2.1 Introduction

From an epidemiologic perspective, retinoblastoma is one of the most interesting childhood tumors to study. Retinoblastoma is a primitive neuroectodermal tumor, and its occurrence in early childhood suggests that incidence can be associated with events affecting development of neuroectodermal tissues during the fetal period. Furthermore, it exists in two genetically distinct forms associated with differing (though not mutually exclusive) clinical presentations (see chapters 3 and 4). This allows the formulation of two distinct, though parallel, mechanisms for disease development. Additionally, there is considerable geographic variation in incidence, suggesting differential genetic susceptibility or environmental exposure(s). The combination of these three factors: a defined and relatively limited temporal window for development; two genetically distinct forms arriving, via different genetic pathways, to essentially identical histologic presentations; and the geographic variation in incidence suggest several handles/angles through which one could examine associations and risk factors for development of this disease. Despite these facts, and due in large part to the rarity of the disease, little is understood about the factors that culminate in the relatively well understood cell cycle and apoptotic pathway defects that define retinoblastoma at a molecular level.

Much has been published regarding the molecular changes that occur during the development of retinoblastoma, and recently, more reports have become available regarding incidence, survival, and treatment in countries outside of northern North America and Europe. Results from these studies point to some potential risk factors underlying disease development;
Chapter 2

Epidemiology

however, few studies have been done to specifically elucidate these factors. Thus, this discussion of the epidemiology of retinoblastoma will, out of necessity, focus on disease incidence and on those few hypotheses that have been explored using population-based study methodology.

2.2 Incidence

2.2.1 Population Differences in Incidence

Global incidence data for retinoblastoma show an approximate 50-fold variation, which is highly atypical for a pediatric tumor. This degree of variation is comparable to that seen in adult malignancy, such as cervical, gastric, and colon cancer, in which variations in environmental exposures – such as infectious agents and diet – are known to play a role. Other pediatric tumors with widely varying incidence rates, such as Hodgkin and non-Hodgkin Lymphomas, are tumors in which infectious agents are known to play a role. For retinoblastoma, when incidence data show separate rates for unilateral and bilateral disease, variation in incidence appears largely restricted to unilateral disease (Stiller and Parkin 1996). The incidence of unilateral retinoblastoma appears to be higher in several less affluent regions of the world, suggesting that environmental factors associated with poor living conditions may increase the risk of mutagenesis in retinal cells (Stiller and Parkin 1996); however, many of the rates that suggest increased incidence in less affluent countries are based on small numbers of cases and thus, need to be interpreted with caution.

Retinoblastoma occurs primarily in children under the age of 5 years, with a median age of diagnosis of 24 months in children with unilateral disease and 9–12 months in children with bilateral disease (Goddard et al. 1999; Butros et al. 2002). Later age at diagnosis is generally reported in areas where there is decreased access to medical care, but diagnosis in children older than 5 years of age is rare. One group in Sao Paulo, Brazil, examined the incidence of retinoblastoma in older children and found that of 453 cases of retinoblastoma, only 3.5% (16) were diagnosed after 60 months of age (Aguirre Neto et al. 2007). Incidence rates are thus frequently expressed as “per million children 0–4 years of age,” rather than as “per million children 0–14 years of age,” as is common for other childhood cancers.

According to population-based registry data compiled and published by the International Agency for Research in Cancer (IARC), incidence rates are generally similar in North America, Europe, and Australia; somewhat higher rates are observed in Central and South America; a wide range of rates are reported in Asia with the highest in one region in India (Chennai); and generally higher rates are observed in Africa (Parkin et al. 1998). This pattern supports the hypothesis of higher retinoblastoma incidence in less industrialized countries, though the highest rates are clearly in Africa and a range of rates are observed in other less industrialized countries. A comparison of the incidence rates for those registries with incidence greater than 15 per million children less than 5 years, using the registry data compiled by IARC, is shown in Table 2.1 (Parkin et al. 1998). Large variation within geographic regions and even within countries is evident within both more and less industrialized regions (comparisons are limited to those countries with multiple registries). Variation within the US is described below. In Europe, although most countries have incidences in the range of 6–12 per million less than 5 years, much lower rates are noted in Bulgaria, and higher rates are noted for several countries (see Table 2.1).

