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ORIGINAL INVESTIGATION

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High parental age is associated with sporadic hereditary retinoblastoma: the Dutch retinoblastoma register 1862–1994

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Abstract We wished to determine the influence of parental age at the birth of a retinoblastoma patient on the risk of sporadic hereditary retinoblastoma. The parental age at birth of 941 patients of the Dutch retinoblastoma register (1862–1994) was identified and compared between sporadic hereditary and nonhereditary patients. In a subcohort (1936–1994), a comparison was made with parental age at birth in the general population, as obtained from the Central Bureau of Statistics. Missing birth dates of the parents of retinoblastoma patients were traced with the help of the municipal registries and the Central Bureau of Genealogy. The mean paternal age was 10.7 months higher and the mean maternal age was 11.0 months higher in the sporadic hereditary retinoblastoma patients than in parents of nonhereditary patients. In the subcohort, the mean paternal and maternal ages of sporadic hereditary patients were also higher (12.4 and 11.5 months, respectively) than those of the general population. All differences were statistically significant. This study shows that a high parental age is associated with an enhanced risk of sporadic hereditary retinoblastoma.

Introduction

Retinoblastoma is a malignant pediatric tumor affecting the retina and occurring about once in 20 000 live births. Retinoblastoma occurs in a hereditary form (30%–40% of the patients) and a nonhereditary form (60%–70%; Vogel 1979). It develops following at least two (Knudson 1971; DerKinderen 1987) mutational events, namely, inactivation of both alleles of the retinoblastoma (RB1) gene at 13q14 (Cavenee et al. 1983; Dryja et al. 1986).

The hereditary form results from a germline mutation which is present in all body cells. Among hereditary cases, a familial hereditary form and a sporadic hereditary form may be distinguished. An indication for the familial hereditary form is a parent with retinoblastoma or a family member with this disease, indicating that one of the parents must be a carrier of the RB1 gene. In the sporadic hereditary form, no other family members are affected, and the patient is the first person in the family with retinoblastoma. In the nonhereditary form, the retinoblastoma mutation is exclusively found in the tumor cells of the retina (Knudson 1971; Vogel 1979).

Epidemiological observations suggest that parental age is related to the genesis of sporadic hereditary disorders such as Down's syndrome and achondroplasia. This is understandable, since the number of new mutations in germline cells increases with age (Penrose 1955; Vogel and Rathenberg 1975; Vogel 1979). The existence of an association of sporadic hereditary retinoblastoma with parental age is still controversial (Falls and Neel 1951; Stevenson and Martin 1957; Smith and Sorsby 1958; Macklin 1960; Matsunaga 1965; Fraser and Friedmann 1967; Tünte 1972; Pellié et al. 1973; Bunin et al. 1989; Matsunaga et al. 1990; DerKinderen et al. 1990). Furthermore, DNA investigations on some patients suggest that new germline mutations are principally of paternal orgin (Ejima et al. 1988; Dryja et al. 1989; Zhu et al. 1989). Therefore, we have studied the possible association between an older age of fathers with a higher incidence of sporadic hereditary retinoblastoma in the Dutch retinoblastoma register. This register has been extensively enlarged since previous analyses concerning this problem (DerKinderen et al. 1990). Furthermore, reliable demographic data are available for analysis in the Netherlands.

The aims of this study have been to compare the mean paternal and maternal ages of parents at birth of sporadic hereditary retinoblastoma patients with (1) the mean pa-

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ternal and maternal ages of parents at birth of nonhereditary retinoblastoma patients and with (2) the mean paternal and maternal ages at birth in the general Dutch population. Furthermore, we have determined the relative risk of older fathers and mothers having a child with sporadic hereditary retinoblastoma.

