Genetic Predispositions and Childhood Cancer

Kevin Shannon

Department of Pediatrics, University of California, San Francisco, California

This article provides an overview of the problem of genetic susceptibility to childhood cancer with a particular emphasis on problems with ascertaining inherited cancer risk and the role of tumor-suppressor gene mutations in cancer predispositions. The association between neurofibromatosis type 1 and childhood leukemia is used to illustrate some of the issues faced by molecular biologists and genetic epidemiologists in identifying and analyzing at-risk individuals. The problem of incomplete penetrance in cancer susceptibility is presented and potential models are discussed. The article concludes with a number of tentative conclusions from existing data and speculations for future studies. — *Environ Health Perspect* 106(Suppl 3):801–806 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-3/801-806shannon/abstract.html*

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Introduction

If identifying genetic susceptibilities is an important challenge facing cancer epidemiologists, particular attention and effort should be directed to childhood cancer. Although inherited cancer syndromes were estimated to account for only 0.1% of all malignant neoplasms (*1*), a known genetic predisposition was observed in 4.2% of pediatric cases (*2*). As new genes and more complex patterns of inheritance and penetrance are uncovered, the proportion of both childhood and adult cancers arising in patients with a known constitutional susceptibility will undoubtedly rise over the next few years. This article begins with a brief overview of inherited cancer susceptibilities. I then use the association between neurofibromatosis type 1 (NF1) and myeloid leukemia as an example of a low penetrance cancer predisposition, discuss a few general principles illustrated by this example, and draw some tentative conclusions.

General Considerations

The Genetic Basis of Cancer

The notion that a particular disorder is genetic in origin usually refers to the fact that it is transmitted within families. Although some human cancers are heritable genetic disorders, most patients have no known susceptibility to cancer, and malignant transformation results from a series of somatic mutations in a target cell. Cancer is therefore unique, in that acquired (somatic) genetic alterations play a major role in its pathogenesis. Many of these genetic alterations are induced by environmental mutagens such as ultraviolet radiation, cigarette smoke, and industrial toxins. In some families, the predisposition to develop cancer is transmitted from parent to child, and children with germline mutations of cancer susceptibility genes are at high risk of developing specific malignant tumors. Familial cancer syndromes have been extraordinarily informative for defining general mechanisms of tumorigenesis and for discovering the responsible genes. In addition, many of the genes that confer a genetic predisposition to childhood cancer play a central role in normal cellular growth control.

Classes of Cancer Susceptibility Genes

A number of distinct genetic mechanisms have been implicated in inherited cancer predispositions including *a*) mutations of cellular protooncogenes, *b*) germline inactivation of both alleles of a recessive gene, *c*) gross chromosomal abnormalities, and *d*) germline inactivation of one allele of a tumor-suppressor gene. The single example of a dominant oncogenic mutation that is associated with a human cancer syndrome involves *RET* gene mutations in multiple endocrine neoplasia type 2 (*3*). Some autosomal recessive disorders carry an increased cancer risk including xeroderma pigmentosum, ataxia telangiectasia, and Fanconi anemia. In these patients, malignant clones arise as a result of acquired mutations at cooperating loci. Down and Turner syndromes are examples of constitutional chromosomal abnormalities that are associated with an increased risk of specific cancers. Although all of these disorders are of general biologic interest, the overall impact of these three genetic mechanisms on cancer incidence is small. In contrast, germline inactivation of one allele of tumor-suppressor genes accounts for the majority of heritable human cancer risk (*4–6*). I will therefore focus on this class of genes in the remainder of this article.

Tumor-Suppressor Genes

In 1971, Knudson developed a hypothesis to explain the peculiar epidemiology of retinoblastoma, a rare malignant eye tumor of childhood. This genetic model predated the development of recombinant DNA by 5 years. Clinically, children with retinoblastoma fall into two categories: a group with early onset disease characterized by frequent bilateral involvement and by an autosomal dominant pattern of inheritance in many families, and a second group that shows later onset, unilateral disease, and a

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Address correspondence to Dr. K. Shannon, Department of Pediatrics, Room HSE 302, Box 0519, University of California, 513 Parnassus Ave., San Francisco, CA 94143. Telephone: (415) 476-7932. Fax: (415) 502-5127. E-mail: kevins@itsa.ucsf.edu

Abbreviations used: GAP, GTPase-activating protein; JMML, juvenile myelomonocytic leukemia; LFS, Li-Fraumeni syndrome; LOH, loss of constitutional heterozygosity; NF1, neurofibromatosis type 1; SMN, second malignant neoplasms.