There is marked variation in rates within Latin America, though data is limited to those countries or cities with registries. Incidence rates cluster into two groups: less than 9.5 per million in countries such as Cuba and Uruguay with the lowest rates, and greater than 15 per million children <5 years in others. Some of this variation appears to correlate with variation in development indices, such as Gross National Product, literacy, and degree of health care system infrastructure. Within countries, there is also a suggestion of variation by economic development, with higher rates in poorer regions of countries such as Mexico (see below) and Brazil, where incidence in Goiania is 1.6 times that of Belem. The regions with higher incidence may differ from those with lower incidences in many other factors including ethnic origin and environmental exposures.
In Asia and Oceania, some registries have documented incidence by ethnic origin, and this has further highlighted populational differences in incidence; for example, in New Zealand, rates differ between Maoris and non-Maoris (see Table 2.1), and in Singapore, where incidence in Malays is one third that of ethnic Chinese. Regional variations in incidence are also present. Notably, incidence in India is markedly higher in Chennai (formerly Madras) than in Delhi, Bombay, Bangalore, or Poona. Incidence in New Zealand is far higher than incidence in Australia. Incidence in Africa also varies greatly, with population based registries in Algeria and Egypt documenting rates of 4–6 per million <5 years, while incidence rates for some sub-Saharan African countries, such as Mali, Uganda, and Zimbabwe, are amongst the highest worldwide. These incidence variations suggest that environmental factors may be playing a role, though genetic susceptibility to particular environmental exposures may explain some of these differences.

Recent articles have further delineated population differences. In Europe, a recent evaluation of incidence

<table>
<thead>
<tr>
<th>Rates per million children ages 0–4</th>
<th>Registry/Ethnicity</th>
<th>Country</th>
<th>Incidence</th>
<th>Total cases (N&lt;1 yr) + (N age 1-4)</th>
<th>Ratio M:F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registries with rates &gt;20.0</td>
<td>Bamako</td>
<td>Mali</td>
<td>42.5</td>
<td>2 + 46</td>
<td>1.5</td>
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<td></td>
<td>Kampala</td>
<td>Uganda</td>
<td>24.0</td>
<td>4 + 15</td>
<td>1.4</td>
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<tr>
<td></td>
<td>Zimbabwe (African ancestry)</td>
<td>Zimbabwe</td>
<td>23.3</td>
<td>1 + 17</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Hawaii (native Hawaiians)</td>
<td>US</td>
<td>22.5</td>
<td>4 + 7</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>IMSS Chiapas</td>
<td>Mexico</td>
<td>21.8</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>Registries with rates 17.5–20.0</td>
<td>Hanoi</td>
<td>Vietnam</td>
<td>19.6(15.9–16.1)</td>
<td>10 + 68</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Singapore (Chinese)</td>
<td>Singapore</td>
<td>18.9</td>
<td>3 + 14</td>
<td>2.3</td>
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<tr>
<td></td>
<td>New Zealand (non-Maori)</td>
<td>NZ</td>
<td>18.6</td>
<td>5 + 24</td>
<td>1.5</td>
</tr>
<tr>
<td>Registries with rates 15.0–17.4</td>
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<td>Philippines</td>
<td>17.4</td>
<td>10 + 68</td>
<td>1.0</td>
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<tr>
<td></td>
<td>Cali</td>
<td>Colombia</td>
<td>17.1</td>
<td>3 + 14</td>
<td>2.3</td>
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<tr>
<td></td>
<td>Quito</td>
<td>Ecuador</td>
<td>16.6</td>
<td>4 + 26</td>
<td>1.5</td>
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<tr>
<td></td>
<td>Ibadan</td>
<td>Nigeria</td>
<td>16.1</td>
<td>4 + 15</td>
<td>1.0</td>
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<tr>
<td>Registries with rates 15.0–17.4</td>
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<td>Costa Rica</td>
<td>15.7</td>
<td>4 + 26</td>
<td>1.5</td>
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<tr>
<td></td>
<td>Lima</td>
<td>Peru</td>
<td>15.5</td>
<td>1 + 29</td>
<td>1.3</td>
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<tr>
<td></td>
<td>Norway</td>
<td>Norway</td>
<td>15.4</td>
<td>11 + 29</td>
<td>0.6</td>
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<tr>
<td>Registries with rates 15.0–17.4</td>
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<td>Brazil</td>
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<td>14 + 24</td>
<td>0.6</td>
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<tr>
<td></td>
<td>Denmark</td>
<td>Denmark</td>
<td>15.3</td>
<td>14 + 24</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*a Annual incidence per million children ages 0–4 years. From Parkin et al 1998 except as otherwise noted
*b Populations with F:M >1.0
*c The rate was 7.4 per million children under 14 years. If their rate were calculated based on children under age 5, their rate would likely surpass 20 per million, though this rate was based on a small number of children
*d Incidence decreased in follow up report based on data from later years
*e From Fajardo Gutierrez et al. 2007
of retinoblastoma in European children (1978–1997) using data collected through the Automated Childhood Cancer Information System project and including data from 60 registries demonstrated an overall incidence that increased by 1% per year over the 20-year period beginning in 1978. The age-standardized incidence rate for the age range 0–14 years was 4.1 per million children, with one third of cases affected with bilateral disease (MacCarthy et al. 2006). In contrast, a recent study by Seregard et al., in Sweden and Finland, where birth cohort analysis was possible, demonstrated that incidence was stable from 1990 to 1998, with a mean incidence rate of 11.8 (95% CI 10.5–13.1) and 11.2 (95% CI 9.4–13.0) per 1 million children less than 5 years of age in Sweden and Finland, respectively, despite the fact that analysis looking at annual incidence rates suggested an increase during that period (Seregard et al. 2004). Similarly, in a study examining incidence rates in Germany over the 20-year period beginning in 1987, incidence based on 732 retinoblastoma cases registered in German Children’s Cancer Registry (one third were bilateral), demonstrated an age-standardized incidence rate/year for all children <15 years of 4.0 per million with stable rates throughout this period (D. Debling, personal communication).

