogenic on the available evidence.

Intra-ocular medulloepithelioma

Intra-ocular medulloepithelioma, also known as dictyoma, is a very rare embryonal tumor usually originating in the ciliary body of the eye that most commonly occurs during childhood.[28] One child with PPB also developed a dictyoma, and has a *DICER1* mutation.

Thyroid non-toxic goiters / cysts

In the PPB Registry thyroid cancers and thyroid hyperplasia are reported in both probands and relatives of PPB cases. We analysed DNA from 88 individuals with thyroid cancer but did not identify any mutations. However, one proband and six relatives of *DICER1* mutation-positive individuals developed thyroid cysts / multinodular colloid goiter between the ages of 9-30 years. All were non-toxic, associated with normal thyroid function and non-malignant. Thyroidectomy was required in four individuals because of recurrent disease.

Other tumors/cysts

We did not identify mutations in any of the other tumor types as detailed in Table 1.

Table 2. Probands and relatives with constitutional *DICER1* mutations

ID	Phenotype	Age at diagnosis (yrs)	Current age (yrs)
Family 1 - c.4403_4406delCTCT			
proband	pleuropulmonary blastoma	1.5	5
parent	none		40
uncle	none		44
grandparent	none		68
Family 2 - c.1716delT			
proband	pleuropulmonary blastoma	0.8	2
parent	none		36
Family 3 - c.1196_1197dupAG			
proband	pleuropulmonary blastoma	4	5
parent	none		30
aunt	none		26
grandparent	none		62
Family 4 - c.3505delT			
proband	pleuropulmonary blastoma	3	3
sibling	none		12
parent	none		36
Family 5 - c.1966C>T p.R656X			
proband	pleuropulmonary blastoma	7	12
parent	none		52
Family 6 - c.2268_2271delTTTG			
proband	pleuropulmonary blastoma	0.9	1
parent	none		38
Family 7 - c.3665delT			
proband	pleuropulmonary blastoma ^a	4.2	died 5.2
parent	none		36
Family 8 - c.3583_3584delGA			
proband	pleuropulmonary blastoma	1.3	7
	intra-ocular medulloepithelioma	6	
parent	none		29
Family 9 - c.2040+1 G>C			
proband	pleuropulmonary blastoma	3	13
parent	thyroid cysts unclassified	25	38
Family 10 - c.3726C>A p.Y1242X			
proband	pleuropulmonary blastoma	4	15
parent	thyroid cysts unclassified	30	37
^b Family 11 - c.5465A>T p.D1822V			
proband	pleuropulmonary blastoma	1.8	9

parent	thyroid cysts unclassified	20	40
Family 12 - c.3288_3289insTTTC			
proband	cystic nephroma	1.5	11
half-sibling	cystic nephroma	0.8	5
parent	multinodular colloid goitre	25	39
Family 13 - c.328_338dupGTGTCAGCTGT			
proband	cystic nephroma	3	5
parent	multinodular colloid goitre	20	35
	thyroglossal duct cyst	30	
grandparent	thyroid cysts unclassified	7	66
great-aunt	none		59
Family 14 - c.5122_5128delGGAGATG			
proband	ovarian Sertoli-Leydig cell tumor L	17	35
	ovarian Sertoli-Leydig cell tumor R	27	
parent	ovarian Sertoli-Leydig cell tumor R	21	71
	melanoma	50	
	endometrial cancer	62	
	breast cancer	68	
Family 15 - c.1966C>T_p.R656X			
proband	ovarian Sertoli-Leydig cell tumor R	12	17
	ovarian Sertoli-Leydig cell tumor L	14	
parent	none		50
Family 16 - c.3793delA			
proband	ovarian sex cord stromal tumor R	6	9
parent	none		29
Family 17 - c.2988-2_2988-1delAGinsCT			
proband	Wilms tumor	8	15
	multinodular thyroid goiter unclassified	9	
	ovarian Sertoli-Leydig cell tumor R	12	
	ovarian Sertoli-Leydig cell tumor L	12	
parent	none		40
Family 18 - c.1153delC			
proband	medulloblastoma/infratentorial PNET	13	18
Family 19 - c.4740G>T p.Q1580H			
proband	seminoma	32	46

^aindividual deceased, no sample available

^bthis family includes a sibling with neuroblastoma, no sample available

Family studies in *DICER1* mutation-positive individuals

We had samples from both parents in 17 families in which a *DICER1* mutation had been identified in an index individual. In each, the mutation had been inherited (Table 2). We had grandparental samples for five families. In two families, the mutation was absent in the respective grandparents indicating that the mutation had arisen *de novo* in the parent. In the other three families, the mutation was present in a grandparent.