Patients and methods

This project was approved by the Medical Ethics Committee of the Free University Hospital, Amsterdam, The Netherlands. The national retinoblastoma register of the Netherlands (Schappert-Kimmijser et al. 1966; DerKinderen 1987) was used. For our study, the register has recently been updated and the data verified (Moll et al. 1995). We extracted the date of birth of the 941 registered Dutch retinoblastoma patients born during the period from 1862 to 1994 and, if available, the date of birth of their parents. The missing birth dates of the parents were obtained with the help of the municipal registers and the Central Bureau of Genealogy in The Hague. Nineteen patients were excluded because data regarding heredity were missing.

We may consider retinoblastoma hereditary if one or more of the following criteria are met: a bilateral retinoblastoma has occurred, a family history for retinoblastoma is known (the related parent will then be a carrier of the defect in the RB1 gene) or a defect in the RB1 gene has been found in a chromosomal/DNA analysis of the patient. Some sporadic unilateral retinoblastoma patients might be classified incorrectly as nonherditary, since approximately 10% of the hereditary retinoblastoma patients are affected unilaterally (Vogel 1979; Blanquet et al. 1995).

Only the mean ages of parents at birth of the general population and the annual total number of live births for the subcohort 1936–1994 were available from the Central Office of Statistics in the Hague. Therefore, it was possible to compare our data regarding parental age at birth with data for the general population and to calculate the relative risk for parents in various age groups of having a child with sporadic hereditary retinoblastoma only for this subcohort.

The mean parental age at birth of sporadic hereditary retinoblastoma patients and the mean parental age at birth of nonhereditary retinoblastoma patients were compared with Student's two-sample *t*-test. The mean parental age at birth of sporadic hereditary retinoblastoma patients was tested against the mean parental age at birth in the general population using Student's one-sample *t*-test (the general mean is considered to be fixed). Since both the general literature concerning germline muatations (Penrose 1955) and the more specific retinoblastoma literature (Pellié et al. 1973; Vogel 1979) indicate that the relationship between parental age and the risk of mutation should be an increasing one, we felt justified in applying the statistical tests one-sidedly, with a level of significance of 0.05. This implies confidence intervals (CI), based on a Gaussian distribution, of 90%.

The incidence of sporadic hereditary retinoblastoma was calculated for different paternal and maternal age categories (< 24, 25–29, 30–34, 35–39, > 40 years). These incidences were compared with incidences in the same age categories in the general population. The relative risk for fathers older than 35 years (compared with those younger than 35 years) and for mothers older than 30 years (compared with those younger than 30 years) was calculated. The 90% CI were computed for incidences and for relative risks.

Results

Data on parental ages at birth, during the period 1862–1994, were obtained for 224 fathers and 221 mothers of 241 sporadic hereditary patients and for 533 fathers and 519 mothers of 602 nonhereditary patients. In the subcohort 1936–1994, 704 patients were listed in the national retinoblastoma registry. Three patients lacking data regarding hereditarity were excluded. We excluded the 82 familial hereditary patients from the analyses for reasons given in the Introduction. With respect to the 182 sporadic hereditary patients, the parental age at birth of 178 fathers and 178 mothers was known. The parental age at birth for 411 fathers and 416 mothers of the 437 nonhereditary patients was available.

For the complete cohort (1862–1994), the mean paternal and maternal ages at birth of sporadic hereditary retinoblastoma patients were significantly higher (10.7 months and 11.0 months, respectively) than those of nonhereditary patients (Table 1). In the subcohort (1936–1994), the mean paternal and maternal ages at birth of sporadic hereditary retinoblastoma patients were signifi-

Table 1 Mean paternal and maternal age at birth of sporadic hereditary retinoblastoma patients compared with nonhereditary retinoblastoma patients (1862–1994). *RB* Retinoblastoma, *CI* confidence interval

	Mean paternal age		Mean maternal age	
	Years	(*n*)	Years	(*n*)
Sporadic hereditary RB	33.01	(224)	30.21	(221)
Nonhereditary RB	32.12	(533)	29.29	(519)
P-value (one-sided)	0.048		0.02	
Difference in months	10.72		11.03	
90% CI in months	0.09–21.35		2.11–19.94	