Some registries have published follow up reports after the compilation of the 1998 IARC monograph. For India, a follow up study done in Chennai showed a slight decrease in incidence of retinoblastoma. From 1990 to 2001 in children <5 years, incidence was 15.9 (males)–16.1 (females) per million, a decrease from that reported earlier, suggesting a change in underlying risk factors. Interestingly, survival in Chennai is noted to be far less than in other parts of the world at 48%; thus, the overall elevated incidence is not likely to result from an increase in inherited familial cases (Swaminathan et al. 2008). In Karachi, Pakistan, registry data also suggests elevated incidence (Bhurgri et al. 2004). Incidence from this registry, however, was reported only as frequency in the IARC compilation because of a lack of actual population estimates (the last census was in 1981). After the data was compiled by IARC, the registry itself published data on cases reported in the 5 years following the IARC publication. They calculated the Age Standardized Incidence for Karachi itself at 5.3 per 100,000 children (or 53 per million) <5 years, which would place its incidence as the highest in the IARC reports. However, given concerns regarding underestimation of the actual source population, it is likely that this incidence is much lower. This group also reports a predominance of males to females (M:F 1.5), but as noted by IARC, this is true for all cancers in Karachi and is thought to represent a relative neglect of illness in girls. Reliable incidence data for Islamabad are also not available, but frequency data there also demonstrates an elevated M:F ratio (1.9) (Parkin et al. 1998). Although incidence estimates for Pakistan are not reliable, there is a suggestion of increased incidence, as the relative frequency of retinoblastoma compared with other pediatric malignancies is 7.4% (compared with 3% in US White populations) (Parkin et al. 1998).

### 2.2.2 Variation Within Countries: Subpopulations with Higher Incidence

The overall incidence of retinoblastoma in the U.S. has been stable over the 20-year period ending in 1995, ranging from 10 to 14 per million children <5 years, with retinoblastoma comprising 3–4% of childhood cancer. However, incidence within the US is not uniformly distributed and there appears to be variation by ethnicity, gender, and geographic region. Within the US, particular subpopulations have unusually elevated incidence. In most of the US registries, the rates amongst African American children are higher than those in “non-black” or “white” children. The difference in rates between ethnic groups is quite variable, with the ratio of incidence of “black” to “white” ranging from 1.43 in the Greater Delaware Valley, to 1.17 in New York State and 1.15 in the combined SEER registries, and 0.47 in Los Angeles. In the Hawaii SEER registry data (see Table 2.1), native Hawaiians appear to have an increased incidence of retinoblastoma. Similarly, Alaska natives had a rate significantly higher than US whites in SEER data (Odds Ratio [OR] 2.8; 95% CI 1.3–5.3). Like the Alaskan Native children, the rate in the New Mexico SEER registry Native Americans was higher (6.8 per million <14 years) compared with “white” children from the combined SEER registries (2.7 <14 years) (OR 2.5; 95% CI 1.4–4.5). Interestingly, the overall rates for childhood cancer in Alaskan Natives and
New Mexico Native Americans were lower than those in SEER registry data for white children, though the rate for osteosarcoma (but not other bone tumors) was also higher among New Mexico Native Americans when compared with US whites (SEER) (OR 1.8; 95% CI 1.0–3.4) (Lanier et al. 2003). Given the biological overlap between osteosarcoma and retinoblastoma, this finding is particularly intriguing. Interpretation of these variations based on data from regional US subpopulations is limited by the small number of cases upon which the incidence rates are based.