negative family history. According to Knudson's model, children in the group with early onset, heritable retinoblastoma were at markedly increased risk of cancer because all of their somatic cells carry an inactive copy of a gene that plays a central role in regulating the growth of immature retinal cells (first hit). An acquired mutation of the single normal allele in any susceptible cell (second hit) inactivates the gene and contributes to the development of cancer. Thus, the Knudson model postulates that both alleles of tumor-suppressor genes must be inactivated by separate events during tumorigenesis. Because every somatic cell contains the first hit, patients with germline mutations of tumor-suppressor genes develop cancer at a relatively young age and are prone to multiple independent tumors. Although loss of growth control is recessive at the cellular level, the cancer predisposition is transmitted as a dominant trait, with offspring having a 50% chance of inheriting the predisposing mutant allele from an affected parent. The Knudson model also correctly predicted that the same tumor-suppressor genes would be involved in the pathogenesis of nonfamilial cases of cancer. In these patients, both copies of the tumor-suppressor gene are affected by independent somatic mutations. Inactivation of tumor-suppressor genes occurs by various genetic mechanisms including structural deletions, point mutations, or gene conversion. Human tumor-suppressor genes identified to date include *RB1* (retinoblastoma, osteosarcoma), *WT1* (Wilms tumor), *p53* (Li-Fraumeni syndrome), *NF1* (neural crest tumors, childhood leukemia), *NF2* (acoustic neurofibroma, meningioma), *APC* (colon cancer), and the breast cancer susceptibility genes *BRCA1* and *BRCA2*. An interesting group of genes that have recently been implicated in human tumorigenesis encode proteins involved in DNA mismatch repair. Although many investigators argue that these *hMUT* genes define a new class of cancer susceptibility genes because of their novel biochemical properties, they behave genetically like tumor-suppressors (i.e., at-risk individuals inherit a single mutant allele in the germline and the malignant clone inactivates the normal copy).

Molecular Analysis

The identification of tumor-suppressor genes has been problematic because of the technical difficulty in finding genes that are inactivated during tumorigenesis. For this reason, most of the existing tumor-suppressor genes were identified by reverse genetics; that is, their protein products were only known after the gene was cloned by ascertaining the position of the gene by linkage. Analysis of tumor specimens for loss of constitutional heterozygosity (LOH) has provided a powerful tool for localizing human tumor-suppressor genes to specific chromosomal regions. The underlying principle is simple. Somatic inactivation of tumor-suppressor genes is frequently caused by structural deletions of DNA and this, in turn, can be detected by finding that a heterozygous locus in normal tissues is reduced to homozygosity in the tumor. This principle is illustrated in Figure 1. The recent availability of a set of highly polymorphic microsatellite markers has provided powerful tools for the studies of LOH and for linkage analysis in affected families. Once a tumor-suppressor gene has been cloned, a variety of techniques exists to analyze specimens from cancer-prone families for mutations including Southern blotting, single-strand conformational polymorphism analysis, protein truncation testing, and DNA sequencing. Caution must be exercised in the use of these methods; in addition to issues of privacy and confidentiality, it may be difficult to ascertain if nucleotide substitutions in tumor-suppressor genes represent true mutations or polymorphisms (*7,8*). Recently, targeted homologous recombination into murine embryonic stem cells has proven invaluable in characterizing how tumor-suppressor genes function in both normal development and in tumorigenesis (*9*).

Penetrance, Phenotypic Variability, and Genetic Heterogeneity

Individuals with germline *RB1* mutations represent perhaps the ideal constellation of clinical and molecular characteristics for ascertaining cancer susceptibility. First, the penetrance of retinoblastoma is greater than 90% in individuals with these mutations. This means that affected families will show a clear dominant pattern of inheritance that can be used to reliably score individuals with respect to carrier status. Second, because retinoblastoma is cured in a majority of cases, many affected individuals reproduce and this provides pedigrees for analysis. Third, children with germline mutations of *RB1* often show a distinctive phenotype of early onset and bilateral involvement that distinguishes them for patients without such alterations. This has proven to be particularly useful in identifying children who are likely to show *de novo* germline alterations. Fourth, biallelic inactivation of *RB1* is a consistent finding in both familial and sporadic retinoblastoma tumors. In summary, retinoblastoma is a particularly well-behaved tumor from the standpoint of genetic epidemiology

