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### Exploring the Importance of Single Nucleotide Polymorphisms of HSPA9 in DNA of Sarcoma Patients

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# **Exploring the Importance of Single Nucleotide Polymorphisms of HSPA9 in DNA of Sarcoma Patients**

**Honors Thesis**

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**Abstract:**

The aim of this project was to identify genetic variants that may influence the risk and progression of sarcoma through targeted genotyping of HSPA9 gene. It is important to look at genetic variants in DNA samples because if a variant is determined to be more likely than another, a screening for the particular variant can be done to identify a patient's risk of sarcoma. The study population was sarcoma patients from the International Sarcoma Kindred Study. These patients had no mutations in p53 or MDM2. Genotyping data from the HapMap project ([hapmap.org](http://hapmap.org)) for HSPA9 was used to identify the polymorphisms needed to tag the entire region. In order to genotype the DNA sample, KASP reagents (KBioSciences, UK) were used. KASP uses a two-set PCR process. Allele specific primers are used to preferentially amplify each allele of a given SNP. The specific genetic variations of HSPA9 in sarcoma patient DNA samples with no mutations in p53 or MDM2 amplification are not more or less likely to occur than in DNA samples with the mutation or amplification. If continued research can show that MDM2 is not amplified, but activated through other mechanisms such as the interaction between polymorphisms of mitochondrial genes, p53, or MDM2, we can propose anti-MDM2 therapies to the patients with these polymorphisms.

**Introduction**

Identifying genetic variants that may influence the risk and progression of sarcoma through targeted genotyping of HSPA9 gene is important because looking at genetic variants in DNA samples and significant relationships of variants may help produce a screening for the particular variant that can be done to identify a patient's risk of sarcoma. A strong candidate biological pathway in cancer susceptibility is the regulation of growth arrest and/or apoptosis in the stressed or damaged cell (Cox et. al., 2007). The tumor suppressor gene p53 and MDM2 are essential in

cell apoptosis. A malfunctioning of p53 or MDM2 amplification will inhibit cell death of a cancerous cell. In normal human cells, the p53 protein has a pivotal role in the regulation of growth arrest and apoptosis when DNA is damaged. In 60% of human cancers, p53 malfunctions and cannot bind to DNA to transcribe downstream genes that initiate apoptosis (Vaseva and Moll, 2008). Many patients do not have mutations in p53 or MDM2 amplification that cause the malfunction in cancer cell, so the target DNA samples were sarcoma patient without p53 or MDM2 amplification. The HSPA9 gene encodes for mortalin. Mortalin has an important role in this pathway because in conditions of high stress, some cancer cell lines have been seen to have an overexpression of mortalin. This over expression causes a binding of mortalin and p53. Once p53 is bound to mortalin, it cannot be used as a checkpoint in the cell cycle to induce apoptosis of cancerous cells. Many variants in the gene for mortalin have been associated with Parkinson's Disease (Lu 2011, Burbulla 2011).

Dr. David Cox has performed research on the polymorphisms of tumor suppressor gene, *p53*, in relation to polymorphisms in *MDM2*, which causes p53 to become biologically active through processing (Cox et.al. 2007). Mutations in the tumor suppressor gene *p53* and amplification of the *MDM2* gene occur in many sarcomas, but these mutations and amplifications are not detected in all patients. Therefore, a hypothesis has been proposed that patients without *p53* mutations and *MDM2* amplifications may have germ line variants or cryptic mutations in genes related to p53, *MDM2*, and mitochondria-mediated apoptosis that are responsible for their sarcoma. SNP tagging can be done to identify polymorphisms that may influence sarcoma risk and progression. The null hypothesis is that there is no association between polymorphisms in *HSPA9* and sarcoma.

## **Material and Methods**

### *Study Population*

The study population were sarcoma patients from the International Sarcoma Kindred Study (ISKS). Patients are recruited by hospitals around the world. DNA samples are both kept locally at each national collaborating center and centralized at the main study center in Australia. DNA from blood samples and tumors is available. Tumors are studied for *p53* mutations and *MDM2* amplifications. DNA samples were requested from the main study center in Australia. There were 53 patients DNA samples and 29 control DNA samples. The 53 patients were cases that did not have mutations in *p53* or *MDM2* amplifications. The 29 control samples were positive for the mutation and amplification.

### *SNP Tagging*

In order to tag the single nucleotide polymorphisms, the desired genetic variants had to be determined. Genotyping data from the HapMap project ([hapmap.org](http://hapmap.org)) for HSPA9 was used to identify the polymorphisms needed to tag the entire region. HapMap is a catalog of common genetic variants that occur in human beings. It describes what these variants are, where they occur in our DNA, and how they are distributed among people within populations and among populations in different parts of the world. *HSPA9* is located on chromosome 5. The gene starts at 137890000 and ends at 137921318. By inputting the location of the gene, HapMap provides a list of all relevant SNPs.

