**BRIEF COMMUNICATION**

**MDM2 as a Modifier Gene in Retinoblastoma**

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Variability in the age of onset and number of tumors is occasionally described among retinoblastoma patients, and possible genetic modifiers might lie in the pRB or p53 pathways, both of which are involved in the development of retinoblastoma. MDM2, which increases p53 and pRB catabolism, is therefore a prominent candidate. The minor allele of MDM2 that includes a 309T>G transversion (single-nucleotide polymorphism rs2279744) in the MDM2 promoter is known to enhance MDM2 expression. Its genetic transmission was studied in 326 individuals including 212 RB1 mutation carriers in 70 retinoblastoma families, and the marker genotype was tested for association with age at diagnosis and disease phenotype. In family-based association analyses, the MDM2 309G allele was found to be statistically significantly associated with incidence of bilateral or unilateral retinoblastoma among members of retinoblastoma families (Z = 3.305, two-sided exact P = .001) under a recessive model (ie, affected patients tend to be homozygous for the G allele); in transmission disequilibrium analyses using the recessive model, the association was also observed (estimated odds ratio = 4.0, 95% confidence interval = 1.3 to 12.0). The strong association of this genotype with retinoblastoma development designates MDM2 as the first modifier gene to be identified among retinoblastoma patients and suggests that enhancement of pRB haploinsufficiency and/or resistance to p53-mediated apoptosis is critical to tumor formation.


Retinoblastoma is the most common intraocular childhood cancer and occurs when both alleles of the RB1 gene are inactivated in the retina. In subjects with a genetic predisposition to retinoblastoma, the first RB1 mutation is found in the germline, and the second appears as a somatic mutation (1–4). Germline carriers usually develop bilateral or multifocal tumors. However, some rare families exhibit low penetrance and variable expressivity of the disease because bilaterally affected, unilaterally affected, and unaffected mutation carriers are known to coexist (5). The existence of genetic modifiers in retinoblastoma therefore appears highly probable and must be considered (6).

Modifiers of retinoblastoma expressivity and penetrance could be components of the RB1 protein (pRB) pathway. Because TP53 is generally not mutated in retinoblastoma, complete loss of RB1 activates p53-mediated apoptosis and would not confer a growth advantage (7). MDM (Murine Double Minute) family proteins such as MDM2 and MDM4, which are both expressed in retinoblastoma cells (8), are key negative regulators of the p53 pathway: their amplification would be expected to inhibit p53-mediated transactivation activity targeting p53 for proteosomal degradation, thereby bypassing p53-mediated apoptosis and conferring a cell growth advantage when RB1 expression has been lost. Amplification of MDM4 (9) has been shown to repress p53-mediated apoptosis and facilitate retinal cell transformation and tumorigenesis associated with inactivation of both RB1 allele in the context of retinoblastoma (10). MDM2 (11) has been shown to enhance proteasome-dependent degradation of p53 (12) and also to promote pRB degradation by ubiquitin-dependent or -independent mechanisms (11,13–15). Changes in MDM2 expression levels may therefore be associated with variable phenotypic expression of retinoblastoma as previously demonstrated in patients with Li-Fraumeni syndrome, who have autosomal-dominant germline mutations in TP53 (16).

A single-nucleotide polymorphism (SNP) located at nucleotide 309 in the MDM2 promoter, the MDM2 309T>G SNP (rs2279744, also referred to as “SNP309”), has been described to enhance transcription of MDM2 and accumulation of MDM2 protein, resulting in attenuation of the p53 pathway. The MDM2 SNP309 G allele has been clearly shown to be associated with earlier age of onset of tumors among Li-Fraumeni patients (16,17). Because MDM2 interacts directly with pRB (13), presence of the SNP309 G allele might similarly be associated with enhanced pRB degradation and accelerated tumorigenesis, explaining the variable expression of pRB in retinoblastoma.

We investigated the possible association of MDM2 SNP309 with retinoblastoma clinical outcome by using software for the family-based allelic association test (FBAT), a generalized version of the original transmission disequilibrium test (TDT) (18–20). We assessed MDM2 SNP309 genotype in 326 individuals from 70 retinoblastoma families, including 212 carriers of a germ-line mutation (113 bilaterally affected patients, 40 unilaterally affected patients, 53 unaffected mutation carriers, and six patients with retinoma) and 114 relatives (Supplementary Table S1, available online). The subjects were included from 650 consecutive retinoblastoma patients, who were tested for germ-line RB1 mutations at the Institut Curie from September 2000 to April 2008, and their relatives. Individual written consent was obtained from all sampled individuals or their legal guardians. MDM2 SNP309 genotype was determined by sequencing, and family-based association analyses were conducted as described...
Table 1. Association of MDM2 SNP309 and tumor incidence in 70 pedigrees with familial retinoblastoma

