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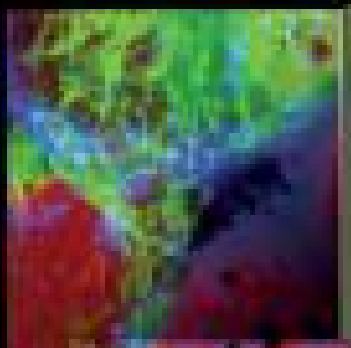
Principles and Practice of Pediatric Oncology

Seventh Edition



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Preface

The five previous editions of the ***Principles and Practice of Pediatric Oncology***, now joined by the sixth, have catalogued and chronicled the extraordinary changes that have taken place in the diagnosis, treatment, and long-term care of children with cancer during the past 22 years. When we began our own personal education(s) in science and medicine two decades before the publication of the first edition of ***Principles and Practice of Pediatric Oncology***, the treatment of cancer was in its infancy and the prospect for cures a distant aspiration and dream. At that time, there was no consideration of long-term consequences, since survival for most patients was measured in months and years. So much has changed—both in the celebration that the majority of children with cancer can become long-term survivors and in the disappointment that their survival is mired by the consequences of treatments configured in the past. Still, in many ways, pediatric oncology stands as the exemplar in codifying the dramatic changes that have taken place in the discovery and application of new medical knowledge as well as a paradigm for what can be achieved through collaborative clinical and translational research.

From the first pioneering physician–scientists who laid the foundation for the discipline of pediatric oncology, to those now at its leading edge of inquiry, there has been a remarkably integrated relationship between basic sciences, clinical research and patient care in the principles and practice of pediatric oncology. As cancer biology evolved from the study of cellular kinetics to its current molecular and genetic underpinnings, pediatric cancer has served as the equivalent of a model organism. The first edition had a primer of the then still new field of molecular biology. Over the past two decades, modern cancer biology, including genetics and genomics, have become integrated into the diagnosis and treatment of childhood malignancies. Trainees in pediatric oncology today are no longer passive observers of molecular medicine—but more often its leaders and innovators.

The concept of multidisciplinary care had its origins in childhood cancer. This concept of teams of physicians, nurses, social workers, and pharmacists working together to optimize patient care has become the signature of 21st century oncology practice. The locus of care also is shifting from largely in-patient to the more frequent outpatient ambulatory setting— including for the administration of heretofore-intensive therapies including stem cell infusions or even the treatment of complications like fever and neutropenia. Indeed, hospitalization is increasingly reserved for the management of the most intense care situations.

Because of its relative rarity and the need to evaluate patients on a larger scale than that available to regional children's hospitals or treatment centers, pediatric oncologists were pioneers in the development of national cooperative groups and closely linking clinical investigation and clinical trials to the delivery of state-of-the-art patient care. Indeed, the discipline of pediatric oncology stands nearly alone in the close partnership of clinical research and patient care—with the vast majority of children who are diagnosed with cancer receiving treatment on clinical protocols. This stands in stark contrast to adult oncology, and it seems clear that those managing many serious and chronic diseases could learn much from how the care of children with cancer has been organized on a national and international basis. Indeed, in an era when innovation defines state-of-the-art patient care and where quality outcomes, excellence in the patient experience, and attention to cost and efficiency will define the future of medicine, the field of pediatric oncology should be used as a prototype, role model, and testing ground—whose principles for study, evaluation, and organized delivery can be extrapolated to many other diseases, whether in children or adults.

The Sixth Edition of ***Principles and Practice of Pediatric Oncology*** has been extensively revised and updated to reflect the continued dramatic and significant changes that are occurring in this discipline. Although authors who have contributed to one or more prior editions have prepared the majority of the chapters, new contributors to this edition have written 40% of the chapters. They share in common that each are leaders in their fields and in shaping the future of care for children with cancer. As with prior editions, we have sought to provide the

fundamental underpinnings of cancer biology, genetics, and immunology as well as the conceptual context of surgery, chemotherapy, and radiation oncology in discrete chapters. Although each provides an informed introduction for those new to the field, the principles they articulate are suffused in virtually every chapter. Because we also recognize that the diagnosis and management of the child with cancer must be framed in the context of the family, school, and community, we continue to provide informed attention to the broad and interdisciplinary supportive care and psychosocial management of children and their families facing the challenge of childhood cancer.

We have been proud to serve as editors for each of the now six editions of ***Principles and Practice of Pediatric Oncology***. In this sixth edition, we are enormously pleased to welcome three associate editors: Peter Adamson, Susan Blaney, and Lee Helman. Each is a national leader in the field and we have had the special privilege of sharing in their education and training at the Pediatric Branch of the National Cancer Institute. We remain indebted to the wonderful support we have received from our staff and assistants, especially Ms. Mira Engel at Stanford University and Ms. Sara Farnum at Texas Children's Hospital. We have also been fortunate in having a continued and outstanding relationship with our publisher, now Wolters Kluwer Health—Lippincott Williams & Wilkins, which has undergone its own evolution over the years. In particular, we want to thank Jonathan Pine, who has worked with us on half of the six editions and also Emilie Moyer who served as our managing editor for the current edition.

The future of books as paper publications is rapidly changing. But in whatever format they appear the power of the knowledge that textbooks contain is transformative. It remains our hope and singular goal that the Sixth Edition of the ***Principles and Practice of Pediatric Oncology*** will help educate the current and future providers of care to children with cancer and through their accrued knowledge and experience, further transform and improve the lives and futures of their patients.

Chapter 1
Epidemiology of Childhood Cancer

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This chapter provides an update on childhood cancer statistics and an overview of epidemiologic methods, including study designs, potential biases, and statistical measures of effect, with examples from the childhood cancer literature to illustrate these concepts. The information in this chapter is meant to help clinicians better understand the approaches used in epidemiologic research on the causes and consequences of childhood cancer and to interpret and communicate research findings to their patients and colleagues.

Central Concepts of Epidemiology

Epidemiology is a key scientific methodology for conducting health-related research. It involves the comparative study of the distribution and determinants of disease and other health-related conditions within defined human populations. Identifying, describing, and interpreting patterns of cancer occurrence (distribution) and studying factors that may cause or contribute to the occurrence, prevention, control, and outcome of cancer (determinants) encompass the activities of epidemiologists.[1,2]

Epidemiology incorporates aspects of research from biologic, clinical, social, and statistical sciences. Two central concepts of epidemiology are as follows:

1. *Disease is not randomly distributed*. Measurable factors influence the patterns and causes of disease within a defined population.
2. *Disease causation is multifactorial*. Few individual agents are necessary or sufficient to cause disease; in fact, disease results from a multitude of endogenous and exogenous factors. Identifying and measuring the relative contribution and interaction of these factors is the principal role of analytic epidemiology.

Surveillance and Descriptive Studies

Public health surveillance involves the systematic collection, analysis, and interpretation of outcome-specific health data and the timely dissemination of the findings to prevent and control disease or injury. Surveillance systems are thus essential to plan, implement, and evaluate public health practice.[3,4] Surveillance systems provide data on disease incidence and mortality on a population basis for policy makers and researchers. In the United States, an exceptionally high-quality cancer surveillance system is funded and coordinated by the National Cancer Institute's (NCI's) Surveillance, Epidemiology, and End Results (SEER) program. The SEER program was established in 1973 and now encompasses nine state and four large metropolitan cancer registries and registries covering the Alaska Native and Arizona American Indian populations (http://www.seer.cancer.gov).

Data from the SEER program enables evaluation, otherwise unachievable, of rare childhood malignancies and of cancer patterns in demographic subgroups. Descriptive analyses from cross-sectional (prevalence) or ecologic (correlational) studies allow investigators to develop hypotheses on the patterns and causes of cancer and then test those hypotheses using analytic approaches.[1,2] The rarity of any specific type of childhood cancer, however, makes it very difficult to recruit enough cases for statistically meaningful studies, even with statewide population-based registries. This problem of conducting good epidemiologic research on rare events has prompted the Children's Oncology Group (COG) to develop a nationwide, volunteer childhood cancer registry, the Childhood

Cancer Research Network (CCRN).[5,6] The CCRN allows newly diagnosed childhood cancer patients and their parents to participate in the data registry with or without the option of being recontacted for future research. Initial pilot studies on the feasibility of the registry showed that 96% of participants agreed to fully participate, and only 1% declined participation.[6] About 90% of children with cancer in the United States are treated on the basis of COG protocols; therefore, the CCRN makes it possible to perform essentially population-based research on childhood cancer etiology.

Childhood Cancer Statistics

Childhood cancer is relatively uncommon, with approximately 1 to 2 children in every 10,000 children aged 14 years and younger diagnosed in the United States each year.[7] Despite the rarity of childhood cancer, approximately 15,100 children and adolescents younger than 20 years will be diagnosed with cancer in the United States (~10,700 cases among children 0 to 14 years of age[8] and ~4,400 cases among 15- to 19-year-olds).[9] These numbers correspond to an average annual incidence rate of 18.8 cases per 100,000 person-years for all cancers for children younger than 20 years. The likelihood of a young person reaching adulthood and being diagnosed with cancer during childhood is approximately 1 in 300 for males and 1 in 333 for females.[6] Childhood cancer remains the leading cause of disease-related mortality among children 1 to 14 years of age (Fig. 1.1A), and there were approximately 1,300 cancer-related deaths in 2006 in the United States among children younger than 15 years. The relative contribution of cancer to overall mortality for 15- to 19-year-olds is lower than that for the younger children (Fig. 1.1B), although

approximately 700 deaths from cancer occurred in 2006 in this age group.

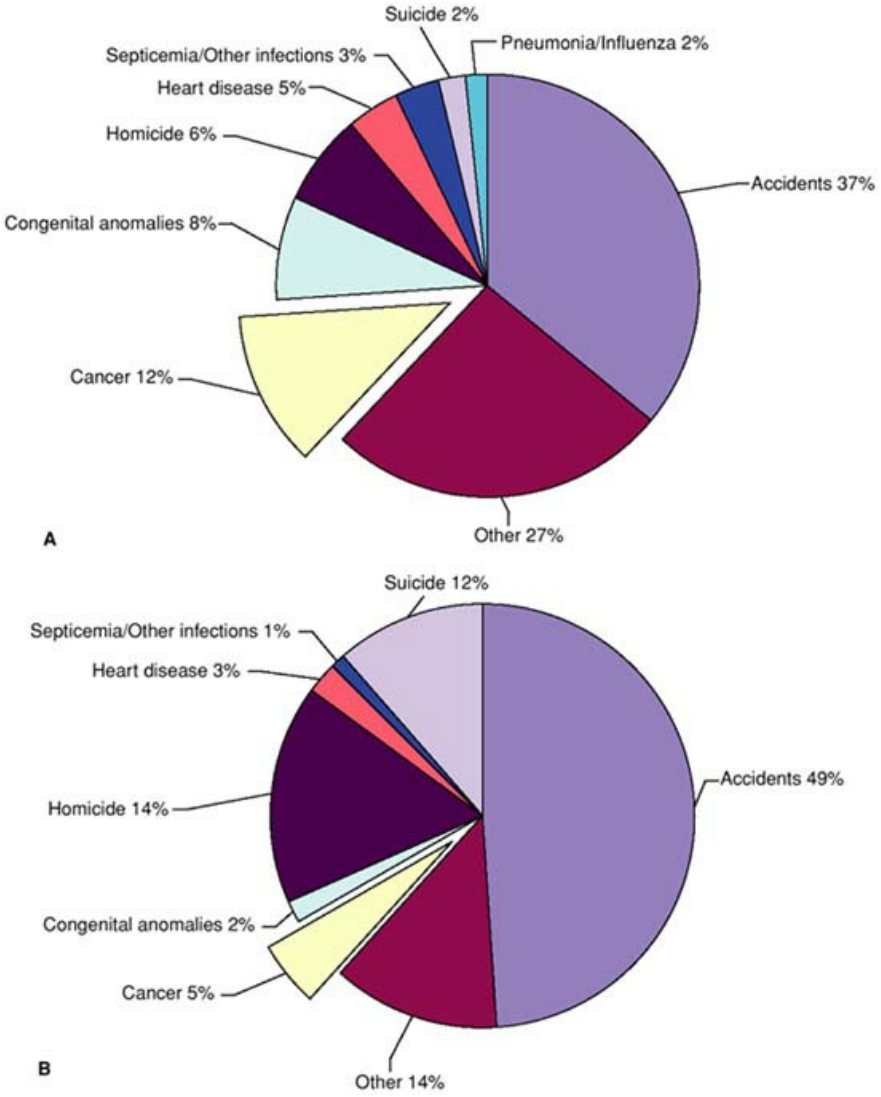

Figure 1.1 Leading causes of death in children in the United States, 2006. Causes of death among (**A**) children 1 to 14 years and (**B**) adolescents 15 to 19 years of age. (Death data are from the National Center for Health Statistics public-use file.)

The population-based data for invasive cancer incidence and survival, unless otherwise indicated, are from the SEER program of the NCI. The SEER data for this chapter are based on 58,316 cases of childhood cancer diagnosed among residents of 17 SEER areas that represent approximately 26% of the U.S. population. (More information on the inclusion of these SEER areas and their contribution to case data is available from the SEER Web site.) The mortality data cover all cancer deaths among children in the United States, as provided by the National Center for Health Statistics. The classification scheme used in this chapter is the International Classification of Childhood Cancer, which allocates tumors into 12 major diagnostic groups that reflect the most prevalent tumors in the pediatric population.[10]

Overall Cancer Frequency and Incidence by type of Cancer for Children and Adolescents

Figure 1.2 compares the distribution by percentages of the cancers that occurred among 0- to 14-year-olds and 15- to

19-year-olds for the years 1973 to 2006, whereas Table 1.1 provides the annual incidence of the major types of cancer in these two age groups by gender. For children aged 0 to 14 years, acute lymphoblastic leukemia (ALL) was the most common cancer, accounting for 25.4% of all cancer diagnoses. Acute myeloid leukemia (AML) was

the next most common type of leukemia in this age group, occurring at a rate one-fifth of that for ALL. Central nervous system (CNS) cancers, primarily occurring in the brain, accounted for 20.6% of cancer diagnoses and together with ALL and AML made up one-half of cancer diagnoses among children younger than 15 years. The most common non-CNS solid tumor in the 0- to 14-year age group was neuroblastoma (7.0%), followed by Wilms' tumor (5.4%) and non-Hodgkin lymphoma (NHL) (5.9%). Other diagnoses that individually represented 2% to 4% of cancer diagnoses in this age group included Hodgkin disease, rhabdomyosarcoma, non-rhabdomyosarcoma soft tissue sarcomas, germ cell tumors, retinoblastoma, and osteosarcoma.

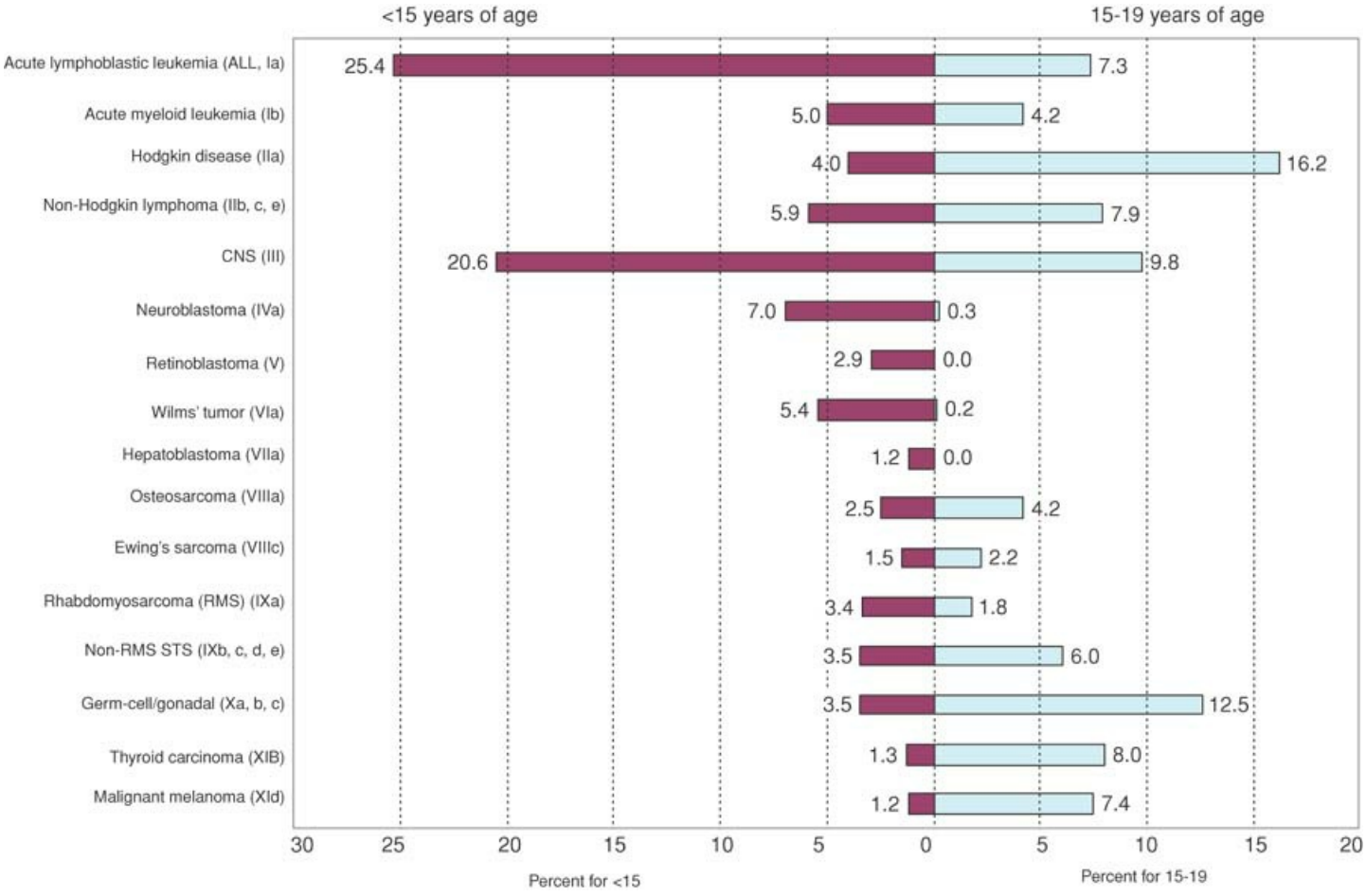

Figure 1.2 Distribution of specific cancer diagnoses for children (aged 0 to 14 years) and adolescents (aged 15 to 19 years), 1973 to 2006. Percentage distribution by International Classification of Childhood Cancer diagnostic groups and subgroups for younger than 15 years and 15 to 19 years of age (all races and both sexes). CNS, central nervous system; RMS, rhabdomyosarcoma; STS, soft tissue sarcoma. (Incidence data are from the Surveillance, Epidemiology, and End Results program, National Cancer Institute.)

The distribution of cancer diagnoses for 15- to 19-year-olds is significantly different (Fig. 1.2). For example, Hodgkin disease (16.2%) and germ cell tumors (12.5%) were the most frequently diagnosed cancers. The percentages of cases represented by NHL (7.9%), melanoma (7.4%), thyroid cancer (8.0%), non-rhabdomyosarcoma soft tissue sarcoma (6.0%), osteosarcoma (4.2%), and Ewing's sarcoma (2.2%) were also higher for 15- to 19-year-olds compared with 0- to 14-year-olds. Although CNS tumors were the third most common tumor type, representing 9.8% of all cancer diagnoses (Fig. 1.2), their incidence was lower for 15- to 19-year-olds compared with 0- to 14-year-olds (Table 1.1). ALL accounted for a much lower proportion of cases among 15- to 19-year-olds (7.3%) compared with children 0 to 14 years of age (25.4%) and occurred only slightly more frequently than AML (4.2% of cases) in this age group. The percentages of cases for rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma were nearly equal for 0- to 14-year-olds, but the percentage for non-rhabdomyosarcoma soft tissue sarcoma was higher than that for rhabdomyosarcoma for 15- to 19-year-olds (Fig. 1.2). Some cancers that are more common in young children (e.g., CNS cancers, neuroblastoma,

retinoblastoma, hepatoblastoma, and Wilms' tumor) occurred at very low rates among 15- to 19-year-olds (Table 1.1).

Variation in Childhood Cancer Incidence by Gender

Table 1.1 shows the incidence of cancer by gender for children (<15 years) and adolescents (15 to 19 years). For both 0- to 14-year-olds and 15- to 19-year-olds, a male predominance

was most apparent for NHL, with males having incidence rates more than 1.5- to 2.0-fold higher than those for females. For children younger than 15 years, other cancer diagnoses that showed a 1.2-fold or higher male predominance were ALL, CNS tumors, hepatoblastoma, Ewing's sarcoma, and rhabdomyosarcoma. For 15- to 19-year-olds, the patterns of incidence by gender were generally similar to those observed in younger children but with the following exceptions: (a) Hodgkin disease among younger children had a higher incidence rate among males, whereas among adolescents Hodgkin disease had a similar rate between males and females; (b) germ cell tumors had a similar rate between males and females among younger children; however, males had a 2.6-fold higher rate among adolescents; (c) osteosarcoma occurred at similar rates in males and females in the 0- to 14-year-old population, although the rate was 2.1-fold higher in males among 15- to 19-year-olds; and (d) the male predominance for Ewing's sarcoma was more pronounced in the 15- to 19-year-old group (1.7-fold higher) than in younger children (1.4-fold higher).