In a recent population-based study by Fajardo-Gutierrez et al in Mexico, in which it was possible to examine regional differences in the incidence of retinoblastoma within the Mexican Social Security system (IMSS), the highest incidence of retinoblastoma occurred in the state with the highest incidence for central nervous system tumors (Chiapas, see Table 2.1) (Fajardo Gutierrez 2007). Although the incidence of retinoblastoma was highest in Chiapas – the poorest state in Mexico and a state in which a high proportion of the population is indigenous – the number of cases on which incidence was calculated is very small.

Some populations with apparent excess of retinoblastoma have a relative paucity of neuroblastoma (Alaskan natives, New Mexico American Indians, Chiapas) as if one occurred in greater numbers at the expense of development of the other. This may indicate a possible shared underlying mechanism of tumor formation in which different factors lead to a bifurcation in the formation of a primitive neuroectodermal tumors (PNET) leading to development of neuroblastoma in one population, and retinoblastoma in another. Alternatively, the factors that lead to development of retinoblastoma may coexist with factors that protect against neuroblastoma.

### 2.2.3 Gender Differences

Comparing incidence worldwide, focusing on those countries with the higher incidence rates, we see some differences in the incidence by gender (Table 2.1). In some regions of the world, there appear to be slightly more male cases than female cases, while in other regions, the difference is markedly in the other direction. In some populations of Latin America and in native American populations of North America, the incidence in girls is much higher than in boys; for example, the Alaskan Native population had a 3:1 Female:Male incidence (Lanier et al. 2003), and in the states in Mexico, in which retinoblastoma figured amongst the five most common pediatric malignancies – Chiapas (where it was second) and Veracruz (where it was fifth) – there was a predominance of female cases, with M:F 0.5 and 0.7, respectively. In Oman, where the incidence is only 8.3 per million <5 years of age, the ratio of M:F is 0.7 as well (Khandekar et al. 2004). However, all of these data are the results of observations on small numbers of cases in each registry. Unfortunately we do not have data on laterality from the majority of these registries, thus it is not possible to see if the ratio of male to female cases varies by laterality.

### 2.2.4 Biologic Underpinnings of Epidemiologic Studies on Retinoblastoma

Hereditary retinoblastoma is characterized by the presence of a germline mutation, earlier clinical manifestation of disease with disease detectable in utero in children with known familial disease, and very elevated risk of developing secondary malignancies. All children with bilateral tumors and roughly 15% of children with unilateral tumors have this form of disease. There are no definitive clinical characteristics that differentiate the 15% of unilateral patients with germline mutations from the 85% who lack them. The ability to differentially examine risk factors for tumors involving germline mutations from those without them by using laterality as a proxy is thus hampered. Ongoing epidemiologic studies, such as one by the Children’s Oncology Group, will be able to bypass this imprecision by evaluating tumor mutations directly.

Studies done to date have relied on the relative distinctiveness of the laterality phenotypes in order to analyze results. Although much is understood about the effects of the \( RB1 \) mutations underlying the formation of retinoblastoma, little is known about the etiology of these mutations in germinal or retinal cells. New germline mutations are known to occur preferentially
on the paternal allele, suggesting the implication of paternal preconception risk factors (Zhu et al. 1989; Kato et al. 1994; Dryja et al. 1989). However, there is no knowledge about the etiology of retinal cell mutations or their time of occurrence, and little is known about the causes of unilateral disease. The crucial period for mutation development in retinoblastoma may be during retinal formation occurring in early embryonal development between the 4th and 8th week of gestation, or during infancy as retinal cells continue to divide until about age 2 years. Although as stated earlier, the molecular changes leading to retinoblastoma are well characterized, the role of risk factors or contributing exposures has been studied only rarely. Below is a summary of studies that explore risk factors or characteristics hypothesized to play a role in disease development.