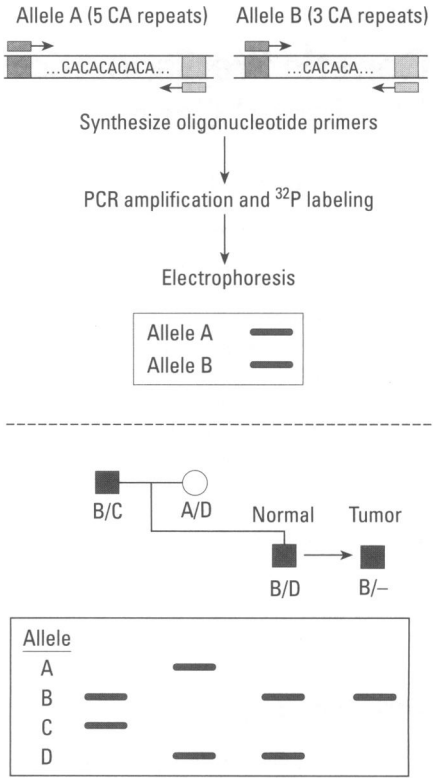

Figure 1. LOH analysis in tumor specimens by analysis of polymorphic microsatellite markers consisting of variable nucleotide tandem repeats. Top panel: Noncoding (intronic) sequences in human DNA frequently contain stretches of short tandemly repeated DNA sequences such as CA. Because the number of repeats is highly variable between individuals, these sequences provide excellent polymorphic markers for genetic linkage and LOH studies. Oligonucleotide primers are designed which flank the repeat on interest and the repeat is amplified and labeled using the polymerase chain reaction. In this illustration, allele *A* contains five CA repeats and allele *B* contains three. This results in a four-nucleotide difference in the size of the amplified fragment which is easily resolved on a sequencing-type gel. Bottom panel: In this ideal family, the filled in squares indicate that the son has inherited a tumor-suppressor gene that predisposes to cancer from his father. Amplification using oligonucleotide primers that detect a polymorphic marker at the disease locus reveals a larger fragment from one of the father's chromosomes (labeled *B*) and a shorter *C* fragment. The repeats amplifed from maternal chromosomes are slightly longer (*A*) and shorter (*D*) that the paternal alleles. The son has inherited a mutant *B* allele from his affected father and an abnormal copy of the gene from his mother (*D*). Deletion of the normal maternal allele of this tumor-suppresor gene during tumorigenesis results in LOH at the locus.

because it shows a high degree of penetrance, is frequently curable, gives a consistent phenotype, and shows limited genetic heterogeneity. As discussed below, as these characteristics deviate from this ideal situation, identifying individuals who inherit cancer susceptibilities and discovering the tumor-suppressor genes that are mutated in the germline becomes progressively more complicated.

NF1 and Childhood Leukemia

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder with an incidence of approximately 1 in 3500 (*10*). Affected individuals are predisposed to specific benign and malignant neoplasms that primarily arise from cells of neural crest origin. These tumors include neurofibromas, neurofibrosarcomas, optic gliomas, and pheochromocytomas (*10*). Young children with NF1 have a 200- to 500-fold excess incidence of malignant myeloid disorders, particularly juvenile myelomonocytic leukemia (JMML) (formally known as juvenile chronic myelogenous leukemia) and monosomy 7 syndrome (Mo 7) (*11–14*). Interestingly, adults with NF1 do not appear to be at increased risk of leukemia. In 1990, the *NF1* gene was identified independently in the laboratories of Francis Collins and Ray White (*15–17*). Almost immediately thereafter, a computer-based sequence alignment showed that *NF1* contains a domain with significant homology to yeast and mammalian GTPase-activating proteins (GAPs) (*18,19*). GAPs negatively regulate the biochemical output of cellular Ras proteins by accelerating conversion of Ras from its active guanosine triphosphate-bound conformation (Ras-GTP) to its inactive guanosine diphosphate-bound state (Ras-GDP) (*20*). Activating point mutations of *RAS* protooncogenes are among the most common molecular alterations seen in human cancer cells, including myeloid leukemias [reviewed in Bos (*21*) and Rodenhuis (*22*)]. Taken together, the finding of activating *RAS* mutations in many human tumors, biochemical evidence that neurofibromin negatively regulates Ras-GTP, and the increased cancer risk observed in individuals with NF1 suggested that *NF1* might function as a tumor-suppressor gene. This hypothesis predicts that the single normal *NF1* allele will be inactivated during tumorigenesis in individuals with NF1. Indeed, LOH at has been detected in neurofibrosarcomas (*23–25*), pheochromocytomas (*26*), and neurofibromas (*27*) from patients with NF1.