We analysed DNA from 51 relatives and we identified 25 relatives with *DICER1* mutations. Of these, as described above, one mother had a Sertoli-Leydig tumor, one half-sibling had cystic nephroma and six relatives had thyroid cysts / goiter. The remaining 17 individuals did not have clinical features or symptoms likely to be related to the *DICER1* mutation; one relative having muscular dystrophy and another Wegener's granulomatosis.

Analysis of tumors from *DICER1* mutation-positive individuals

We obtained eight tumors from six *DICER1* mutation-positive individuals. This included three PPB, four Sertoli-Leydig tumors and one cystic nephroma. We analysed each tumor for the relevant mutation. Each was heterozygous for the mutation, i.e. the tumor showed a similar mutational profile as in the blood and there was no loss of the wild-type allele in any tumor.

***DICER1* mutation analysis in cancer cell lines**

We screened 781 cancer cell lines (Supplementary Table 1) for *DICER1* mutations. These represent an extensive cross-section of cancers, but do not include many of the cancers in which we identified germline *DICER1* mutations. 206 of the cell lines have previously been shown to have loss of heterozygosity of 14q encompassing *DICER1*.

We identified 4 truncating mutations in the 781 cell lines and these were in microsatellite unstable lines. We identified 22 non-synonymous variants that were either proven to be somatic or in which normal DNA was not available for evaluation. None were predicted to be deleterious (Supplementary Table 4).

DISCUSSION

In 2009, germline *DICER1* mutations were identified in familial pleuropulmonary blastoma, adding to the list of rare familial cancer syndromes that have yielded critical evidence linking essential biological processes with cancer causation.[15] In this study we have expanded knowledge of the link between *DICER1* and cancer. Firstly, we demonstrate that germline *DICER1* mutations are the major cause of PPB. In the UK, 20 cases of PPB have been registered in the National Registry of Childhood Tumors over the last 35 years. We were able to include 14 in the current study, none of whom had a family history of PPB. We identified *DICER1* mutations in 11 of these cases. [15] It is uncertain whether cryptic *DICER1* mutations account for any of the negative cases, whether mutations in another gene can also cause PPB, or whether the remaining cases are not due to germline predisposition genes. However, it is clear that germline *DICER1* mutations are the major cause of both familial and non-familial PPB.

We have also demonstrated that *DICER1* mutations cause a range of phenotypes; not all families include PPB and a high proportion of mutation carriers are clinically well. In view of this we suggest that '*DICER1* syndrome' is a preferable term to 'PPB familial tumor syndrome' which has previously sometimes been used. The range of different tumors that can occur in individuals with *DICER1* mutations is broad and it is likely that more associated tumors will be identified as further mutation testing is undertaken. The contribution of *DICER1* mutations to different tumors is very variable. The major tumors that occur in *DICER1* syndrome appear to be PPB, cystic

nephroma and ovarian Sertoli-Leydig tumor. Evidence for this comes from our mutational data, our clinical and mutational investigation of relatives of mutation-positive probands and from the spectrum of tumors that have been documented in relatives of PPB cases in the PPB Registry.[17, 18] The contribution of *DICER1* mutations to different tumors is also very variable. Whilst germline *DICER1* mutations may contribute significantly to cystic nephroma, ovarian Sertoli-Leydig tumor and intra-ocular medulloepithelioma they are unlikely to have a major impact on the incidence of Wilms tumor, medulloblastoma/infratentorial PNET or neuroblastoma.

In addition to tumors, *DICER1* mutations also appear to confer a risk of thyroid cysts. One proband and six *DICER1* mutation-positive relatives developed thyroid cysts in childhood or young adulthood. While histology was only available for two cases, both were non-toxic multinodular colloid goiters. This is of particular interest as the gene for familial non-toxic multinodular thyroid goiter has been previously shown to localise to 14q.[29]

The mutation analyses in cancer cell lines suggest that somatic *DICER1* mutations do not make a substantial contribution to cancer. This is in contrast to recent reports hypothesising that the 14q hemizygosy observed in 206/761 cell lines is targeted at *DICER1*. [19, 20, 21] In the great majority of the cell lines the 14q loss of heterozygosity extends over a very large area and includes many genes. If loss of *DICER1* were the main driver, one would anticipate that somatic *DICER1* mutations would occur in at least some of the cell lines with normal 14q chromosomes. However, we only identified four truncating mutations and these were in microsatellite unstable lines and therefore are unlikely to be driver mutations. Thus the somatic mutational profile of *DICER1* appears to differ from that of other ubiquitously expressed, critically important genes such as *TP53* and *RB1* that also predispose to rare childhood cancers when mutated in the germline.