Table 1.1 Incidence of Different Cancers by Gender for The 0- to 14-Year-Old and 15- to 19-Year-Old Populations (1992 to 2006)

	Age (years)							
Diagnosis	<15 (Both sexes rate)	<15 (Male rate)	<15 (Female rate)	<15 (Male to female ratio)	<15-19 (Both sexes rate)	<15-19 (Male rate)	<15-19 (Female rate)	<15-19 (Male and Female ration)
Total	147.7	156.8	138.1	1.1	204.9	215.0	194.3	1.1
Acute lymphoblastic leukemia (Ia)	38.9	42.5	35.1	1.2	15.7	20.6	10.5	2.0
Acute myeloid leukemia (Ib)	7.6	8.1	7.1	1.1	9.3	9.6	8.9	1.1
Hodgkin disease (IIa)	5.4	5.9	4.7	1.3	29.7	27.8	31.7	0.9
Non-Hodgkin lymphoma (IIb,c,e)	8.4	11.2	5.5	2.1	17.2	20.8	13.3	1.6
Central nervous system (III)	30.7	32.8	28.5	1.2	19.6	22.4	16.6	1.3
Neuroblastoma (IVa)	10.3	10.6	9.9	1.1	0.3	0.4	0.2	1.9

Retinoblastoma (V)	4.8	4.9	4.7	1.1	0.1	0.0	0.1	0.9
Wilms' tumor (VIa)	7.6	6.9	8.3	0.8	0.2	0.1	0.3	0.5
Hepatic tumors (VII)	2.4	2.8	2.0	1.4	1.3	1.5	1.1	1.4
Hepatoblastoma (VIIa)	2.0	2.3	1.7	1.4	0.0	0.0	0.0	0.0
Malignant bone tumors (VIII)	5.9	6.1	5.7	1.1	15.5	20.0	10.7	1.9
Osteosarcoma (VIIIa)	3.5	3.2	3.7	0.9	8.8	11.8	5.7	2.1
Ewing's sarcoma (VIIIc)	1.9	2.2	1.6	1.4	4.6	5.7	3.5	1.7
Rhabdomyosarcoma (RMS) (IXa)	4.8	5.4	4.1	1.3	3.7	4.1	3.3	1.2
Non-RMS soft tissue sarcoma (IXb,c,d,e)	5.2	5.4	5.1	1.1	12.4	13.0	11.8	1.1
Germ cell/other gonadal tumors (Xa,b,c)	5.7	5.4	5.9	0.9	27.4	39.3	14.9	2.6
Thyroid carcinoma (XIb)	1.9	1.0	2.9	0.3	16.5	5.3	28.5	0.2
Malignant melanoma (XId)	1.7	1.4	2.0	0.7	15.1	12.2	18.2	0.7

Rates are per 1,000,000 and the <15-year rates are age adjusted to the 2000 U.S. standard. The Roman numerals in parentheses represent the International Classification of Childhood Cancer category for each tumor type.

Variation in Childhood Cancer Incidence by Race and Ethnicity

For many adult cancers, black Americans have higher incidence rates than do white Americans. However, for children and adolescents, the incidence of cancer among white children was approximately 40% higher than that for black children (Table 1.2; Fig. 1.3). The largest difference in absolute incidence between white children and black children was for ALL (32.5 vs. 15.6 per million). This difference was primarily due to the approximately 2.3-fold higher incidence rate for ALL among 0- to 4-year-old

white children compared with 0- to 4-year-old black children. The higher rates for leukemia were limited to ALL, as white children and black children had identical rates for AML (Table 1.2). The incidence of Ewing's sarcoma in white children was 12 times higher than that for black children. For melanoma, white children had incidence rates 21 times higher than those for black children (Table 1.2).

Table 1.2 Incidence of Different Cancers for White, Black, and Hispanic Children, 0 to 19 Years Old (1992 to 2006)

Cancer type	White	Black	Hispanic	W:B ratio	W:H ratio	B:H ratio
Total	176.9	123.2	153.1	1.4	1.2	0.8
Acute lymphoblastic leukemia (Ia)	32.5	15.6	43.7	2.1	0.7	0.4
Acute myeloid leukemia (Ib)	7.4	7.3	8.8	1.0	0.8	0.8
Hodgkin disease (IIa)	14.0	9.9	9.2	1.4	1.5	1.1
Non-Hodgkin lymphoma (IIb,c,e)	11.6	9.6	8.4	1.2	1.4	1.1
Central nervous system (III)	33.0	22.6	22.7	1.5	1.5	1.0
Neuroblastoma (IVa)	9.2	6.9	6.0	1.3	1.5	1.2
Retinoblastoma (V)	3.2	3.5	4.1	0.9	0.8	0.8
Wilms' tumor (VIa)	6.3	6.9	5.1	0.9	1.2	1.4
Hepatoblastoma (VIIa)	1.5	0.7	1.6	2.1	0.9	0.4
Osteosarcoma (VIIIa)	4.4	5.6	4.8	0.8	0.9	1.2
Ewing's sarcoma (VIIIc)	3.6	0.3	2.1	12.0	1.7	0.1
Rhabdomyosarcoma (RMS) (IXa)	4.8	5.9	3.8	0.8	1.3	1.5
Non-rhabdomyosarcoma STS (IXb,c,d,e)	7.4	7.4	5.8	1.0	1.3	1.3
Germ cell (Xa,b,c)	11.4	5.8	11.7	1.9	1.0	0.5
Thyroid carcinoma (XIb)	6.7	2.0	4.1	3.4	1.6	0.5
Malignant melanoma (XId)	8.4	0.4	1.0	21.0	8.8	0.4

CNS, central nervous system tumors; STS, soft tissue sarcoma.

Rates are per 1,000,000 and are age adjusted to the 2000 U.S. standard. The Roman numerals in parentheses represent the International Classification of Childhood Cancer category for each tumor type.

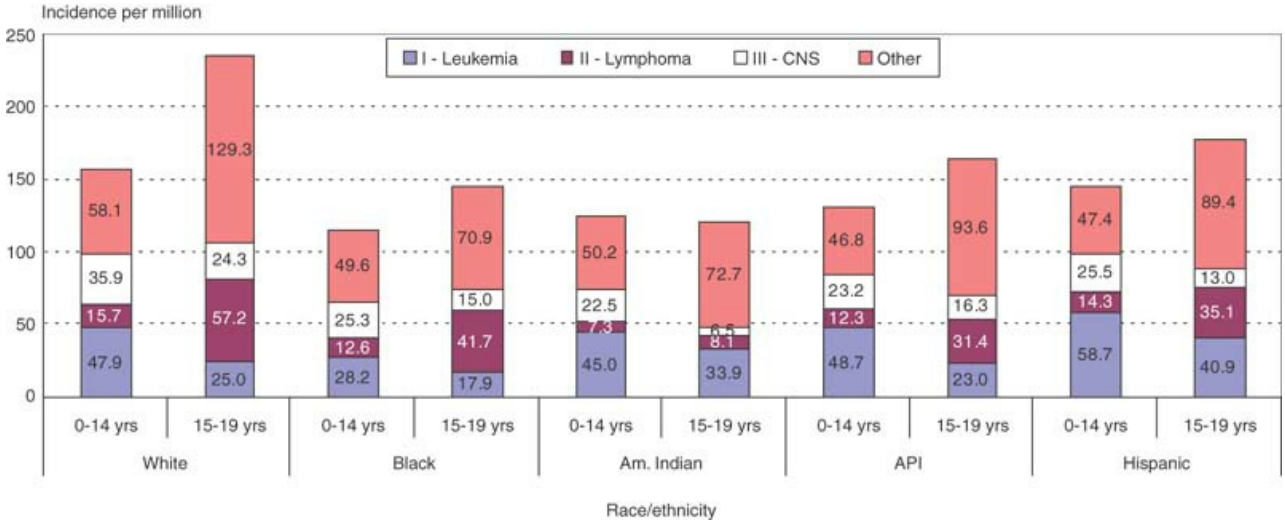

Figure 1.3 Age-adjusted incidence rates for childhood cancer by race and ethnicity, 1992 to 2006. Data are for International Classification of Childhood Cancer diagnostic groups (age 0 to 19 years and both sexes). Am. Indian, American Indian or Native American; API, Asian/Pacific Islander; CNS, central nervous system; Hispanic, Hispanic of any race and does not overlap other categories. (Incidence data are from the Surveillance, Epidemiology, and End Results program, National Cancer Institute, and are adjusted to the 2000 U.S. standard population.)

In contrast to black children, Hispanic children had higher rates of ALL than did white children (43.7 per million vs. 32.5 per million) (Table 1.2). Hispanic children had a higher rate of AML (8.8 per million) compared with both white (7.4 per million) and black (7.3 per million) children. However, overall cancer incidence for Hispanic children

was lower than that for white children because of lower rates for CNS tumors, lymphomas, and other tumors. The incidence of leukemia (Fig. 1.3) was similar for Asian/Pacific Islander children and white children, but Asian/Pacific Islander children had lower rates for CNS tumors and lymphomas.

Survival and Mortality Rates for Children with Cancer

Survival rates for children 0 to 14 years of age have improved dramatically since the 1960s when the overall 5-year survival rate after a cancer diagnosis was estimated as 28%.[9] Improvements in survival rates continued into the early 2000s in the United States (Fig. 1.4), with 3-year survival rates exceeding and 5-year survival rates nearing 80% for children and adolescents diagnosed during this period (Fig. 1.4). In fact, 10-year survival rates have exceeded 75%, looking at those diagnosed in 1996 (the most recent data available for this rate).

The increase in survival rate was most dramatic for ALL, a virtually incurable disease in the early 1960s and for which 5-year survival rates exceeded 80% from 1989 to 1996 (Fig. 1.5A). Survival rates for childhood NHL increased to nearly 80% from 1989 to 1996, up from 20% to 25% in the early 1960s (Fig. 1.5B), and survival rates for Wilms' tumor increased from 33% to 92% during the same period (Fig. 1.5B). Five-year survival rates at or above 90% have also been achieved for Hodgkin disease, thyroid cancer, and melanoma (Fig. 1.6), whereas 5-year survival rates for AML remain approximately 50% (Fig. 1.5A).

Five-year survival rates for 15- to 19-year-olds were similar to those for younger children for most cancer types, including brain tumors, NHL, osteosarcoma, Hodgkin disease, Ewing's sarcoma, AML, and germ cell tumors (Fig. 1.6). Survival rates for 15- to 19-year-olds with ALL were lower than those for younger children, which could be

due in part to a higher proportion of cases with unfavorable biology among 15- to 19-year-olds. A similar explanation may explain the lower survival rates for 15- to 19-year-olds with rhabdomyosarcoma. Five-year survival rates near or above 90% were observed for the most common cancers among 15- to 19-year-olds: Hodgkin disease, germ cell tumors, thyroid cancer, and melanoma.

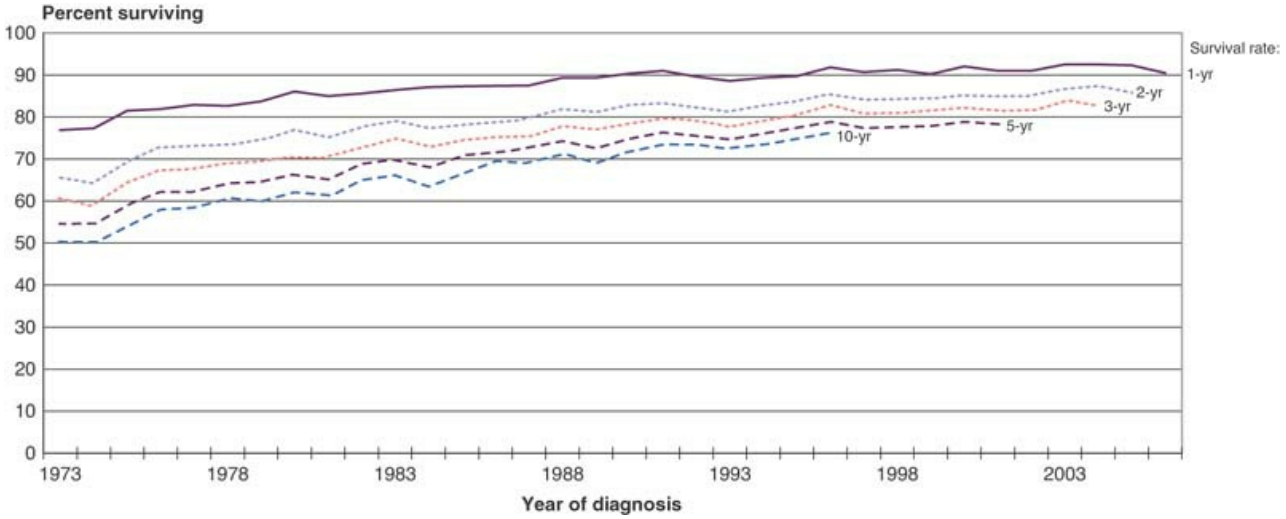

Figure 1.4 Trends in relative survival rates for all childhood cancers, age 0 to 19 years (all races and both sexes) for Surveillance, Epidemiology, and End Results (SEER) program regions, 1973 to 2006. (Data are from the SEER program, National Cancer Institute.)

As a result of improved survival, the cancer mortality rates have decreased for children since the 1950s. Mortality rates for all cancers and selected tumors from 1969 to 2004 are shown in Figure 1.7A and B. In the 1950s, childhood cancer mortality rates were stable at approximately 80 per million. The cancer mortality rate for 0- to 19-year-olds began declining in the 1960s and by 1995 had decreased to less than 30 per million. Declines in mortality for leukemias began in the early 1960s, with rates decreasing from 30 to 35 per million to less than 7 per million by 2004 (Fig. 1.7A). For NHL, decline in mortality began in the late 1960s, with rates decreasing from 6 to 7 per million to less than 2 per million by 1994. Mortality due to kidney tumors (primarily Wilms' tumor) decreased by 80% over a similar time period from approximately 4 per million to less than 1 per million by 1989. Mortality rates also declined for Hodgkin disease (data not shown), with rates decreasing from approximately 3 per million in the 1950s and early 1960s to approximately 0.4 per million in the mid-1990s.[7] The brain cancer mortality rate was approximately 10 per million in 1970 and had decreased to approximately 7 per million by 1997 (Fig. 1.7B), remaining fairly constant since then.

Figure 1.8 shows the distribution of causes of cancer death for 0 to 19-year-olds in 2006. Overall, these proportions have remained fairly constant over time. Approximately one-third of cancer-related deaths were caused by leukemias, with ALL accounting for an estimated 50% to 60%, AML for 30% to 40%, and chronic myeloid leukemia for approximately 5% of leukemia-related deaths. CNS tumors were the second leading cause of cancer mortality among children and adolescents, accounting for 24% of cancer-related deaths. The other primary causes of cancer-related mortality were neuroblastoma (classified under endocrine tumors), bone tumors, soft tissue sarcomas, and NHL.

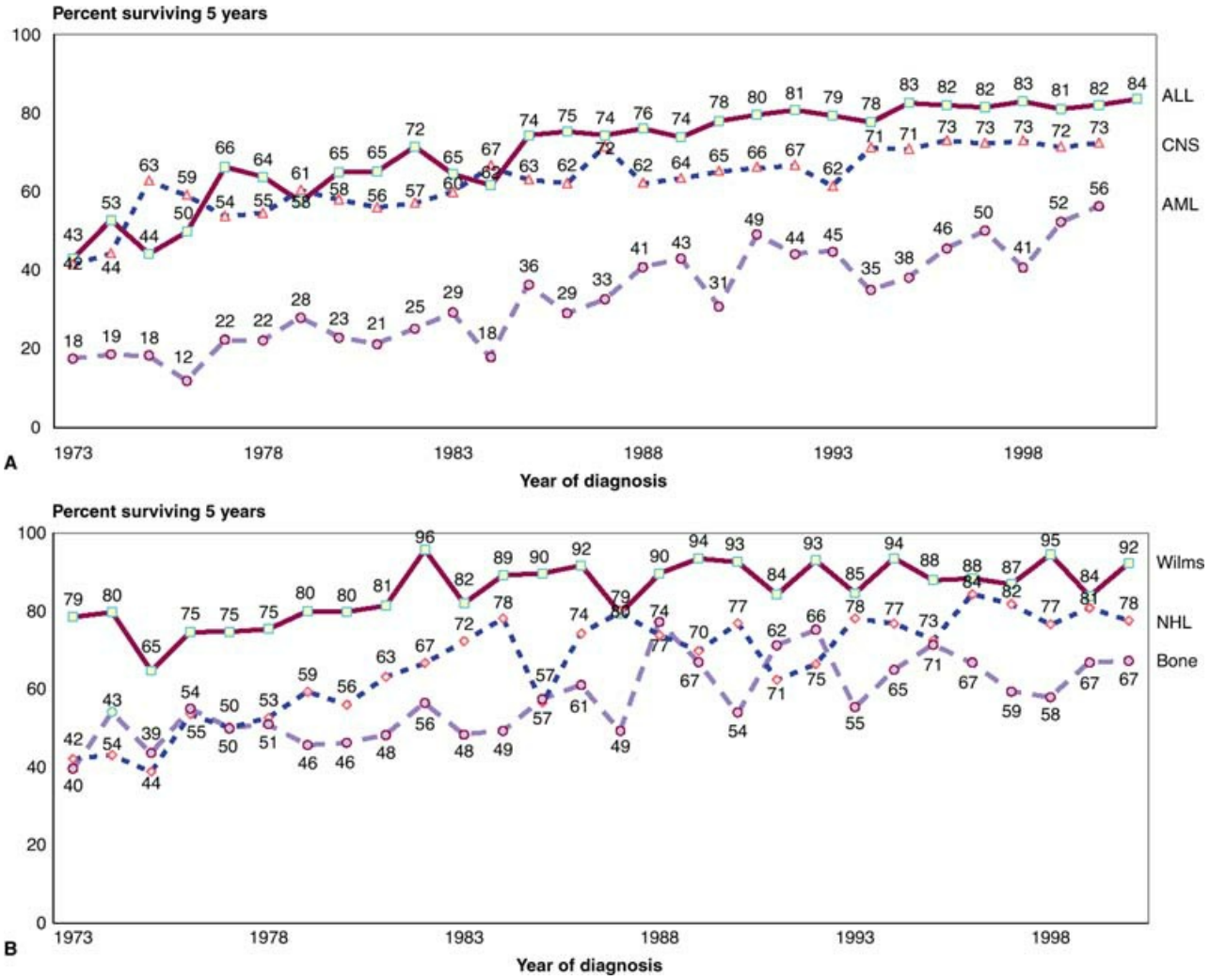

Figure 1.5 Five-year relative survival rates for specific cancers of children (aged 0 to 19 years), 1973 to 2006. Data are from the Surveillance, Epidemiology, and End Results (SEER) program regions (nine areas). **A:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CNS, central nervous system. **B:** Bone tumors; NHL, non-Hodgkin lymphoma; and Wilms' tumor.

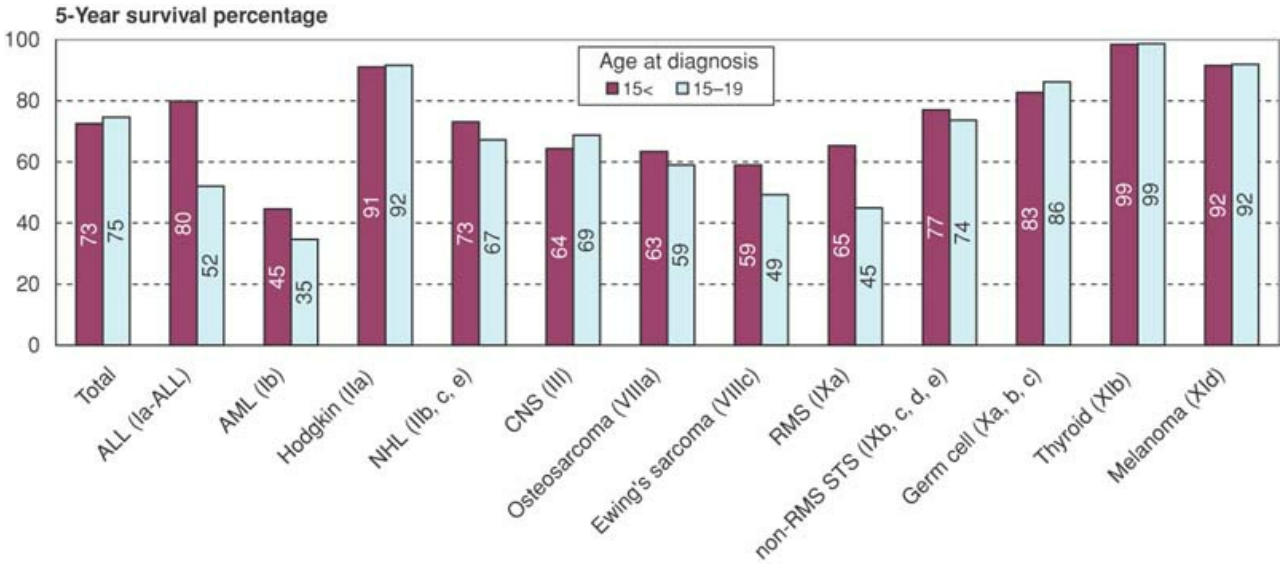

Figure 1.6 Five-year survival rates for 0- to 14-year-olds and for 15- to 19-year-olds in Surveillance, Epidemiology, and End Results (SEER) program regions, 1973 to 2006. Rates are for all races and both sexes. ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CNS, central nervous system; NHL, non-Hodgkin lymphoma; non-RMS STS, non-rhabdomyosarcoma soft tissue sarcoma; RMS, rhabdomyosarcoma.

(Data are from the SEER program, National Cancer Institute.)