2.3 Potential Hypotheses

2.3.1 Parental Occupations

Because retinoblastoma occurs during infancy and early childhood, the examination of risk factors and environmental exposures has focused primarily on potential contributions from parental exposures. Given the low incidence of retinoblastoma, traditional epidemiologic studies have been limited to case-control and case-series studies. The largest and most extensively reported study has been a case-control telephone interview study conducted by the Children’s Cancer Study Group in 67 bilateral and 115 unilateral cases who did not have a family history of the disease (Bunin 1990). In this study, paternal employment in the military (OR 2.8; 95% CI 1.1–8.8, \( p = 0.04 \)) or in the metal industry (OR infinity; 95% CI 1.4–infinity, \( p = 0.02 \)) was associated with having a child with bilateral disease, and paternal employment as a welder or machinist was associated with having a child with unilateral disease (OR 4.0; 95% CI 1.1–22.1, \( p = 0.04 \)). These findings are similar to those from studies done on other PNETs of childhood (Olshan et al. 1999). Interestingly, this study also examined potential transgenerational exposures and found that maternal grandparental employment in farming was also associated with unilateral disease (OR 10.0; 95% CI 1.4–433, \( p = 0.02 \)).

One retrospective cohort study of Norwegian agricultural workers noted an increased incidence of retinoblastoma in children whose parents had worked with pesticides (Kristensen et al. 1996). This is particularly intriguing given the findings of the above noted association between unilateral retinoblastoma and maternal grandparental farm work (Bunin et al. 1990), as well as because work done earlier as part of a case-control study in Mexico found increased risk with paternal occupation as a farm worker (bilateral disease) (OR 2.7; 95% CI 1.1–6.5) (Orjuela et al. 2000a) and with maternal and infant exposure to chickens and pigs (unpublished data); these trends echo findings in epidemiologic studies of other PNETs. For example, maternal occupation as a farm worker has also been associated with development of neuroblastoma (Olshan et al. 1999). For brain tumors, increased risk has been found for agricultural workers, or for maternal exposure to farm animals or farm residence (Preston-Martin et al. 1993; Bunin et al. 1994; Holly et al. 1998; Kristensen et al. 1996; Cordier et al. 2001). In those studies of brain tumors in which farm animal exposure has been analyzed by species, exposure to chickens and pigs conferred increased risk of PNET (medulloblastoma) (Holly et al. 1998; Kristensen et al. 1996).

2.3.2 Parental Age

Increased parental age has been associated with greater risk for bilateral retinoblastoma. Advanced paternal age is hypothesized to be associated with an increased risk of new germ cell mutations by way of increased opportunity for mutation formation in dividing spermatocytes. Since new germline mutations occur preferentially on the paternal allele, this hypothesis has some biological plausibility. Earlier studies from the Dutch population based registries found that increased parental age (of both fathers and mothers) was significantly associated with increased Relative Risk of having a child with bilateral (but not unilateral) retinoblastoma (Moll et al. 1996). However, these studies were hampered by relatively small sample sizes. One case-series study from India found that fathers...
of children with nonfamilial bilateral disease were older than those of children with unilateral disease (Sivakumaran et al. 2000). Two recent European studies attempted to address this question using the case data available from larger national registries. One such study in the UK matched cases ascertained from the national registry (diagnosed from 1968–1986) with birth record controls matched on date of birth, sex and origin. The OR for retinoblastoma resulting from assumed new germ cell mutations among children of fathers who were at least 45 years old at the time of the child’s birth was 3.0 (95% CI 0.2–41.7) (Dockerty et al. 2001). Although this finding was not significant, it was suggestive and consistent with results of earlier studies. The authors did not hypothesize an increased risk from advanced maternal age, nevertheless, the effect observed in women between 30 and 34 years of age was also elevated, with an OR 2.03 (95% CI 0.87–4.74) when compared with women 25–29 years of age. The authors were able to eliminate cases with family history and further restricted their analysis to bilateral cases. However, because of the high degree of correlation between maternal and paternal ages, the authors were unable to separate the effects of parental age. A more recent study done in Sweden is the only population-based cohort study thus far with the power to examine the independent effect of parental age on incidence of childhood cancer (Yip et al. 2006). In this study, the authors were able to separate the effects of maternal from paternal age and found that advanced maternal age is actually significantly predictive of increased risk of having a child with retinoblastoma. Although a modest increased risk associated with advanced paternal age was apparent, the effect disappeared after adjusting for maternal age. This Swedish study assessed parental ages via national registries by examining 4.3 million children (and their parents) born between 1961 and 2000, including 226 cases of retinoblastoma. Yip et al. found that for children <5 years of age, increased maternal age (after adjustment for paternal age) was associated with elevated risk of retinoblastoma (when women older than 40 years at age of childbirth were compared with women younger than 25 years at childbirth), the Incidence Rate Ratio was 2.39; 95% CI 1.17–4.85) (Yip et al. 2006) This finding suggests that maternal risk factors can contribute to likelihood of developing hereditary (bilateral) retinoblastoma despite the fact that new mutations were expected to occur on the paternal allele given prior findings. When comparing these results with those of the UK study, it is important to note that in the latter case, women younger than 25 years of age had lower incidences of having children with retinoblastoma than the designated reference group (25–29); thus, if these younger mothers had been used as a reference group, it is likely that results in the UK study might have been similar those from the Swedish study.