We have been working to understand the molecular basis of the increased risk of leukemia seen in children with NF1. We first showed that their bone marrows frequently acquired LOH at the *NF1* locus (*28,29*). In children with familial *NF1*, the copy of the *NF1* gene that was inherited from the unaffected parent was invariably deleted in the leukemia, whereas the copy from the parent with NF1 was retained. This result is consistent with the Knudson model. To ascertain if both copies of the *NF1* gene were functionally inactive in these leukemias and to ask if specific germline mutations were associated with a strong risk of cancer, we adapted a coupled *in vitro* transcription/translation assay to screen for mutations that lead to premature termination of protein translation, and then performed DNA sequencing to confirm suspected mutations. These studies revealed nine different *NF1* gene mutations, all of which are predicted to destroy protein function. The bone marrows of five patients with these truncating *NF1* mutations also showed LOH at *NF1* and have therefore inactivated both *NF1* alleles (*30*). These findings prove that *NF1* functions as a tumor-suppressor gene in childhood leukemia.

The data presented above are consistent with the proposal that neurofibromin (the protein encoded by the *NF1* gene) negatively regulates the growth of myeloid cells by acting as a GAP for Ras. This model predicts that acquired *RAS* mutations will be restricted to children who do not have *NF1* because an oncogenic Ras protein would be biochemically redundant in cells with defective neurofibromin GAP activity. We and our collaborators tested this hypothesis in children with malignant myeloid disorders and found activating *RAS* mutations in 15 of 72 samples from children without NF1 but in 0 of 19 patients with NF1 ($p = 0.02$) (*31,32*). These genetic data suggesting that neurofibromin restrains the growth of immature myeloid cells by regulating Ras were further supported by biochemical experiments showing that the leukemic cells of children with NF1 show a reduction in neurofibromin-associated GAP activity and increased levels of Ras-GTP (*33*). A final line of evidence that *NF1* functions as a tumor-suppressor gene in immature hematopoietic cells is derived from experiments performed in mice in which one copy of the murine *NF1* gene (*Nf1*) was disrupted by targeted homologous recombination (*34,35*). Heterozygous *Nf1* mice (*Nf1+/–*) are phenotypically normal; however, these animals are predisposed to pheochromocytoma and myeloid leukemia (*35*). Molecular investigation has shown loss of the wild-type *Nf1* allele in the murine leukemias studied to date, a finding that parallels our experience in children with *NF1*. *Nf1–/–* embryos die *in utero* by day 13 of gestation from developmental abnormalities of the cardiopulmonary system (*34,35*). However, fetal liver cells from *NFf1–/–* embryos have been used to reconstitute hematopoiesis in irradiated recipients. Mice transplanted with *Nf1–/–* cells consistently develop a syndrome reminiscent of JMML characterized by a marked overproliferation of myeloid cells that infiltrate the spleen and liver (*36*).

In addition to the predilection to primary malignant tumors of the central nervous system, rhabodomyosarcomas, fibrosarcomas, and myeloid leukemia, recent data suggest that children with NF1 who are successfully treated with mutagenic agents are at high risk of developing second malignant neoplasms. We have investigated five such patients who developed secondary leukemia with Mo 7 after being treated with multimodal therapy for other cancers (*37*). Each received alkylating agents as part of their initial cancer therapy. Our collaborator John Maris also reviewed the Tumor Registry at the Children's Hospital of Philadelphia and found an incidence of second cancer of 10% in children with NF1. Of eight patients with embryonal cancers (neuroblastoma, Wilms tumor, or rhabdomyosarcoma), six developed a second cancer (*37*). This is perhaps due to the fact that these young children typically receive aggressive multimodal therapy that includes both chemotherapy and radiation.

General Questions Raised by Data from NF1-Associated Leukemias

Problems Posed by Low Penetrance Cancer Genes

Of 509 children with cancer identified in the study of Narod et al. (*2*), four major genetic associations accounted for 90% of the cases: bilateral retinoblastoma (162 patients), Down syndrome (135 patients), NF1 (90 patients), and hereditary forms of Wilms tumor (70 patients). I believe that these associations represent the tip of the iceberg with respect to the true influence of genetic factors in childhood cancer. Retinoblastoma and some cases of Wilms tumor follow a classic pattern of autosomal dominant inheritance with high penetrance.

