ASSOCIATIONS OF RB1 GENETIC VARIANTS WITH CLINICAL TRAITS IN RETINOBLASTOMA PATIENTS FROM A BRAZILIAN SERVICE (INCA - RIO DE JANEIRO)

Authors: Mireille Caroline Silva de Miranda Gomes[1], Bruna Palma Matta[1], Valdirene Lima[1], Leila Leontina Couto[1], Barbara Nasr[1], Hector Nicolas Seuanez de Abreu[1], Miguel Angelo Moraes Moreira[1], Cibele Rodrigues Bonvicino[1], Maria Angelica de Faria Domingues de Lima[2], Fernando Regla Vargas[3,4], Anna Claudia Evangelista dos Santos[1]

1- Instituto Nacional do Cancer (INCA), Rio de Janeiro - RJ, Brasil. 2- Universidade do Grande Rio (UNIGRANRIO), Rio de Janeiro - RJ, Brasil. 3- Hospital Universitário Gaffrée e Guinle (HUGG), Rio de Janeiro - RJ, Brasil. 4 Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro - RJ, Brasil.

AIMS

To estimate the frequency of genetic variants in *RB1* gene and to test possible associations with different clinical traits, in patients with Retinoblastoma (RB) diagnosis referred to INCA's Clinical Genetics Clinic.

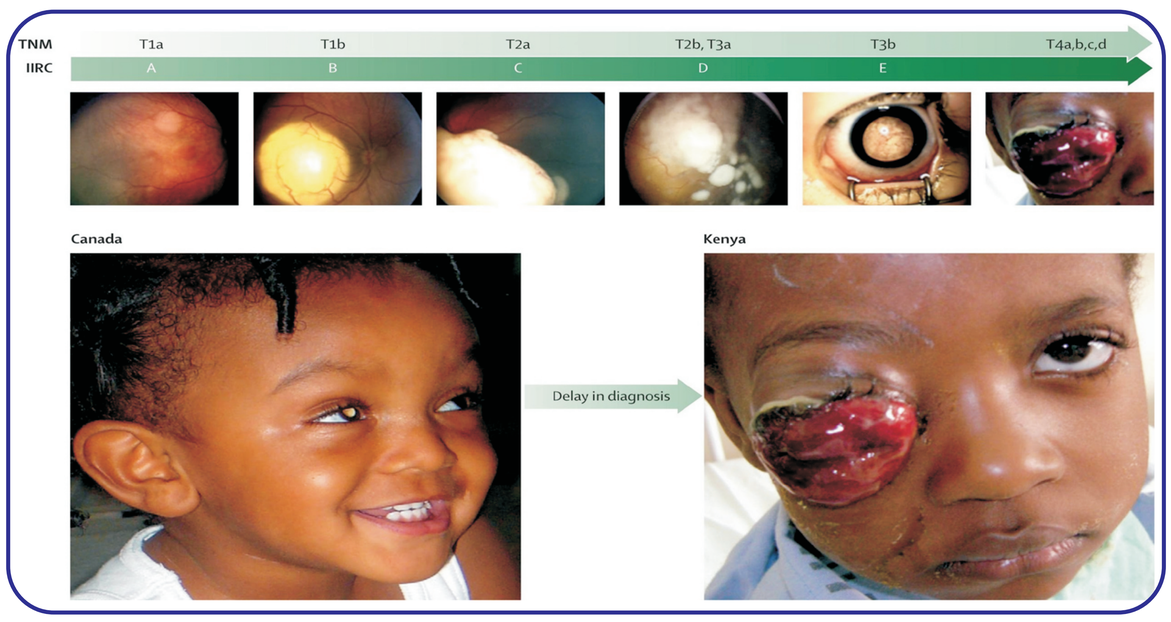

Figure 1: Progression of retinoblastoma from small intraretinal tumours to massive orbital retinoblastoma probably extending into the brain. **Original from: The Lancet 2012 379(9824): 1436-1446. DOI: (10.1016/S0140-6736(11)61137-9). Copyright © 2012 Elsevier Ltd.**