The SNP information on HapMap was extracted and uploaded to the Haploview program. The Tagger functionality of the Haploview program was used to select the tagSNPs. A tagSNP represents single nucleotide polymorphisms in a region of the genome with high linkage disequilibrium. The group of SNPs that a tagSNP represents is called a haplotype. This makes it

possible to identify genetic variation and association to phenotypes without genotyping every SNP in a chromosomal region. After using this program with the data from the HapMap project four tagSNPs were chosen for HSPA9. The tagSNPs were rs256008, rs256001, rs1042665 and rs11740501. These determined the primers that were going to be used with the KASP reagent.

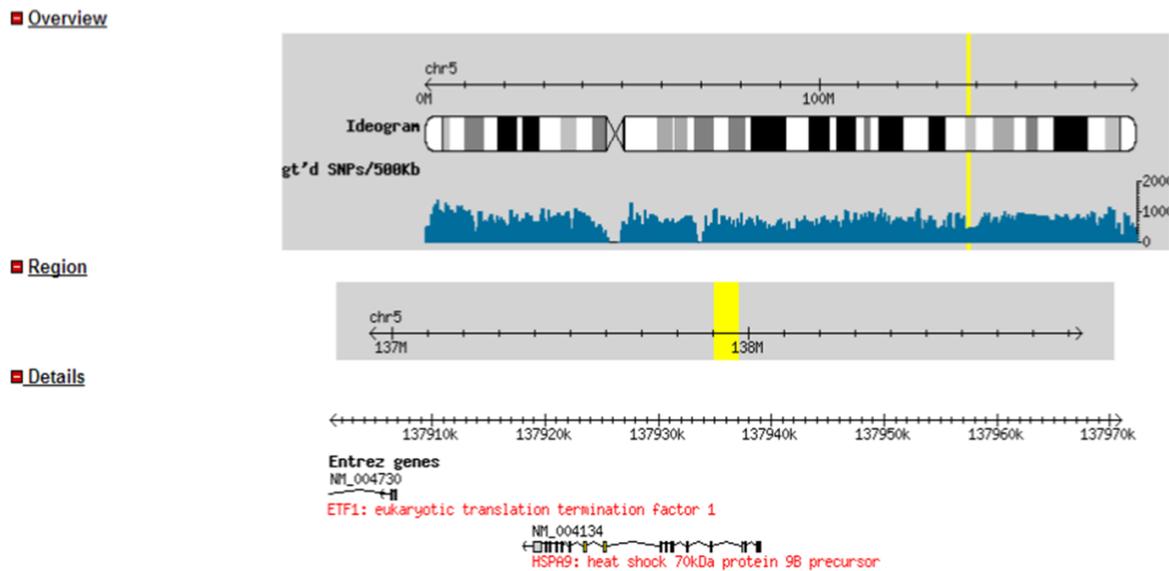


Figure 1. Representation of the location of the gene HSPA9 on chromosome 5. This image also illustrates the number of SNPs at the region of the chromosome within HSPA9. This information was exported and the tagSNPs were determined. The four tagSNPs represent all of the SNPs at this particular location (Hapmap.org).



## Results

### *Genotype Data*

<b>rs256008</b>	<b>Allele 1/Allele1</b>	<b>Allele1/Allele2</b>	<b>Allele2/Allele2</b>	<b>Total</b>
Cases	0	5 (10%)	45 (90%)	50
Control	0	0 (0)	27 (100)	27
Total	0	5	72	77
<b>rs256001</b>	<b>Allele 1/Allele1</b>	<b>Allele1/Allele2</b>	<b>Allele2/Allele2</b>	<b>Total</b>
Cases	43 (89.6%)	5 (10.4%)	0	48
Control	27 (100)	0 (0)	0	27
Total	70	5	0	75
<b>rs1042665</b>	<b>Allele 1/Allele1</b>	<b>Allele1/Allele2</b>	<b>Allele2/Allele2</b>	<b>Total</b>
Cases	1 (2%)	21(43%)	27 (55%)	49
Control	4 (14.8%)	12 (44.4%)	11 (40.7%)	27
Total	5	33	38	76
<b>rs11740501</b>	<b>Allele 1/Allele1</b>	<b>Allele1/Allele2</b>	<b>Allele2/Allele2</b>	<b>Total</b>
Cases	31 (60.8%)	17 (33.3%)	3 (5.9%)	51
Control	19 (65.6%)	9 (31%)	1 (3.4%)	29
Total	50	26	4	80

Table 1. Number of patients for homozygous for allele1, allele 2 or heterozygous for the tagSNPs rs256008, rs256001, rs1042665, rs11740501.

