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RNase L Variants Do Not Appear to Impact on Clinical Features of Sporadic Prostate Cancer Patients*

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ABSTRACT

Introduction: Prostate cancer is the most common non-cutaneous male cancers, contributing to significant mortality rates globally. Mutations of RNase L, an enzyme involved in inflammatory and immunological pathways, have been speculated to predispose to cancer. This study assesses three different mutations of the RNase L gene in Irish prostate cancer patients, including one linked with general cancer susceptibility never investigated before in prostate cancer (rs3738579), and reports on links with aggressive cancer. **Methods:** 134 patients had their RNase L mutation status determined by polymerase chain reaction (PCR) of serum DNA. Complementary clinical details for each patient are statistically analysed. **Results:** No link to age of diagnosis, high grade disease or prostate specific antigen (PSA) level at diagnosis was demonstrated with any of the studied single nucleotide polymorphisms (SNP). The SNP variation was consistent with that of published international series. **Conclusion:** SNP genotypic frequencies in Ireland are consistent with international findings. The studied RNase L mutations including rs3738579 do not appear to have a significant impact on our patient population.

Keywords: RNase L; $R_{462}Q$; $D_{541}E$; Prostate Cancer

1. Introduction

RNase L is a gene found on the hereditary prostate cancer (HPC) locus of chromosome 1 that codes for a latent endoribonuclease. This enzyme participates in an interferon inducible RNA decay pathway that has a role in inflammation and cellular immunity against viral infection. Sustained activation will lead to apoptosis. The gene is 741 amino acids long with 8 exons and is roughly 13 kilobases. It is converted from an inactive monomeric form to a potent dimeric structure by the action of a series of 2' to 5' linked oligodenylates, commonly known as 2 - 5 A.

RNase L has been a candidate gene for prostate cancer researchers for a number of years and many variants including $R_{462}Q$ [1], $E_{265}X$ [1], M_1I [2] and 471ΔAAAG [3] have been described.

The $R_{462}Q$ RNase L mis-sense mutation (rs486927) arises when a substitution of the "G" to "A" base along exon 1 at mRNA position 1552 gives an amino acid change of argenine to glutamine at amino acid position 462. The consequence of this is a variant of the gene that produces an enzyme functioning at a third of the wild-type enzyme's efficiency [4]. It has been of interest to researchers since it was linked to prostate cancer in 2002 [2]. Subsequent studies have been mixed with some showing a link with this variant and familial prostate cancer [5,6], whilst others have not [7]. A meta-analysis of 7 papers has failed to show a significant link between this mutation and prostate cancer regardless of ethnicity or family history [8].

$D_{541}E$ (rs627928) comes about following a substitution of the "T" base at mRNA position 1790 with "G", causing a change in the protein at amino acid position 541 from aspartate to glutamic acid. This area of the gene codes for a region within the protein kinase domain of the enzyme, an area with an important role in dimerization of the protein into its active state. Despite the mutation, this enzyme has comparable catalytic activity with the wildtype variant [4]. Its significance is the source of some debate within the literature. Studies have linked

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this change to an increased risk of familial prostate cancer [7] and sporadic, metastatic disease [9], but in a Japanese study [10] the wildtype variant was actually linked to an increased risk of familial prostate cancer. Other investigators have found no significant associations with it [1,5,11].

An undescribed mutation (rs3738579) located in the 5' untranscribed region (5'-UTR) has been identified as a general indicator of increased cancer susceptibility [12]. The exact role of this variant on expression is unclear but mutations are seen disproportionally in cervical as well as head and neck squamous cell cancers.

Work in the fields of cervical cancer (human papillomavirus) [13], gastric cancer (helicobacter pylori) [14], B-cell lymphomas (Epstein-Barr virus) [15] and osteogenic sarcomas (chronic osteomyelitis) [16] have all found definite links between chronic infection and neoplasia. Whilst no such links have yet been established in prostate cancer, there are inconsistent reports in the literature of positive associations of the disease with sexually transmitted infections (STIs) [17-19], and one study finding men with 25 or more sexual partners being 2.8 times more likely to be diagnosed with prostate cancer than a man with 5 or less partners [20]. If inflammation or viral infection plays a role in prostate cancer development, it is speculated that polymorphisms in genes involved in the inflammatory and infectious disease pathways like RNase L could be important.

