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Parental age and seasonal variation in the births of children with sporadic retinoblastoma: a mutation-epidemiologic study

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Summary. Statistical analysis of parental age data from 225 sporadic cases of bilateral retinoblastoma, plus ten sporadic cases of chromosome deletion or translocation involving 13q14 that was identified as of paternal origin, revealed no evidence of paternal or maternal age effect. Parental exposure to ionizing radiation or chemical mutagens, the effect of which is accumulated with advancing age, does not seem to play a major role in the production of germinal mutations at the responsible (RB) locus. Furthermore, analysis of variation in the month of birth of 753 children with sporadic unilateral retinoblastoma did not show any significant deviation from the controls or a cyclic trend. The occurrence of nonheritable retinoblastoma is not likely to be associated with certain viruses such as human adenovirus 12 whose activity varies markedly with season. These results, together with the fairly uniform pattern in the incidence of this tumor among different populations, suggest that most, if not all, cases of sporadic retinoblastoma are caused by some intrinsic biological mechanisms, and not by environmental mutagens that may vary with respect to time and place.

Introduction

Whereas our knowledge about the molecular mechanism of the development of retinoblastoma and the nature of the responsible (RB) gene has been extended tremendously over the last few years (Murphree and Benedict 1984; Friend et al. 1986; Fung et al. 1987; Lee et al. 1987), few epidemiological studies have been conducted to investigate possible environmental factors associated with the first mutational event in germinal or somatic cells leading to heritable or nonheritable retinoblastoma. Etiologically, all sporadic bilateral cases and about 10% of sporadic unilateral cases arise from germinal mutations, the great majority of sporadic unilateral cases being the result of somatic mutations (Vogel 1979). The rarity of this disease obviously makes it difficult to collect a sufficiently large number of sporadic bilateral and unilateral cases separately to give an epidemiologically sound conclusion. In the present paper, we report the results of analysis of parental age data from 225 children with sporadic bilateral retinoblastoma, plus ten cases of sporadic retinoblastoma associated with 13q14 chromosome abnormalities of paternal origin, and seasonal variation in the births of 753 children with sporadic unilateral retinoblastoma.

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Materials and methods

Using medical records of children with retinoblastoma ascertained by a nationwide registry initiated in 1975 (Minoda 1975), information was obtained about the date of birth of 1110 children with sporadic retinoblastoma (753 unilateral and 357 bilateral cases), who were born during the period from 1965 to 1982, and the name and address of their parents. A letter was sent to the ward office of each administrative district where the children were living, asking for information about the date of birth of the parents. Precise data on parental ages were thus obtained for 633 cases (408 unilateral and 225 bilateral) born during 1965–1968 and 1975–1982; these periods were selected because of the availability of data from Japanese population statistics concerning distributions of all legitimate live-births by paternal and maternal ages. In addition, data on parental ages of ten cases of sporadic retinoblastoma associated with either deletion or translocation involving 13q14 (Ejima et al. 1988; Sasaki 1989) were analyzed; in these cases, paternal origin of the chromosome abnormalities was identified by examination of Q-band heteromorphism and esterase D phenotypes.

Variations in the month of birth of these children and those in parental ages were compared with respective controls constructed on the basis of data from the population statistics in all Japan, adjusted by the year of birth of the children.

Results

Parental age

As shown in Table 1, the distributions of both paternal and maternal ages for the 225 children with sporadic bilateral cases were close to those in the general population; both mean and variance in the paternal and maternal ages were almost identical to the controls. For paternal age, the relative incidence (Observed/Expected) remains at unity before the fathers reached 35 year of age, and then fluctuates slightly for older fathers whose number is very small. The same relationship is true of the relative incidence with advancing maternal age. Table 2 represents parental age data from the 408 chil-

Table 1. Parental age distribution, observed (obs.) and expected (exp.), for 225 children with sporadic bilateral retinoblastoma

Age (years)	Paternal age			Maternal age		
	Obs.	Exp.	Obs./ Exp.	Obs.	Exp.	Obs./ Exp.
<19	1	0.4	1.0	1	1.9	1.0
20–24	17	16.9		53	48.4	
25–29	89	90.6	1.0	114	119.6	1.0
30–34	90	86.2	1.0	52	45.9	1.1
35–39	19	25.1	0.8	5	8.1	0.6
40–44	7	4.7	1.6	0	1.0	
45–49	2	0.9		0	0	
50+	0	0.2		0	0	
Total	225	225.0	1.0	225	224.9	1.0
Mean age	30.2	30.1		27.3	27.3	
Variance	20.6	20.3		12.6	15.9	