PATIENTS AND METHODS

Retrospective chart review of 206 patients was performed. So far we reviewed three continuous traits – age of diagnosis (DX), age of first symptom (1S) and lag-time between 1S and DX (LAG) – and the following categorical traits: laterality, survival, family history of RB, family history of other cancers, intra/extraocular tumors, enucleation, radio/chemotherapy, secondary tumors, and change in laterality or intra/extraocular tumors. We performed complete Sanger sequencing and/or Multiplex Ligation-dependent Probe Amplification (MLPA) of *RB1* gene from blood samples of 127 patients. *RB1* molecular diagnosis was defined as Positive (pathogenic variant in Sanger sequencing and/or MLPA abnormality) or Inconclusive (variants with benign or unknown clinical significance). To test the effects of *RB1* molecular diagnosis, laterality and survival on the square root (SQRT) of each continuous trait we used three-way ANOVA. Chi-square tests were used to test possible associations between pairs of categorical traits (alpha was adjusted for multiple testing).

Table 1: Descriptive statistics and three-way ANOVA of continuous age traits in Retinoblastoma patients from INCA's Clinical Genetics Clinics. * P < 0.05; ** P < 0.01; ND = non-determined

CATEGORICAL TRAITS		CONTINUOUS AGE TRAITS (IN MONTHS)		
		DX: age of diagnosis mean ± SE (n)	1S: age of 1st symptom mean ± SE (n)	LAG: 1S to DX mean ± SE (n)
Laterality	Bilateral	**11.49 ± 0.26** (64)	**4.67 ± 0.30** (60)	4.83 ± 0.25 (60)
	Unilateral	**26.41 ± 0.24** (142)	**15.74 ± 0.44** (135)	6.22 ± 0.21 (135)
Molecular diagnosis	Positive	**10.95 ± 0.29** (49)	4.13 ± 0.41 (47)	4.40 ± 0.25 (47)
	Inconclusive	**23.19 ± 0.30** (78)	14.97 ± 0.43 (74)	4.75 ± 0.23 (72)
Survival	Deceased	24.05 ± 0.47 (36)	11.00 ± 0.70 (34)	**10.28 ± 0.41** (34)
	Alive/ND	20.52 ± 0.26 (170)	11.77 ± 0.41 (161)	**4.69 ± 0.14** (159)
THREE-WAY ANOVA FACTORS		DX: age of diagnosis mean squares	1S: age of 1st symptom mean squares	LAG: 1S to DX mean squares
df = 1	Laterality (L)	**17.01****	**18.15****	0.14
df = 1	Molecular diagnosis (M)	**10.27***	8.79	2.62
df = 1	Death (D)	3.55	0.11	**19.20****
df = 1	L × M	1.22	**13.29****	4.14
df = 1	L × D	2.88	0.20	0.03
df = 1	M × D	3.25	2.24	1.86
df = 1	L × M × D	0.10	1.19	5.50
df = 113	Error	2.29	3.13	1.72

RESULTS

ANOVA of SQRT age data showed that patients with bilateral RB have, on average, significant earlier diagnosis (DX = 11.5m ± 0.3) and first symptom (1S = 4.7m ± 0.3) than unilateral ones (DX = 26.4m ± 0.2; 1S = 15.7m ± 0.4); $P < 0.01$. Patients with pathogenic variants also presented significant earlier DX average (11.0m ± 0.3) than inconclusive ones (23.2m ± 0.3); $P < 0.05$. These results are in accordance with RB literature. Significant effect of survival was found only for LAG time, in which deceased patients had greater LAG average (10.3m ± 0.4) than living/non-determined ones (4.7m ± 0.1); $P < 0.01$. Chi-square tests were non-significant for all pairs of categorical traits (alpha = 0.01), except for: *RB1* molecular diagnosis (M) x laterality; M x family history of RB; M x change in laterality; survival x intra/extraocular tumors. For instance, pathogenic variants were associated with higher frequency of bilateral tumors (31 cases) than unilateral ones (18 cases). Most frequent types of pathogenic *RB1* variants were nonsense (20 cases) and splicing variants (9 cases). Interestingly, all patients with splicing variants also had bilateral tumors. We are currently reviewing other chart data, like focality and tumor staging, as well as finishing Sanger sequencing and MLPA analysis of remainder patients, which might bring more information about the effects of *RB1* molecular diagnosis on clinical traits.