### 2.3.3 In Vitro Fertilization (IVF)

Recently, another risk factor has emerged, which may be closely linked to the risk factor of increased maternal/paternal age. A study done through the Dutch Retinoblastoma registry found that children conceived by means of in vitro fertilization (IVF) had a 5–7-fold increased risk of retinoblastoma (Moll et al. 2003); however, these results were based on only 5 cases (2 bilateral, 3 unilateral). Although studies done in birth cohorts of children born after IVF in the UK, Denmark, and Australia observed no increase in incidence of retinoblastoma (Bradbury and Jick 2004; Lidegaard et al. 2005; Bruinsma et al. 2000), it is possible that the increases in incidence found in Holland might in part be related to parental age. None of the birth cohort studies restricted analysis to a subpopulation with older parental ages in order to examine the effect on risk of retinoblastoma. Further examination of the incidence of retinoblastoma in children conceived through IVF will be essential to determine if the effect is indeed independent of parental age.

### 2.3.4 UV Exposure

One explanation posited for the geographic variations in retinoblastoma incidence has been the inherent geographic differences in ultraviolet (UV) radiation exposure. One group of investigators had suggested that the geographic differences that are found in the incidence of unilateral disease can be attributed to the annual environmental exposure to UV rays, but that
the incidence of bilateral tumors is unrelated to UV exposure. Increased UV exposure to the retina would lead to increased probability of mutations and thus tumors in the retina. Hooper et al found that incidence of retinoblastoma falls significantly with increasing geographic latitude, and that increased incidence of unilateral disease (but not bilateral disease) is significantly associated with increased annual ambient exposure to UV rays (Hooper et al. 1999). However, a subsequent analysis by the NCI found that although incidence of retinoblastoma was significantly correlated with UV-B radiation exposure levels, this association disappeared after adjusting for climate, race, and socioeconomic development. As a result, they concluded that geographic variation is not explained by variations in UV exposure, but rather that tropical climate and exposure related to ethnic susceptibility and economic development may be confounding the association found with UV exposure (Jemal et al. 2000). Studies done on this topic have only looked at the UV exposure of a given geographic region but have not taken into account individual differences in duration of exposure.

2.3.5 Nonoccupational Parental Exposures

Other parental exposures have been examined as potential risk factors for retinoblastoma. In a study by Bunin et al., gestational exposure to X-ray OR 2.3; \( p = 0.08 \), a morning sickness medication (which is no longer available) (OR 2.8; \( p = 0.02 \), and low maternal educational level (not finishing high school) (OR 5.5; \( p = 0.03 \) were associated with increased risk for having a child with unilateral disease. Two other exposures were found to be significantly protective: multivitamin use during pregnancy (for both unilateral and bilateral forms of the disease) and periconceptional use of barrier contraceptive (OR 0.1; \( p = 0.02 \) or spermicide (OR 0.2; \( p = 0.02 \). The protective effects of these two factors were further explored in the context of another study (see below).

In a later study of 106 children with retinoblastoma and 198 hospital-based controls at the Instituto Nacional de Pediatria (INP), a public tertiary care hospital in Mexico City, a significantly increased risk for the development of unilateral retinoblastoma was associated with factors related to maternal poverty during pregnancy, including poor nutrition (OR 2.3; 95% CI 1.2–2.4), lack of prenatal care (OR 2.6; 95% CI 1.1–5.9), delivery at home (OR 4.8; 95% CI 2.1–11.0), low level of maternal education (not finishing secondary school) (OR 3.7; 95% CI 1.4–9.5), birth outside of the capital (OR 2.5; 95% CI 1.3–5.0), and breast feeding for longer than 6 months (OR 2.4; 95% CI 1.3–4.3) (Orjuela et al. 2000a). None of these risk factors appeared to play a role in development of bilateral disease, although analysis was hampered by the small number of cases with bilateral disease. Per capita and household income did not appear to contribute to risk. These factors echoed in part the findings from the US study. Together, these socioeconomic indicators are more suggestive of an underlying mechanism that could be correlated with lower maternal socioeconomic status in Mexico rather than suggesting that a lower socioeconomic status per se increases risk. Among the potential underlying factors that could contribute risk, nutrient intake is one of the mechanistically more interesting possibilities.

