A POLYMORPHIC GGC REPEAT IN THE *NPAS2* GENE AND ITS ASSOCIATION WITH MELANOMA

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BACKGROUND

Circadian rhythms are influenced by the expression of clock genes, which are controlled through a feedback mechanism that allows a rhythmic variations during 24 hours. Various evidences indicate that clock genes are important in neoplastic transformation.

NPAS2, the largest circadian gene, is located on chromosome 2 (2q11.2) and is mainly expressed in the forebrain, as well as in peripheral organs such as liver and skin. *NPAS2* forms heterodimers with *BMAL1*: the circadian pattern of mRNA levels produced by *NPAS2* is synchronized with those of *BMAL1* in various tissue. *NPAS2* is involved in the cell cycle, specifically in the mechanisms of DNA damage repair, therefore assumes a putative activity as tumor suppressor. Moreover, *NPAS2* influences the activity of other genes involved in tumorigenesis and epidemiological evidence suggests an association between polymorphisms in *NPAS2* and cancer risk. *CDKN2AIP* (*p16IP*), a gene influenced by *NPAS2*, is involved in the regulation of the cell cycle, activating *p53/TP53* through pathways *CDKN2A*- dependent. Gene *CDKN2A* (*p16*) contains somatic mutations in almost all melanomas, and is present in the 40% of hereditary melanomas.

Based on these notions, an association between *NPAS2* and melanoma is assumed, in terms of regulation of gene expression. Thus we investigated the presence of polymorphisms in the putative promoter and 5' untranslated region of *NPAS2* gene.

METHODS

Study included 72 melanoma patients and 77 control subjects. A polymorphic GGC repeat in the untranslated (first) exon of *NPAS2* was found using Sanger sequencing and capillary electrophoresis (Tab. 1).

	PCR forward	PCR reverse	Sequencing forward	Sequencing reverse
RORE	GACCTTTCCCCTCTCCCCG	CCTAGTGTAGACCCTCGCAG	GGCACCCGGGGATTATTC	CCTGTCCTAAGGCGAACGTA
Fr. 1	ACCTTGGTAAAATCCTCCCTGT	CTCCAAGTGCCCGCTCCT	ACCTTGGTAAAATCCTCCCTGT	CTCCAAGTGCCCGCTCCT
Fr. 2	AAAAGGAGAGGAGGGCAGC	TGACAGTCGCGTGCTCGT	AAAAGGAGAGGAGGGCAGC	TGACAGTCGCGTGCTCGT
Fr. 3	GAGGACAGTGTGGAGGGGG	CTGCGTGCGGAAGAGTTTG	GAGGACAGTGTGGAGGGGG	CTGCGTGCGGAAGAGTTTG
Exon 1	GTGGAGAGGGAGGAGGGT	AGGGTGGGTAGTACGTGCG	GTGGAGAGGGAGGAGGGT	AGGGTGGGTAGTACGTGCG
Sizing GGC repeat	[6FAM]GTGGAGAGGGAGGAGGGT	AGGGTGGGTAGTACGTGCG	-	-

Tab. 1: Primers for direct sequencing and sizing analysis.

RESULTS AND CONCLUSIONS

We found a polymorphic GCC repeat in the untranslated region (first) exon of *NPAS2* (Fig.1). In both groups (melanoma subjects and controls), four alleles where present, with 7, 9, 12 and 13 GGC repeats (Fig.2). Alleles 7 and 9 were the most frequent, showing a frequency >40% in controls and melonoma subjects.

In both groups allele and genotype frequencies were in Hardy-Weinberg equilibrium.

In terms of allele frequencies, no statistical difference was found between melanoma and control subjects. In contrast, significant differences were found in genotype frequencies (Fig.3). In particular, the genotype 7/9 was more frequent in controls than in melanoma subjects (57.1% vs 34.7%; p=0.0084); the genotype 9/9 was more frequent in melanoma subjects than in controls (26.3% vs 9.0%; p=0.0087). No statistical difference was found for other genotypes.

Therefore, the homozygous genotype of the GCC repeat of the *NPAS2* gene could be a susceptibility factor for melanoma.