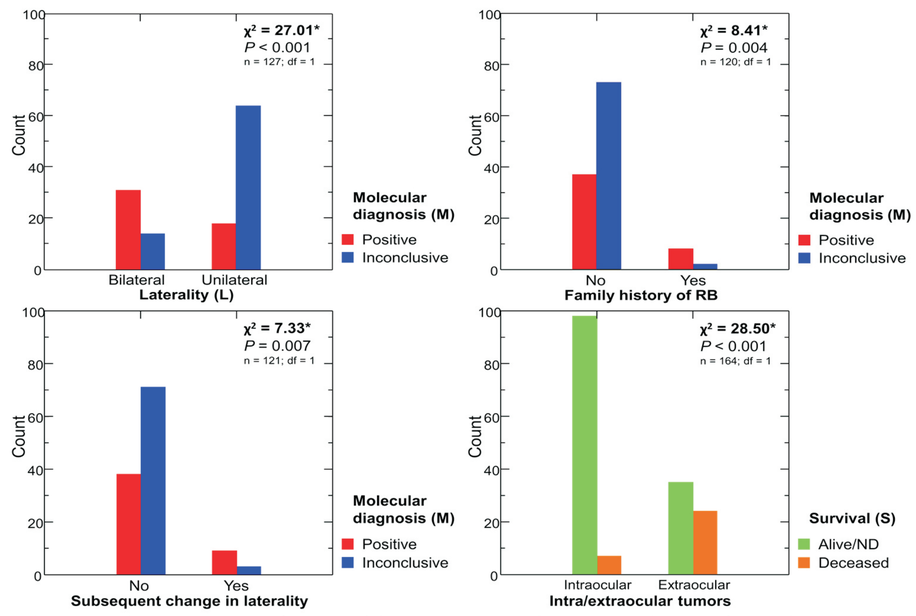

Figure 2: Bar plots and chi-square tests (α = 0.01) for some pairs of categorical traits; * significant.

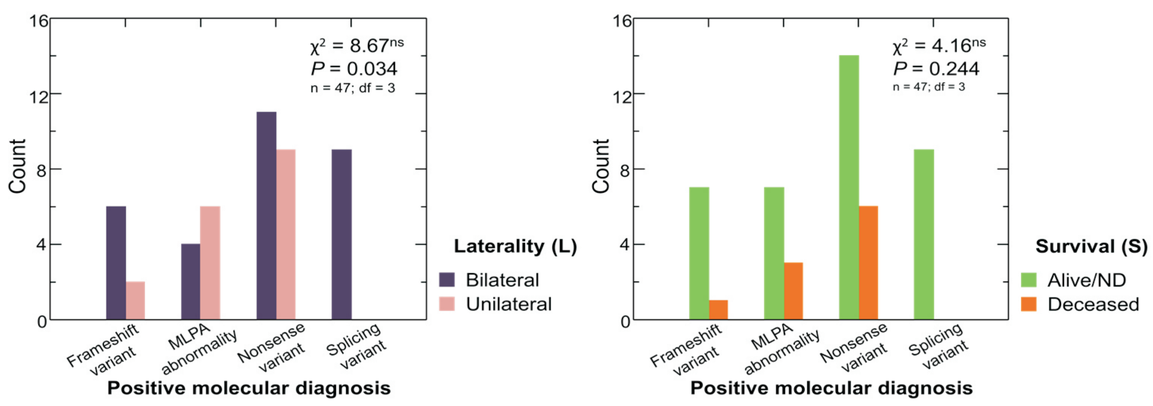

Figure 3: Bar plots and chi-square tests for the most frequent classes of Positive (pathogenic) molecular diagnosis; missense and 5'UTR variants are omitted (2 cases); ns non-significant.

CONCLUSION

Frequencies of *RB1* genetic variants were similar to those in RB literature. Pathogenic variants were more frequent in patients with bilateral tumors. And a possible trend in which patients with splicing variants develop bilateral RB was detected.

REFERENCES

1. Dimaras H., et al. (2012) The Lancet 379(9824): 1436-1446.
2. Aerts I., et al. (2016) Archives de Pédiatrie, 23(1):112-116
3. Brichard B., et al. (2006) European Journal of cancer, 42(1):65-72.
4. Ramírez-Ortiz M.A., et al. (2014) Cancer Epidemiolgy, Biomarkers & Prevention, 23(5):784-792.
5. Selistre S.G.A., et al. (2016) BMC Pediatrics, 16:48.

Meeting: XXIX CONGRESSO BRASILEIRO GENÉTICA MÉDICA 2017

Financial support: CNPq CAPES

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA