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### Title and Subtitle
Locating a Prostate Cancer Susceptibility Gene on the X Chromosome by Linkage Disequilibrium Mapping Using Three Founder Populations in Quebec and Switzerland

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### Abstract
The funded proposal has not yet begun at all sites. We have yet to receive the SPA for the Chicoutimi site but we are confident that this will happen shortly as all the necessary documents have been sent for final review by the Army. Due to the delay with ethics approval, we applied and received a "no cost extension" for this proposal. At the Montreal site, 153 participants have consented to participate and their blood was drawn. We have another 20 men that have agreed to participate and their blood will be drawn in the next few months. We are continuing to ascertain new cases as via hospitals' tumor registries. We have recruited a total of 29 controls. The pedigrees for all controls and cases have been drawn. Ishihara charts were shown to all cases and controls and the results were recorded. At the Switzerland site, case ascertainment is underway. To date, four physicians have given their support to this project and we anticipate the participation of more physicians. The cancer registry in Bern has been contacted to request a patient list and a favorable decision is expected in March or April 2003. Seven patients have been contacted so far and 5 patients consented and gave blood. Repeated contacts have lead us to believe we can expect many more over the next few months. We have published work in the Journal of Medical Genetics for the testing of RNASL471delAAAG on our cases.

### Subject Terms
prostate cancer, X chromosome, linkage disequilibrium mapping

### Security Classification
- **Unclassified**
- **Unclassified**
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Locating a prostate cancer susceptibility gene on the X chromosome using linkage disequilibrium mapping using three founder populations in Quebec and Switzerland.

Dr. William Foulkes

New Investigator Award: DAMD 17-00-1-0033

Introduction

In this study, we are funded to localize a prostate cancer susceptibility gene to the X chromosome (Xq) by linkage disequilibrium mapping. We plan to use three founder populations 1) French Canadian inhabitants of the Saguenay-Lac St Jean region of Quebec; 2) The Ashkenazi Jewish population of greater Montreal; 3) the population of the Swiss Canton of Valais. The DNA is also available for other prostate cancer genetic studies by the PI.

We have chosen these three populations because they have all been shown to contain founder mutations in various disease-associated genes and because they are accessible to us and have participated in research in the past.

Body of text

Task 1: Case ascertainment, contact, consent, interview, DNA extraction and pathology confirmation, years 1-3 (aim to complete in second quarter of year 3).

- Obtain approval for this study from relevant IRBs and submit appropriate documentation for review to start the project at the Chicoutimi and Switzerland sites.

In progress

The goals established in Task 1 have not yet been achieved at all sites and human subject research cannot commence at the Chicoutimi site until the Army has granted approval. Please see appendix for a full description on how we have worked to achieve this goal.

However, some of the goals have been achieved by the Sion (Switzerland) and McGill University Hospital (Quebec) sites.

- Identify all prevalent cases of prostate cancer at hospitals serving the three populations under the study.
This goal was achieved at the McGill University Hospitals last year and has been ongoing this year. Twenty-five more cases were ascertained since March 2002.

Because of the private health care system in Switzerland this has been a difficult and time-consuming task. Four physicians at the Sion site have given their support to this project and ascertainment of cases has begun through these physicians. The Cancer Registry in Bern has also been contacted to ask for their support in ascertaining cases. We expect a favourable response from the Cancer Registry in March or April of 2003. We believe that this registry will play a significant role in the ascertainment of patients.

- Identify incident cases through urology clinics at the three centres. Method of contact as for prevalent cases.

This goal has not yet been achieved at any of the sites but we estimate that this goal will take place within the next three months.

- Consent all eligible patients.

153 patients at the McGill University Hospital sites have given their consent to participate. A total of 29 controls have given their consent to participate. A total of 45 affected men refused to participate and none of the controls have refused to participate to date.

5 patients at the Sion Hospital site have given their consent to participate. There have been no controls recruited to date.

- Interview and construct three-generation pedigree for each case and control.

153 pedigrees have been drawn for cases and 29 have been drawn for controls at the McGill University Hospital site.

- Extract DNA locally at each participating centre, transfer aliquots of DNA to PI laboratory for quality check and storage.

DNA has been extracted at the McGill University Hospital and the Switzerland site. We are waiting till more cases have participated before DNA from the Sion site is transferred to the PI laboratory.

- Transfer representative slides and blocks to Montreal for central pathology review *(NB this will take place after ascertainment as we except few cases will be reclassified and subsequently excluded).*
Slides and blocks from patients ascertained at the McGill University Hospital site have been transferred to a central pathologist for his review. The pathologist is currently reviewing material form 150 of the participating patients. We will wait for more cases to be recruited at the Sion site before the central pathologist reviews them. It would be more cost efficient to transfer these in bunches rather than a few at a time.

➢ Create a central database at the MGHRI.

We are currently developing a database at the McGill University Hospital sites and it should be ready to enter data associated with this proposal within the next month.

Key Research Accomplishments

➢ 113 cases recruited at the Montreal site
➢ 5 cases recruited at the Sion (Switzerland) site
➢ Publication in the Journal of Medical Genetics from this research project.
➢ We have found 10% of the cases to be colour blind in comparison to 8% of controls.