2.3.6 Diet

Recent studies have suggested that gestational nutrient intake may be relevant to the development of PNET tumors, such as neuroblastoma, medulloblastoma, and retinoblastoma. The case–control study by Bunin et al in the US found a significantly protective effect against both unilateral (OR 0.4; \( p = 0.02 \) and bilateral disease (OR 0.2, \( p = 0.02 \) for mothers who consumed multivitamins during pregnancy (Bunin et al. 1989). The case–control study in Mexico found that maternal diets low in vegetables, folate, lutein, and vitamin B6 intake during pregnancy were associated with a 2–4-fold increased risk of having a child with sporadic retinoblastoma (Orjuela et al. 2005). The significantly increased risk for developing unilateral disease that was associated with breast feeding for longer than 6 months also suggests an increased risk associated with prolonged dependence on breast milk for nutrients, and possibly a protective effect of substituting formula (which has synthetic vitamins added) or other foods. Breast fed infants receive folate and other B vitamins in breast milk, however, the levels
of these in breast milk necessarily depend on maternal diet. Decreased intake of these nutrients, necessary for DNA methylation and synthesis, as well as retinal function, may increase risk for having a child with sporadic retinoblastoma. Other studies have found similar increased risk for development of neuroblastoma and medulloblastoma associated with lower maternal micronutrient intake during pregnancy (Bunin et al. 1993; Olshan et al. 2002; French et al. 2003).

### 2.3.7 Viral Agents

The geographic variations in patterns of incidence of retinoblastoma have led investigators to wonder about the possibility of involvement by an infectious agent. The retinoblastoma protein, pRb, which is generally absent or truncated and ineffective in retinoblastoma as a result of RB1 mutations, can be inactivated by three viral proteins, which share sequence homology for a region that binds and inactivates pRb. These three viral proteins are the E7 protein of the human papilloma virus (HPV), the T antigen of the SV40 virus, and the E1A antigen of adenovirus. Their potential to inactivate pRb suggested a possible role for these viruses in development of a subset of retinoblastoma. The distribution of areas of increased incidence of retinoblastoma shared some similarity with areas of increased incidence of cervical carcinoma known to be causally associated with HPV. The US-based study’s finding of the tenfold protective effect of periconceptional use of barrier contraceptives also suggested the possible involvement of a sexually transmitted agent.

DNA sequences from oncogenic HPV subtypes (16 and 18) were detected in about one third of fresh frozen retinoblastoma tumor samples studied in central Mexico, suggesting a role for HPV infection (Orjuela et al. 2000b). Tumors containing HPV had significantly lower proliferative indexes and clinically less invasive behavior. Similarly, in Brazil, Palazzi, Villa et al examined paraffin-embedded tumor tissue from 43 children with unilateral retinoblastoma for HPV DNA using PCR/ dot blot hybridization and also found high risk oncogenic HPV DNA types 16 and 35 in 12 (27.9%). A higher frequency of differentiated tumors (63.3%) was observed among the HPV-positive tumors (Palazzi et al. 2003). Montoya Fuentes in Northern Mexico examined 51 paraffin embedded samples collected between 1985 and 1997 and found that 35% of tumors contained oncogenic HPV subtypes (by PCR and immunohistochemistry); 31.4% (16 cases) with HPV 33 and 5.9% (3) with HPV 31,35, or 51. Interestingly, no tumors with HPV 16 or 18 were found, and 2 of the 18 cases with High Risk HPV were bilateral. Their study found a slightly higher proportion of advanced stage disease at diagnosis (St. Jude stage 3 or greater) in children whose tumors were HPV negative when compared with those that were HPV positive (18% vs. 13%) (Montoya-Fuentes et al. 2003). More recently, a study in India has found HPV16 in 27% of 44 tumors. Tumors that were HPV positive were more likely to have detectable pRb and to have occurred in children diagnosed before 18 months of age (Krishnakumar 2008). Oncogenic HPV subtypes found in various studies are among those causally associated with the development of cervical cancer; however, one study has not found presence of HPV or other possible causal viruses in retinoblastoma tumors from the US and Canada (Gillison et al. 2007). Thus, the evidence for a role of HPV is not conclusive. More molecular evidence of a possible role for HPV in development of retinoblastoma has recently been published by Ponce-Castaneda et al who have found that gene expression profiles made from tumor tissue from retinoblastoma differ by HPV status. Tumors with differing HPV status (presence versus absence by PCR) differ significantly in their expression of certain genes involved in inflammatory responses, while their gene expression profiles do not differ as clearly when comparing unilateral and bilateral tumors (Ponce-Castaneda et al. 2008). Together, these studies suggest an intriguing possible mechanism that may contribute as a cofactor for incidence of retinoblastoma in some areas of the world, but further work to better elucidate a potential infectious etiology is needed.