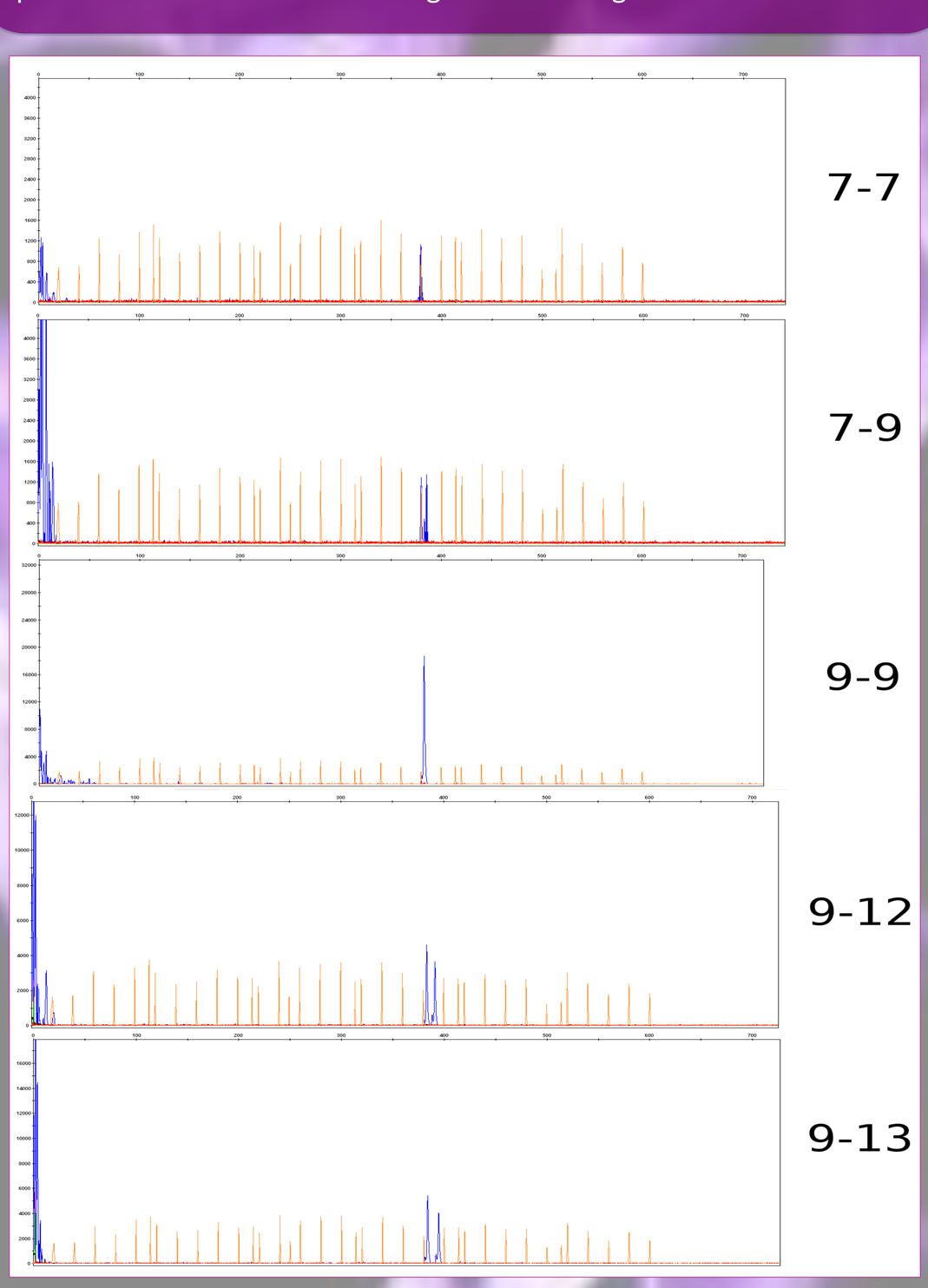

Fig. 2: Alleles revealed by capillary electrophoresis analysis.

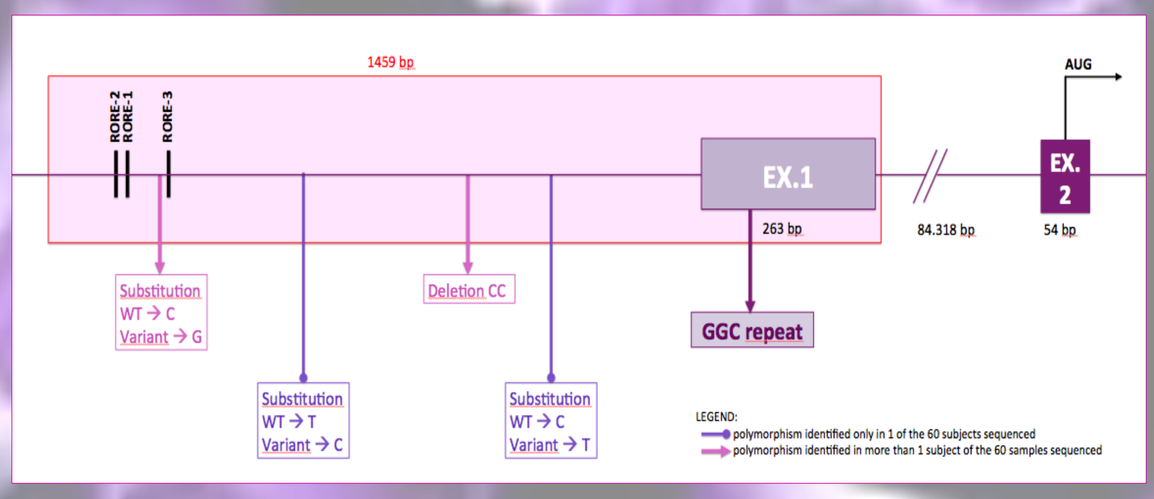

Fig. 1: Polymorphisms found in the putative promoter and 5' untranslated region.

Fig. 3: Genotype frequencies in the cases analyzed.

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