The remaining Wilms tumor patients and children with Down syndrome or NF1 show characteristic physical findings. These observations suggest that it will be difficult to ascertain heritable cancer risk if only a small proportion of affected individuals develop a specific cancer (incomplete penetrance) or if at risk individuals do not show characteristic phenotypic findings. NF1 is an interesting case in point. The best epidemiologic study of cancer risk in NF1 estimated a relative risk of 2.5 in affected individuals followed in Denmark for over 40 years (*38*). Similarly, in a comprehensive survey of the Japan Children's Cancer Registry, Matsui et al. (*39*) found that disproportionate numbers of children with NF1 developed neurofibrosarcoma, optic glioma, rhabdomyosarcoma, and myeloid leukemia. However, although the relative risk of all of these malignant neoplasms was greatly increased, the absolute risk was generally small. For example, the overall population-based risk of rhabdomyosarcoma has been estimated to be 4.5 cases per million per year (*40*) and the data of Matsui suggests that this is 54-fold higher in children with NF1. However, this translates to an risk of 243 cases per million children with NF1 (0.02%). Because the penetrance of rhabdomyosarcoma is very low in children with NF1, it is highly unlikely that this relationship would be known if NF1 were not associated with a set of diagnostic physical features. Similar arguments apply to myeloid leukemia in children with NF1 or Down syndrome. Individuals with NF1 illustrate another potentially problematic aspect of inherited cancer susceptibility: alterations of the same gene may predispose to many different tumors. Unlike patients who inherit germline *RB1* or *APC* mutations and almost invariably develop a specific type of cancer as the initial malignant tumor, individuals with NF1 are at risk for a range of different cancers. Li-Fraumeni syndrome (LFS) is perhaps the best-known example of an inherited tumor-suppressor gene alteration that is associated with a spectrum of different tumor types. With *p53* gene mutations now well established as the predominant cause of LFS, families with atypical clinical findings (including variable penetrance) have been identified. Furthermore, screening studies of patients with LFS-associated tumor types have revealed individuals with germline *p53* mutations who were not suspected of having LFS (*41–43*).

Potential Models to Explain Cancer Susceptibilities with Incomplete Penetrance

There are a number of potential models that might explain variable clinical expression of inherited cancer predispositions. The first and most likely is that homozygous inactivation of most tumor-suppressor genes represents an important step in tumorigenesis but is not sufficient for full malignant transformation. Colon cancer is the best-characterized example of multistep tumorigenesis; in that system two known heritable cancer genes (*APC* and *p53*) are frequently mutated in both familial and sporadic cases (*44*). The leukemias of children with NF1 not only inactivate the normal copy of *NF1*, but frequently show deletions of chromosome 7 (*13,45*). In addition, epigenetic factors such as the sex of the parent transmitting NF1 and the sex of the affected child also appear to modulate the risk of leukemia (*13,29*). These observations suggest that the relatively low penetrance of childhood leukemia in children with NF1 is because additional genetic lesions are required in addition to loss of *NF1* gene function. A second potential explanation for relatively low penetrance is that specific tumor-suppressor gene mutations differ with respect to the associated cancer risk. There is limited evidence in support of this model. For example, certain families with a low penetrance pattern of retinoblastoma inheritance show mutations in the *RB1* promoter that presumably do not completely abrogate gene transcription (*46*). A final genetic mechanism to explain variable penetrance is that interacting genetic factors strongly influence the clinical expression of many inherited cancer susceptibilities. A strong influence of cooperating loci has been shown clearly in mice which carry germ line mutations of *APC* (*47–50*). With respect to NF1, two lines of evidence argue strongly that specific *NF1* mutations are not associated with a high incidence of childhood leukemia. First, although we have investigated over 25 affected children (many with siblings who have NF1), none has had a first-degree relative with leukemia. Second, each of the mutations that we have identified in leukemic cells are expected to abrogate neurofibromin function and none has previously been associated with cancer. On the other hand, one study suggested that cooperating loci may modulate the severity of nonmalignant NF1-associated complications (*51*). How inherited mutations of tumor-suppressor genes cooperate with other genetic factors such as alterations in the levels of enzymes that detoxify environmental carcinogens is an important area of ongoing investigation in human beings and in animal models.