Table 2. Parental age distribution, observed and expected for 408 children with sporadic unilateral retinoblastoma

Age (years)	Paternal age			Maternal age		
	Obs.	Exp.	Obs./ Exp.	Obs.	Exp.	Obs./ Exp.
<19	2	0.7	1.3	6	3.4	1.1
20–24	38	30.6		97	87.8	
25–29	153	165.3	0.9	208	216.9	1.0
30–34	155	155.5	1.0	84	83.3	1.0
35–39	48	45.4	1.1	11	14.7	0.8
40–44	8	8.4	1.2	2	1.8	
45–49	3	1.6		0	0.1	
50+	1	0.4			0	0
Total	408	407.9	1.0	408	408	1.0
Mean age	30.2	30.1		27.2	27.3	
Variance	22.4	20.3		14.0	15.9	

Table 3. Parental ages at the birth of children with sporadic retinoblastoma associated with chromosome abnormality of paternal origin. B, Bilateral; U, unilateral

Patient	Sex	Laterality (B or U)	Chromosome abnormality	Age (years)	
				Father	Mother
1	F	B	del(13)(q12q22)	23	24
2	M	B	del(13)(q14)	26	23
3	F	B	t(13;10)(q14;q22)	26	24
4	F	B	del(13)(q14q21)	36	26
5	M	B	t(13;6)(q14;q13)	35	32
6	M	U	del(13)(q12q14)	34	29
7	F	B	del(13)(q14q21)	29	24
8	F	B	t(13;X)(q13p22)	22	23
9	M	U	del(13)(q12q14)	41	33
10[a]	F	B	t(13;X)(q12.3;p11.21)	30	30
			Mean age	30.2	26.8
			Controls	30.3	27.5

[a] Previously reported by Kajii et al. (1985)

Table 4. Distribution of 753 children with sporadic unilateral retinoblastoma by month of birth

Month of birth	Obs. no.	Exp. no.	Difference obs. – exp.
January	74	65.4	8.6
February	62	59.4	2.6
March	56	62.7	−6.7
April	52	62.6	−10.6
May	57	63.4	−6.4
June	67	60.8	6.2
July	63	66.4	−3.4
August	66	65.9	0.1
September	60	63.5	−3.5
October	67	62.3	4.7
November	60	58.2	1.8
December	69	62.5	6.5
Total	753	753.1	−0.1

dren with sporadic unilateral cases. As expected, there was no difference in the distribution of either paternal or maternal age between the cases and the controls. It should also be noted that both the mean and variance of paternal and maternal ages for the unilateral cases were close to those for the bilateral cases.

Table 3 gives parental age data from the ten cases with 13q14 chromosome abnormality of paternal origin. For each case, mean paternal or maternal age of the general population in the same year of birth was taken as a control. Here again, the mean paternal and maternal ages of the cases were close to the controls.

Seasonal variation

Table 4 compares the distribution of month of birth for the 753 cases of sporadic unilateral retinoblastoma with that in the general population. Although there was no significant deviation from the control ($\chi^2 = 6.49$, $df = 11$, $P > 0.80$), there appears to be a slight excess of affected births beginning from October through February (332 observed vs 307.8 expected) and a slight deficit of births in the months from March to May (165 observed vs. 188.7 expected), although the difference was not significant ($\chi^2 = 4.88$, $df = 2$, $P > 0.05$). Assuming a homogeneous distribution through the months and applying Edward's method (Edward 1961) of testing for a cyclic trend, θ was found to be 303°. This implies that the maximum incidence was in early November and the minimum in early May, but the ratio of the highest to the lowest incidence was only 1.15. The discrepancy from a homogeneous distribution was not statistically significant ($\chi^2 = 1.82$, $df = 2$, $P > 0.3$).