The mechanism of *DICER1* tumor predisposition also appears to differ from the majority of known cancer predisposition genes and is likely to operate by a haploinsufficiency mechanism (Figure 1). Our analysis of tumors showed no loss of the wild-type allele and Hill *et al* showed retained *DICER1* expression in tumor cells.[15] Data from mice studies are also consistent with a haploinsufficiency model and indicate that whilst monoallelic *DICER1* inactivation promotes tumorigenesis, biallelic loss is inhibitory.[20, 21] Our data further suggests that although inactivation of one *DICER1* allele is the initiating event in *DICER1* syndrome, presumably because it leads to dysregulation of miRNAs levels, other events must be required for cancer to occur. It is not known what these additional events are, or how many are required for oncogenesis to proceed. However, the low frequency of tumors in *DICER1* mutation carriers suggests that either more than one additional event is required, and/or the likelihood of the event(s) occurring is small.

Our data demonstrate that the risk of tumors in *DICER1* mutation carriers is low and most mutation-carriers do not develop tumors. This modest penetrance and the variable phenotype of *DICER1* syndrome raise significant clinical challenges. We suggest that diagnostic *DICER1* testing should be considered in individuals with possible PPB, cystic nephroma, ovarian Sertoli-Leydig tumor or medulloepithelioma. The prevalence of mutations in these conditions may be considerable and identification of a *DICER1* mutation can aid diagnosis and management, particularly for PPB which can show significant clinical overlap with other types of lung cyst.[18, 30]

The issue of surveillance in a pleiotropic condition of modest penetrance is also complex. To date, ad-hoc, variable screening for PPB has been undertaken in individual families, often utilising lung CT, which can require an anaesthetic, involves

radiation exposure and is of unproven efficacy.[31] Moreover, the natural history and appropriate management of such screen-detected lesions in a well child is unknown. In view of these considerations, and the modest penetrance, we are currently operating an 'open-door' management policy with early investigation of potential tumor-related symptoms, but we are not undertaking routine surveillance in healthy mutation-positive individuals. This policy will be under continuous review, particularly over the next few years, when extensive expert discussions about the optimal management of *DICER1* mutation-carriers are likely to occur.

In this study we clarify the phenotypes associated with constitutional *DICER1* mutations and propose that the condition should be called '*DICER1* syndrome'. In the future, additional research will hopefully further clarify the clinical features, tumor risks and optimal management of this condition and will illuminate the mechanisms by which *DICER1* haploinsufficiency predisposes to human disease.

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WEB RESOURCES

The URLs for data presented herein are as follows:

OMIM, <http://www.ncbi.nlm.nih.gov/Omim/>

PPB Registry, http://www.ppbregistry.org/pdf/Doc_D.pdf

Cancer Genome Project, Cell Line Project,
<http://www.sanger.ac.uk/genetics/CGP/CellLines/>

Polyphen, <http://genetics.bwh.harvard.edu/pph/>

SIFT, <http://blocks.fhcrc.org/sift/SIFT.html>

NNSplice, http://fruitfly.org:9005/seq_tools/splice.html

Cancer Genome Project, Catalogue of Somatic Mutations in Cancer,
<http://www.sanger.ac.uk/perl/genetics/CGP/cosmic>

ACCESSION NUMBERS

The cDNA Genbank accession number for the *DICER1* Sequence reported in this paper is NM_030621.2.

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Competing Interests

None to declare.

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FIGURE LEGEND

Figure 1. Different mechanisms of cancer predisposition resulting from germline mutations in tumor suppressor genes

A: The classic two-hit mechanism, exemplified by retinoblastoma, involves a germline/constitutional mutation which constitutes the first-hit and is present in every cell. A second mutation (hit) targeting the wild-type allele in a retinoblast has to occur for oncogenesis to proceed.

B: Haploinsufficiency mechanism, exemplified by *DICER1* syndrome. A germline/constitutional mutation predisposes to tumors. One or more additional events are required for oncogenesis to proceed but does not appear to include inactivation of the wild-type *DICER1* allele.

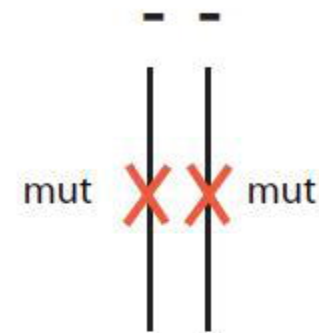
Germline

Tumor

Penetrance

gene function - +

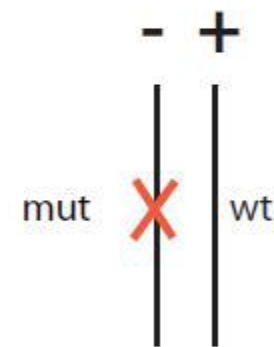
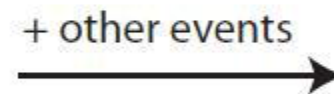
mut X wt



High

gene function - +

mut X wt



Lower

**A. Classic two-hit
mechanism
e.g. *RB1***

**B. Haploinsufficiency
mechanism
eg. *DICER1***

Figure 1. Different mechanisms of cancer predisposition resulting from germline mutations in tumor suppressor genes