This study aims to sequence RNase L single nucleotide polymorphisms (SNPs) in men with known cases of sporadic prostate cancer and correlate to the clinical aspects of the cancer.

2. Methods and Materials

Patient samples were obtained from the Prostate Cancer Research Consortium (PCRC) bio-repository. This is a collaboration of three University institutions affiliated with five major hospitals. Complementary clinical details relating to each patient are also logged into a password protected, secure database. All volunteers were pre-operative radical prostatectomy patients with localised prostate cancer, enrolled with full consent in line with ethical advice. All cases of prostate cancer analysed were sporadic.

Using the database of known SNPs published online at the National Centre for Biotechnology Information (NCBI), the sequence of code surrounding the SNPs of interest were obtained.

Primers were designed using *Primer Express* (*Applied Biosystems*, USA). Details of the actual sequence of interest and the primer set are given in **Table 1**. Primers were supplied by MWG and were purchased at 0.01 μmol scale, HPS purification.

DNA was extracted from peripheral blood DNA by the Autopure automated system (*Qiagen*, USA) which uses puregene chemistry. The product was purified (Qiaquick PCR purification kit from *Qiagen*, USA) and sequencing reactions with a fluorescent dye terminator (BigDye v3.1, *ABI*) were performed. Results were interpreted with *Sequencing Analysis* v5.1 from *Applied Biosystems*.

Polymerase chain reaction (PCR) was carried out with the product initially being exposed to 94˚C for thirty seconds, followed by 35 cycles where the temperature alternated from 94˚C for 30 more seconds, then [53.1˚C ($R_{462}Q$)/53˚C ($D_{541}E$)/55˚C (5'-UTR)] for 30 seconds followed by a minute at 72˚C. The reaction culminated in 30 minutes exposure to 72˚C.

3. Results

Table 2 illustrates the frequencies of each RNase L SNP studied in the prostate cancer population. These figures are in keeping with those published on the NCBI database.

$\underline{R_{462}Q}$

9% of patients had the homozygous AA genotype ($R_{462}Q$), which corresponds to the less efficient variant of the RNase L enzyme, while 52.2% were heterozygous for this SNP. **Table 3** outlines $R_{462}Q$ status versus histological disease aggression. On initial inspection it would appear that a disproportionate amount of men with the AA (*i.e.* the $R_{462}Q$) genotype have tumours that are of Gleason score 7 or greater (7 out of 11). This association, whilst a trend did not demonstrate statistical significance ($p = 0.258$). No association was seen between this SNP and either percentage gland involvement by tumour ($p = 0.57$) or number of affected first-degree relatives ($p = 0.69$). Furthermore, anova 1-way p values for age and prostate specific antigen (PSA) levels at diagnosis also failed to demonstrate any statistical significance (p =

Table 1. Description of primers selected for each SNP studied.

DbSNP ID	Base	Amino acid	Primer	Sequence
rs486907	G/A	R462Q	Forward	5'-TGGAAGCGTGTTTGGATGTG-3'
			Reverse	5'-TGCAGATCCTGGTGGGTGTA -3'
rs627928	T/G	D541E	Forward	5'-TTGATTTATGGCTTTTGTGCAGG-3'
			Reverse	5'-TGAGGTCCTTAGTTTCCTCATCT-3'
rs3738579	C/T	-	Forward	5'-GTGGAAT GTCAGAAGAC TGAGAAC-3'
			Reverse	5'-AATGCCACCTGCTACCACTT-3'

Table 2. Summary of sequencing results for each SNP in men with prostate cancer.