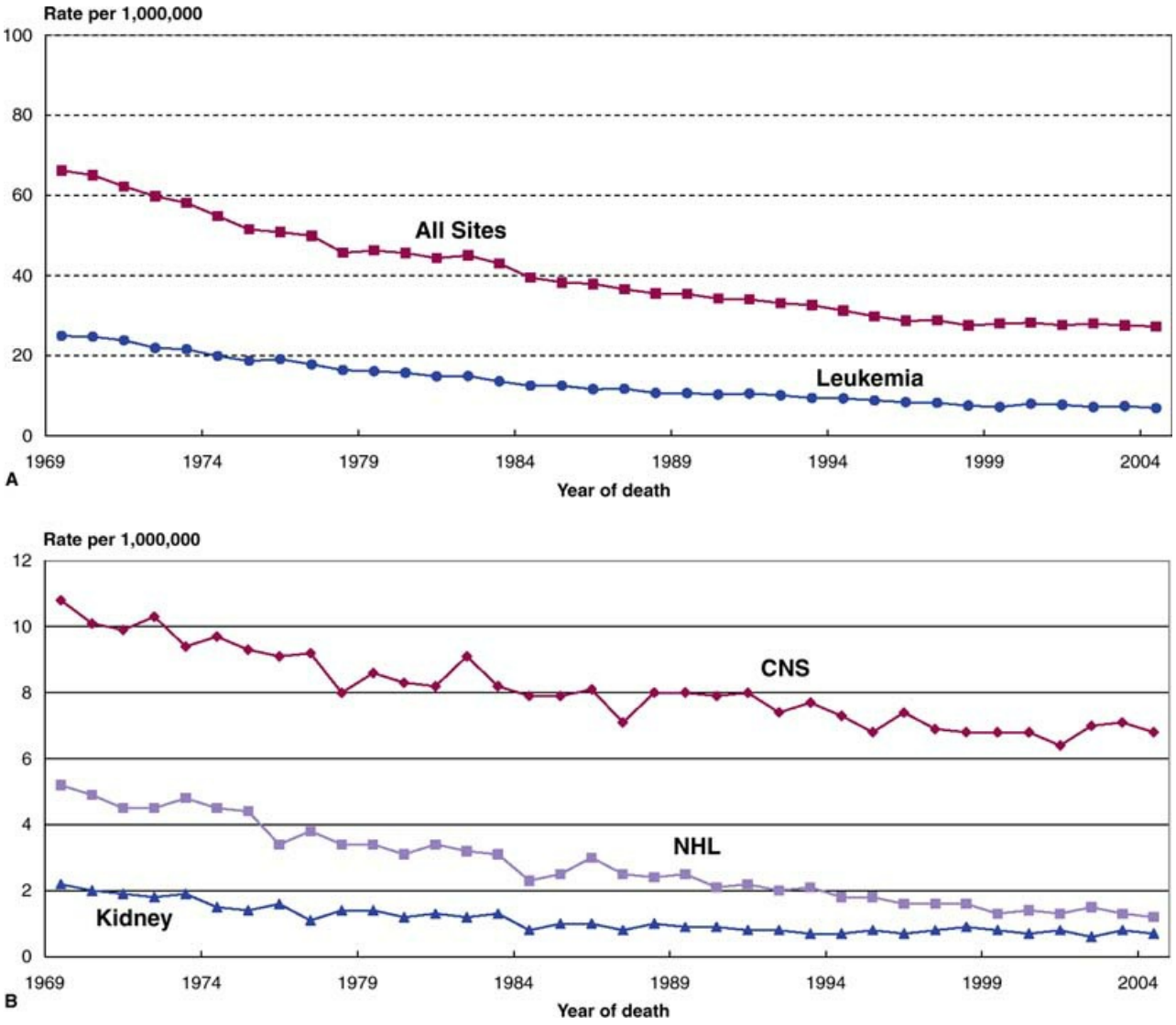

Figure 1.7 Mortality rates for children and adolescents (aged 0 to 19 years) in the United States, 1969 to 2004. **A:** Mortality rates for all cancers and for leukemia. **B:** Mortality rates for non-Hodgkin lymphoma (NHL), central nervous system (CNS) tumors, and kidney tumors. (Death data are from the National Center of Health Statistics public-use file.)

Analytic Study Designs

Some epidemiologic studies, such as randomized intervention trials and randomized controlled clinical trials, follow the principles of scientific experimentation in which a treatment or intervention of interest and the control condition are randomly assigned.[11] Childhood cancer clinical trials compare one treatment regimen with another, such as the recent study of prophylactic cranial irradiation in children with ALL. This collaborative study from St. Jude Children's Research Hospital and Cook Children's Medical Center showed that prophylactic cranial irradiation could be safely omitted from the standard treatment regimen for children with ALL. Cranial irradiation is beneficial for those ALL patients at high risk for CNS relapse; however, this treatment can lead to a number of untoward late effects (e.g., second malignancies, cognitive deficits, and endocrinopathy) in this patient population.[12] Well-designed and well-conducted nonexperimental (observational) studies can also provide accurate estimates of treatment effects.[13],[14],[15]

Observational analytic studies assess the causal influence of potential risk factors that cannot be evaluated

experimentally because the experiment would be unethical or impractical. An obviously unethical experiment would, for example, randomize pregnant mothers to ingesting different kinds and amounts of organophosphate pesticides to measure subsequent incidence rates of NHL in their offspring. As another example, it would be impractical, even if ethical, to randomly allocate newly pregnant mothers to receive high daily doses of vitamins C and E to assess their efficacy in preventing childhood brain cancer. To provide an accurate and reliable

conclusion, the trial would require thousands, if not hundreds of thousands, of preconceptual mothers and their children to be followed for many years. Thus, epidemiologists must use several nonexperimental study designs to identify causal risk factors and quantify the contribution those risk factors have on disease incidence in populations with "naturally" occurring exposures varied enough to be useful in comparisons. An example is a national childhood leukemia study from Canada that found evidence to suggest a protective effect of immunosuppressant medications (e.g., prednisone, mercaptopurine) taken during childhood.[16] Cohort studies and case-control studies are two analytic observational approaches commonly used by epidemiologists to assess such nonexperimental associations.

Cohort Studies

Cohort studies evaluate subjects initially free of a specific disease of interest and whose exposure status can be classified. Subjects are followed for a defined time period to ascertain differences in rates of end points attributable to exposure, such as new events in or death from a specific disease. The disease rate in the exposed group is then compared statistically with the rate in the unexposed group. A prospective cohort study resembles a clinical trial, but subjects are not randomly allocated to an exposure arm. Rather, as mentioned previously, exposure (or lack of exposure) occurs "naturally" and the investigator uses variations in natural exposure levels to evaluate differences in the risk of subsequent disease occurrence during some follow-up period.

Cohort studies permit efficient study of relatively common diseases with a reasonably short latency period from exposure to disease onset. Cohort studies are usually impractical for rare diseases, such as childhood cancer, as statistically meaningful results could be achieved only by assembling and following for a very long time a huge number of at-risk subjects. One notable exception, however, is a cohort of 3,268 people who were *in utero* and 15,899 who were younger than age 5 and living in Hiroshima or Nagasaki at the time of the atomic bombing during World War II. Studies on the effects of the atomic bombing on survivors' health are being conducted using a detailed and complicated exposure reconstruction procedure,[17] in which each child's radiation dose was estimated. Children with a dose of greater than 1 Gy had a cumulative cancer death rate of approximately 26 per 1,000, compared with 6.5 per 1,000 among those with a dose of 0.1 Gy or less.[18] The ratio of these rates, 4.0, is a type of relative risk (RR; described later) and a measure of how strong is the association between ionizing radiation exposure and death from cancer. The study found a fourfold higher cumulative cancer death rate for those children exposed to higher compared with lower levels of ionizing radiation. A more recent study examined the effects of whether the exposure was *in utero* versus during early childhood.[19] The rates of solid cancer incidence during adolescence and young adulthood (12 to 29 years) were much higher in males (RR = 5.0) and females (RR = 10.2) who were exposed *in utero* to radiation doses of 0.2 Sv or more. Even though an elevated risk was also seen among males (RR = 2.3) and females (RR = 2.8) who were younger than age 6 at the time of exposure, the association was not as strong. The effect was also not as strong for persons who developed their cancer later in life (ages 30 to 54).

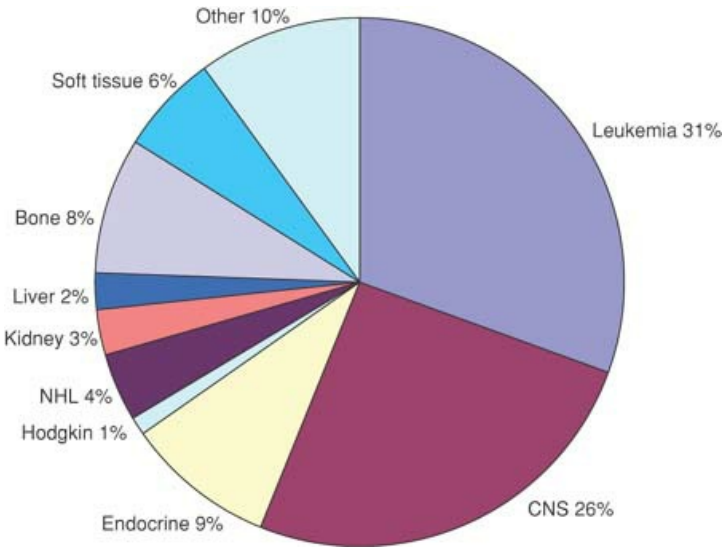

Figure 1.8 Percentage distribution by cause of cancer death in children and adolescents 0 to 19 years of age, 2006. The endocrine category primarily represents neuroblastoma. CNS, central nervous system; NHL, non-Hodgkin lymphoma. (Death data are from the National Center for Health Statistics public-use file.)

Cohort studies can be prospective or retrospective in nature. Prospective cohort studies involve active follow-up of subjects in real time, as in clinical trials. Retrospective cohort studies use historical records to identify the study population and to reconstruct their exposure and subsequent disease experience. Examples of retrospective cohorts surround the evaluation of excess cancer incidence among those exposed to Salk poliovirus vaccine contaminated with simian virus 40 (SV-40). One birth cohort was inadvertently exposed to the contaminated vaccine during infancy (born 1956 to 1962), one was exposed later in childhood (born 1947 to 1952), and one was unexposed to SV-40 (born 1964 to 1969). Using cancer registry and mortality records, age-specific cancer incidence rates can be calculated for each birth cohort. A study conducted in the United States found no meaningful differences in overall cancer rates or for any specific type of malignancy among the three cohorts.[20] Similar

studies have shown no increased incidence of childhood cancers in Denmark[21] or of childhood medulloblastoma in the United States.[22]

The Childhood Cancer Survivors Study (CCSS) was established in 1994 to evaluate medical late effects and psychosocial outcomes as a function of cancer treatment.[23],[24] The CCSS includes both retrospective and prospective components and has currently recruited more than 14,000 childhood cancer survivors (or their parents for those deceased) from a consortium of 27 medical centers in the United States and Canada. Eligible subjects survived at least 5 years after diagnosis between 1970 and 1986; efforts are currently underway to extend this cohort to include those diagnosed between 1987 and 1999. This study addresses the important question of the long-term consequences of childhood cancer and its treatment among survivors and serves as a resource for researchers dedicated to identifying modifiable risk factors that could aid in reducing the incidence of these late effects of treatment. To date, the CCSS has produced more than 100 manuscripts on various topics of importance to childhood cancer survivors (i.e., health care utilization, health behaviors, health status, quality of life, etc.) and has contributed invaluably to the development and refinement of epidemiologic methods related to survivorship research.

Case-Control Studies

For rare exposures, such as pesticides or some medications, cohort studies provide the best study design. However, for rare outcomes, such as childhood cancer, case-control studies provide a more efficient strategy to

evaluate potential causal associations. A case-control study of childhood cancer identifies and recruits children (or their parents) who are diagnosed within a defined population and time period. A similar group of children without the disease, but from the same defined population (in time, location, and eligibility criteria) that gave rise to the cases, is recruited to serve as controls. The investigator, as completely and accurately as possible, uses self-report, health records, environmental measures, and biologic specimens to reconstruct the cases' prediagnosis exposure experience. Similarly, a “reference” date substituting for a diagnosis date is assigned to each control child, whose exposure experience before that date is reconstructed. The exposure frequency among the case group is then compared statistically with the exposure frequency among the control group. The resultant statistic, known as an *odds ratio* (OR), is analogous to an RR and is a measure of the strength of the association between the exposure and the disease. For example, a population-based case-control study of childhood ALL evaluated household dust in 184 cases and 212 controls of similar birth date, sex, race, and Hispanic ethnicity from northern and central California.[25] Levels of organochlorine pesticides and polychlorinated biphenyls (PCBs) measured in carpet dust from the residences of the cases and controls suggested an increasing risk of childhood ALL with increasing concentrations of PCBs. Relative to homes with the lowest levels of total PCBs in carpet dust, the adjusted OR was 2.8 (95% confidence interval [CI]: 1.4–5.5) for exposure levels of 15.5 ng/g or higher. Thus, in this study, children presumably exposed in their homes to PCB levels of 15.5 ng/g or more were found to have a nearly threefold risk for ALL than were children not so exposed. This study in no way proves a causal relationship between childhood ALL and exposure to PCBs in the home; however, it suggests a possible etiologic agent that should be further explored.

Cluster Investigations

It is common for clinicians to encounter parental concern about multiple cancer occurrences in their child's community. The implication, of course, is that a shared environmental exposure is responsible for the cluster of cancer cases. Cluster investigations use standard epidemiologic study designs, primarily case-control studies, to ascertain whether an unusual number of cancer cases occurred in a specific area (spatial cluster), time (temporal cluster), or both (space-time cluster).[26] The latter, for instance, would be an excess of childhood leukemia in a neighborhood or school over a specific time period. Public health agencies have the responsibility to investigate cancer clusters and communicate findings to the public.[26] Clinicians are well advised to refer cluster inquiries to local health departments or the Centers for Disease Control and Prevention (http://www.cdc.gov or http://www.atsdr.cdc. gov). Such investigations, however, rarely produce evidence that a true childhood cancer cluster exists.[27],[28],[29],[30]

Molecular Epidemiology

Classical or traditional epidemiology, as discussed previously, permits epidemiologists to evaluate risks and causal roles of environmental factors in cancer. Molecular epidemiology, a hybrid of epidemiology and molecular biology, enables researchers to assess biologic or genetic characteristics that may influence cancer susceptibility. The concept that risk of cancer from a given exposure differs between subgroups of a population is known in epidemiology as *effect modification*; biostatisticians often refer to this heterogeneity of effect as *interaction*. With the advent of polymerase chain reaction and other advanced laboratory methods, epidemiologists can incorporate molecular markers into their studies to identify specific suspect endogenous or exogenous host factors at the biochemical or molecular level.[31],[32],[33] Such studies aim to determine the roles, including interactions, of environmental and genetic factors in the initiation and progression of the carcinogenic process. The approach of incorporating molecular markers in epidemiologic studies of childhood cancer etiology shows promise for reducing cancer risk and providing strategies for prevention.

The addition of molecular parameters to population-based studies aids in the identification of genes and

pathways involved in cancer development due to environmental exposures and in the identification of susceptible or resistant subpopulations. In turn, information about molecular mechanisms of carcinogenesis helps improve risk assessment. Past studies of childhood cancer were limited to the examination of only a few candidate genes. However, the exponential growth of scientific technology and the creation of consortia to facilitate the need for larger sample sizes have led to the ability to analyze multiple polymorphisms in the same gene (e.g., haplotypes and diplotypes), multiple genes along the same molecular pathway, and multiple polymorphisms in multiple genes across the entire genome (i.e., genome-wide association studies [GWAS]). In fact, GWAS are quickly becoming the approach of choice for performing association studies of common genetic susceptibility alleles in cancer epidemiology. However, because of the rarity of pediatric cancers, the majority of this work has been conducted for adult tumors. On the other hand, GWAS have proven helpful in identifying genes associated with risk[34] of and treatment outcomes[35] in childhood ALL. It is likely that, as consortia form to investigate other rare childhood cancers, such studies will also contribute to new findings for these other tumors.

In addition to identifying causal factors, studies of childhood cancer etiology aim to determine the critical period of exposure and disease susceptibility. Exposures *in utero* and during the early years of life can disproportionately increase the risk of cancer later in life.[36],[37],[38] Laboratory and epidemiologic evidence suggests that differential exposure response or physiologic immaturity raises the risk for infants and children far above that for adults experiencing the same environmental insults. The underlying mechanisms combine to proportionately increase exposure to toxicants and lessen the ability of the child in early stages of development to detoxify or repair damage. The cancer can be initiated *in utero*, with subsequent genetic mutational events and clonal progression occurring later. Adolescence and young adulthood are also sensitive times because of such proliferative surges as hormone outflow and rapid bone growth.

Current studies of molecular epidemiology are based on an understanding of the complex, multistage process of carcinogenesis and heterogeneous responses to carcinogenic exposures. Quantitative methods to measure human exposures to carcinogens improve continuously and have been successfully applied in a number of epidemiologic studies. Genetic predispositions to cancer, both inherited and acquired, have been, and continue to be, identified. The combined approach of correlating genetic polymorphisms with other cancer risk factors is showing considerable promise. For instance, activity of the glutathione *S*-transferase (GST) enzymes is involved in the detoxification of carcinogens such as epoxides and alkylating agents. GST genes are polymorphic, and lack of enzymatic activity potentially increases cancer risk. The *GSTM1* null genotype has been shown to increase the risk of childhood AML and myelodysplasia (AML/MDS),[39] and polymorphisms in *GSTP1* have been associated with ALL/AML/NHL and soft tissue tumors[40] using the case-control study design. These studies illustrate the future of molecular epidemiology as the leader in developing individual risk profiles for patients, including assessment of multiple biomarkers. The field has the near-term potential to have a significant impact on regulatory quantitative risk assessments, which may aid in the determination of allowable exposures and the identification of individuals who will most benefit from cancer prevention strategies.

Molecular epidemiology also holds the key to identifying not only those at risk for the development of a malignancy but also those at risk for adverse events due to the treatment of their primary tumor. Survivorship issues have come to the forefront of cancer epidemiology, as survival for childhood cancers continues to increase and these children move into adulthood. This importance is evidenced by the creation and maintenance of the CCSS (described earlier) as a resource for conducting this type of research. Using the GST enzymes as an example again, researchers have found that ALL patients with the *GSTM1* null genotype will experience less hepatotoxicity from methotrexate[41]; however, they are also at greater risk of severe infection following glucocorticoid administration.[42]

Investigators who conduct molecular epidemiology studies use traditional designs, including case-control and cohort studies, with inclusion of one or more molecular markers to determine exposure associations with disease outcome. Therefore, the methodologic challenges of epidemiologic studies (described later), such as accurate measurement of disease and exposure, appropriate selection of study samples, reduction of potential confounding, and optimization of precision of effect measures, also apply to studies in the rapidly growing and promising field of molecular epidemiology. A serious concern lies with assuring an adequate sample size for study; this is especially true for studies of childhood tumors. Often, the prevalence of a genetic polymorphism or other biomarker is either quite low or quite high. Hence, the number of cases required to detect an association tends to be very large. Because childhood cancers are rare, it is often necessary to combine data from several studies to obtain adequate statistical power to draw meaningful conclusions. For all of these reasons, it is necessary for investigators to exercise caution when interpreting their study data and the implications of their results.[43]

Bias and Causal Inference

All human studies are susceptible to bias of varying degrees (i.e., producing inaccurate measures of the effect of a treatment or exposure on disease). An important goal of any study is to make every effort feasible to minimize the effect of bias.

Three general types of bias can occur:

1. *Selection bias*, when subjects who are sampled, recruited, enrolled, and complete the study are unrepresentative, in that they inaccurately reflect the exposure-disease relationship in the target population
2. *Information (misclassification) bias*, when information collected on exposure, treatment, disease, or other study factors is inaccurate or incomplete
3. *Confounding bias*, when an extraneous factor distorts (increases or decreases) the true magnitude of the exposure-disease association

Selection Bias

Because all human studies include some element of sampling from larger (target) populations and require recruitment from the sample identified, selection bias is a potential source of error. Selection bias may occur when exposure or disease frequency among those in the study is unrepresentative of the target population. Case-control studies are susceptible because it is difficult to identify and recruit controls who provide an accurate accounting of baseline exposure frequency in the population that gave rise to the cases. For instance, selection bias is suspected in the apparent association of some childhood cancer–electromagnetic field (EMF) studies.[44] If lower-income persons are proportionately less likely to participate as controls than higher-income persons, and lower-income persons live in areas with proportionately more high-current power lines, baseline exposure (high EMF) will be underestimated. Unlike controls, if case participation is independent of power line status, the odds of exposure among cases will appear higher than that for controls, resulting in a positive association when none really exists. Cohort studies and randomized trials, on the other hand, are susceptible to selection bias from attrition. If participants lost to the study during the follow-up period represent a different outcome experience than those who remain in the study to completion, the final results may be biased. For this reason, great effort must be expended in prospective studies to assure the most complete follow-up possible of study subjects.

Information Bias

The most important threat to the validity of epidemiologic research of childhood cancer is inaccurate or incomplete

information on study participants' exposure relevant to etiology. It is usually impossible, especially in retrospective studies, to directly measure exposure dose and duration during a time thought biologically relevant to cancer initiation or progression. As such, indirect or surrogate measures of exposure are used in lieu of direct measures. Indirect exposure tools include, for instance, self-reported recall of diet, smoking, and alcohol consumption during pregnancy; 24-hour food intake diaries; parental occupational job titles; recall of household pesticide use or inventory of household pesticide products; power line configurations, personal dosimeters or 24-hour measurements of EMF levels in the child's bedroom; pharmacy records among those in self-contained health maintenance organization plans; census tract information; urinary cotinine levels for smoking intake; and medical records.[45]

These proxy measures may usefully approximate real exposure but provide only imprecise information on dose, duration, and exposure time period. When exposure measures are equally inaccurate between study groups (nondifferential error), as is often the case, the cause-effect relationship may be attenuated or completely obscured. Nondifferential misclassification of exposure has no doubt been one reason why few environmental agents are known risks for childhood cancer occurrence.

Differential information bias occurs when accuracy and completeness of exposure information differ between comparison groups. Recall bias in case-control studies, for example, can occur if mothers of children with brain cancer (cases) are more motivated than mothers of healthy children (controls) to recall accurately their history of using household pesticides. This may happen because case mothers want to discover the cause of their children's disease. The control mothers may have hazier memories, and their incomplete or inaccurate recall can lead to underestimates of exposure frequency in the control group, and thus cause exaggeration of the strength of the association between disease and exposure. From a practical standpoint, however, recall bias may be more theoretical than factual. One method sometimes advocated to minimize recall bias is to choose a control group of children with a chronic disease, rather than disease-free. Control mothers might then have equal incentive to recall exposure accurately and completely. Using this approach, one must be sure that the control group's disease is not causally related to the exposure under evaluation, or the resultant risk estimate will be biased as to whether the exposure is causally related to the childhood cancer in question.