Table 2 **a** Mean paternal and maternal age at birth of sporadic hereditary retinoblastoma patients compared with nonhereditary retinoblastoma patients (1936–1994). **b** Mean paternal and maternal age at birth of sporadic hereditary retinoblastoma patients compared with the general population (1936–1994). *RB* Retinoblastoma, *CI* confidence interval

a	Mean paternal age		Mean maternal age	
	Years	(*n*)	Years	(*n*)
Sporadic hereditary RB	32.96	(178)	30.18	(178)
Nonhereditary RB	31.86	(411)	28.97	(416)
P-value (one-sided)	0.025		0.005	
Difference in months	13.23		14.55	
90% CI in months	1.48–24.97		5.00–24.10	
b	Mean paternal age		Mean maternal age	
	Years	(*n*)	Years	(*n*)
Sporadic hereditary RB	32.96	(178)	30.18	(178)
Nonhereditary RB	31.98		29.25	
P-value (one-sided)	0.03		0.015	
Difference in months	12.35		11.48	
90% CI in months	3.23–21.47		3.71–19.25	

Table 3 **a** The incidence of sporadic hereditary retinoblastoma per 100 000 live births stratified for parental age (1936–1994). **b** Relative risk (*RR*) for sporadic hereditary retinoblastoma of high versus low parental age (1936–1994)

a	< 20	20–29	30–34	35–39	> 40
Paternal	–	1.35	1.43	2.06	1.54
90% CI	–	1.05–1.62	1.10–1.76	1.53–2.59	0.97–210
Maternal	1.32	1.33	1.62	2.04	–
90% CI	0.97–1.68	1.04–1.62	1.23–2.01	1.50–2.58	–

b		RR	90% CI
Paternal age	(age < 35 vs age > = 35	1.34	1.04–1.74)
Maternal age	(age < 30 vs age > = 35	1.35	1.06–1.72)

cantly higher (13.2 months and 14.6 months, respectively) than those of the nonhereditary retinoblastoma patients (Table 2a) and also significantly higher (12.3 months and 11.5 months, respectively) than in the general population of the Netherlands (Table 2b).

The incidences of sporadic hereditary retinoblastoma in the different paternal and maternal age categoriesare shown in Table 3a. There appears to be a trend indicating higher incidences for higher ages at birth, both for fathers and mothers. The relative risk for fathers older than 35 years and for mothers older than 30 years, and their 90% CI were computed (Table 3b).

Discussion

This study has shown that the mean age of parents at the birth of children with sporadic hereditary retinoblastoma is significantly higher than the mean age of parents at birth of children in the general population. This applies to both the paternal and maternal age at birth. In the literature, the relationship between parental age at birth and retinoblastoma remains controversial (Table 4). Most of the eleven available studies are based on a small number of cases. We have found only three studies with more than 100 sporadic bilateral cases, viz., those of Pellié et al. (1973; $n = 155$), DerKinderen et al. (1990; $n = 104$), and Matsunaga et al. (1990; $n = 225$). Until recently, it has been difficult to obtain appropriate control data of paternal age. Pellié et al. (1973) compare paternal ages using French population statistics for 1956. The year of birth of those children extends over more than 10 years (1951–1960), during which time the paternal age distribution in the general population is likely to have changed gradually. Matsunaga et al. (1990) obtained precise data on parental age from Japanese population statistics only for the periods 1965–1968 and 1975–1982. In the Netherlands, data regarding parental age are annually provided by the Central Bureau of Statistics from 1936 onwards. DerKinderen et al. (1990) have used these data to establish a parental age effect in their patient cohort (1945–1970). These three large studies have led to different results: Pellié et al. (1973) find a paternal age effect, DerKinderen et al. (1990) have established a parental age effect, and Matsunaga et al. (1990) mention only an age effect for fathers older than 35 years. In summary, all three studies suggest a paternal age effect, whereas a maternal age effect is only revealed by DerKinderen et al. (1990).