SNP	Genotype	Frequency (%)
$R_{462}Q$	AA (Mutation)	12/134 (9%)
	AG	70/134 (52.2%)
	GG (Wildtype)	52/134 (38.8%)
$D_{541}E$	GG (Mutation)	35/91 (38.5%)
	GT	38/91 (41.7%)
	TT (Wildtype)	18/91 (19.8%)
5'-UTR (rs3738579)	CC (Mutation)	18/136 (13.2%)
	CT	55/136 (40.4%)
	TT (Wildtype)	63/136 (46.4%)

Table 3. Gleason score and SNP status at the exon 1/$R_{462}Q$ SNP.

Gleason Score	AA	AG	GG	Total numbers	P value
4 - 6	4	39	23	66	
7 - 9	7	26	23	56	
Total	11	65	46	122	p = 0.258

0.60 and 0.44 respectively).

$D_{541}E$

38.5% of prostate cancer patients in the study were shown to carry the $D_{541}E$ mutation. Analysis of complementary clinical data revealed no association with either percentage gland involvement (p = 0.58), number of first-degree relatives affected (p = 0.058) or age at diagnosis (p = 0.68). Analysis of PSA at diagnosis (on **Table 4**) revealed that men with the mutation had a much higher PSA at diagnosis however this fell just short of statistical significance (p = 0.06).

Analysis of Gleason score of tumour and SNP status failed to show any statistically significant association (p = 0.68).

5'-UTR

Statistical analysis of Gleason score (p = 0.24), percentage gland involvement (p = 0.5), family history (p = 0.49), age (p = 0.29) or PSA at diagnosis (p = 0.77) failed to reveal any association with this SNP (see **Table 5**).

4. Discussion and Review of the Literature

To our knowledge this is the first study of the RNase L SNP rs3738579 in the context of prostate cancer and also adds to the debate about the significance of $D_{541}E$ and $R_{462}Q$.

There are a number of studies in the literature examining prostate cancer and the $R_{462}Q$ mutation. The results of these are summarised in **Table 6** and a comparison of the frequency that the mutation is detected across these series are displayed in the Forest plot given in **Figure 1**. This shows our findings to be consistent with internationally published series.

Table 4. Breakdown of PSA values (given as ng/ml) versus genotype at $D_{541}E$ SNP.

Genotype	Mean ± SD	Median	Anova 1-way p value
GG ($D_{541}E$)	8.42 ± 3.13	8.25	
GT	7.08 ± 3.26	6.25	0.0634
TT	6.84 ± 2.07	6.6	

Table 5. Analysis of low and high grade tumours versus 5'-UTR locus SNP status.

Gleason score	CC	CT	TT	Total numbers	p value
4 - 6	8	29	29	66	
7 - 9	8	22	26	56	
Total	16	51	55	122	0.24

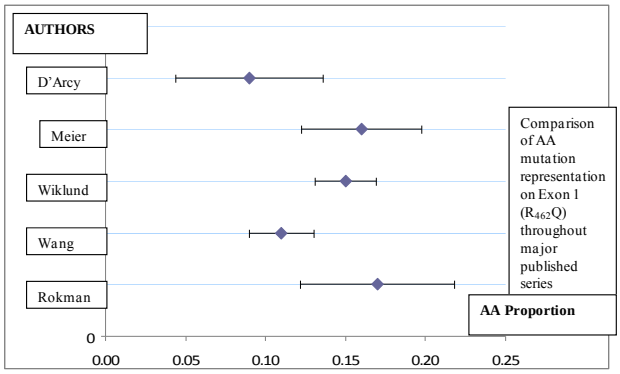

Figure 1. Expression of mutated form of Exon 1, AA ($R_{462}Q$), as a proportion of total numbers examined for all major studies, expressed on a Forest plot. Y-axis has study's author's name whilst X-axis contains proportion of AA genotype given with 95% confidence limits.

$D_{541}E$ mutation has been the focus of multiple studies. We fail to demonstrate a significant clinical association with this mutation. Results summary and comparison of frequency of mutation are given in **Table 7** and **Figure 2** respectively.