After the DNA samples were added to the KASP reagents and SNP primers, the genotypes at the SNP locations were determined. For the rs256008, no cases or controls were homozygous for allele 1, five cases were heterozygous, and 45 cases and 27 controls were

homozygous for allele 2. For rs256001, 43 cases and 27 controls were homozygous for allele 1, 5 cases were heterozygous, and no cases or controls were homozygous for allele 2. For rs1042665, 1 case and 4 controls were homozygous for allele 1, 21 cases and 12 controls were heterozygous, and 27 cases and 11 controls were homozygous for allele 2. For rs11740501, 31 cases and 19 controls were homozygous for allele 1, 17 cases and 9 controls were heterozygous, and 3 cases and 1 control were homozygous for allele 2. It was necessary to determine the genotypes of the in order to configure haplotype frequencies.

*Haplotype Data*

<b>Haplotypes</b>	<b>Hap.freq</b>	<b>Case hap.freq</b>	<b>Control hap.freq</b>	<b>Odds Ratio</b>	<b>Confidence Intervals</b>
1	0.471757	24.9 (0.4886)	12.81 (0.44201)	1	1
2	0.2125	11.5 (0.22549)	5.5 (0.18966)	0.985	0.39875, 2.4332
3	0.283401	12 (0.23504)	3.93 (0.36834)	1.85	0.76716, 4.4614

Table 2. Haplotype frequency and likelihood of occurrence.

With the genotype information, the haplotypes were determined as well as the haplotype frequencies using the Haplo.stats package in R. With the genotypes determined, three haplotypes were statistically configured. Each haplotype represents a group of SNPs that may be associated. Each of the haplotypes contain different SNPs based on the tagSNPs that were genotyped. Based on Table 2 the odds ratio for all three of the haplotypes include one when adding the confidence intervals. The haplotype frequency for haplotype 1 is 0.47 when comparing the case and control. The haplotype frequency for haplotype 1 for just the case is 0.49. The haplotype frequency for haplotype 1 for the control is 0.44. The haplotype frequency for haplotype 2 is 0.21. The haplotype frequency of haplotype 3 is 0.28.

## Discussion

Based on the results, all three of the odds ratios, with confidence intervals, included one. If an odds ratio is one the data set is not statistically significant because including one means that there is not a haplotype that is more or less likely to be a haplotype in a case than in a control. This exhibits that the specific genetic variations of HSPA9 in sarcoma patient DNA samples with no mutations in p53 or MDM2 amplification are not more or less likely to occur than in DNA samples with the mutation or amplification. This information is important because describes the relationship of the genetic variations in HSPA9 with the risk of sarcoma.

To continue the exploration of genetic variations in this biological pathway, it is important to now look at the genetic variations in p53 and MDM2 and their association with the risk and progression of sarcoma. This is important because there is little known about the mechanisms behind what causes a risk for sarcomas, with the exception of certain translocation mutations. By doing research on SNPs of selected gene sequences we can advance the field by providing more mechanistic descriptions of the biology behind sarcoma tumorigenesis. Today, only patients that have amplification of the MDM2 gene can be treated with anti-MDM2 therapies. If continue research can show that MDM2 is implicated in sarcomas, and not amplified, but activated through other mechanisms such as the interaction between polymorphisms of mitochondria, p53, MDM2 or we can propose anti-MDM2 therapies to the patients with these polymorphisms.

In order to do so, the next objective would be to use standard statistical modeling to examine statistical interactions between the Arg72Pro allele of *p53* and the G309T polymorphism in *MDM2*. These include the Likelihood Ratio Test, which compares the model that includes only main effects for the two polymorphisms to the model containing the main

effects as well as an interaction term. This test should be analogous to the Wald test for interaction, which is simply the p-value of the interaction term in the latter model.

The mitochondrial genome also encodes for a number of genes, mainly proteins that are involved in the electron transport chain. Reactive oxygen species (ROS) generated through the electron transport chain can lead to DNA damage, initiating a feedback loop that, if unchecked, will lead eventually to apoptosis. Humanin, encoded by a mitochondrial 16SRNA, can block the translocation of Bax to the mitochondrial membrane, blocking the release of cytochrome C leading to apoptosis (Guo 2003). This would be an area of interest for further research. To understand the significance phylogenetic trees can then be used to describe populations based on their mitochondrial polymorphisms. The distribution of subjects in trees based on different phenotypes (i.e. different forms of sarcoma, *p53* or *MDM2* polymorphisms) can be compared across trees, in order to identify potential associations between mitochondrial variants and these phenotypes.

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