Confounding Bias

Randomization in clinical trials, if enough people are in the study, greatly reduces the probability that an extraneous factor will cause bias in the results because such “nuisance” factors should be randomly and evenly distributed among treatment groups. Absent randomization, however, confounding is a threat to validity in observational studies. Confounding requires a variable to be associated with, or a marker for, the disease of interest and for it to occur at a differing frequency between the exposure (or treatment) groups. When these two conditions hold, the extraneous factor may bias the exposure-disease association. Few exogenous risk factors, however, have been identified in the etiology of childhood cancer, and those few represent fairly weak associations. Thus, confounding bias has not been shown empirically to be of major concern in epidemiologic research of childhood cancer, although this possibility cannot be ruled out. Partly because of the implausibility of a biologic connection between nonionizing EMFs and cancer, for instance, some scientists hypothesized that the associations found between power lines and childhood leukemia and brain cancer in early EMF studies were due to confounding by unidentified etiologic agents.[46] A recent methodologic study that carefully examined that possibility found little support for the theory.[47]

Statistical methods to control (adjust for, or correct) confounding, such as stratified analysis or multivariable regression analysis, are at hand, but effective only if data on the potentially confounding variables are collected and accurate. Thus, for statistical analysis, observational studies often collect data on many factors not directly related to the cause-effect relationship being investigated. Design strategies can also minimize or eliminate

confounding. A study of asbestos exposure and lung cancer, for example, could minimize confounding from smoking status by recruiting only nonsmokers, although residual confounding may still be present if frequency, duration, or intensity of passive smoke exposure differs between those exposed to asbestos and those not exposed.

Causal Inference

Epidemiologic studies strive to provide the most accurate and precise risk estimate of an exposure-disease association. Concerns about potential bias of effect measures, however, contribute to the critical approach using inference and judgment to evaluate exposure-disease causal relationships. Criteria commonly used to evaluate study results and to help guide judgments on the likelihood that an association indeed is causal and not merely statistical include the following:

1. *Strength of the exposure-disease association*. Large RRs are less likely than small RRs to result from chance or uncontrolled confounding (although this does not preclude other sources of error).
2. *Temporal relationship between exposure and disease onset*. Studies are stronger when they can establish that the exposure appropriately preceded the biologic onset of disease.
3. *Biologic coherence*. When a plausible biologic mechanism or when experimental evidence from animal studies, or both, supports the hypothesized relationship, there is greater confidence in the observed association.
4. *Dose-response gradient*. If exposure intensity or duration is associated with increased disease frequency when it is hypothesized that such a dose gradient should exist, the results appear more coherent and believable.
5. *Consistency of results within and across studies*. If multiple sources of the same exposure type show similar effects, if multiple studies using different target populations and study designs show consistent results, or both, there is greater evidence to favor a true relationship.

These concepts, which are widely applied, were originally derived from two papers by Sir Austin Bradford Hill and reprinted in a monograph on philosophy and epidemiologic reasoning in causal inference.[48]

Statistical Measures in Epidemiology

Epidemiologic analyses generally focus on estimating effect measures, the strength (magnitude) of an exposure-disease association, rather than statistical hypothesis testing using a

p value.[2] The *p* values provide a measure of probability for observing the study results or results more extreme than those observed, if indeed there is no true association. No direct information from *p* values is given, however, on the strength, direction, or precision of an effect measure, nor do *p* values supply information on the extent to which an association (or lack of an association) can be explained by confounding or other bias.

Effect measures for dichotomous outcomes, such as disease occurrence versus no disease, are often estimated using one of several ratio measures of RR.[1],[2],[49] In a cohort study, in which disease rates can be directly calculated, the ratio of the incidence rate of leukemia among those exposed to an agent can be compared with the rate of leukemia among those not so exposed. The ratio is 1:1 if the rates are the same in the two comparison groups, an RR of 1.0, suggesting no association between exposure and disease. If the exposed group has a higher rate than that of the unexposed group, the ratio will be larger than 1, suggesting an excess risk due to exposure. If the rate is lower in the exposed compared with the unexposed groups, the ratio will be less than 1, suggesting a protective effect from exposure. The further the effect measure is away from the “null” value of 1.0 in either direction, the stronger the association. Notice that an RR of 2.0 (double the risk compared with the reference group) is equivalent in strength to an RR of 0.5 (one-half the risk of the reference group). Rates of

disease cannot be calculated directly in case-control studies. Alternatively, exposure frequencies are compared between diseased groups and nondiseased groups. The resultant OR is an effect measure on a ratio scale and, as mentioned previously, functionally equivalent to an RR. Other types of ratio-based RRs are rate ratios, hazard ratios, standardized mortality ratios, standardized incidence ratios, and proportional mortality ratios. CIs are used to measure the precision of an effect measure. Similar to *p* values, CIs are functions of the variability of the data and the size of the sample. Roughly speaking, a CI provides a likely range in which the true effect measure lies within some level of confidence (often calculated as 95% CI).

RRs are important to help judge whether an association is causal and to estimate the degree to which risk of disease is increased (or decreased) by exposure. RRs, however, do not measure the "absolute" risk from exposure. In other words, an RR does not measure the number of excess cancers that are likely caused by an exposure. Attributable risk measures provide estimates of the actual rate (or number, or percentage) of cases "due to" exposure, assuming there is a causal relationship.[1,2,48] Thus, attributable risks indicate the proportion of the disease preventable if the exposure were removed from the population at risk. Assume for the sake of argument, for example, that living within 50 ft of a high-current power line increases a child's risk of ALL by a factor of 2. The annual rate of ALL in the United States is approximately 34 per million children younger than age 15 years. If 10% of children in the United States lived near high-current power lines, the percentage of childhood ALL cases that could be attributed to the power lines would be 9%. This attributable risk of 9% (sometimes called an *etiologic fraction*) translates to an excess of three ALL cases per million children per year, which is the leukemia rate that hypothetically would be prevented if all children lived away from high-current power lines. Even very large RRs may explain little of the total disease incidence within a population. Children with Down syndrome have an estimated 20-fold excess risk of ALL,[50] but because the prevalence of Down syndrome is only approximately 1.3 per 1,000 live births, the percentage of ALL in children that can be attributed to Down syndrome is only approximately 2.5%.

Risk Factors for Childhood Cancer Occurrence

Environmental risk factors for adult cancer generally involve long latency periods from exposure commencement to clinical onset of disease. Cigarette smoking illustrates this point: Smoking usually starts during adolescence, but associated malignancies do not become apparent until many decades after smoking is initiated. The genetic processes that go awry and lead to childhood cancer are likely different from that of adult malignancies; at the least, the carcinogenic process in children is much shorter in time. Infancy, when embryonal neoplasms such as neuroblastoma predominate, is the age when cancer incidence rates are highest during childhood.[51,52] It is reasonable to surmise, therefore, that many childhood cancers result from aberrations in early developmental processes.

To our dismay from a prevention standpoint, the current evidence to support a major etiologic role for environmental or other exogenous factors in childhood cancer is minimal. A comprehensive review of epidemiologic studies of childhood cancer is available elsewhere[39] and will not be reproduced here. The major types of childhood cancer and the few risk factors that are reasonably well documented are shown in Table 1.3. Many other factors are suspected to increase or decrease risk but are not well established. Even the known risk factors shown in the table explain only a small proportion of childhood cancer cases.

Summary and Future Considerations

Although knowledge about childhood cancer continues to increase, there is much work to be accomplished before reliable preventative measures can be recommended. In this brief overview, we have discussed the essentials of epidemiologic research approaches in childhood cancer, the role epidemiology plays in understanding the public health impact of childhood cancer, and the ongoing efforts to improve knowledge on the

causes of these diseases and the consequences to the children who experience them.

Many of the epidemiologic studies performed to date have provided important clues to the etiology of childhood cancer. The field of molecular epidemiology will continue to expand and take advantage of new "–omic" technologies to elucidate the risk factors for childhood cancer with the goal of preventing these diseases. Current investigations seek to determine the most reliable biomarkers of exposure and disease. Likewise, GWAS have already proven helpful in identifying genes associated with risk of many adult tumors and of childhood ALL. In the near future, these studies will likely contribute to new findings for other childhood cancers. Epigenetic gene expression and the use of copy number variants have been identified as potentially important sources of genetic variation. The possibility of genome-wide sequencing and epigenomics may also become feasible in population-based studies. Such technologies will ultimately offer complete interrogation of genetic variation in the human genome and provide insights into the biology of these tumors, allowing the development of preventive and treatment strategies.

Table 1.3 Known Risk Factors for Selected Childhood Cancers

Cancer type	Risk factor	Comments
Acute lymphoid leukemia	Ionizing radiation	Although primarily of historical significance, prenatal diagnostic x-ray exposure increases risk. Therapeutic irradiation for cancer treatment also increases risk.
	Race	White children have a twofold higher rate than do black children in the United States.
	Genetic conditions	Down syndrome is associated with an estimated 20-fold increased risk. Neurofibromatosis type 1, Bloom syndrome, ataxia telangiectasia, and Langerhans cell histiocytosis, among others, are associated with an elevated risk.
	Birth weight	>400 g increases risk.
Acute myeloid leukemias	Chemotherapeutic agents	Alkylating agents and epipodophyllotoxins increase risk.
	Genetic conditions	Down syndrome and neurofibromatosis 1 are strongly associated. Familial monosomy 7 and several other genetic syndromes are also associated with increased risk.
Brain cancers	Therapeutic ionizing radiation to the head	With the exception of cancer radiotherapy, higher risk from radiation treatment is essentially of historical importance.
	Genetic	Neurofibromatosis 1 is strongly associated with optic

	conditions	gliomas, and, to a lesser extent with other central nervous system tumors. Tuberous sclerosis and several other genetic syndromes are associated with increased risk.
Hodgkin disease	Family history	Monozygotic twins and siblings of cases are at increased risk.
	Infections	Epstein-Barr virus is associated with increased risk.
Non-Hodgkin lymphoma	Immunodeficiency	Acquired and congenital immunodeficiency disorders and immunosuppressive therapy increase risk.
	Infections	Epstein-Barr virus is associated with Burkitt's lymphoma in African countries.
Osteosarcoma	Ionizing radiation	Cancer radiotherapy and high radium exposure increase risk.
	Chemotherapy	Alkylating agents increase risk.
	Genetic conditions	Increased risk is apparent with Li-Fraumeni syndrome and hereditary retinoblastoma.
Ewing's sarcoma	Race	White children have approximately a ninefold higher incidence rate than do black children in the United States.
Neuroblastoma		No known risk factors.
Retinoblastoma		No known nonhereditary risk factors.
Wilms' tumor	Congenital anomalies	Aniridia and Beckwith-Wiedemann syndrome, as well as other congenital and genetic conditions, increase risk.
	Race	Asian children reportedly have approximately one-half the rates of white and black children.
Rhabdomyosarcoma	Congenital anomalies and genetic conditions	Li-Fraumeni syndrome and neurofibromatosis 1 are believed to be associated with increased risk. There is some concordance with major birth defects.
Hepatoblastoma	Genetic conditions	Beckwith-Wiedemann syndrome, hemihypertrophy, Gardner's syndrome, and family history of adenomatous polyposis increase risk.
Malignant germ cell	Cryptorchidism	Cryptorchidism is a risk factor for testicular germ cell

tumors	tumors.

Derived from Ries LAG, Smith MA, Gurney JG, eds. Cancer incidence and survival among children and adolescents: United States SEER program 1975–1995. Bethesda, MD: National Cancer Institute, SEER Program, 1999. NIH Pub. No. 99-4649. The publication and additional data are available on the SEER Web site: http://www.seer.cancer.gov.

As research in the field of genomics advances, well-designed studies and new analytic techniques will be critical. With the increasing volume of genomic data comes the need to collect high-quality data on environmental exposures and focus studies onto the evaluation of gene-environment interactions. Such studies require very large sample sizes, which could be accomplished through large consortial studies. In fact, such epidemiologic consortia are now charged with the sole purpose of evaluating risk factors for childhood malignancies.[53,54]

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Chapter 2
Childhood Cancer and Heredity

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Questions often arise in the minds of parents when their children are newly diagnosed with cancer: “Did this happen because of something I did or passed on to my child?” and “What are the chances that my other children will develop cancer?” In this chapter, we outline the scientific and clinical evidence that is available to answer these questions with regard to genetic susceptibility. Overall, it is the minority of childhood cancers that are caused by a clearly inherited predisposition. However, the percentage varies significantly with individual tumor types and is a composite of several different genetic factors. Ongoing identification of the genes that are mutated in cancer susceptibility syndromes provides opportunities for genetic testing. After reviewing these syndromes, we discuss the special issues to be considered in genetic testing for cancer susceptibility for the pediatric patient.

Inherited Predisposition to Pediatric Cancers

Overwhelming evidence demonstrates that cancer is the result of multiple changes in the DNA of the tumor cell, including point mutations, larger-scale copy number changes, and silencing of genes by epigenetic changes. Many of these somatic alterations are discussed in Chapter 3 and in the disease-specific chapters. The proportion of pediatric cancers that have a clear hereditary component is small. *Hereditary* in this case implies a genetic alteration that has been passed on to the child from a parent or that was a new constitutional mutation that occurred in the oocyte or sperm before fertilization. A child therefore can have a hereditary predisposition to cancer despite a negative family history of cancer because of a constitutional chromosome disorder such as trisomy 21 (Down syndrome [DS]) or a *de novo* mutation in a cancer predisposing gene, such as *RB1*.

Estimates of the fraction of hereditary predisposition for an individual cancer were originally based on epidemiologic studies of the number of familial cases and studies of associated syndromes. More recently, these estimates rely on direct molecular testing of a series of cancer patients for mutations as the particular gene involved in a tumor type is discovered. The percentage of cases due to hereditary factors varies widely among tumor types, as illustrated in Table 2.1, with adrenocortical carcinoma, optic glioma, and retinoblastoma having 40% or higher and many other tumor types including leukemia falling in the range of 1% to 10%.[1] Thus, some of the most common pediatric cancers have the lowest hereditary fraction.

Geneticists categorize disorders by the molecular mechanism underlying the cancer susceptibility, including constitutional chromosomal abnormality; mendelian autosomal dominant, recessive, or X-linked patterns; and nonmendelian inheritance, including polygenic, mitochondrial, and imprinting disorders. For any given tumor type, the overall inherited fraction maybe the sum of several different genetic mechanisms. In the following sections, we describe the major types of hereditary disorders that result in genetic susceptibility to childhood cancers.

Constitutional Chromosomal Abnormalities

Children with constitutional chromosomal abnormalities (abnormal number [i.e., aneuploidy] or structural rearrangements) present with defined clinical phenotypes that can include dysmorphic features, congenital abnormalities, growth failure, and developmental delay. Most chromosome abnormalities result from errors that occurred during male or female meiosis, with both the parents having a normal chromosomal count of 22 pairs of

autosomes and the sex chromosome pair. Rarely, these disorders can result when a parent is a carrier for a balanced translocation who has offspring with an unbalanced karyotype. The increased association of specific chromosome disorders with malignancy risk was recognized early.

Down Syndrome

One of the most striking predispositions to cancer caused by a constitutional chromosome abnormality is the increased risk of leukemia in children who have trisomy 21 (reviewed by Rabin and Whitlock[13]). An analysis of the Danish population reveals an estimated cumulative risk for developing leukemia of 2.1% by 5 years and 2.7% by 30 years.[14] This represents at least a 20-fold increase compared with the risk for the general population. Trisomy 21 is also a common finding in the karyotype of leukemia cells from patients without DS. Thus, the presence of an extra chromosome 21 appears to be leukemogenic and may be acquired in the germline or somatically.

In children with DS, the ratio of leukemia subtypes is shifted to 60% lymphoid and 40% myeloid from the ratio in the general population of 80% lymphoid and 20% myeloid.[13] This shift is principally due to the increased incidence of myeloid leukemias in children younger than 2 years. Most striking is the distribution of types of acute myeloid leukemia (AML) among DS children.[15] Approximately 30% of DS children with AML develop acute megakaryocytic leukemia (AMKL or M7). This results in an almost 400-fold excess of AMKL in DS children compared with non-DS (NDS) children. AMKL from children with and without DS show different cytogenetic characteristics, for example, an absence of the characteristic t(1;22)(p13;q13) translocation seen in a proportion of NDS children with AMKL.[16] NDS

AMKL children tended to present in early infancy and have significant hepatomegaly, but the DS children, on average, presented at 23 months, and a high proportion had myelofibrosis.

Table 2.1 Hereditary Component of Several Pediatric Malignancies

Tumor type	Hereditary component (%)[a]
Adrenocortical carcinoma[2,3]	50–80
Optic gliomas[4]	45
Retinoblastoma[5]	40
Pheochromocytoma[6]	40
Rhabdoid/ATRT[7]	25
Wilms' tumor[8,9]	3–5
Central nervous system neoplasms[1,10,11]	<1–3[b]
	2.5–5

Leukemia[1,12]	

ATRT, atypical teratoid/rhabdoid tumor.

[a]These percentages are approximations from large population studies and may include familial cases and associated syndromes such as Down syndrome.

[b]Studies of pediatric brain tumors vary considerably in detection of a hereditary fraction.

In infancy, children with DS can develop transient myeloproliferative disorder (TMD) that can appear similar to leukemia but that is self-limited.[17,18] However, 25% of DS children with this syndrome eventually develop frank AML. Children who are mosaic for trisomy 21 in their blood and bone marrow have also developed TMD and subsequent leukemia.[19] Similarly, children with DS have a higher rate of occurrence of myelodysplasia syndromes (MDS), which are characterized by thrombocytopenia, abnormal megakaryocytopoiesis, and an abnormal karyotype, most commonly trisomy 8.[13]

AMKL samples from DS patients have a distinct pattern of somatic mutation compared with AMKL samples from NDS patients. In particular, somatic mutations in the *GATA1* gene are frequently detected in this group of patients.[20,21] *GATA1* encodes a transcription factor that is essential for maturation of erythroid cells and megakaryocytes.[22,23] *GATA1* mutations are also found in the bone marrow of the majority of TMD patients, suggesting that *GATA1* mutagenesis is an early event in DS myeloid leukemogenesis, probably occurring *in utero*. In contrast *GATA1* mutations are not detected in leukemic cells of NDS patients with AMKL or in DS patients who develop other forms of leukemia.[20,24]

Data from several large Pediatric Oncology Group protocols were compared for the presentation and result of therapy for acute lymphoblastic leukemia (ALL) in children with and without DS.[25] There were no children with DS and t(9;22), t(1;19), or t(4;11) chromosomal translocations, compared with an expected frequency of 10% to 13% in the NDS population. However, the DS children suffered more toxic effects from the chemotherapy, and their overall outcome therefore was not better than that of the NDS patients. Analysis of children with DS and leukemia in the United Kingdom treated between 1980 and 1994 also found a decreased five year disease free survival (57% vs. 75%) for the children with DS.[26] Similar to the *GATA-1/AMKL* story, recently, activating mutations in the *JAK2* kinase have been found in 20% of DS-ALL samples.[27,28]

Despite the well-documented increase in the risk of leukemia in children with DS, a study based on exhaustive analysis of the Danish population found no increased risk of solid tumors in children or adults with DS including significantly fewer cases of breast cancer compared with an age-matched population.[14]

Sex Chromosome Abnormalities

Sex chromosome abnormalities comprise a large group of disorders that result from numerical and structural problems with the X and Y chromosomes. The overall incidence of sex chromosome abnormalities is high, with 47,XXY and Turner syndrome (45,X) each affecting approximately 1 in 2,000 individuals. The diagnosis of these disorders, unlike DS, is often not made until late adolescence or young adulthood, when problems with the transition through puberty and fertility become apparent. However, children with these disorders are at increased risk for certain malignancies during childhood, arguing for earlier diagnosis.

Y Chromosome

Any phenotypic female child with part or all of a Y chromosome is at risk for development of gonadoblastoma. Recent studies suggest that the risk can be as high as 25% for individuals in the late second or third decade and can include gonadoblastoma and dysgerminomas.[29] Children with this problem include girls with androgen resistance syndromes (i.e., testicular feminization) who have a 46,XY karyotype, children with gonadal dysgenesis, and girls with Turner syndrome and a mosaic 45,X, 46,XY karyotype. Mosaicism describes an individual with several different populations of cells, presumably due to a 46,XY zygote losing a Y chromosome in an early mitotic division during development. Approximately 25% of girls with Turner syndrome have some evidence for mosaicism.[30] The *TSPY* gene on the Y chromosome has been implicated as the gene responsible for gonadoblastoma in these conditions (reviewed by Lau[31]).

Phenotypic girls with a Y chromosome component should have prophylactic surgery to remove their gonads (reviewed by Saenger[32]). In most circumstances, these gonads are nonfunctional, and removal does not affect the girls medically. However, the discovery of a sex chromosome karyotype that is not consistent with their phenotypic sex can be devastating for patients and their parents and should be carefully handled by a medical team familiar with these disorders and psychosocial aspects of gender assignment.[33]

47XXY

The clinical phenotype of boys with a 47,XXY karyotype (Klinefelter syndrome) is variable and includes tall stature, infertility, decreased secondary sex characteristics, and gynecomastia. Men with 47,XXY are often not diagnosed until adulthood, making epidemiologic studies of the increased risk of malignancy in childhood difficult. Nonetheless, some studies suggest an increased risk of dysgerminomas[34] and extragonadal germ cell tumors.[35] Men with 47,XXY have an increased risk of breast cancer.[36] There is controversial evidence for an increased risk of leukemia in men with a 47,XXY karyotype, and one large cytogenetic study of men with leukemia demonstrated no increased incidence of 47,XXY.[37]

Structural Chromosomal Abnormalities

Detection and Impact

As cytogenetic techniques were improved in the 1970s, it became clear that many of the complex dysmorphic syndromes were the result of large cytogenetically visible deletions. During the next two decades, detection of deletions by Southern blot analysis and fluorescent *in situ* hybridization (FISH) permitted further progress in identifying the underlying cause of these syndromes. A new method, array comparative genomic hybridization (array CGH), allows detection of small deletions by comparative hybridization of fluorescently labeled DNA from patient and normal control samples onto glass slides containing gridded arrays of human genomic DNA contained in bacterial artificial chromosomes, long oligonucleotides, or cDNAs. Array CGH allows the entire genome to be sampled for deletions or amplification in one experiment.[38] Array CGH is now widely available and has rapidly increased our ability to identify both inherited and somatic interstitial deletions in pediatric cancer.[39]

Interstitial deletions can result in the loss of several contiguous genes, and the varied features of a particular disorder may result from the loss of unrelated neighboring genes, with the size of the deletion impacting how many genes are lost and how many features the child may manifest. Chromosomal deletions may be *de novo* events or inherited from either parent. Deletion syndromes overlap with autosomal dominant disorders that are the result of smaller mutations affecting a single gene within the deleted segment. For example, retinoblastoma can be transmitted as result of an autosomal dominant disorder due to point mutations in the *RB1* gene or can be

associated with a cytogenetically visible deletion in a small percentage of cases.[40]

WAGR: Wilms' Tumor, Aniridia, Genital Abnormalities, and Mental Retardation

Patients with Wilms' tumor (WT) commonly exhibit a spectrum of congenital abnormalities and susceptibility to the tumor derives from several different underlying molecular mechanisms.