Rokman's study focused on 492 patients with prostate cancer, 47 of whom reported a positive family history. They were consecutive cases diagnosed at the University Hospital, Tampere, Finland, over a three-year period. This population would have differed from the one we are reporting on by including men with both organ confined and metastatic disease. Control samples came from 566 healthy male blood donors. This study failed to show any significant difference in $D_{541}E$ status between prostate cancer, BPH and normal control groups and concluded that this mutation doesn't have an important role in prostate cancer within their population. It did however demonstrate an association between $R_{462}Q$ status and hereditary prostate cancer.

Wang's study came from Minnesota in the United States and included a total of 825 patients with prostate

Table 6. Comparison of results of analysis of $R_{462}Q$ mutation ("AA" genotype) throughout different studies within the literature.

Study Author	Population	Patient group	AA (Mut) Genotype	AG Genotype	GG Genotype	Total
Rokman [1]	Finnish	Prostate cancer	39 (16.7%)	106 (45.5%)	88 (37.8%)	233
		Control	23 (13.1%)	84 (47.7%)	69 (39.2%)	176
Wang [5]	Hispanic and White. USA	Prostate cancer	102 (11.1%)	427 (46.5%)	389 (42.4%)	918
		Control	67 (13.5%)	233 (47.3%)	193 (39.1%)	493
Wiklund [7]	Swedish	Prostate cancer	247 (15.2%)	778 (48.0%)	597 (36.8%)	1622
		Control	115 (14.4%)	384 (48.2%)	297 (37.4%)	796
Maier [11]	German	Prostate cancer	59 (16.3%)	171 (47.1%)	133 (36.6%)	363
		Control	37 (17.9%)	97 (46.9%)	73 (35.2%)	207
D'Arcy	Irish	Prostate cancer	12 (9.0%)	70 (52.2%)	52 (38.8%)	134

Table 7. Comparison of results of analysis of $D_{541}E$ mutation ("GG" genotype) throughout different studies within the literature.

Study Author	Population	Patient group	GG (Mut) Genotype	GT Genotype	TT Genotype	Total
Rokman [1]	Finnish	Prostate cancer	78 (33.5%)	126 (54.1%)	29 (12.4%)	233
		Control	56 (31.8%)	91 (51.7%)	29 (16.5%)	176
Wang [5]	Hispanic and White. USA	Prostate cancer	181 (19.5%)	476 (51.2%)	272 (29.3%)	929
		Control	107 (21.1%)	228 (44.9%)	173 (34.0%)	508
Wiklund [7]	Swedish	Prostate cancer	462 (33.9%)	668 (49.0%)	233 (17.1%)	1363
		Control	257 (32.5%)	372 (47.0%)	162 (20.5%)	791
Maier [11]	German	Prostate cancer	125 (34.4%)	176 (48.5%)	62 (17.1%)	363
		Control	69 (33.3%)	97 (46.9%)	41 (19.8%)	207
Noonan-Wheeler [9]	European-Americans	Prostate cancer	55 (36.7%)	73 (48.7%)	22 (14.6%)	150
		Control	44 (25.7%)	94 (55.0%)	33 (19.3%)	171
Nakazato [10]	Japanese	Prostate cancer	51 (50.5%)	32 (31.7%)	18 (17.8%)	101
		Control	59 (56.2%)	43 (40.9%)	3 (2.9%)	105
D'Arcy	Irish	Prostate cancer	35 (38.5%)	38 (41.7%)	18 (19.8%)	91

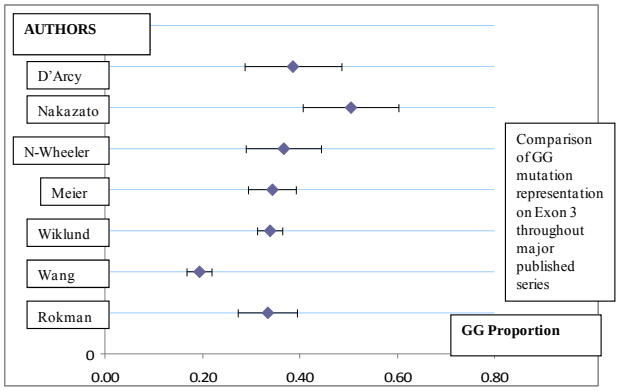

Figure 2. Expression of mutated form of Exon 3, GG ($D_{541}E$), as a proportion of total numbers examined for all major studies, expressed on a Forest plot. Y-axis has study's author's name whilst X-axis contains proportion of GG genotype given with 95% confidence limits.