As mentioned above, DNA investigations of a few patients have shown that new germline mutations causing retinoblastoma are of paternal origin. Our study has established an association between high paternal and high maternal age and sporadic hereditary retinoblastoma and thus cannot confirm an exclusive association for high paternal age only. Isolated paternal age effects are difficult to find in traditional Western communities, where the ages of fathers and mothers are usually similar (Central Office of Statistics 1936–1994). Therefore, any possible paternal age effect would be confounded by maternal age and vice versa. A theoretical solution would be a correction for this

Table 4 Review of the literature: mean parental age at birth (in years) of sporadic hereditary retinoblastoma patients

Publication	Cases (*n*)	Controls	Mean paternal age		Mean maternal age		Conclusion
			obs.	exp.	obs.	exp.	
Falls ’51	23	pop. Michigan	?	?	?	?	Paternal trend NS
Stevenson ’57	5	matched-controls	34.2	25.8	30.3	23.8	Paternal age effect S
Smith ’58	56	pop. England	32.6	32.2	29.1	28.9	NS
Macklin ’60	?	?	?	?	?	?	No association
Matsunaga ’65	21	pop. Japan	34.4	32.2	28.9	28.8	Paternal age effect S
Fraser ’67	37	pop.	33.6	31.7	30.9	28.6	Parental age effect S
Tünte ’72	17	?	33.5	31.5	31.7	28.7	Parental trent NS
Pellié ’73	155	pop. France	32.3	31.1	28.2	27.6	Paternal age effect S
Bunin ’89	67	matched-controls	29.8	28.6	27.4	26.4	NS
DerKinderen ’90	104	pop. Netherlands	33.7	32.5	31.2	29.5	Parental age effect S
Matsunaga ’90	225	pop. Japan	30.2	30.1	27.3	27.3	Only fathers older than 35 effect S
Moll ’96	178	pop. Netherlands	33.0	32.0	30.2	29.3	Parental age effect S

cases = cases of the hereditary retinoblastoma, obs. = observed, exp. = expected, pop. = population based, NS = not significant, S = Significant

confounding effect by means of stratification and multivariance analysis. This is problematic because of multicolliniarity, viz., the strong association between the ages of fathers and mothers. Furthermore, in The Netherlands, it is rarely possible to form subgroups of "old mothers with young fathers" and "old fathers with young mothers". Therefore, the age effect of the father cannot be readily separated from the age effect of the mother. In contrast, it is much more customary that older men marry much younger women in Saudi Arabia. Senft et al. (1994) have indeed found a higher incidence of sporadic bilateral retinoblastoma in Saudi Arabia, specifically attributable to the higher paternal ages.

In the last few decades, the mean parental age has changed in The Netherlands (Central Office of Statistics 1936–1994). Der Kinderen et al. (1990) have found a gradual decrease of the mean parental age in The Netherlands, and a parallel decrease of the incidence of sporadic hereditary retinoblastoma over the period 1945–1979. The distribution of hereditary and nonhereditary cases over the period 1862–1995 has not changed significantly (Moll et al. 1996).

As mentioned above, some sporadic unilateral retinoblastoma patients might be classified incorrectly as nonherditary, since approximately 10% of the hereditary retinoblastoma patients are affected unilaterally (Vogel 1979; Blanquet et al. 1995). Such possibly incorrect classifications could influence statistical analysis. However, we have previously classified a number of unilateral sporadic retinoblastoma patients as hereditary retinoblastoma patients when they had affected offspring or when a defect in the RB1 gene was found in chromosomal/DNA analysis of the patient. Thus, the misclassification would be less than 10%. On the other hand, this influence should be a conservative one, since wrongly classified unilateral hereditary patients would decrease the difference between the hereditary and nonhereditary mean parental age, as wrongly classified unilateral patients (with a high mean parental age associated with hereditary retinoblastoma) would increase the mean parental age of nonhereditary patients.

We conclude that, in the Netherlands, the mean age of parents (both fathers and mothers) at birth of children with sporadic hereditary retinoblastoma is significantly higher than the mean age of parents at birth of children in the general population for the period 1936–1994. This confirms our hypothesis that age is a risk factor for sporadic hereditary retinoblastoma.

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