Discussion

It is known that paternal age has a profound effect upon the production of mutations leading to certain dominant bone anomalies such as achondroplasia, acrocephalosyndactly and Marfan's syndrome (Penrose 1961). With respect to sporadic retinoblastoma, Vogel and Rathenberg (1975) reviewed the literature and concluded that there seems to be a paternal

age effect in bilateral but not in unilateral cases, although the extent of the effect is much smaller than in the dominant bone anomalies. A smaller paternal age effect, if verified, is consistent with the hypothesis that most mutations occur as the result of "copy-error" at mitotic division in male spermatogenesis. However, epidemiologic evidence for the paternal age effect is not strong. First, previous studies (Matsunaga 1965; Tünte 1972; Czeizel and Gárdonyi 1974) were based on a small number of cases, with the exception of Pellié et al. (1973). Secondly, it is generally difficult to obtain appropriate control data for paternal age; until recently, data for paternal age were seldom available in population statistics in most countries. Pellié et al. (1973) compared paternal ages of 155 cases of sporadic bilateral retinoblastoma using French population statistics for 1956. The year of births of those children, however, extended over more than 10 years including 1951 to 1960 (Briard-Guillemot et al. 1974), during which paternal age distribution in the general population is likely to have changed gradually. In England and Wales, for example, mean paternal age in the general population declined gradually from 30.2 years in 1961 to 28.6 years in 1974 and then recovered to 29.8 years in 1983 (Emery 1986); in Japan, mean paternal age rose from 29.6 years in 1975 to 31.1 years in 1987. Therefore, in order to construct an appropriate control, it is necessary to adjust possible variation by year of birth of the patients.

We analyzed parental age data from the 225 children with sporadic bilateral retinoblastoma and found no difference in the mean paternal or maternal age, not only from the controls adjusted by year of birth of the children, but also from the mean for the 408 cases of sporadic unilateral retinoblastoma. Furthermore, there was no increase in the mean paternal age for the ten cases of sporadic retinoblastoma associated with either deletion or translocation involving 13q14 that was identified as of paternal origin. This was unexpected in view of the recent reports that most of the 13q14 abnormalities were of paternal origin (Ejima et al. 1988) and that structural deletions within the RB gene were commonly noted in retinoblastomas (Fung et al. 1987). The negative finding may be a results, in part, of diminishing variance in parental ages in the general population during the last 30 years in this country, resulting from an increasing concentration of childbearing at around 30 and 27 years of paternal and maternal ages, respectively. Therefore, our results do not necessarily exclude the possibility of a slight paternal age effect, but they do suggest that parental exposure to ionizing radiation or chemical mutagens, which should have an accumulated overall effect with advancing age, does not play a major role in the production of germinal mutations, both genic and chromosomal, at the RB locus.

The first mutational event in the somatic cells of children with nonheritable retinoblastoma may be caused by maternal exposure during pregnancy to ionizing radiation, certain oncogenic viruses or chemical mutagens. Of particular interest is possible viral etiology, which has been suggested from time to time not only for nonheritable retinoblastoma but also for the appearance of what has been called delayed mutation (Zimmerman 1970; Vogel 1979; Albert 1980). In experiments with rodents and baboons, retinoblastoma-like tumors can be produced by intraocular injection of human adenovirus 12 (Mukai et al. 1980), and the viral DNA can induce malignant transformation of human embryonic retinal cells in vitro (Byrd et al. 1982). Recently, the RB gene product, p105-RB, has been shown to form stable protein complexes with the oncoproteins of three DNA tumor viruses, the adenovirus E1A proteins, simian virus 40 large T antigens, and human papilloma virus-16 E7 (Whyte et al. 1988; DeCaprio et al. 1988; Dyson et al. 1989). It is possible that these oncoproteins inactivate the product of the RB gene, which presumably has a tumor suppressing effect. However, the viral hypothesis has never been tested by epidemiological studies or by an examination of patients for antiviral antibodies.

Seasonal variation in births of children with nonheritable retinoblastoma would suggest that the disease is influenced by certain environmental agents such as viral infection. Earlier reports (Falls and Neel 1951; Vogel 1954; Suckling et al. 1982) failed to show clustering in specific seasons. Nevertheless, this would be expected, because they were based on a small number of patients. We were able to analyze seasonal variation in the births of 753 children with sporadic unilateral retinoblastoma; about 90% of them can be assumed to be the result of somatic mutations, which, judging from the fairly early onset of the disease, must have occurred some time during the fetal or early postnatal period. The frequency of these cases fluctuated to some extent by month of birth, but there was no statistically significant deviation from the control or no cyclic trend. Thus, the occurrence of nonheritable retinoblastoma is not likely to be associated with certain viruses such as human adenovirus 12 whose activity varies markedly with season.

In conclusion, our epidemiological survey based on a large body of data failed to provide evidence for environmental risk factors in sporadic retinoblastoma. The negative results, together with the fairly uniform pattern in the incidence of this tumor among different populations (Parkin et al. 1988), suggest that most, if not all, cases of sporadic retinoblastoma are caused by some intrinsic biological mechanisms, and not by mutagens that are found in our daily environment and that vary with respect to time and place. If this proposition is correct, then primary prevention of this tumor would pose an extremely difficult problem, as is true for Down's syndrome in children born to younger mothers.