| Marker | Allele | Frequency† | Family‡ | S§ | $E(S)$ || Var(S)¶ | Z# | Asymptotic P** | Exact P†† |
|--------|--------|------------|---------|----|--------|--------|--------|----------|-----------|
| Additive model | | | | | | | | | |
| SNP309 | T      | 0.604      | 39      | 53 | 60    | 14.36  | −1.847 | .065     | .085      |
| SNP309 | G      | 0.396      | 39      | 49 | 42    | 14.36  | 1.847  | .065     | .085      |
| Recessive model | | | | | | | | | |
| SNP309 | T      | 0.604      | 30      | 17 | 17.17 | 8.84   | −0.056 | .562     | 1.0       |
| SNP309 | G      | 0.396      | 16      | 15 | 8.17  | 4.28   | 3.305  | <.001    | .001      |

* Values shown are from family-based allelic association test (FBAT) analyses that tested associations of bilateral or unilateral tumor presentation vs no tumor presentation among familial retinoblastoma patients carrier of the segregating RB1 mutation with either allele of MDM2 single-nucleotide polymorphism (SNP) 309. Familial retinoblastoma was defined as families with two or more carriers of an RB1 gene mutation in a family. In the additive model, cumulative effect of both transmitted alleles of SNP309 was tested for association. In the recessive model, effect of homozygous genotype of SNP309 was tested for association. P values for the dominant model are the inverse of those of the recessive model. The dominant asymptotic P value for SNP309 are .0009 for the T allele and .562 for the G allele.
† Frequency represents the single allele frequency.
‡ Number of informative families for the specific allelic test (ie, families with at least one heterozygous parent).
§ S represents the test statistic of FBAT and expresses the observed number of transmitted alleles to the affected mutation carrier offspring.
|| E(S) is the value expected for S under the null hypothesis of no biased transmission.
¶ Var(S) is the asymptotic variance.
# Z-score (S normalized using E(S) and Var(S)).
** Asymptotic two-sided P values as calculated by FBAT, uncorrected for multiple testing.
†† Exact two-sided P values as calculated by exact family-based association tests, uncorrected for multiple testing.

SNP309 G allele with retinoblastoma development remained statistically significant after Bonferroni correction for multiple testing (the threshold for a 5% significance level would be 0.0125). By contrast, FBAT analysis did not demonstrate any statistically significant association between the presence of SNP309 and a patient’s age at diagnosis of retinoblastoma (Supplementary Table S2, available online). However, age at diagnosis may represent a poor marker of disease severity in familial retinoblastoma due to changing ophthalmologic follow-up in more recent years.

Association of MDM2 SNP309 with retinoblastoma development was then tested using a subset composed of 52 retinoblastoma families with a high penetrance mutation in RB1. This analysis also demonstrated a statistically significant association

Table 2. Association of MDM2 SNP309 and tumor incidence in the subset of 52 pedigrees with familial retinoblastoma and a high penetrance mutation in RB1

| Marker | Allele | Frequency† | Family‡ | S§ | $E(S)$ || Var(S)¶ | Z# | Asymptotic P** | Exact P†† |
|--------|--------|------------|---------|----|--------|--------|--------|----------|-----------|
| Additive model | | | | | | | | | |
| SNP309 | T      | 0.631      | 29      | 43 | 48    | 10.86  | −1.517 | .129     | .17       |
| SNP309 | G      | 0.369      | 29      | 37 | 32    | 10.86  | 1.517  | .129     | .17       |
| Recessive model | | | | | | | | | |
| SNP309 | T      | 0.631      | 23      | 14 | 13.67 | 7.08   | 0.125  | .90      | 1.0       |
| SNP309 | G      | 0.369      | 12      | 11 | 5.67  | 3.03   | 3.066  | <.002    | .003      |