The WAGR syndrome is named for the components of the disorder: WT; aniridia; genital abnormalities, including hypospadias; and mental retardation associated with cytogenetically detectable deletions at 11p13. Surveys of children with WT in the United Kingdom[41] and France[8] revealed that 3% and 1%, respectively, of children with WT had aniridia.

WT1, the gene responsible for the WT phenotype, lies within the WAGR interval and encodes a zinc finger transcription factor (reviewed by Little[42]). All or part of *WT1* is deleted in children with WAGR and WT.[43] In contrast, point mutations in *WT1* including missense mutations are found in children with the Denys-Drash syndrome, a disorder characterized by severe urogenital abnormalities and WT.[43],[44] This is an example where total loss of a gene product results in a less severe disease than does production of a mutant protein due to a missense mutation. It is hypothesized that the mutant protein may have a dominant negative impact on genital development, which may not occur when the gene is deleted. Surprisingly, somatic mutations in the *WT1* gene in sporadic WT are found in only 10% of cases.[42]

PAX6 is the gene responsible for the aniridia phenotype and is deleted in children with WAGR,[45] with point mutations found in isolated aniridia.[46] Array CGH or FISH analysis is performed for infants with aniridia to map out whether the deletion includes the *WT1* gene in order to determine the risk of developing WT and need for surveillance. Screening for the development of WT in children with WAGR or Denys-Drash syndrome is often performed by abdominal ultrasound examinations every 4 months until the age of 5 years, with decreasing frequency of examinations at later ages.[47] The recommendation for serial ultrasound scans is controversial and is based on small numbers of children screened by varying protocols. The National Wilms Tumor Study found more stage 1 tumors in children who had been screened.[47] However, the Childhood Cancer Research Group in Oxford found that eight children who had their WT diagnosed by ultrasound screening did not have more favorable outcomes than those in the group that was not screened, although the screening interval was variable.[9] As discussed later, for Beckwith-Wiedemann syndrome (BWS), there are additional data that show that children who were screened had fewer cases of advanced WT than those unscreened.[48] Parents should be counseled to bring the child in for evaluation if they suspect any change in abdominal girth or feel a mass, regardless of whether ultrasound screening is performed as interval tumors can develop. A long-term analysis of children with WT and either Denys-Drash or WAGR found 62% and 38% rate of renal failure, respectively, 20 years after the diagnosis of WT.[49] Therefore, children with constitutional mutations in the *WT1* gene require long-term follow-up for evidence of declining renal function.

Overgrowth Disorders and Imprinting Errors

Beckwith-Wiedemann Syndrome

The relationship between disorders of increased growth and predisposition to cancer are evident in BWS and hemihyperplasia (HH, previously termed *hemihypertrophy*) linked to a significantly increased risk of developing abdominal tumors, including WT and hepatoblastoma.[50] HH can be a feature of BWS or an isolated finding. HH is defined as asymmetric growth due to overgrowth of one side relative to the other. It can be limited to a limb or the face or can include the whole side. Of 183 children in the BWS Registry, 13 had developed a tumor by age

4.[51] BWS is characterized by excessive intrauterine and postnatal growth, organomegaly, hypoglycemia at birth, macroglossia, and unusual linear ear creases and pitting.[50] For children with isolated HH, the risk of WT is approximately 3%.[47] Children with both BWS and HH had a higher risk of WT than did children with either condition alone.[51],[52] Cohort studies have also demonstrated that nephromegaly is associated with an increased risk of WT in children with BWS.[53]

The genetic basis of BWS and HH is complex (reviewed by Weksberg[54]). Rare families have an apparent autosomal dominant pattern that maps to 11p15 with BWS more likely to be inherited from mothers than fathers.[55] Cytogenetically visible rearrangements that result in paternal duplications of 11p15 are also seen. The mechanisms behind these unusual genetics results from *imprinting*, which refers to the fact that certain genes are expressed differently, depending on whether they were inherited from the maternal or paternal chromosome. Disorders of imprinted genes result in unusual pedigrees (e.g., unaffected sisters who can pass on a mutation in an imprinted

gene to their children, resulting in affected cousins). Apparently, cytogenetically normal children with BWS may inherit two copies of a paternal chromosome 11 and no maternal copy, termed *uniparental disomy* (UPD).[56]

Table 2.2 BWS Genetic and Epigenetic Subgroups

Region	DNA	RNA	Karyotype	Frequency (%)	Inheritance
A. Regional	Paternal 11p15 UPD		Normal	10–20	Sporadic
			11p15 Duplication	1	Sporadic
	Disruption of KCNQ1OT1		11p15 Transl/Inver	1	Sporadic
B. Domain 1	H19 Hypermethylation	IGF2 LOI	Normal	2	Sporadic
	Normal H19 methylation	IGF2 LOI	Normal	25–50	Sporadic
C. Domain 2	CDKN1C mutation		Normal	5–10	Sporadic
	CDKN1C mutation		Normal	25	AD
	KvDMR1LOM	KNQ1OT1 LOI	Normal	50	Sporadic

D. Other	Unknown		Normal	5	AD
	Unknown	Unknown	Normal	10–20	Sporadic

BWS, Beckwith-Wiedemann syndrome; UPD, uniparental disomy; LOM, loss of methylation; LOI, loss of imprinting; AD, autosomal dominant.
Adapted from Weksberg R, Smith AC, Squire J, et al. Beckwith-Wiedemann syndrome demonstrates a role for epigenetic control of normal development. Hum Mol Genet 2003;12(spec no 1):R61–R68.

Significant effort has been made to identify which imprinted genes in 11p15 are disrupted in BWS. Imprinted genes implicated in the etiology of BWS include the paternally expressed genes (maternally imprinted) insulin-like growth factor 2 gene (*IGF2*) and RNA transcript, *KCNQ1OT1(LIT1)*,[57],[58] and the maternally expressed (paternally imprinted) genes *H19* and *CDKN1C*. Among BWS patients, 25% to 50% have loss of imprinting (biallelic expression) of *IGF2*. Another 50% have an epigenetic mutation that results in loss of imprinting of *KCNQ1OT1*. There are also rare cases of patients with BWS who carry mutations in the *CDKN1C/p57^{KIP2}* gene.[59] These different etiologies may be particularly important in defining the cancer phenotype of different BWS patients. Children with BWS who develop embryonal tumors such as rhabdomyosarcoma (RMS) and hepatoblastoma are more likely to have epigenetic changes in domain 2,[60] whereas WT is more strongly associated with epigenetic alterations in domain 1 or UPD.[61],[62] A summary of the molecular defects found in children with BWS is found in Table 2.2.

The risk of having a child with BWS is increased when the pregnancy was initiated by assisted reproductive technologies (ART), including *in vitro* fertilization (IVF). A study of children in the BWS Registry revealed that 4.6% were the result of ART, which appeared increased compared with the general population.[63] A population-based case control study from Australia confirmed a 17-fold relative risk of BWS in pregnancies initiated by IVF.[64] However, the absolute risk of BWS in an ART-associated pregnancy is still very low at 1 in 4,000 pregnancies. BWS in ART pregnancies appears to result from abnormal methylation of *KCNQ1OT1/LIT1*.[65]

Molecular testing for alteration in the genes implicated in BWS is now available in clinically certified laboratories (www.genetests.org). The results of testing improve prediction of cancer risk and the likelihood of the parents having another child with BWS. For example, parents of a child with BWS due to UPD have a very low risk (much less than 1%) of recurrence in another pregnancy.

Overall, given the increased risk of WT and other abdominal malignancy in these conditions, screening by regular serial abdominal ultrasound examinations and serial α-fetoprotein (AFP) levels is recommended for children with BWS, HH, or both (see the preceding section on WAGR for details about screening). Children with BWS who were screened for WT were much less likely to present with advanced disease than those who were not screened (0 of 12 vs. 25 of 59).[48] Screening until age 9 will detect the majority of children with BWS who will develop WT. Although the risk of a second tumor is low (typically WT in the contralateral kidney), it is recommended that children with BWS and WT or hepatoblastoma continue with routine screening by ultrasound and serum AFP levels until age 9.

Other Wilms' Tumor Loci

Using array CGH, a novel gene termed *WTX* was identified on chromosome Xq11.1.[66] *WTX* is inactivated in one-third of WTs, and tumors with *WTX* mutations lack *WT1* mutations. WTX binds WT1 in the nucleus, suggesting a role for WTX in nuclear pathways implicated in the transcriptional regulation of cellular

differentiation programs.[67] Whereas autosomal tumor suppressor genes undergo biallelic inactivation, *WTX* is inactivated by a monoallelic "single-hit" event that targets the single X chromosome in WTs in males and the active X chromosome in tumors in females.[68] However, germline mutations of *WTX* do not appear to be associated with WT susceptibility. Rather, this causes a form of X-linked sclerosing bone dysplasia, osteopathia striata congenital with cranial sclerosis.[69] These observations suggest the existence of temporal or spatial constraints on the action of *WTX* during tumorigenesis. Overall, bilateral WT or a family history of WT occurs in 1% to 5% of patients. Although linkage studies have indicated that the gene for familial WT must be distinct from *WT1* and *WTX* and from genes that predispose to BWS, to date, no familial WT gene has been identified.[70]

Autosomal Dominant Disorders

Autosomal dominant syndromes comprise the majority of families with single-gene disorders that convey an increased risk of cancer. The features of autosomal dominant inheritance include equal transmission from the father or the mother to a son or daughter, in contrast to an X-linked disorder. Often, there is a multigenerational pattern, and a variable expression of the disorder within a family, with "skipped"

generations (at the phenotypic level) because of incomplete penetrance. *Penetrance* is defined as the probability that a person inheriting the mutation will have the disease.

Retinoblastoma

Much of our knowledge of autosomal dominant cancer families was gained from the study of retinoblastoma. In a series of landmark papers in the early 1970s, Knudson and Strong performed statistical analysis of children with retinoblastoma and other pediatric malignancies.[71],[72] Knudson hypothesized that bilateral retinoblastoma represented the hereditary form, and those patients had already acquired one "hit" or mutation.[71] The best model consistent with his data indicated that the bilateral form required only one additional hit after birth but that the unilateral form required two hits. Prior to this work, in 1968, Nicholls[73] had proposed that the skin lesions in neurofibromatosis type 1 (NF1) represented two mutational events in the same gene, with the first mutation being inherited and the second mutation occurring somatically.

The most striking features of autosomal dominant cancer predisposition disorders are those initially observed by Knudson: hereditary forms of retinoblastoma present earlier and with a greatly increased percentage of bilateral and multiple primary tumors. Importantly, some patients (about 15%) with unilateral retinoblastoma carry a constitutional mutation. An even milder form, retinoma or retinocytoma, which spontaneously regress, can also be seen in apparently unaffected adults. Approximately 10% of people with a germline mutation in *RB1* do not develop retinoblastoma (i.e., incomplete penetrance).[74] However, the penetrance varies among families, with specific mutations (often missense changes or splice abnormalities) resulting in mutation carriers having a higher likelihood of developing unilateral (as opposed to bilateral) disease. These types of families are said to demonstrate attenuated or low penetrant retinoblastoma.[75],[76]

Individuals carrying germline mutations in the *RB1* gene are at increased risk for development of other primary tumors, including osteosarcoma and malignant melanoma in childhood. In a U.K. cohort of long-term survivors, children with bilateral retinoblastoma were found to have 48% risk of developing a second neoplasm by age 50.[77] Further follow-up of this cohort (up to age 84) identified a 68% cumulative incidence of second cancers including many epithelial cancers, for example, lung cancer, at later ages.[78] Few individuals in the U.K. cohort received radiation therapy, confirming that there is a significant risk of second primary cancers in all bilateral retinoblastoma patients. Recent data from a U.S. cohort looking at cumulative cancer mortality (as opposed to

incidence) identified 25% and 1% risk for hereditary and nonhereditary retinoblastoma, respectively.[79] For children with hereditary retinoblastoma treated with radiation, there is a substantial increased risk of sarcomas with one estimate of a 13% cumulative risk of developing a sarcoma by age 50.[80]

On the basis of rare cases of patients with cytogenetically visible deletions, the gene mutated in retinoblastoma, *RB1*, was mapped to chromosome 13q14 and eventually isolated.[81] Molecular studies confirmed Knudson's two-hit hypothesis. Retinoblastoma requires loss of both copies (i.e., two hits) of the *RB1* gene for a tumor to develop (Fig. 2.1). Loss of the normal tumor suppressor function of RB1 is consistent with loss of cell cycle control (see Chapter 27 for details). In the familial form, a mutation in one *RB1* gene is inherited; and therefore, all the cells in the body have only one normal allele. If during development that normal copy is mutated or lost, then cell cycle control is disrupted and retinoblastoma can develop. The most common mechanisms by which the second copy is lost are loss of the whole chromosome, large deletions, and gene conversion, normally resulting in loss of heterozygosity (LOH) for markers near the *RB1* locus or silencing of the gene by epigenetic methylation of the *RB1* promoter. In the sporadic form, mutation or loss of both *RB1* genes must

occur in the same somatic retinal cell for retinoblastoma to develop.

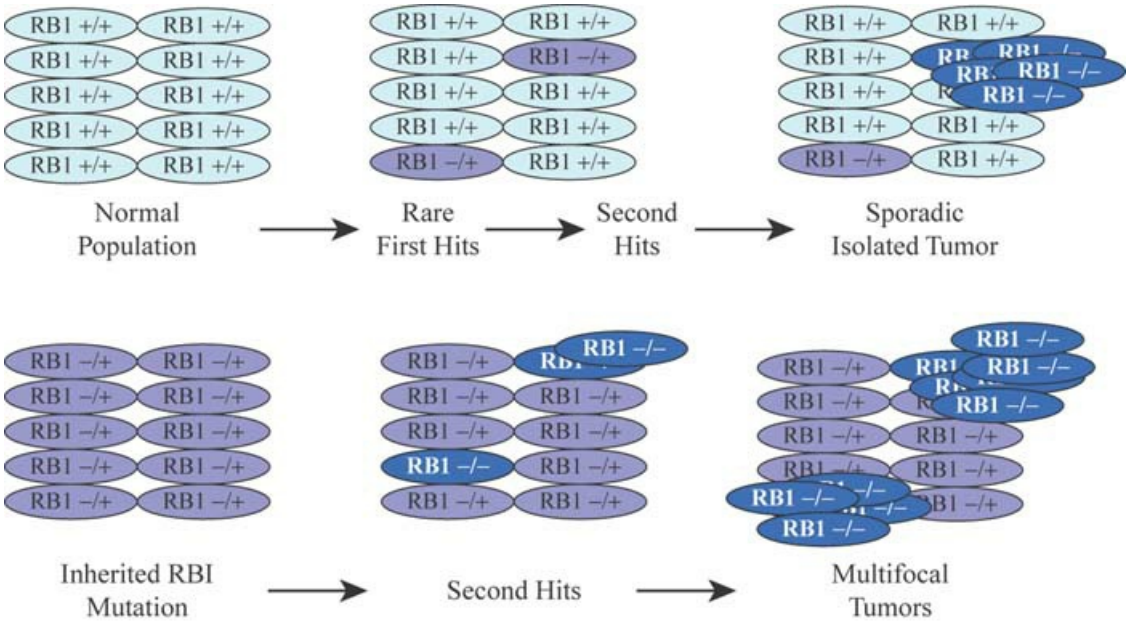

Figure 2.1 Knudson's two-hit hypothesis. In all tumors, the same cell must undergo at least two mutations in the *RB1* gene to become malignant. In sporadic, nonhereditary tumors (**top**), the first hit occurs at low frequency, with a rare cell having a second hit in the same gene yield isolated tumors. In hereditary tumors (**bottom**), the first mutation is in a germ cell, such that all body cells have the first mutation. When a second mutation or inactivating event occurs in *RB1*, tumors develop. (Modified from Plon SE. "Cancer Genetics and Molecular Oncology" by Plon, SE in Principles of Molecular Medicine, 2nd Edition, 2006 (Eds. Runge, M.S. and Patterson, C.) The Humana Press, Inc., Totowa, New Jersey. With kind permission of Springer Science+Business Media).

Table 2.3 Empirical Recurrence Risks in Families with Retinoblastoma in the Absence of Genetic Testing

Clinical scenario	Retinoblastoma risk (%)
Offspring of bilateral cases	45

Offspring of unilateral cases	7.5
Sibling of bilateral cases (with unaffected parents)	5–7
Sibling of unilateral cases (with unaffected parents)	1
Sibling of bilateral or unilateral cases (if either parent is affected)	45

Although bilateral retinoblastoma results from constitutional mutations in the *RB1* gene, 80% of patients will have no family history of retinoblastoma. This is due to the majority being the result of a *de novo* mutation in the *RB1* gene. In Table 2.3, the risk is given of having a second child with Rb for parents of a child newly diagnosed with either unilateral or bilateral Rb.[74] Surprisingly, parents of a child with bilateral retinoblastoma who have normal eye examination results retain a 5–7% risk to have a second affected child due to the *de novo* mutation occurring in the father's germline and with a variable percentage of the sperm carrying the mutation (germline mosaicism).[82] More rarely, the mutation occurs during oogenesis. Therefore, if genetic testing is not pursued, all siblings of children with bilateral retinoblastoma should have ophthalmologic surveillance beginning at birth.

The discovery of the *RB1* gene[81] allowed for clinical molecular diagnostics. Several different approaches are used to identify mutations.[83],[84] Larger-scale deletions of the entire *RB1* gene (detected by cytogenetics, FISH, or array CGH) are found in fewer than 5% of germline mutations.[40] The remaining mutations are scattered throughout the gene and require full DNA sequence and copy number analysis, which is clinically available in several certified laboratories. The majority of these mutations result in a truncated protein or disrupt specific functional domains of the RB protein. Assays of *RB1* promoter methylation are performed to look for epigenetic silencing.

With extensive testing, clinical laboratories can identify the causative mutation in about 95% of bilateral cases. Recent studies suggest that the remaining 5% of patients are most likely mosaic for the causative mutation with too few blood cells containing the mutation to be detected.[85] Patients with a negative family history and unilateral disease have only a 15% *a priori* chance of having a germline *RB1* mutation. Thus, a negative test from a blood sample from a unilateral case can be difficult to interpret. For unilateral cases, where enucleation has been performed, molecular diagnostic laboratories first identify mutations in the *RB1* gene in a fresh frozen tumor specimen and then determine whether the mutation can be identified in constitutional DNA from the blood.[84] Examples of genetic test results for unilateral retinoblastoma patients are shown in Table 2.4. Testing of a tumor sample is not necessary for bilateral cases where testing is done directly from a blood sample.

Genetic evaluation and testing is recommended for all retinoblastoma patients. Unaffected parents of a child with bilateral disease often are concerned about their risk for having additional children with retinoblastoma. The physician first looks for the mutation in the affected child and then studies both parents and all siblings to ascertain whether they carry the mutation. If prenatal testing is not pursued, then siblings of the proband should have a careful eye examination at birth and a blood sample sent for analysis of the specific *RB1* mutation found in the affected child. Only those siblings who carry the mutation need subsequent surveillance for retinoblastoma. The adult survivors of childhood retinoblastoma can also use DNA testing for prenatal diagnosis or immediate postnatal diagnosis of their children. Current recommendations for ophthalmic surveillance include examination in the first few days of life and then serial examinations every 3 to 4 months until 3 years of age (see Chapter 27).

Table 2.4 Examples of *RB1* Genetic Test Results for Two Patients with Unilateral Retinoblastoma

Sample	Allele 1	Allele 2
Patient 1—Hereditary form of RB		
Tumor 1	Q347X	Loss
Blood 1	Q347X	Normal
Patient 2—Sporadic Rb due to somatic mutation		
Tumor 2	Methylation of promoter	567delAG
Blood 2	Normal	Normal

In contrast, if genetic testing demonstrates that the child did not inherit the mutation found in the affected relative, the surveillance and anesthesia required for ophthalmic examinations could be avoided, decreasing costs and potential morbidity.[86] Genetic testing of unilateral pediatric patients can be particularly informative for parents. If it can be documented that the child does not carry a constitutional *RB1* mutation then (1) the child is not at substantial risk for secondary malignancies, (2) radiation therapy is associated with less hazard, and (3) the parents have a negligible risk of having another child with retinoblastoma. The child with unilateral retinoblastoma may carry some residual risk of having an affected child, given the possibility of mosaicism.[85] For adult long-term survivors of unilateral retinoblastoma, testing of tumor sample is not possible. A positive test of a blood sample is found in approximately 12% of cases and is informative of a hereditary form of Rb. If comprehensive *RB1* analysis of the blood is negative, then there is approximately 0.5% to 1% residual risk for each offspring to develop retinoblastoma. Appropriate screening guidelines in this circumstance should be discussed in detail with a pediatric ophthalmologist. A coordinated team of oncologists, ophthalmologists, pathologists, and genetics professionals facilitates optimal care of families with a child diagnosed with retinoblastoma.

Inherited p53 Mutations, the Li-Fraumeni Syndrome and Its Variant Phenotypes

In 1969, an inherited cancer predisposition syndrome was reported by Li and Fraumeni on the basis of characterization of four families in which at least two cases of sarcoma occurred in early life.[87],[88] These investigators subsequently defined the “classic” syndrome as a proband with sarcoma diagnosed under age 45 years, with a first-degree relative with any cancer under 45 years, plus another first- or second-degree relative with either any cancer under 45 years or a sarcoma at any age.[89],[90] An example of a pedigree from a Li-Fraumeni syndrome (LFS) family is shown in Figure 2.2. The list of LFS tumors includes premenopausal breast cancers, brain tumors, leukemias, adrenocortical carcinomas, gastric cancers, lymphomas, and possibly

early-onset lung cancers, choroid plexus carcinomas, and colorectal cancers.[91],[92] More recently, the criteria for families that do not quite meet criteria, termed *LFS-like* (LFS-L), are generally accepted to include those outlined by Chompret et al[93] to include all children with adrenocortical carcinomas; a family in which the proband has multiple tumors, two of which are classical LFS tumors and the first occurred before age 36 years; and a family in

which the proband has a characteristic LFS tumor diagnosed under age 36 and has at least two first- or second-degree relatives with an LFS component tumor (other than breast cancer if the proband had breast cancer). Hisada and colleagues[94] showed that gene carriers are at significant risk of developing multiple synchronous or metachronous non–therapy-induced neoplasms. In particular, the overall relative risk of occurrence of a second cancer was 5.3 (95% CI = 2.8–7.8), with a cumulative probability of second cancer occurrence of 57%.