Second Malignant Tumors

A feature of inherited cancer predispositions that has received limited attention is that susceptible individuals are not only at risk of developing an initial cancer, but also second malignant neoplasms (SMNs). Two broad categories of SMN can be distinguished: neoplasms that arise in prior radiation fields or in patients who previously received systemic mutagenic chemotherapy, and a second primary *de novo* cancer that does not appear to be related to the previous malignancy or its treatment. Although ascertaining the relative contributions of treatment and of a possible inherited susceptibility to the development of SMN is not straightforward in patients who have been exposed to mutagenic therapies, it appears that individuals with germline mutations of *RB1*, *p53*, and *NF1* are each at increased risk of developing SMN (*5,37,52*). As myeloid leukemia is one of the most common types of SMN overall (*53*) it is not surprising that it accounts for a significant proportion of SMNs that occur in children with NF1 (*37*). We recently used heterozygous *Nf1* knockout mice to directly demonstrate an interaction between chemotherapeutic agents that alkylate DNA and germline inactivation of *Nf1* in leukemogenesis (*54*).

Conclusions and Implications for Epidemiologic Studies

I close with a few thoughts that might be relevant to epidemiologists with an interest in childhood cancer.

I believe that we have only seen the tip of the iceberg with respect to the influence of genetic predispositions on the development of childhood cancer. Uncovering the submerged hunk of the iceberg poses a formidable task for genetic epidemiologists and molecular biologists because the underlying genetic alterations are likely to show incomplete penetrance and will probably not be associated with a constellation of known clinical syndromes.

On the basis of what we now know, it is likely that germline inactivation of tumor-suppressor genes will account for most inherited cancer predispositions that will be discovered in the future. Individuals who inherit a mutation of one allele of a given suppressor gene will likely be especially susceptible to environmental mutagens.

Interactions between different genetic loci, as well an environmental factors, will likely be important in determining which patients with inherited genetic susceptibilities actually develop cancer, what type of cancer appears, and the age at disease onset.

Many of the cancer predispositions that remain to be discovered will probably be similar to LFS and NF1 in that they will show a wide range of tissue expression.

Patients who develop second malignant neoplasms may prove a valuable resource for uncovering new cancer susceptibilities.

With respect to discovering new cancer susceptibility genes, there is both good news and bad news. The good news is that the experimental methodologies and genomic resources available for the positional cloning of tumor-suppressor genes are vastly better than even a few years ago—good news for molecular biologists. The bad news is that the tumor-suppressor genes that remain to be discovered will almost certainly behave badly in that they will be less penetrant, show less tissue specificity, and will not be associated with a constellation of characteristic physical findings to readily identify at risk individuals—bad news for genetic epidemiologists. All of this is succinctly summarized in a recent review by King (*55*).

Given this complexity and the immense technical, ethical, legal, and insurance issues that surround testing people for genetic susceptibilities to cancer, it is clear that studies need to be planned and conducted very carefully with appropriate review. Multidisciplinary teams of committed investigators are required for this important task.

I would emphasize the central role of genetic epidemiologists who are not molecular biologists in *a*) recognizing subtle and/or complex cancer predispositions, *b*) identifying at risk families and assembling pedigrees and biologic specimens, *c*) developing strategies to investigate modifying genetic and environmental factors that affect cancer risk, and *d*) generating testable hypotheses.