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References

Albert DM (1980) The role of viruses in the development of ocular tumors. Ophthalmology 87:1219–1225

Briard-Guillemot ML, Bonaïti-Pellié C, Feingold J, Frézal J (1974) Étude génétique de rétinoblastome. Humangenetik 24:271–284

Byrd P, Brown KW, Gallimore PH (1982) Malignant transformation of human embryonic retinoblasts by cloned adenovirus 12 DNA. Nature 298:69–71

Czeizel A, Gárdonyi J (1974) Retinoblastoma in Hungary, 1960–1968. Humangenetik 22:153–158

DeCaprio JA, Ludlow JW, Figge J, Shew J-Y, Huang C-M, Lee W-H, Marsilio E, Paucha E, Livingston DM (1988) SV40 large tumor antigen forms a specific complex with the product of retinoblastoma susceptibility gene. Cell 54:275–283

Dyson N, Howley PM, Münger K, Harlow E (1989) The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243:934–940

Edwards JH (1961) The recognition and estimation of cyclic trends. Ann Hum Genet 25:83–86

Ejima Y, Sasaki MS, Kaneko A, Tanooka H (1988) Types, rates, origin and expressivity of chromosome mutations involving 13q14 in retinoblastoma patients. Hum Genet 79:118–123

Emery AEH (1986) Methodology in medical genetics. Churchill Livingstone, Edinburgh

Falls HF, Neel JV (1951) Genetics of retinoblastoma. Arch Ophthalmol 46:367–389

Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 323:643–646

Fung Y-K, Murphree AL, T'Ang, Qian J, Hinricks SH, Benedict WF (1987) Structural evidence for the authenticity of the human retinoblastoma gene. Science 236:1657–1661

Kajii T, Tsukahara M, Fukushima Y, Hata A, Matsuo K, Kuroki Y (1985) Translocation (X;13)(q11.21;q12.3) in a girl with incontinentia pigmenti and bilateral retinoblastoma. Ann Génét (Paris) 28:219–223

Lee W-H, Bookstein R, Hong F, Young L-J, Shew J-Y, Lee EY-H (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science 235:1394–1399

Matsunaga E (1965) Parental age and sporadic retinoblastoma. Annu Rep Natl Inst Genet 16:121–123

Minoda K (1976) National registration of retinoblastoma in 1975 (in Japanese). Acta Soc Ophthalmol Jpn 80:1648–1657

Mukai N, Kalter SS, Cummins LB, Matthews VA (1980) Retinal tumor induced in the baboon by human adenovirus 12. Science 210: 1023–1025

Murphree AL, Benedict WF (1984) Retinoblastoma: clues to human oncogenesis. Science 223:1028–1033

Parkin DM,Stiller CA, Draper GJ, Bieber CA (1988) The international incidence of childhood cancer. Int J Cancer 42:511–520

Pellié C, Briard ML, Feingold J, Frézal J (1973) Parental age in retinoblastoma. Humangenetik 20:59–62

Penrose LS (1961) Mutation. In: Penrose LS, Brown HL (eds) Recent advances in human genetics. J & A Churchill, London, pp 1–18

Sasaki MS (1989) Cytogenetic aspects of cancer-predisposing genes. Jpn J Hum Genet 34:1–16

Suckling RD, Fitzgerald PH, Stewart J, Wells E (1982) The incidence and epidemiology of retinoblastoma in New Zealand: a 30-year survey. Br J Cancer 46:729–736

Tünte W (1972) Human mutations and paternal age. Humangenetik 16:77–82

Vogel F (1954) Über Genetik und Mutationsrate des Retinoblastomas (Glioma retinae) nebst einigen allgemeinen Bemerkungen über die Methoden zur Mutationsratenschätzung beim Menschen. Z Menschl Vererb Konstitutionslehre 32:308–336

Vogel F (1979) Genetics of retinoblastoma. Hum Genet 52:1–54

Vogel F, Rathenberg R (1975) Spontaneous mutation in man. Adv Hum Genet 5:223–318

Whyte P, Buchkovich KJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, Harlow E (1988) Association between an oncogene and an antioncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature 334:124–129

Zimmerman LE (1970) Changing concepts concerning the pathogenesis of infectious disease. Am J Ophthalmol 69:947–964

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