* Values shown are from family-based allelic association test (FBAT) analyses that tested associations of bilateral or unilateral tumor presentation vs no tumor presentation among familial retinoblastoma patients carrier of the segregating RB1 mutation with either allele of MDM2 single-nucleotide polymorphism (SNP) 309. Familial retinoblastoma was defined as families with two or more carriers of an RB1 gene mutation in a family. In the additive model, cumulative effect of both transmitted alleles of SNP309 was tested for association. In the recessive model, effect of homozygous genotype of SNP309 was tested for association. The RB1 mutation was classified as either a "low penetrance mutation" (18 families) or a "high penetrance mutation" (52 families), depending on the documented effect of the mutation in the literature (see supplementary information on line).
† Frequency represents the single allele frequency.
‡ Number of informative families for the specific allelic test (ie, families with at least one heterozygous parent).
§ S represents the test statistic of FBAT and expresses the observed number of transmitted alleles to the affected mutation carrier offspring.
|| E(S) is the value expected for S under the null hypothesis of no biased transmission.
¶ Var(S) is the asymptotic variance.
# Z-score (S normalized using E(S) and Var(S)).
** Asymptotic two-sided P values as calculated by FBAT, uncorrected for multiple testing.
†† Exact two-sided P values as calculated by exact family-based association tests, uncorrected for multiple testing.
under a recessive model (Z = 3.066, P = .003; Table 2) and showed that the variable penetrance of germline RB1 mutation cannot account for the observed association of the MDM2 SNP309 G allele with tumor status. The analysis was not performed in low penetrance families due to an insufficient number of informative families. However, when the classical TDT test was performed on the whole set of retinoblastoma patients, a statistically significant association of SNP309 with tumor status was also observed (asymptotic P = .007; empirical P = .019, with an estimated odds ratio = 4.0, 95% confidence interval = 1.3 to 12.0; supplementary information, available online). The TDT test performed in the subset of retinoblastoma patients with a high penetrance mutation in RB1 also yielded a statistically significant result (asymptotic P = .008; empirical P = .016, with an estimated odds ratio = 4.7, 95% confidence interval = 1.3 to 16.2; supplementary information, available online).

Since we obtained these results, two recent publications have suggested that certain SNPs in the human MDM4 gene might also modify the efficacy of the p53 pathway (22,23). MDM4 is considered to be an important component of p53 regulation (24), although its precise role is less clear than that of MDM2 (there are 120 PubMed references focusing on MDM4 vs 3599 on MDM2). No statistically significant association was found between the presence of the MDM4 C>T SNP rs1563828 and tumor incidence (Supplementary Table S3, available online). This complementary investigation, therefore, did not support a role for MDM4 in hereditary predisposition to retinoblastoma in humans. MDM4 amplification may be restricted to proliferating cells in the tumor, as previously suggested (25).

By contrast, our results do show that the MDM2 SNP309 G/G genotype could accelerate tumor formation in RB1+/- cells. Several mechanisms can be proposed based on the role of pRB on genomic instability and apoptosis. Several lines of evidence indicate that RB1 inactivation enhances DNA double-strand break accumulation and participates in genetic instability in a dose-dependent manner (26). Specifically, in mouse embryonic stem cells, loss of one RB1 allele causes an increase in genetic instability (27). A recent publication (28) proposed that human retinoblastoma originates in L/M cone precursors of the retina in the context of constitutive overexpression of MDM2 mediated by activation of its promoter. In an L/M cone precursor that is genetically predisposed to retinoblastoma, overexpression of MDM2 could enhance RB1 haploinsufficiency and therefore the acquisition of DNA double-strand breaks and genetic instability. Furthermore, pRB has recently been described as a stress-induced activator of apoptosis (29), prompting those authors to suggest that germline RB1 carriers might have a higher risk of cellular transformation because apoptosis in response to DNA damage is compromised, thereby enabling the acquisition of other mutations. In the context of genetic predisposition to retinoblastoma, therefore, the MDM2 SNP309 G/G genotype could exacerbate all of the effects of RB1 haploinsufficiency (13,14).

Consequently, genomic instability could arise, and somatic mutations could accumulate in RB1+/- apoptosis-resistant cells, prompting the emergence of clonal RB1+/- tumor(s).

One limitation of our study is that the contribution of MDM2 to disease severity was measured with just three phenotypes, depending on the number of affected eyes (unaffected, unilateral, and bilateral disease). This categorization obviously represents a clinical marker of disease severity but not a marker for the biological aggressiveness of retinoblastoma per se. Such marker should indeed include the number of affected eyes but also other parameters (eg, histopathological data, age at diagnosis, and occurrence of secondary nonretinal tumors). However, histopathological evaluation cannot always be used because (fortunately) not all retinoblastoma patients are enucleated. Moreover, a young child with an early-stage tumor could indeed be categorized as having a true “aggressive tumor” but could also be found to represent early detection of a “nonaggressive tumor” on ophthalmologic follow-up. These limitations may be partially overcome with a prospective study in fully genotyped retinoblastoma families followed by homogeneous protocols.

However, our findings may have therapeutic implications. Nutlin-3 (30) is an inhibitor of the MDM2 and MDM4 gene products that has an antitumor activity (31) that has been shown to be more effective in chronic lymphocytic leukemia cells carrying the MDM2 SNP309 G allele than in similar cells carrying the T allele (32). The MDM2 SNP309 genotype should therefore be considered in clinical trials of MDM2 inhibitors among retinoblastoma patients.

Overall, this study demonstrates a clear association between the presence of the MDM2 309G allele and retinoblastoma development in predisposed patients with RB1 mutations. Therefore, we propose MDM2 to be the first modifier gene identified to date in retinoblastoma.

References


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