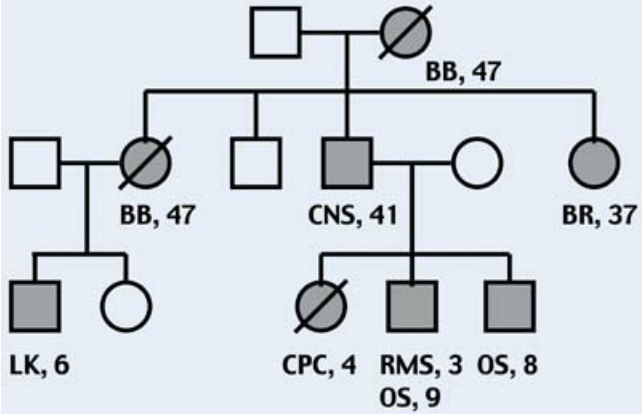

Figure 2.2 Pedigree of a family with Li-Fraumeni syndrome. Filled circles/squares represent affected members; circles with slashes represent deceased family members. Numbers represent age at diagnosis. BB, bilateral breast cancer; CNS, brain tumor; BR, unilateral breast cancer; LK, leukemia; CPC, choroid plexus carcinoma; RMS, rhabdomyosarcoma; OS, osteosarcoma.

Given the high mortality rate for affected members of LFS families, it was not possible to obtain DNA from extended pedigrees to perform linkage analysis. In 1990, Malkin and colleagues[95] took a candidate gene approach based on data from somatic mutation and mouse models of *p53*. These investigators detected heterozygous point mutations in the *P53* gene (also referred to as *TP53*) in constitutional DNA of LFS kindreds.[95] However, numerous subsequent studies have shown that only 60% to 80% of "classic" LFS families harbor detectable germline *p53* mutations,[92],[96] while the majority of LFS-L families do not have detectable *p53* mutations (see, e.g., Tinat et al[97]). Mutations occur throughout the *p53* gene, though they are primarily confined to highly conserved regions. More recently, intragenic deletions of the *p53* gene have been reported in a subset of families that had negative sequencing studies.[98] The cancer phenotype in LFS is quite diverse. While specific p53 genotype:phenotype correlations have not been clearly demonstrated, several genetic modifier effects are reported. In particular, the mean age of onset of tumors is significantly less in *p53* mutation carriers who carry an *MDM2* SNP309 G allele compared with those homozygous for the T allele.[98] Similarly, carriers of the *p53* codon 72 arginine allele have an earlier tumor onset than those who harbor a homozygous proline allele.[98] These provide examples of how polymorphisms affecting p53 protein degradation can act as genetic modifiers of mendelian predisposition to cancer. In addition, accelerated telomere shortening as measured in peripheral blood lymphocytes is predictive of earlier tumor onset.[99],[100] The cumulative combination of *MDM2* SNP309 and *p53* codon 72 status, telomere length, and possibly specific *p53* mutations may eventually be used as a predictive biomarker for cancer type and the age of onset in LFS.[101] DNA copy number variation (CNV) is strikingly enriched in the constitutional DNA of *p53* mutation carriers, and these CNVs can be inherited and frequently encompass other cancer genes, suggesting that the genomic instability conferred by the *p53* mutation can be transmitted from generation to generation.[102] Regions of DNA showing variability in a number of subjects likely identify other genetic factors that may modulate the cancer phenotype.

Several groups have failed to identify mutations in other tumor suppressor genes, for example, *PTEN*, *p16INK4*

a, and *p19Arf*, or genes in p53-mediated regulatory pathways in LFS, LFS-L families and individuals with the occurrence of multiple tumors who are negative for *P53* mutations. Heterozygous germline mutations in the checkpoint kinase *hCHK2/CHEK2* in one LFS family and one LFS-L family were reported.[103] However, several subsequent studies have failed to identify *CHK2* mutations in a large number of LFS families.[104] Thus, the other genes that result in LFS or LFS-L families are unknown.

Although *p53* behaves as a classic tumor suppressor gene, less than 50% of tumors from *p53*-heterozygous mice and LFS patients have evidence of LOH.[105],[106] It remains unclear in these patients how the retained wild-type *p53* allele is functionally inactivated *en route* to malignant transformation of the cell.

A number of studies have analyzed groups of patients with tumors characteristic of LFS, yet lacking characteristic family histories of cancer, for germline *p53* mutations. Such mutations have been identified in approximately 50% to 80% of children with adrenocortical carcinoma,[2],[3] 10% of children with osteosarcoma,[107] and 10% of children with RMS.[108],[109] The age of onset of tumors in the latter group of patients is strikingly lower (average age approximately 22 months) than in RMS patients with intact constitutional *p53*.[108] One-third of children with sarcomas plus either multiple primary tumors or a significant family history of cancer have germline *p53* mutations. A study of patients with adrenocortical carcinoma in Brazil revealed that 35 or 36 patients carried a specific germline *p53* mutation, R337H, without a family history of cancer, suggesting that it may represent a lower penetrance mutation that imparts a distinct susceptibility to adrenocortical carcinoma.[110]

Presymptomatic molecular testing for *p53* germline mutations in members of Li-Fraumeni kindreds has been met with significant controversy. Because of the variable expressivity, the diverse tumor spectrum, and lack of clear clinical surveillance and preventative or treatment recommendations, it is unclear how to manage the detection of a *p53* mutant carrier. However, women who carry *p53* mutations should begin screening for breast cancer with magnetic resonance imaging (MRI) in their mid-20s, given that the average age of onset is 31 years.[111] Recently, the use of positron emission tomography-computed tomography as a clinical surveillance modality has been reported, identifying presymptomatic lesions in adults,[112] and anecdotal reports of presymptomatic detection of childhood cancers, in particular, adrenocortical carcinoma, have also been noted.[113],[114] Furthermore, the concept of predictive genetic testing

of a child for a disease that may (or may not) occur in young adulthood poses significant challenges to our perception of the ethics of disclosure of genetic test results, where the potential beneficiary of these results may wish to uphold the right to “not know.” Notwithstanding these considerations, presymptomatic and even prenatal genetic testing for *p53* mutation is being performed in carefully selected and counseled situations, taking into account the particular balance of beneficence and harm. These issues are discussed further in the last section of this chapter.

Inheritance of a Mutation in an Oncogene: RET and ALK

A large series of autosomal dominant cancer susceptibility syndromes were identified that resulted from deleterious mutations in tumor suppressor genes. Beginning with multiple endocrine neoplasia (MEN) type 2 in 1993 and continuing with familial neuroblastoma in 2008, it is now clear that some autosomal dominant cancer susceptibility syndromes result from inheritance of a mutation (typically missense mutations) that converts a proto-oncogene to an activated oncogene. These conditions do not require a somatic mutation of the other allele (no “second hit”) and do not demonstrate LOH in flanking markers in tumor specimens.

Multiple Endocrine Neoplasia

The MEN disorders represent at least three different diseases, which are all autosomal dominant cancer family syndromes that affect different endocrine organs. MEN type 1 (MEN1) is characterized by parathyroid, pancreatic islet cell, and pituitary gland involvement. Parathyroid involvement is found most frequently, and individuals from MEN1 families can also have their disease complicated by Zollinger-Ellison syndrome. The gene for MEN1 acts as a typical tumor suppressor gene, with inactivating mutations passed down in families.[115] Although most MEN1-associated tumors present in adulthood, by age 15, 28% of mutation carriers have either biochemical or clinical evidence for disease.[116]

Both MEN2A and MEN2B syndromes present in the pediatric period. MEN2A is associated with medullary thyroid carcinoma (MTC), parathyroid adenomas, and pheochromocytomas. MEN2B is a related disorder but with the onset of tumors in infancy, ganglioneuromas of the gastrointestinal (GI) tract, and skeletal abnormalities. Additional families appear to show autosomal dominant MTC without the other features of MEN2A. Because of the life-threatening potential of metastatic MTC, treatment for MEN2 is prophylactic thyroidectomy in childhood.[117]

Analysis of *RET*, at chromosome 10q11.2, in constitutional DNA from multiple MEN2A families revealed a set of highly consistent missense mutations that replace one of a set of conserved cysteines with another amino acid in the extracellular domain of the protein encoded by exons 10 and 11 (reviewed by Raue and Frank-Raue[118]). Families that have isolated MTC or those with the full MEN2A syndrome share the same mutations. However, there is a correlation between disease phenotype and the specific mutation, for example, a mutation in cysteine 634 results in a high risk of pheochromocytomas. Studies of individuals from multiple MEN2B patients demonstrated two specific missense mutations, M918T and A883F, or the association of V804M (with other mutations) in the highly conserved tyrosine kinase domain of the *RET* gene.

These findings are of both scientific and clinical importance. Unlike tumor suppressor genes, predisposition to cancer in MEN2A and MEN2B families is due to inheritance of a mutation that activates the *RET* proto-oncogene as demonstrated by both *in vivo* and *in vitro* assays.[119]

Clinically, the screening, the preventive surgery, and the treatment of MEN2A and MEN2B families have been significantly improved by these genetic discoveries. DNA-based screening results in greatly increased sensitivity and specificity compared with calcitonin assays, particularly for young children.[120] All individuals with MTC (either sporadic or familial) should have DNA analysis performed for *RET* mutations. If the results are positive for mutation, then family members should be tested to determine whether they carry the *RET* mutation. Children found to be mutation positive will need prophylactic thyroidectomy by age 5 for MEN2A and by age 1 for MEN2B.[117] In addition, they will require lifelong surveillance for development of pheochromocytoma and parathyroid disease. Knowledge of RET biology is impacting treatment as the Ret inhibitor, vandetanib, has shown excellent activity in treatment of metastatic MTC and may be incorporated as frontline treatment of this disorder.[121]

Familial Neuroblastoma

Neuroblastoma has been reported to cluster in very rare families and a family history is documented only in 1% to 2% of newly diagnosed cases.[122] Initially, deleterious mutations in one gene, *PHOX2B*, was reported in two families with multiple cases of neuroblastoma.[123] Mutations in *PHOX2B* are also associated with Hirschsprung's disease and congenital central hypoventilation syndrome. Review of larger series of familial neuroblastoma revealed only rare mutations in the *PHOX2B* mutations. By using dense array hybridization techniques that allow genotyping of thousands of single nucleotide polymorphisms, Mosse et al. identified linkage for familial neuroblastoma to chromosome 2p23.[124] Sequence analysis of genes in the interval revealed specific missense

mutations in the *ALK* proto-oncogene, resulting in activation of the gene and predisposition to neuroblastoma.[124] These families demonstrate incomplete penentrance (many mutations carriers did not develop tumors) and the grade of tumor varies among family members, from ganglioneuroma to advanced stage IV neuroblastoma. Several groups also identified both constitutional and somatic mutations in *ALK* in approximately 10% of neuroblastoma tumors resulting in the development of clinical trials with inhibitors of Alk kinase.[39],[125],[126] Clinical testing for *ALK* mutations is now available and is being incorporated into evaluation of familial neuroblastoma.

Atypical Teratoid and Malignant Rhabdoid Tumors and the Rhabdoid Predisposition Syndrome

Malignant rhabdoid tumor (MRT) of the kidney is a rare, aggressive childhood cancer, and it was noted early on that 10% to 15% of presentations in infants are associated with separate primary tumors of the central nervous system (CNS).[127] Although infants' kidney is the most common site for rhabdoid tumors, they occasionally are observed in other sites and in older children and even adults. The tumor is histologically defined by large cells of unknown origin that may resemble benign or malignant skeletal muscle cells. These histologically resemble primitive neuroectodermal tumors (and were previously diagnosed as medulloblastoma or pineoblastoma) or rhabdoid tumors. Because of its potential to differentiate into heterologous elements at the cellular level, this tumor type has been termed *atypical teratoid/rhabdoid tumor* (ATRT).[128]

Cytogenetic analyses of ATRT of the CNS and MRT of the kidney revealed abnormalities of chromosome 22, in particular, loss of one entire copy of the chromosome or deletion or translocation involving 22q11.2.[129],[130] In 1998, the *SMARCB1/hSNF5/INI1* gene was isolated from chromosome band 22q11.2 and several rhabdoid tumor cell lines were shown to harbor truncating mutations of this gene.[131] *SMARCB1* encodes a protein that is part of a multiprotein complex involved in chromatin remodeling, an essential process for regulation of gene expression. Biegel and colleagues have reported *hSNF5/INI1* mutations in virtually all

MRTs/ATRTs examined.[7] In this early study, they found that approximately 20% of children with apparently sporadic tumors harbored constitutional mutations of the gene, suggesting a potential hereditary component to the etiology of the disease. Subsequent studies by Severet et al. confirmed this finding and, in sum, approximately 25% of such tumors arise in the context of a constitutional mutation.[132] These studies suggest that *SMARCB1* acts as a classic tumor suppressor gene, namely complete loss of *SMARCB1* function in tumors, resulting either from two somatic events in sporadic cases or an inherited mutation followed by a second somatic silencing event. The dominantly inherited syndrome termed *rhabdoid predisposition syndrome* includes a spectrum of tumors including renal and extrarenal MRT, choroid plexus carcinoma, central PNET, and medulloblastoma.[132] The likelihood of developing a tumor is very high at a very young age and these tumors are often lethal. Thus, in most cases, the constitutional mutation represents a *de novo* dominant mutation in the child diagnosed with the tumor with unaffected siblings or parents. This same paradigm of *de novo* mutation may apply to other rare pediatric tumors with high mortality. A few families have been reported with adult *SMARCB1* mutation carriers without a cancer history, suggesting incomplete penetrance.[133] Unexpected was the recent discovery that a completely different condition, familial schwannomatosis, is also the result of mutations, typically splice site changes, in the *SMARCB1* gene.[134] These families have late childhood and adult onset of multiple schwannomas and occasional meningiomas without evidence for rhabdoid tumors. One family was described with overlap of the two otherwise distinct clinical phenotypes.[135]

Familial Leukemia

Acute leukemias are the most frequent malignancy of childhood. However, knowledge about genetic predisposition to leukemia is very limited compared with many less common malignancies. Some well-described

autosomal dominant syndromes, including LFS and NF1, demonstrate an increased risk of leukemia as one of many features as described elsewhere in this chapter. But families that demonstrate a specific predisposition to leukemia are extremely rare (reviewed by Horwitz[136]).

Some progress has been made in mapping loci responsible for these rare families with one leukemia susceptibility gene identified. Familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) is an autosomal dominant syndrome, characterized by both neonatal thrombocytopenia and a very high propensity to develop AML associated with inheritance of deleterious mutations in the *RUNX1*/*CBFA2/AML1* gene as identified by the laboratory of Gilliland and colleagues.[137]

Many more families and *de novo* cases have been described with *RUNX1* mutations, spanning from point mutations to deletion of the whole gene.[138] Children with large deletions at 21q22 have thrombocytopenia, susceptibility to AML, congenital anomalies and developmental delay.[139] In several cases the AML cells have acquired a trisomy 21 karyotype with the duplicated chromosome 21 carrying the deletion of *RUNX1*. Thus, a gene other than *RUNX1* on chromosome 21 must play a role in tumorigenesis. A recent analysis of multiple leukemia samples from FPD/AML patients reveals second somatic mutations in *RUNX1*, thus following the two-hit hypothesis.[140]

Childhood Cancers Associated With Familial Colon Cancer Syndromes

Although not generally considered a pediatric disease, children of familial colon cancer kindreds can present with GI manifestations in the adolescent period.[141] In addition, there is an increased prevalence of a variety of pediatric malignancies, including hepatoblastoma and brain tumors. The familial colon cancer syndromes are divided into those associated with polyposis (i.e., familial adenomatous polyposis [FAP]) and hereditary nonpolyposis colon cancer (HNPCC).

Familial Adenomatous Polyposis

FAP, also known as *adenomatous polyposis coli* (APC), is associated with carpeting of the colon with thousands of polyps with onset in the second or third decade of extensive polyposis (Fig. 2.3) and a nearly 90% rate of development of malignant colorectal carcinoma (reviewed by Haggitt and Reid[142]). Extracolonic manifestations in some kindreds with polyposis, including desmoid tumors, epidermoid cysts and osteomas of the mandible, was referred to as *Gardner syndrome*. However, with the discovery of the APC gene, these “different” disorders were found in some cases to be caused by identical mutations, and different members of the same family might demonstrate features of Gardner syndrome or isolated colonic polyposis. Thus, the terms Gardner syndrome and FAP are now often used interchangeably.

In addition to the greatly increased risk of colorectal carcinoma, carriers of this disorder have additional cancer risks. Upper GI tract tumors include duodenal and periampullary adenocarcinomas and can result in increased mortality in FAP patients postcolectomy.[143] Approximately 1% of FAP patients develop thyroid cancer, and some authors recommend beginning surveillance at age 15 for thyroid cancer.[144] Of particular importance for pediatric oncologists, approximately 1 child

per 250 children with FAP develops hepatoblastoma, compared with 1 per 100,000 in the general population.[145] Familial cases of FAP may not be obvious because the parents of a young child with hepatoblastoma may have unrecognized polyposis even though the parent is at an age when it is essential to perform prophylactic colectomy to prevent invasive colorectal cancer. Thus, a careful family history of colon cancer and polyposis should be taken for any child diagnosed with hepatoblastoma.

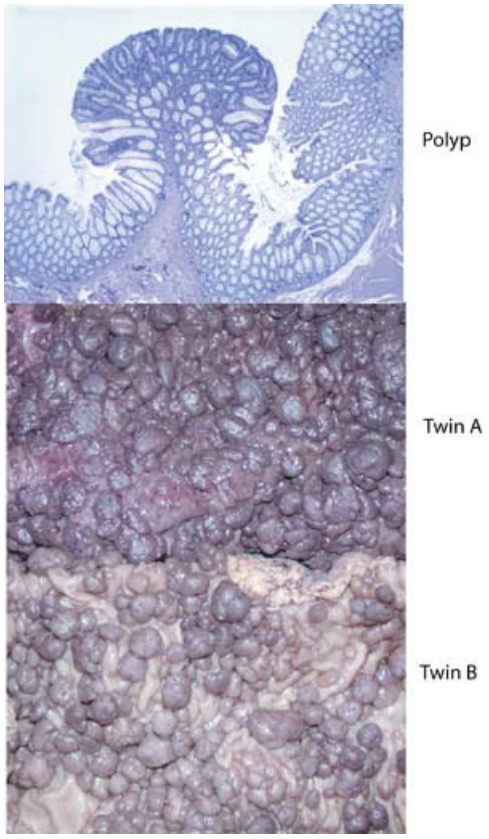

Figure 2.3 Carpeting of colonic epithelia with adenomatous polyps. Shown are samples from total colectomy of two twin brothers who were diagnosed with familial adenomatous polyposis due to a *de novo* APC mutation after Twin A presented with abdominal pain and rectal bleeding. (Photographs courtesy of M. Finegold, MD, Baylor College of Medicine/Texas Children's Hospital).

Reports from cohorts of patients in Germany and the Children's Oncology Group in the United States suggest that approximately 10% to 15% of hepatoblastoma patients carry mutations in *APC*.[146,147] Thus, some centers now offer *APC* mutation testing (both full sequencing and deletion/rearrangement analysis, described below) for all hepatoblastoma patients, independent of family history. There has not been enough clinical experience to know the mutation yield although one more recent report found mutations in zero of 29 probands with hepatoblastoma.[148]

Deleterious mutations and deletions are found spread throughout the *APC* gene as the causative mutation in 85% to 90% of FAP families.[149] In the workup of a child at risk for polyposis due to an affected parent, it is important to test the affected parent first to identify the specific mutation causing FAP in that family. Subsequently, at-risk family members are tested for that specific mutation. One must also consider a recessive form of colonic polyposis (MYH-associated polyposis) due to inheriting mutations in the *MYH/MUTYH* excision repair gene from both parents.[150] This condition results in somewhat later ages of polyposis and colon cancer compared with classic FAP.

Unlike the situation for LFS, there are clear surveillance and prophylactic surgery guidelines for FAP, and testing of children is considered standard of care.[151,152] Screening by colonoscopy is recommended to begin between the ages of 8 and 10 years for mutation carriers. Prophylactic surgery that includes total colectomy with removal of the rectal mucosa is recommended after extensive polyposis develops or by late adolescence. Modern surgical techniques allow the maintenance of fecal continence in these patients.[153,154] Surgery recommendations are modified if the family is demonstrated to carry a low penetrance or attenuated mutation. After prophylactic surgery, carriers need screening of their upper GI tracts and rectums (if rectal mucosa is left in

place) for development of malignancy. Data on the efficacy of nonsteroidal anti-inflammatory drug in reducing colonic polyp risk are controversial and treatment should be conducted in conjunction with a pediatric gastroenterologist.[155]

Familial Juvenile Polyposis

Familial juvenile polyposis (JP) results in multiple hamartomatous polyps in the rectocolon of young children. These lesions often manifest with abdominal pain and rectal bleeding.[156] Recent studies estimate a 39% lifetime risk of colorectal carcinoma with some difference based on the gene involved.[157] This clearly inherited condition stands in contrast to a child with a single, isolated hamartomatous polyp who does not demonstrate cancer risk.[158] JP is inherited as an autosomal dominant trait due to mutations in one of three genes: *SMAD4/MAD4H*, *BMPR1A1*, or rarely *PTEN*.[159],[160] Genetic testing for mutations in these three genes is available clinically, although a significant percentage of JP patients still do not have an underlying mutation identified.[159]

Surveillance recommendations for JP include annual complete blood cell count (to detect anemia due to GI blood loss) and semiannual colonoscopy. Prophylactic colectomy is *not* recommended because the risk of colorectal cancer is lower than that seen in FAP. Thus, it is very important to distinguish patients with JP from those with FAP. In addition, there are a group of *SMAD4* families with JP that also demonstrate clinical features of hereditary hemorrhagic telangiectasias syndrome and require surveillance for visceral and CNS telangiectasias.[161]

Hereditary Nonpolyposis Colon Cancer

The HNPCC or Lynch syndrome describes families with an increased risk of colon cancer and in the absence of polyposis.[162] Extracolonic malignancies include uterine, ovarian, ureteral, biliary tract, and in the upper GI tract cancers. Malignancies can rarely manifest in the second decade of life, and for families with particularly early onset, screening beginning 5 years before the earliest cancer diagnosis or otherwise screening biannually by colonoscopy is recommended to begin around age 25.[163]

Tumors from patients with HNPCC display an unusual DNA pattern, termed *microsatellite instability*, which was identified as changes (both increases and decreases) in the length of repetitive sequences spread throughout the genome.[164] This pattern suggested that the tumor cell is mismatch repair (MMR) deficient and deleterious mutations in one of four different MMR genes, *MSH2*, *MLH1*, *MSH6*, and *PMS2*, were found to be causative for HNPCC (reviewed by Bocker[164]).

Analysis of 25 children younger than 18 years presenting with colorectal carcinoma demonstrated a pattern of colon and uterine cancer in relatives suggestive of HNPCC.[165] A more recent molecular study of patients in a population-based registry found that for those presenting under age 24, more than 75% had microsatellite instability and 50% of those with available germline DNA have documented HNPCC mutations.[166] Thus, mutation in one of the MMR genes (either heterozygous or biallelic) is the predominant cause of childhood and very young adult onset of colon cancer.

Turcot and Mismatch Repair Deficiency Syndrome

Turcot syndrome was first reported as the unusual finding of multiple pediatric brain tumors in families with polyposis and colon cancer.[167] In one study of 14 Turcot syndrome families, there were mutations in *APC* (10 families) or HNPCC loci (4 families).[168] In the families with APC-related mutations, there were more medulloblastomas (92-fold relative risk compared with the general population), and three families with glioblastoma multiforme had microsatellite instability in their tumor specimens, as did the original family studied

by Turcot. Two of these families had detectable mutations in the MMR genes *MLH1* and *PMS2*. Thus, the clinical phenotype of these disorders should be enlarged to include pediatric brain tumors. Conversely, careful attention should be paid to a history of colon cancer in relatives of pediatric brain tumor patients.

There is also a third form of Turcot syndrome with autosomal recessive inheritance demonstrating childhood onset of brain tumors (predominantly gliomas), hematopoietic cancers with T cell predominance, and colon cancer. The affected children in these families carry biallelic mutations in one of the MMR genes (reviewed by Wimmer and Etzler[169]). The condition is now referred to as *mismatch repair deficiency syndrome* given that biallelic mutations result in the absence of MMR function and genetic instability in all tissues. These children show a neurofibromatosis-like phenotype (*café au lait* spots and axillary freckling) thought to be due to somatic mutations in the *NF1* gene.[170] Clearly, the identification of this syndrome highlights the need for a careful skin examination in children being diagnosed with gliomas and T cell leukemia/lymphoma. If molecular testing confirms the diagnosis of MMR deficiency, then surveillance by colonoscopy should begin in early childhood.

Table 2.5 Diagnostic Criteria for Neurofibromatosis Type 1 (NF1)

The diagnosis is confirmed if the patient has two or more of the following features:

- Six or more café-au-lait spots
  - 1.5 cm or larger in postpubertal individuals
  - 0.5 cm or larger in prepubertal individuals
- Two or more neurofibromas of any type
- One or more plexiform neurofibromas
- Freckling of armpits or groin
- Optic glioma (tumor of the optic pathway)
- Two or more Lisch nodules (benign iris hamartomas)
- A distinctive bony lesion
  - Dysplasia of the sphenoid bone
  - Dysplasia or thinning of long bone cortex
- First-degree relative with NF1

From Gutmann DH, Aylsworth A, Carey JC, et al. The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. JAMA 1997;278:51–57, with permission.

The Phakomatoses

The word *phakomatosis* refers to multiple phacomas (Greek for tumor of the lens) and mato (Greek for spot or spotty) that refers to the patchy nature of these disorders. Although these disorders share many features of the other autosomal dominant disorders, their frequency in the pediatric population and their pleomorphic symptoms deserve additional comment.

Neurofibromatosis Type 1

NF1 is one of the most common genetic disorders in the general population (reviewed by Gutmann and colleagues[171]). Approximately 1 in 2,500 people is affected by this disorder. Table 2.5 lists the diagnostic criteria for *NF1* that were formulated at National Institutes of Health conferences in 1988 and 1997.[171] Many of the

criteria, including *café au lait* spots, axillary freckling, and neurofibromas, are detectable by general physical examination. Lisch nodules of the iris, which do not impact vision, are particularly useful in diagnosing NF1 in older children and adults. A careful slit lamp examination reveals Lisch nodules in more than 80% of adults older than 20 years with NF1.[172]

The hallmark of NF1 is the development of benign tumors, including peripheral neurofibromas, plexiform neurofibromas, gliomas of the optic tract, other low-grade gliomas, and pheochromocytomas. The peripheral neurofibromas often do not begin to develop until adolescence and rarely cause significant cosmetic problems until adulthood.[173] In contrast, plexiform neurofibromas are believed to be congenital in nature and can develop within the first few years of life.[174] Plexiform neurofibromas develop most commonly in the craniofacial and paraspinous regions, mediastinum, and retroperitoneum.[173],[175] They are deep masses that can be covered by hyperpigmented skin. They can be invasive and can cause significant disability, depending on the structures they invade. Malignant transformation of a plexiform neurofibroma is discussed later. There is no satisfactory treatment for plexiform neurofibromas; partial resection is used if they become too disabling or invade the spinal tract. Clinical studies to determine the efficacy of farnesyl transferase inhibitors in the treatment of plexiform neurofibroma are under way (reviewed by Widemann[176]). Studies of a mouse model of plexiform neurofibromas revealed infiltration of activated mast cells that are sensitive to inhibitors of the c-Kit pathway, such as imatinib.[177],[178] Trials of imatinib in patients with NF1 and symptomatic plexiform neurofibromas are under way.

Development of gliomas, especially involving the optic tract, is frequent in young children with NF1. Approximately 15% to 20% of children with NF1 have some optic tract involvement when assayed by MRI or computed tomography scanning.[179] About one-third of these children have lesions that grow large enough to interfere with vision. Conversely, a large percentage (30%–70%) of children with a new finding of optic glioma have NF1.[180] Because of the difficulty in detecting visual changes in young children, MRI of the brain and optic pathway is often performed for a young child with NF1.[181] However, performing scans in asymptomatic children is controversial.[182] If a child does not show any sign of optic pathway involvement by age 6, the prognosis for lack of eye involvement is excellent.[183] Treatment of enlarging optic tract gliomas is described in subsequent chapters. Although treatment guidelines are controversial for patients with NF1, several large series demonstrate that NF1-associated optic gliomas have a more favorable course over long-term follow-up.[184] Children with NF1 are more likely to demonstrate cerebrovascular dysplasia, which should be taken into account when making treatment decisions.[185]

Gliomas can also develop in other parts of the CNS, ranging from very low-grade to high-grade tumors. Indications for imaging include change in headache pattern, seizures, and new neurologic deficits. In several small studies, the presence of an optic glioma in childhood may predispose the person to the later development of other gliomas.[186]

Because NF1 is a common disease, cases of NF1 and malignancy are likely to happen coincidentally. The clearest associations between NF1 and pediatric malignancies are the increased risk of optic gliomas and malignant peripheral nerve sheath tumors (MPNST).[187],[188] A large population study of 26,084 children younger than 15 years from Japan revealed a 6- to 8-fold increased incidence of cancer, in particular, gliomas, MPNST, RMSs, and myelogenous leukemia in NF1 patients compared with the non-NF1 patients.[189] In particular, 50% of the patients with MPNST had NF1, a percentage similar to that found in a large Dutch study.[190] In one study, survival among patients with NF1 and MPNST was worse than those with sporadic tumors (33% and 63%, respectively).[191]

The likelihood of a child with NF1 developing MPNST has varied among series with a population-based study from the United Kingdom, demonstrating as high as 8% to 13% lifetime risk.[192] For this reason, physicians, parents, and patients should be particularly concerned and seek prompt evaluation for malignant transformation in a NF1 patient with a plexiform neurofibroma that demonstrates a significant change in growth rate or pain. Although less common than children with MPNST, children with NF1 also have an increased risk of developing GI stromal tumors.[193]

Children with NF1 have an increased risk of several myelogenous disorders, including AML and MDS.[194],[195] Moreover, bone marrow from children with NF1 and malignant myeloid disorders shows a loss of the normal *NF1* gene in the malignant cells.[196] Thus, *NF1* appears to be a tumor suppressor gene with regard to malignant myeloid disease.

The gene *NF1*, found at 17q11.2, is a large gene with mutations spread throughout.[197] The *NF1* gene encodes a protein, neurofibronin, which is homologous to the GTPase-activating protein called Gap. This relationship suggests that the NF1 protein normally inhibits the activity of the Ras protein (an oncogene). NF1 follows the two-hit hypothesis in that tumors associated with *NF1*, such as pheochromocytomas,[187],[198] show a loss of the remaining normal copy of the *NF1* gene.

Practical molecular testing for *NF1* mutations with detection of more than 95% of NF1 patients is now available clinically.[197] Because of the high *de novo* mutation rate in NF1, most individuals have different mutations, called *private mutations*, scattered throughout a very large gene. Although most patients are diagnosed on the basis of clinical criteria (Table 2.5), molecular testing is useful in some clinical situations. The first is affected adults requesting prenatal diagnosis, which requires knowing their specific mutation. The second is apparently unaffected parents of affected children who are concerned about recurrence risk. Documenting a negative mutation in the parents lowers their risk of having a second child with NF1, although negative skin and eye examinations can already make this likelihood low. A third clinical scenario relevant to pediatricians is a child with a negative family history and multiple *café au lait* spots with or without axillary freckling. The majority of these children will eventually be diagnosed with NF1. However, several groups have recently identified that mutations in *SPRED1* result in Legius syndrome, which demonstrates clinical overlap with NF1, including *café au lait* spots with variable expression of axillary freckling, macrocephaly, and learning disabilities.[199],[200] Importantly, these individuals do not demonstrate neurofibromas or CNS tumors. Thus, molecular confirmation of the diagnosis of NF1 versus Legius syndrome by genetic testing is recommended in children with *café au lait* spots and axillary freckling as their only diagnostic criterion. A positive molecular diagnostic study provides the correct diagnosis and the appropriate surveillance.

Neurofibromatosis Type 2

Neurofibromatosis type 2 (NF2) represents a distinct and much rarer disorder than NF1. Because most of the manifestations of NF2 occur in adulthood, they are not discussed in detail here. NF2 is characterized by *café au lait* spots, bilateral vestibular schwannomas, central neurofibromas, and meningiomas (reviewed by Gutmann et al[172]). The disease has a high degree of morbidity and is difficult to treat because of the multiple tumors that develop. Treatment modalities include microsurgery, radiosurgery, and radiation therapy. The gene that is mutated in NF2, also called *NF2*, is found on chromosome 22 and encodes a protein, called *merlin* or *schwannomin*, that is homologous to the band 4.1 protein and appears to play a role in cytoskeletal architecture.[201]

Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is diagnosed clinically on the basis of the characteristic features including benign and neoplastic growths. The classic triad of seizures, mental retardation, and facial angiofibromas (previously called *acne sebaceum*) occur in fewer than 50% of patients with TSC.[202] There is a wide range of phenotypes between and within families, with some adults with TSC having very high degrees of intelligence. Two-thirds of patients are affected due to *de novo* mutations and thus do not have a family history of the disease. Part of the clinical heterogeneity results from the underlying genetic heterogeneity, with causative mutations in two different genes, *TSC1* and *TSC2*, both of which acts as a tumor suppressor gene (reviewed by Kwiatkowski and Manning[203]). *TSC1* is located at chromosome 9q34 and encodes hamartin. *TSC2*, on chromosome 16p13.3, encodes tuberin, which has Rag1-Gap activity. Hamartin and tuberin can physically interact. Thus, the protein products of genes mutated in both NF1 and TSC participate in the regulation of Ras or Ras-related GTPase activity, which may respond to inhibitors of the mTor pathway including rapamycin.[203] A comprehensive analysis of mutations in *TSC1* and *TSC2* in 150 TSC patients revealed 120 mutations, 22 in *TSC1* and 98 in *TSC2*.[204] The majority of *TSC1* mutations are truncating, while for *TSC2*, there are both missense mutations in conserved domains and truncating mutations. Clinically, the degree of mental disability was greater for patients with *TSC2* mutations (67% vs. 31%). It is also not unusual for the first person in the family with TSC to have a milder phenotype due to mosaicism for a TSC mutation. The offspring of this individual may be more severely affected as the child will inherit the mutation in all somatic cells.[205]

TSC is characterized by the growth of normally benign tumors in several different organs. Cardiac rhabdomyomas normally develop *in utero* and are often detected during prenatal ultrasound. The morbidity and mortality associated with these tumors reflect the potential for flow abnormalities in the heart if these tumors grow large enough. They typically regress postnatally and become clinically insignificant.[206] In one study, 50% of children with cardiac rhabdomyomas developed clinical criteria for TSC during childhood.[207]

Later in childhood and early adulthood, individuals with TSC are at risk for the development of giant cell astrocytomas.[202] During adulthood, there is often the slow growth of renal angiomyolipomas. In the British population-based study of childhood cancer,[1] TSC was found to be significantly overrepresented due to an increased risk of brain tumors and RMSs.[208] The TSC consensus conference made specific recommendations for the diagnosis and surveillance of children with TSC including periodic MRI of the brain and renal ultrasounds.[209]

Nevus Basal Cell Carcinoma Syndrome or Gorlin-Goltz Syndrome

Gorlin and Goltz described a number of individuals who had multiple nevoid basal cell epithelioma, odontogenic jaw cysts, and bifid ribs, with the syndrome being inherited in an autosomal dominant fashion.[210] Gorlin-Goltz syndrome or Nevus basal cell carcinoma syndrome (NBCCS) includes the aforementioned features and characteristic palmar and plantar pits, mild facial dysmorphisms including frontal and biparietal bossing, calcification of the falx cerebri, and short fourth metacarpal bones (reviewed by Gorlin[211]). Careful clinical examination and radiographs of ribs, skull, and spine are often sufficient to make the diagnosis. The basal cell carcinomas (BCCs) develop around the time of puberty and can eventually number in the hundreds. There are differences in the number of BCCs in different racial groups, with significantly fewer found in individuals of African American descent.[212] It is estimated that 29% of individuals with a BCC under age 18 have NBCCS syndrome.

Medulloblastoma is a significant feature of NBCCS. Analysis of 105 patients with NBCCS evaluated at National Institutes of Health found four children with the diagnosis of medulloblastoma diagnosed at a mean age of 2.3

years.[212] Conversely, it is estimated that approximately 10% of patients with medulloblastoma diagnosed at age 2 years or under have NBCCS.[213] Because of the high frequency of medulloblastoma, children with NBCCS are recommended to have biannual neurologic examinations and annual MRI examinations up to age 7 for early detection of medulloblastoma.[212] Examination of the parents of medulloblastoma patients may aid in identifying NBCCS in the family.

In children receiving radiation therapy, the skin within the field can become severely affected with hundreds of nevi and BCCs with a latency of approximately 5 years.[212] There have also been reports of secondary meningiomas and ependymomas in the radiation-exposed field of children with NBCCS.[212,214] Thus, use of radiation therapy for treatment of tumors in NBCCS syndrome should be limited when possible.

The gene for NBCCS syndrome, *PTCH*, encodes a homologue of the *Drosophila melanogaster* segment polarity gene.[215,216] Mutations in *PTCH* are found in the majority of NBCCS families and in a large percentage of sporadic BCCs, making it one of the most frequently mutated genes in human cancers.[217] In contrast, analysis of sporadic medulloblastomas has identified *PTCH* mutations in only approximately 10% of cases and rare mutations in other members of the same pathway.[218] Mutations in the *SUFU* gene, another member of the *PTCH* pathway, underlay familial medulloblastomas in the absence or presence of other features of NBCCS.[219,220]

Von Hippel-Lindau Disease

The hallmark of von Hippel-Lindau (VHL) disease is the development of multiple benign and highly malignant tumors in the absence of specific dermatologic or developmental abnormalities. Diagnosis typically occurs during second or third decade, when tumors become clinically apparent. VHL disease is characterized by four common tumor types: cerebellar hemangioblastomas, retinal angiomas, renal cell carcinoma, and pheochromocytomas.[221] Affected individuals also have increased rates of pancreatic carcinoma, epididymal cysts, and endolymphatic sac tumors (ELSTs), which can result in hearing loss.[222] The two leading causes of early mortality are cerebellar lesions and renal cell carcinomas.[221]

The retinal and cerebellar lesions typically develop during the second and third decade of life although they can occur in the first decade and should prompt genetic and clinical evaluation for VHL-associated tumors.[223,224] MRI of both the brain and the spinal cord can reveal the presence of isolated or multiple lesions with signal intensities characteristic of a hemangioblastoma. Multiple cerebellar hemangioblastomas or a first-degree relative with VHL disease and an isolated lesion is sufficient for the diagnosis of VHL disease.

VHL disease is classified into subcategories, depending on the patients' likelihood of developing pheochromocytoma. Type 1 patients have a low risk of developing pheochromocytoma but a high risk of developing RCC. Type 2 patients have a high risk of developing pheochromocytoma, with type 2A patients having an additional lower risk of developing RCC, whereas type 2B patients possess a high risk of developing clear cell RCC. Types 1, 2A, and 2B patients also develop cerebellar and retinal hemangioblastomas. Type 2C patients develop only pheochromocytoma (reviewed by Lonser[221]).

Retinal angiomas can often be asymptomatic and diagnosed on yearly dilated eye examinations. If sufficient in size, they can manifest with new visual defects. Treatment of the retinal lesions at an early stage can yield excellent long-term results.[223] Renal cysts accompanied by renal cell carcinoma are one of the hallmarks of the VHL syndrome. The tumors often develop in the third or fourth decade, but the risk of renal cell carcinoma is lifelong.[225] It is necessary to balance curative intent, tumor removal, maintenance of renal function, potential for

transplantation, and the knowledge that the patient is likely to develop other tumors when creating a treatment plan.[221] In particular, the approach to renal cell carcinoma with the avoidance of nephrectomy in VHL patients is designed to preserve as much renal function as possible.[225]

Pheochromocytoma as part of VHL disease can be singular or multiple and may be benign or malignant. They are most likely diagnosed in the second or third decade but can present earlier, and screening for pheochromocytoma is recommended to begin from age 2.

Given the predilection in VHL disease to develop a specific group of tumors, several comprehensive screening protocols have been developed (www.vhl.org).[221,226] The important features are annual surveillance examinations for renal masses, pheochromocytoma, and retinal angiomas, with biannual examination for cerebellar lesions. Screening for pheochromocytoma is improved by use of plasma metanephrines[227] as opposed to urine catecholamines. Any change in hearing or balance or tinnitus should prompt evaluation for an ELST, including computed tomography imaging of the inner auditory canal.[222]

The *VHL* gene, at chromosome 3q25, was cloned in 1994 by using positional methods.[228] The Type 2 VHL families with pheochromocytoma risk tend to have clustering of missense mutations in specific codons.[229] DNA diagnostic assays have been optimized such that more than 98% of patients with VHL disease have a detectable mutation in the *VHL* gene.[230] Testing is used to identify relatives who have not inherited a *VHL* mutation and do not require a surveillance protocol and those who have inherited the mutation and need full screening prior to development of malignancy.

The VHL protein is part of an E3 ubiquitin ligase complex that targets a hypoxia-inducible transcription factor (HIF1α) for ubiquitin-mediated destruction selectively in the presence of oxygen.[231,232] The loss of VHL function results in the overexpression of genes required for angiogenesis under normal oxygen conditions. This has led to clinical trials of thalidomide and antiangiogenesis agents to inhibit tumor growth in patients with VHL.[233,234]

Pheochromocytomas and Paragangliomas

Benign and malignant pheochromocytomas can cause significant morbidity due to the secretion of active catecholamines including norepinephrine, epinephrines, and metanephrines. They typically present in adulthood but can be seen in children, particularly when associated with a genetic predisposition syndrome. As described in this chapter, inherited syndromes associated with a significant risk of pheochromocytoma include VHL, NF1, and MEN2. Extensive mutation analysis of the *RET* and *VHL* genes in case series of patients with pheochromocytoma (both adults and children) suggested that 20% to 30% of patients carry a constitutional mutation in one of these genes.[235] More recent analyses that include *VHL*, *RET*, *SDHD*, and *SDHB* genes (described later) further confirm a high prevalence of 30% to 40% of constitutional mutations.[6,236] Therefore, it is recommended that all patients with pheochromocytoma or paraganglioma should have a genetic evaluation including testing to determine the patient's risk for other tumors (including recurrent pheochromocytoma) and to identify other family members who may be at risk for pheochromocytoma and associated cancers.

Familial Paraganglioma

Paragangliomas arise from chemoreceptor organs distributed throughout the body and are referred to as *glomus tumors*, *chemodectomas*, and *carotid body tumors*. Familial paragangliomas may occur either unilaterally or bilaterally and are transmitted with autosomal dominant inheritance with incomplete penetrance and both intra- and inter-familial variability.[237] Four loci, initially named *PGL1-4*, have been linked to hereditary paraganglioma. Three of the genes, *SDHD (PGL1)*, *SDHC (PGL3)*, and *SDHB (PGL4)* encode subunits of the mitochondrial

enzyme II complex (succinate dehydrogenase).[237] This enzyme complex plays a key role in the oxygen-sensing system of paraganglionic tissue.[238] For example, chronic hypoxic stimulation at high altitude has a modifying effect on the development of carotid body paragangliomas due to *SDHD* mutations.[239] Interestingly, even though the SDH genes all function

to encode succinate dehydrogenase subunits, only *SDHD*[237] demonstrates maternal imprinting such that children who inherit a *SDHD* mutation from their father but not from their mother develop paragangliomas. Germline mutations in SDH genes account for 6% and 9% of apparently sporadic paraganglioma and pheochromocytomas, respectively, 29% of pediatric cases, 38% of malignant tumors, and more than 80% of familial aggregations of these tumors.[237] Mutations in one family in the recently identified *SDH5* gene indicate that more genes may yet be implicated in this broad cancer phenotype.[240] Mutations in *SDHD* primarily predispose to mostly benign head and neck paragangliomas[238] and mutations in *SDHB* predispose to abdominal paragangliomas (pheochromocytomas) that may be malignant.[241] The complex inheritance pattern associated with imprinted disorders mandate that genetic evaluation and recurrence risk estimates be performed by a clinician with experience interpreting this pattern of inheritance.

Autosomal Recessive Disorders

This last category of genetic disorders that predispose to cancer has distinct characteristics when compared with the autosomal dominant disorders. Autosomal recessive disorders are much rarer in the general population. Specific ethnic or geographic groups may have an increased risk of autosomal recessive disorders because of a founder effect or increased prevalence of consanguinity. Given the requirement for two mutant alleles, these disorders normally occur in sibships and are not evident in multiple generations. Within a sibship, there is only a one in four chance that a sibling will have the disorder. For this reason, single affected individuals from a small family may appear to be a sporadic case and physicians should not discount the potential for an autosomal recessive condition in a child with a negative family history. Even more complicated, some genetic disorders such as dyskeratosis congenita (associated with bone marrow failure in childhood and adolescent or early adult onset of cancer) can be inherited as autosomal dominant, autosomal recessive, or X-linked disorder, depending on the underlying gene involved.[242]

Generally, the range of expressivity in an autosomal recessive disease may be more limited and the symptoms often more severe than in autosomal dominant disorders. Most of these disorders manifest in childhood, presumably because of the severe nature of the genetic defect. Many of the autosomal recessive cancer syndromes are caused by mutations in genes that encode DNA repair enzymes or DNA damage checkpoint genes, and they are often referred to as *chromosome breakage syndromes* or *chromosome instability syndromes*. These deficiencies result in increased sensitivity to spontaneous and exogenous DNA damage, which may impact treatment decisions. Significant progress has been made identifying the genes mutated in these disorders. We describe three classes of autosomal recessive disorders later as examples of the severe early-onset presentation and multiple different clinical features of this group of disorders.

Xeroderma Pigmentosum, Cockayne Syndrome, and Trichothiodystrophy

Xeroderma pigmentosum (XP) represents the classic DNA repair defect syndrome. The clinical features of this disorder have been extensively reviewed by Kraemer and colleagues.[243],[244] Patients present with cutaneous sensitivity, as revealed by photosensitivity, telangiectasias, and freckling in the first few years of life. Ocular abnormalities are common and are found in ultraviolet light–exposed areas of the cornea, lids, and conjunctivas, including corneal clouding and ocular malignancies. There is a several thousandfold increased risk of basal and

squamous cell skin carcinomas in sun-exposed areas, which begin developing around 8 years of age (Fig. 2.4), compared with around the age of 50 for the general U.S. population. There is also a significant increase in melanoma (approximately 5% lifetime risk), and significant but smaller increase in the risk of internal malignancies has also been observed for XP patients.[243]

At the cellular level, the ultraviolet sensitivity in XP patients was found to result from defects in excision repair.[245] This form of repair is essential for repair of the thymine dimers and other structures that results from ultraviolet damage. XP is a group of disorders caused by mutations in at least seven different genes.[246] The determination that multiple genes caused the same clinical disorder was based on complementation assays, in which fibroblasts from different patients are fused together and the heterokaryon cell is then assayed for complementation of the repair defect (reviewed in Bootsma and Hoeijmakers[247]).

Some XP patients have neurologic abnormalities. One group, first reported by DeSanctis and Cacchione,[248] has XP-like dermatologic features and progressive neurologic degeneration beginning around the age of 2 years and accompanied by immature sexual development.[248] These patients tend to cluster in complementation group A. Overall, Kraemer and colleagues[244] found that 18% of reported XP patients had neurologic

abnormalities, some of which resemble the DeSanctis-Cacchione syndrome and others that have a later onset of neurologic difficulties and that cluster in complementation group D.

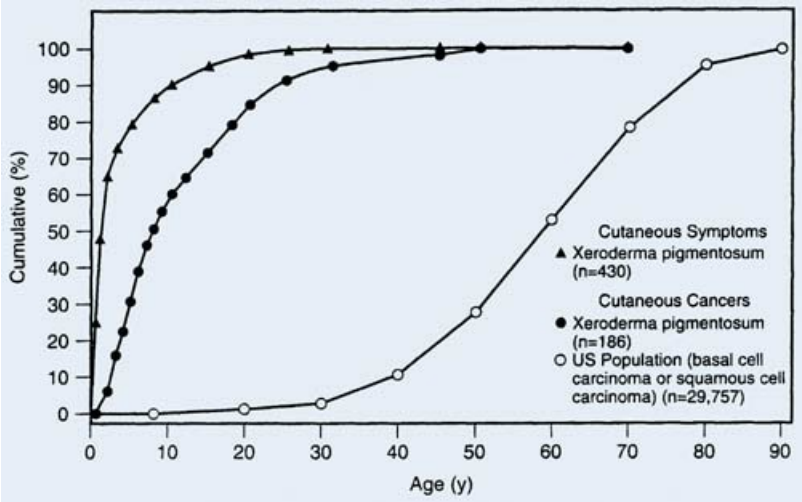

Figure 2.4 Age of onset of xeroderma pigmentosum symptoms. The ages at onset of cutaneous symptoms (generally sun sensitivity or pigmentation) was reported for 430 patients. The age at diagnosis of the first skin cancer was reported for 186 patients and is compared with distribution for 29,757 patients with basal cell carcinoma and squamous cell carcinoma in the U.S. general population. (From Kraemer, Myung L, Scotto J. Xeroderma pigmentosum. Arch Dermol 1987;123:241, with permission.)

Two other disorders can manifest with findings of XP. Trichothiodystrophy (TTD) is a rare disorder that shares an increased risk of skin cancer and repair defects and the findings of brittle hair and ichthyosis. The XP/TTD patients fall in the XP complementation group D.[246] Cockayne syndrome shares some of the ultraviolet hypersensitivity of XP but is also characterized by neurologic deficits, including developmental delay without skin cancer risk. There are at least three complementation groups for Cockayne syndrome.[249]

Utilizing the specific biochemical defects in cells from patients with either XP or Cockayne syndrome, almost all of the genes responsible for this condition have been cloned (reviewed by Cleaver and colleagues[250]). In a normal cell, DNA damage in actively transcribed genes is preferentially repaired before DNA from inactive parts of the

genome, a process termed *transcription-coupled repair* (TCR). Cells from patients with Cockayne syndrome are deficient in TCR due to a mutation in the *ERCC2* DNA helicase, which is also a component of active transcription complex TFIIH. The fact that global repair rates are normal in Cockayne cells may explain the lack of cancer predisposition.

A third group of patients who clinically demonstrate ultraviolet sensitivity but have normal nucleotide excision repair *in vitro* are termed *XP^{V}*. This disorder is due to mutation of a specialized DNA polymerase, polymerase eta, which places two adenine residues opposite a thymine dimer photoproduct, thus restoring the normal base sequence.[251,252]

The Helicase Disorders: Bloom, Werner, and Rothmund-Thomson Syndromes

Three autosomal recessive disorders, although distinct, share some clinical features including a predisposition to malignancy (Table 2.6). Children with Bloom syndrome are very small at birth and remain small[253] and have a photosensitive rash, immunodeficiency, and a very high predisposition to develop a wide variety of malignancies including leukemias/lymphomas and solid tumors.[254] This disorder is more common in children of Ashkenazi descent. Cells from these patients exhibit increased recombination manifested as increased sister chromatid exchange. Werner syndrome is characterized by premature aging (including early-onset atherosclerosis, diabetes, and cataracts beginning in the second decade) with increased incidence of soft tissue sarcomas.[255] The premature aging is manifested at a cellular level as early senescence in fibroblasts from these patients. The third disorder in this group, Rothmund-Thomson syndrome (RTS), is characterized by a very distinct rash termed *poikiloderma* (Fig. 2.5), which begins in infancy, and skeletal dysplasias including radial ray abnormalities and

cataracts. Children with RTS have a distinct predisposition to the development of osteosarcoma and less frequently skin cancers.[256] Osteosarcoma occurring in individuals with RTS has a similar histologic spectrum and treatment response as that seen in the general population.[257]

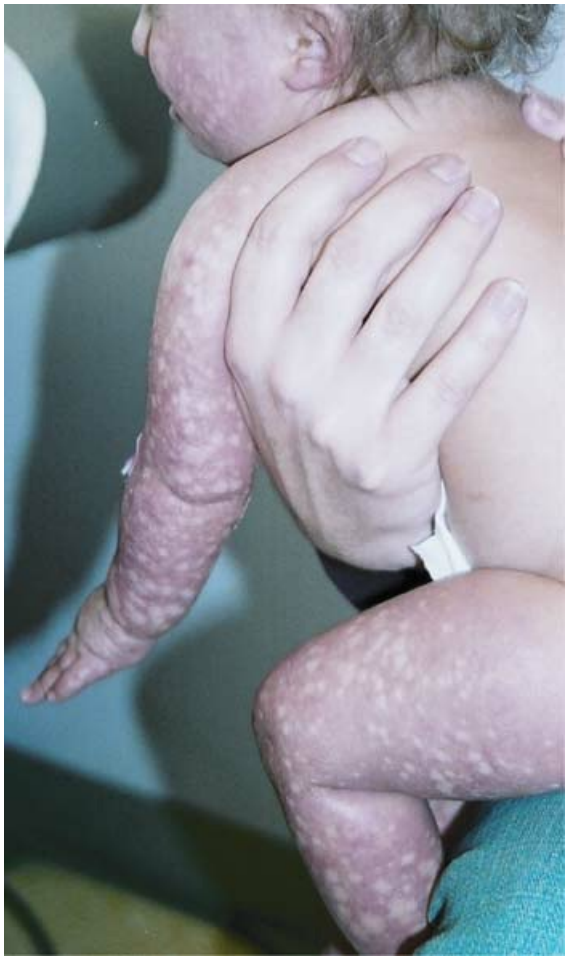

Figure 2.5 Poikiloderma rash in Rothmund-Thomson syndrome. Shown is the typical poikilodermatous rash, which begins on the cheeks and spreads to the extremities, sparing the trunk of a child with RTS who carries two deleterious mutations in the *RECQL4* gene. (Photograph courtesy of L.L. Wang, MD, Baylor College of Medicine/Texas Children's Hospital.)

Table 2.6 Features of the Chromosome Instability Syndromes Due to Mutations in Genes Encoding RECQ Helicases

Syndrome	Clinical features	Cancer predisposition	Gene/chromosome location
Bloom	Small stature, photosensitive rash, immunodeficiency	Multiple tumor types including leukemia/lymphoma and solid tumors	*BLM* 15q26.1
Werner	Premature ageing, cataracts, diabetes, atherosclerosis	Soft tissue sarcomas and skin cancers	*WRN* 8p11
Rothmund-Thomson	Poikiloderma rash, radial ray defects, cataracts	Osteosarcoma and skin cancers	*RECQL4* 8q24.3[a]

[a]Although *BLM* and *WRN* are the genes mutated in the majority of Bloom and Werner syndrome patients, respectively, approximately 60% of patients with Rothmund-Thomson syndrome carry mutations in the *RECQL4* gene. The risk of osteosarcoma is found in the subset of patients with *RECQL4* mutations.

From Wang LL, Gannavarapu A, Kozinetz CA, et al. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. J Natl Cancer Inst 2003;95:669–674.

Table 2.7 Cancer Genetic Disorders that Require Modified Treatment Regimens

Condition	Major clinical features	Diagnostic test	Treatment requiring adjustment
Ataxia-telangiectasia	Cerebella ataxia, telangiectasias, immunodeficiency, leukemias, lymphomas and solid tumors	Increased α-fetoprotein, sensitivity to ionizing radiation	Radiation therapy, chemotherapeutic agents that produce double strand breaks
Nijmegen breakage syndrome	Microcephaly, immunodeficiency, developmental delay, lymphomas	Sensitivity to ionizing radiation, Polish founder mutation in NBS1	Radiation therapy, chemotherapeutic agents that produce double strand breaks
Ligase IV deficiency	Microcephaly, immunodeficiency, anemia, developmental delay, lymphomas	Sensitivity to ionizing radiation	Radiation therapy, chemotherapeutic agents that produce double strand breaks
Fanconi anemia	Bone marrow failure, radial ray anomalies, microphthalmia, renal anomalies, bronzing of the skin	Chromosome breakage assay after exposure to diepoxybutane	Specialized conditioning regimen prior to bone marrow transplant, sensitivity to cross-linking agents
Bloom syndrome	Short stature, butterfly rash on face, GI intolerance, immunodeficiency	Increased sister chromatid exchange	Some evidence for increased toxicity to chemotherapeutic agents
Gorlin syndrome	Palmar pits, calcification of the falx, odontogenic cysts, basal cell carcinomas, medulloblastoma	Mutation analysis of the *PTCH* gene	Radiation therapy causes development of large numbers of BCC in radiation field

GI, gastrointestinal; BCC, basal cell carcinoma.

All three disorders have been shown to be the result of mutations in RecQ helicase genes which are conserved to bacteria: the *BLM* gene in Bloom syndrome,[258] the *WRN* gene in Werner syndrome,[259] and the *RECQL4* gene in a subset of patients with RTS.[260] Analysis of *RECQL4* mutations in a cohort of RTS patients reveals that there are two types of RTSs. Type 1 RTS (approximately 30% of patients) is not associated with *RECQL4* mutations and does not appear to have an increased risk of osteosarcoma. Type 2 RTS is associated with

deleterious mutations in *RECQL4*, with skeletal abnormalities, and with significant osteosarcoma risk.[261,262] Genetic testing for *RECQL4* mutations is now clinically available and will identify those RTS patients at increased risk for developing osteosarcoma.

Ataxia-Telangiectasia

Children with ataxia-telangiectasia (AT) develop ataxia during early years of childhood, with truncal ataxia appearing before appendicular ataxia and eventually requiring a wheelchair for mobility (reviewed by Gatti and colleagues[263]). Choreoathetosis and ocular motor apraxia are also common neurologic findings. Intelligence does not appear to be affected. The oculocutaneous telangiectasias normally begin with the conjunctivas and develop between the ages of 3 and 5 years. Useful biochemical markers for diagnosis include elevated AFP and carcinoembryonic antigen in children with AT.[264]

There is a very high rate of malignancy, particularly the development of leukemias and lymphomas,[265] in AT children. Individuals with AT have immunodeficiency characterized by diminished immunoglobulin G2 and A levels and increased risk of sinopulmonary infections. The major causes of mortality of those with this syndrome are sinopulmonary infection (especially after significant neurologic degeneration) and malignancy (reviewed by Gatti and colleagues[263]).

In addition to the risk of cancer in the AT homozygous children, heterozygotes carrying one AT mutation appear to have approximately a twofold increased risk of breast cancer.[266] Mothers of children with ATM (a gene mutated in AT) should be advised of this moderate increased risk of breast cancer.

Fibroblasts and lymphocytes from AT patients have multiple cellular defects including increased sensitivity to DNA-damaging agents, particularly ionizing radiation due to defects in DNA repair and cell cycle checkpoints (reviewed by Shiloh[267]). The gene mutated in AT (called *ATM*) was cloned in 1995.[268] After DNA damage, the ATM protein signals through the p53 and BRCA1 tumor suppressor gene products.[269] The finding of ATM in the same molecular pathway as other breast cancer genes further substantiates the epidemiologic data with regard to breast cancer predisposition in heterozygotes.

Children with AT have significantly increased sensitivity to chemotherapy and radiation treatments. Specific treatment regimens have been developed for these children.[270] Specialized clinical centers that are familiar with recommended regimens for these unique children are available through the support of the A-T Children's Project (www.atcp.org). Table 2.7 provides a list of cancer susceptibility syndromes for which alteration in cancer treatment regimen needs to be considered.

Issues in Genetic Testing for the Pediatric Oncology Patient

Several studies have suggested that 4% to 10% of childhood cancers result from inherited genetic mutations, making it essential for pediatricians and pediatric oncologists to recognize clinical criteria suggestive of familial cancer syndromes.[1,271] Table 2.8 includes the cancer diagnoses that are frequently the result of genetic susceptibility for which genetics

evaluation should be considered regardless of family history. In addition, as discussed in this chapter, an accurate diagnosis requires an accurate and detailed family history, including all cancers and the age at which they are diagnosed. This is because many cancer predisposition syndromes lack an associated recognized phenotype to identify at-risk individuals. For example, although hemihypertrophy and other features of BWS raise the clinician's alertness to embryonal cancer risk, no known physical features are associated with LFS. It is increasingly important for the pediatric oncologist to recognize that children are part of a network of family

members who may be indirectly affected by genetic diagnosis and testing.

Table 2.8 Cancer Diagnoses that Merit a Genetics Evaluation Independent of Family History

Diagnosis	Genetic loci
Retinoblastoma	*RB1*
Adrenocortical carcinoma	*P53*
Pheochromocytoma/paraganglioma	*VHL, NF1, RET, SDHB, SDHD*
Retinal or cerebellar hemangioblastoma	*VHL*
Endolymphatic sac tumors	*VHL*
Hepatoblastoma/desmoid tumors	*APC*
Optic pathway tumor	*NF1*
Medullary thyroid cancer	*RET*
Atypical teratoid and malignant rhabdoid tumor	*SMARCB1/INI1/SNF5*
Acoustic or vestibular schwannomas	*NF2 [F 36] [F 37]*

In the practice of pediatrics, DNA-based tests for a large number of noncancer conditions, including cystic fibrosis, muscular dystrophy, and hemophilia, have been developed and are currently in use. It has been recommended that as children grow and acquire cognitive and moral skills, they should be permitted to participate in decisions concerning testing.[272] For genetic testing for conditions associated with childhood onset cancers, it is generally accepted that cancer predisposition testing is most helpful for highly penetrant diseases in which individuals at risk for cancer can be identified and followed closely for the development of highly specific tumors,[273] for example, VHL, FAP, and MEN2. For each of these, clear guidelines for clinical surveillance or prophylactic medical interventions in childhood have been established for mutation carriers as discussed in this chapter.

However, for a variety of other cancer-predisposition disorders, the clinical management of carriers is less well defined. Such diseases include LFS. Although predisposition testing may identify asymptomatic carriers, and allow institution of preventive or surveillance programs where available, such testing is associated with the following caveats that must be taken into consideration: (a) the genetic heterogeneity of cancer predisposition, (b) the technical difficulty inherent to gene testing and to test interpretation, and (c) the pyschosocial impact of testing. Both variable degrees of penetrance and expressivity for many conditions, including LFS, suggest that other genetic events play an important role in defining the particular cancer phenotype of individual members of families.

The technical aspects involved in predisposition gene testing and interpretation are complex. Some tests for rare

disorders are available only through participation in research studies where results are made less immediately available and confirmation of results is less well controlled than in clinically certified laboratories. Databases are now available to facilitate identification of clinical and research laboratories performing specific genetic tests, for example, www.genetests.org. Furthermore, such testing, particularly of novel genes, tends to be expensive, with different laboratories performing different assays with extra effort by the physician sometimes required to obtain insurance coverage of testing. Given the complexity, genetic testing should be undertaken only by a physician or genetic counselor aware of different testing options and fully capable of interpreting these results. For example, a significant percentage of physicians ordering a genetic test for FAP incorrectly interpreted a negative result in an affected proband.[274]

Genetic testing for any disease, which should now include cancer, has been demonstrated to have profound psychologic and emotional impact on patients and may be further complicated by relationships with parents and other family members.[275] Issues of the “vulnerable child syndrome” in affected carriers and “survivor guilt” in unaffected, noncarrier siblings raise complex psychosocial concerns that may be beyond the general purview of the pediatric oncologist. Furthermore, lessons from studies in adults have demonstrated that although overall patients learning of their increased risk of disease do well, they may experience feelings of shock, depression, grief, altered self-esteem, or even guilt. Limited studies in children, parents and families have yet to clarify the impact of predictive testing for cancer in children or the appropriate timing of testing with regard to cancer diagnosis.

A recent comprehensive study from France explores the perceptions of two groups of genetic services providers for the usage of prenatal diagnosis and preimplantation genetic diagnosis. As parents from cancer susceptibility families are now routinely discussing these options in planning future pregnancies, the need to engage a multidisciplinary team in these discussions is key to providing parents and families the necessary tools with which to make these ethically challenging decisions.[276,277,278]

In an attempt to address these issues, guidelines for testing have been established by both the American Society of Human Genetics in a statement and the American Society of Clinical Oncology.[151,279,280] These guidelines form a useful foundation on which to build practical testing parameters as better defined genotype:phenotype correlations are generated. While some studies suggest that the benefits to predictive genetic testing for children are still not substantial, further evaluations from different perspectives will continue to evolve in this field.[281]

Based on many of the aforementioned arguments, a number of recommendations established in 1992 for LFS[282] are still applicable to genetic testing in family cancer syndromes that include children. The quality of information provision on cancer genetics is directly related to the knowledge of professionals and their ability to communicate this to a patient and family regardless of their specialty.[283,284] This requirement exists in

the face of a relative lack of in-depth education in genetics in medical schools and postgraduate education, which then place pediatricians and pediatric oncologists in a difficult position of integrating rapidly evolving technologies with patient care and unfamiliar and complex genetic testing issues.[285] This unfamiliarity extends to more recent issues with respect to physicians' duty to warn “third parties” (i.e., members of extended families who may be at risk of avoidable harm from a genetically transmissible condition), and its legal ramifications.[286] Therefore, it is incumbent upon pediatric oncologists without additional training to identify appropriate patients and families for referral to a geneticist or genetic counselor with training in cancer genetics. Recently, the multidisciplinary approach taken by several groups[277,287] involving pediatric oncologists, clinical geneticists, genetic counselors, psychologists, and ethicists in establishing cancer genetics clinics and programs whose primary focus is to serve children with cancer and their families provides an intriguing and novel mechanism to

optimize care of these families and advance our understanding of the role of genetics in the etiology of childhood cancer.

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