### 2.3.8 Diagnostic Interval

Most reports on incidence of retinoblastoma have not discussed disease stage, in large part because registries generally do not collect these data. Relative
prevalence of metastatic disease is limited to reports originating from clinical treatment centers: For example, in a report of 141 children with retinoblastoma followed in Istanbul, 9.9% presented with metastatic disease (Ozkan et al. 2006), while in Ankara, 20.9% of 91 children had metastatic disease (Ozdemir et al. 2007). A report on the incidence amongst Swiss children recorded over a period of 42 years showed that the incidence of more advanced intraocular disease, group E, has decreased. The authors noted an association with decreasing interval of time between the first symptoms (usually by parents) and retinoblastoma diagnosis. Over the 42-year period, this time interval decreased significantly only for unilateral disease (Wallach et al. 2006). Other reports from Argentina and Brazil have noted that children with a longer period of time between noting of symptoms and diagnosis of disease were also more likely to have more clinically advanced disease (Erwenne and Franco 1989; Chantada et al. 1999). These findings suggest a benefit of diagnosing disease closer to the time that symptoms are first noted, implying a linear relationship between this interval and disease progression. However, more recent work in a public hospital serving uninsured patients from central Mexico has not demonstrated a significant association between longer diagnostic delay and more advanced clinical stage (by either ABC or St Jude’s staging) for 125 newly diagnosed patients without a family history who had either bilateral or unilateral disease (Orjuela et al. unpublished data). It is thus less certain that increased delay in diagnosis necessarily leads to progression and development of more advanced disease.

### 2.3.9 Screening and Media Campaigns

Some experts have advocated the importance of establishing retinoblastoma screening programs, as well as media campaigns to increase public awareness and thus empower parents to seek medical attention earlier. Given retinoblastoma’s incidence of 1 in approximately 16,000 live births, campaigns for public awareness will need to weigh its incidence and potential public health impact when prioritizing time for retinoblastoma in public service announcements. Screening campaigns will also need to proceed with caution, given the inherent difficulties of mounting effective screening for rare diseases. The screening campaigns mounted for neuroblastoma, another primitive neuroectodermal tumor of early childhood, have not been successful in decreasing disease related mortality nor in decreasing incidence of clinically more advanced disease (Schilling et al. 2002; Woods et al. 2002; Yamamoto et al. 2002; Maris and Woods 2008). This lack of success may in part be ascribed to inherent biologic differences between more invasive and less invasive forms of neuroblastoma (Maris 2007). During the intervening time since the screening programs for neuroblastoma were first created, investigators have developed a better understanding of the biology of neuroblastoma (Hiyama et al. 2008). The underlying assumption, that the degree of invasiveness is linearly related to the amount of time that the disease remains untreated, now appears to underestimate the true biologic complexity of the disease. Advances in the understanding of the biology of retinoblastoma may help better elucidate the potential benefit derived from a screening program for retinoblastoma. For this disease, the question remains: is more aggressive disease biologically distinct from less aggressive or invasive disease? And, from the epidemiologic perspective, are risk factors the same for more invasive and less invasive disease?

### 2.4 Summary

Risk factors for development of sporadic (without family history of this disease, either laterality) retinoblastoma are poorly understood. Here, we have presented variations in incidence of retinoblastoma and risk factors that have been proposed as potentially important for development of retinoblastoma. In aggregate, the geographic and ethnic variations in incidence in retinoblastoma are suggestive of underlying risk factors for development of disease. Closer examination of factors that may differ between populations with different rates could improve our understanding of disease development. Studies that focus on the likely windows of susceptibility and utilize information from genetic analysis in order to differentiate forms of the disease will inform our understanding of retinoblastoma and
may potentially inform therapy and strategies for earlier detection. Future epidemiologic studies may help elucidate whether more aggressive disease is associated with the same risk factors as less aggressive disease. Such an understanding would inform efforts aimed at earlier disease detection.

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