REFERENCES AND NOTES

1. Easton D, Peto J. The contribution of inherited predisposition to cancer incidence. Cancer Surv 9:395-416 (1990).
2. Narod SA, Stiller C, Lenoir GM. An estimate of the heritable fraction of childhood cancer. Br J Cancer 63:993–999 (1991).
3. Eng C. The RET proto-oncogene in multiple endocrine neoplasia type 2 and Hirschpring's disease. N Engl J Med 13:943–951 (1996).
4. Friend SH, Dryja TP, Weinberg RA. Oncogenes and tumor-suppressing genes. N Engl J Med 318:618–622 (1988).
5. Weinberg R. Tumor suppressor genes. Science 254:1138–1146 (1991).
6. Knudson AG. All in the (cancer) family. Nature Genet 5:103–104 (1993).
7. Report AA. Points to consider: ethical legal, and psychosocial implications of genetic testing in children and adolescents. Am J Human Genet 17:1233–1241 (1995).
8. Nowak R. Gentic testing set for takeoff. Science 265:464–467 (1994).
9. Capechi M. Altering the genome by homologous recombination. Science 244:1288–1296 (1989).
10. Riccardi VM, Eichner JE. Neurofibromatosis. Baltimore, MD: Johns Hopkins University Press, 1986.
11. Castro-Malaspina H, Schaison G, Passe S, Pasquier A, Berger R, Bayle-Weisgerber C, Miller D, Seligmann M, Bernard J. Subacute and chronic myelomonocytic leukemia in children (juvenile CML). Cancer 54:675–686 (1984).
12. Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B, Reeves B, Chessells J. Pediatric myelodysplasia: a study of 68 children and a new prognostic scoring system. Blood 85:1743–1749 (1995).
13. Shannon KM, Watterson J, Johnson P, O'Connell P, Lange B, Shah N, Kan YW, Priest JR. Monosomy 7 myeloproliferative disease in children with neurofibromatosis, type 1: epidemiology and molecular analysis. Blood 79:1311–1318 (1992).
14. Stiller CA, Chessells JM, Fitchett M. Neurofibromatosis and childhood leukemia/lymphoma: a population-based UKCCSG study. Br J Cancer 70:969–972 (1994).
15. Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, Fountain JW, Brereton A, Nicholson J, Mitchell AL, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 249:181–186 (1990).
16. Cawthon RM, Weiss R, Xu GF, Viskochil D, Culver M, Stevens J, Robertson M, Dunn D, Gesteland R, O'Connell P, et al. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 62:193–201 (1990).
17. Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, Culver M, Carey JC, Copeland NG, Jenkins NA, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell 62:187–192 (1990).
18. Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, Wigler M, Collins F. The NF1 locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell63:851–859 (1990).
19. Xu G, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, Dunn D, Stevens J, Gesteland R, White R, et al. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 62:599–608 (1990).
20. Boguski M, McCormick F. Proteins regulating Ras and its relatives. Nature 366:643–653 (1993).
21. Bos JL. *ras* Oncogenes in human cancer: a review. Cancer Res 49:4682–4689 (1989).
22. Rodenhuis S. *ras* and human tumors. Semin Cancer Biol 3:241–247 (1992).
23. Skuse GR, Kosciolek BA, Rowley PT. Molecular genetic analysis of tumors in von Recklinghausen neurofibromatosis: loss of heterozygosity for chromosome 17. Genes Chrom Cancer 1:36–41 (1989).
24. Glover TW, Stein CK, Legius E, Andersen LB, Brereton A, Johnson S. Molecular and cytogenetic analysis of tumors in von Recklinghausen neurofibromatosis. Genes Chrom Cancer 3:62–70 (1991).
25. Menon AG, Anderson KM, Riccardi VM, Chung RY, Whaley JM, Yandell DW, Farmer GE, Freiman RN, Lee JK, Li FP, et al. Chromosome 17p deletions and *p53* gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis. Proc Natl Acad Sci USA 87:5435–5439 (1990).
26. Xu W, Mulligan L, Ponder MA, Liu L, Smith BA, Mathew CG, Ponder BA. Loss of alleles in pheochromocytomas from patients with type 1 neurofibromatosis. Genes Chrom Cancer 4:337–341 (1992).
27. Colman SD, Williams CA, Wallace MR. Benign neurofibromas in type 1 neurofibromatosis (NF1) show somatic deletions of the *NF1* gene. Nature Gen 11:90–92 (1995).
28. Shannon KM, O'Connell P, Martin GA, Paderanga D, Olson K, Dinndorf P, McCormick F. Loss of the normal *NF1* allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330:597–601 (1994).