cancer, made up of 326 familial prostate cancer patients and 499 cases of sporadic prostate cancer, all of the latter group comprising of organ confined disease. Control group came from men of a similar age from the local population (Rochester Epidemiology Project), who were invited to enrol and subsequently had a careful history, physical examination and if necessary a TRUS biopsy. Once prostate cancer had been ruled out, they were incorporated as a control group. In total there were 510 such men. This study also linked $R_{462}Q$ status with hereditary prostate cancer and in such men disease was associated with an earlier age of onset. Like Rokman's work, this study also failed to demonstrate a correlation between the $D_{541}E$ mutation and prostate cancer risk.

Wiklund's series came from patients registered on *Cancer Prostate*, a Swedish nationwide database of prostate cancer patients younger than 79 diagnosed between July 1st 2001 and 30th September 2002. Patients were invited to take part in the study and a blood sample for analysis was taken. In total, 1636 prostate cancer patients with a full spectrum of disease from early to advanced were included. Control samples were matched to similar people from the Swedish Population registry, who were invited to take part. This involved filling a questionnaire and giving a blood sample. In total 801 controls were analysed. No significant association between $R_{462}Q$ status and prostate cancer risk was demonstrated in this study. It did reveal a significant link ($p = 0.03$) between familial

prostate cancer risk and $D_{541}E$ mutation, however no link between this variant and either age at diagnosis or tumour aggressiveness was found.

Maier's patients were recruited from the *Prostate Cancer Genetics Project*. This is a database made up of men from predominately from the South of Germany, the majority of whom would have undergone radical prostatectomies. Patients are encouraged to enrol in this study by their urologist and there are no selection criteria. At risk families are identified by interview of the patient. There were a total of 303 of such patients. 227 sporadic cases were also sequenced as well as 207 control samples. These samples came from healthy, elderly men with no history of prostate cancer and negative DRE and/or normal PSA levels. $D_{541}E$ or $R_{462}Q$ failed to demonstrate any significant association with prostate cancer in this study.

Noonan-Wheeler's study originated in Missouri, USA. It examined RNase L in men with aggressive, metastatic cancer and healthy controls. Patients were recruited from the outpatient department and were required to have a PSA over 50 ng/ml or radiological/pathological evidence of metastatic disease. The control group consisted of men older than 75 with normal PSA levels and rectal examination with no background of prostate cancer. There were a total of 150 patients and 171 controls examined. It was shown that $D_{541}E$ was over-represented ($p = 0.045$) in patients with metastatic disease, and concluded that such patients were at an increased risk for sporadic, metastatic disease (OR = 1.68).

Nakazato's study came from Japan and examined RNase L in familial prostate cancer cases and healthy controls. It comprised 101 patients with a positive family history of cancer (29 of whom had 3 or more affected family members) and 105 controls. Prostate cancer patients ranged in age from 40 to 88 years, encompassed organ confined and metastatic disease and had 76 men with a Gleason score of 7 or greater and the remaining 26 with a Gleason score of 6 or less. Control cases were recruited from the outpatients department and were of similar age. Patients with an elevated PSA or abnormal DRE were excluded from this group. Interestingly this study demonstrated a significant link between the wild-type DD variant and familial prostate cancer ($p = 0.0004$, OR = 7.37), possibly an observation unique to the Japanese population.

5. Conclusion

In conclusion, no statistically significant correlation was proven in the Irish population between Gleason score, percentage gland involvement, patient age, PSA or family history with any of the studied SNPs. In particular the SNP rs3738579 failed to highlight men with significant clinical cancer.

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