29. Miles DK, Freedman MH, Stephens K, Pallavicini M, Sievers E, Weaver M, Grunberger T, Thompson P, Shannon KM. Patterns of hematopoietic lineage involvement in children with neurofibromatosis, type 1, and malignant myeloid disorders. Blood 88:4314–4320 (1996).
30. Side L, Taylor B, Cayouette M, Connor E, Thompson P, Luce M, Shannon K. Homozygous inactivation of the NF1 gene in bone marrow cells from children with neurofibromatosis type 1 and malignant myeloid disorders. N Engl J Med 336:1713–1720 (1997).
31. Neubauer A, Shannon KM, Liu E. Mutations of the *ras* protooncogenes in childhood monosomy 7. Blood 77:594–598 (1991).
32. Kalra R, Paderanga D, Olson K, Shannon KM. Genetic analysis is consistent with the hypothesis that *NF1* limits myeloid cell growth through $p21^{ras}$. Blood 84:3435–3439 (1994).
33. Bollag G, Clapp DW, Shih S, Adler F, Zhang Y, Thompson P, Lange BJ, Freedman MH, McCormick F, Jacks T, Shannon KM. Loss of *NF1* results in activation of the Ras signaling pathway and leads to aberrant growth in murine and human hematopoietic cells. Nature Genet 12:144–148 (1996).
34. Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, Buchberg AM, Jenkins N, Parada L, Copeland N. Targeted disruption of the neurofibromatosis type 1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Gene Develop 8:1019–1029 (1994).
35. Jacks T, Shih S, Schmitt EM, Bronson RT, Bernards A, Weinberg RA. Tumorigenic and developmental consequences of a targeted *NF1* mutation in the mouse. Nature Genet 7:353–361 (1994).
36. Largaespada DA, Brannan CI, Jenkins NA, Copeland NG. *NF1* deficiency causes Ras-mediated granulocyte-macrophage colony stimulating factor hypersensitivity and chronic myeloid leukemia. Nature Genet 12:137–143 (1996).
37. Maris JM, Wiersma SR, Mahgoub N, Thompson P, Geyer RJ, Lange BJ, Shannon KM. Monosomy 7 myelodysplastic syndrome and other second malignant neoplasms in children with neurofibromatosis type 1. Cancer 79:1438–1446 (1997).
38. Sorensen SA, Mulvihill JJ, Nielsen A. Long-term follow-up of von Recklinghausen neurofibromatosis. N Engl J Med 314:1010–1014 (1986).
39. Matsui I, Tanimura M, Kobayashi N, Sawada T, Nagahara N, Akatsuka J. Neurofibromatosis type 1 and childhood cancer. Cancer 72:2746–2754 (1993).
40. Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Cancer 75:2185–2195 (1995).
41. Toguchida J, Yamaguchi T, Dayton SH, Beauchamp RL, Herrera GE, Ishizaki K, Yamamuro T, Meyers PA, Little JB, Sasaki MS, et al. Prevalence and spectrum of germline mutations in the *p53* gene among patients with sarcoma. N Engl J Med 326:1301–1308 (1992).
42. McIntyre JF, Smith-Sorensen B, Friend SH, Kassell J, Borresen A-L, Yan YX, Russo C, Sato J, Barbier N, Miser J, et al. Germline mutations of the *p53* tumor suppressor gene in children with osteosarcoma. J Clin Oncol 12:925–930 (1994).
43. Diller L, Sexsmith E, Gottlieb A, Li FP, Malkin D. Germline *p53* mutations are frequently detected in young children with rhabdomyosarcoma. J Clin Invest 95:1606–1611 (1995).
44. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 61:759–767 (1990).
45. Kaneko Y, Maseki N, Sakuri M, Shibuya A, Shinohara T, Fujimoto T, Kanno H, Nishikawa A. Chromosome patterns in juvenile chronic myelogenous leukemia, myelodysplastic syndrome, and acute leukemia associated with neurofibromatosis. Leukemia 3:36–41 (1989).
46. Sakai T, Ohtani N, McGee T, Robbins P, Dryja T. Oncogeneic germ-line mutations in Sp1 and ATF sites in the human retinoblastoma gene. Nature 353:83–86 (1991).
47. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, Borenstein N, Dove W. Genetic identification of *Mom-1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell 75:631–639 (1993).
48. Laird PW, Jackson-Grusby L, Faseli A, Dickinson SL, Jung WE, Li E, Weinberg RA, Jaenisch R. Suppression of intestinal neoplasia by DNA hypomethylation. Cell 81:197–205 (1995).
49. MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM. The secretory phospholipase *A2* gene is a candidate for the *Mom1* locus, a major modifier of Apc^{Min}-induced intestinal neoplasia. Cell 81:957–966 (1995).
50. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803–809 (1996).
51. Easton D, Ponder M, Huson S, Ponder B. An analysis of variation in expression of neurofibromatosis type 1 (NF1): evidence for modifying genes. Am J Hum Genet 53:305–313 (1993).
52. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. Br J Cancer 53:661–671 (1986).
53. Levine E, Bloomfeld C. Leukemias and myelodysplastic syndromes secondary to drug, radiation, and environmental exposure. Semin Oncol 19:47–84 (1992).
54. Mahgoub N, Taylor B, Le Beau M, Gratiot M, Carlson K, Jacks T, Shannon KM. Unpublished data.
55. King M-C. Leaving Kansas... finding genes in 1997. Nature Genet 15:8–10 (1997).