Reportable Outcomes

1. Published work (see appendix 2)


2. Conclusions

At the present time, our results suggest that the RNASEL 471delAAAG variant is not associated with greatly increased risk of prostate cancer. We are continuing to look for other possible variants associated with an increased risk for prostate cancer such as MSR1 and CHEK2.
Appendix 1

Log of activities at the McGill University Hospital, Chicoutimi and Sion sites.

Abbreviations:

JGH-Sir M.B. Davis-Jewish General Hospital
MGH-Montreal General Hospital
RVH-Royal Victoria Hospital
MUHC-McGill University Health Centre
MGHRI-Montreal General Research Institute

Continued to work towards getting approval at all sites. March 2002-present.

Contacted the remaining physician that had not given their support previously. Arranged meetings with such individuals and showed them patient list to approve.

In March 2002, we worked particularly hard to aid the Chicoutimi site with the “Answers to Questions for foreign institutions” document. Once we received this document form that institution, it had to be translated from French to English.

In April 2002, the consent form for the Sion site had to be revised to add a necessary medical clause. This consent form then had to be sent to the Sion site ethics board for approval. This consent form then had to be translated from French to English before being sent to the Army for approval.

In April 2002, the “Questions for foreign institutions document was faxed and sent to the Army along with Dr. Jorvis’ C.V that was missing for the necessary review.

April 2002, we had to request a letter stating re-approval of the project by the Sion IRB. This document had to be translated once it was received and both the document and the translation were sent to the Army the first week of May.

In July 2002, We have addressed a request to the Institut Central des Hôpitaux Valaisans (Sion, Switzerland) about additional financial support to run the study in Switzerland, as the amount allocated to our centre was not sufficient to cover all costs of the groundwork administration and initial set up. A grant of 60'000 CHF for 3 years was
allocated to this study in July 2002. This amount will cover the costs of a research nurse in charge of visiting the patients with difficulties of mobilization at home, presenting the study, gathering clinical information and collecting blood samples. The name of the research nurse we have recruited is Mrs Marie-Mathilde MEIER. The grant will also allow the secretary of the Unit of Genetics at the Central Institute of Hôpitaux Valaisans, Mrs Marie-Thérèse JENTSCH, to work at part time on this project (to draw pedigrees, classify the data collected by the nurse, run the database and assume various mailing activities).

In September 2002, upon the suggestion from the Army, we applied for a "no cost extension" and we received approval for this extension. (This was necessary due to the delay in obtaining ethics approval).

October - November 2002, we received the necessary IRB re-approval for the project from the Chicoutimi site.

We obtained the SPA for the Sion site and are now waiting for the response for the Chicoutimi site. It appears that all the necessary documents were sent for the review.

While trying to obtain the ethics for the Sion and Chicoutimi sites, we were contacting patients (approx. 200) to participate in the study at the McGill University site.

113 cases have donated blood and answered question about their family medical history. Ishihara charts were shown to all these patients and the results were recorded. A total of 113 pedigrees were drawn for the cases. (March 2002-February 2003)

25 controls consented to participate in the study. Their pedigrees were also drawn. Ishihara charts were shown to the controls and these results were recorded. (March 2002-February 2003)

During this time we continued to contact tumour registries to obtain updated lists of patients who had been diagnosed with prostate cancer.

In November 2002, pathology material for all cases at the McGill University Hospital site were ordered and is being reviewed by a pathologist (Dr. L. Begin) to confirm the diagnosis of prostate cancer. We are waiting for these results.

On December 19, 2002, we have asked the Commission d'experts du secret professionnel en matière de recherche médicale in Bern for the authorization to access to nominative data from the Cancer Registry of the canton du Valais. This is currently under
examination and we expect a favourable decision in the Spring 2003. This will allow us to access to the name of patients who developed prostate cancer in the canton du Valais, to select patients with family names of Swiss origin in order to contact their private physicians (generalists, internists, oncologists...). Thus we should be able to recruit more candidates.

In January 2003, recruitment of patients diagnosed with prostate cancer from the canton du Valais began. To date, seven patients were invited to participate. One patient was excluded because of his origin (not originating from the Valais on one parental side), and one declined the study. Five patients have agreed to participate in the study; blood sampling and DNA extraction is currently being carried out for these patients.

RNASEL 471delAAAG analysis was done for 122 cases and 13 of the controls. These results were published (see published work).

At the McGill University site, another 40 patients have agreed to participate and we have arranged to meet most over the next three months. We anticipate more controls participating and are working actively towards recruiting many more.
The RNASEL 471delAAAG allele and prostate cancer in Ashkenazi Jewish men

K Kotar, N Hamel, I Thiffault, W D Foulkes

There is compelling evidence that genetic factors play an important role in prostate cancer, but no unequivocally disease causing mutations in prostate cancer susceptibility genes have been identified. In 2002, Carpent et al. reported that variants in the gene RNASEL were present in some multiple case prostate cancer families. RNASEL maps to the region of chromosome 1q that has been linked to prostate cancer in multiple case prostate cancer families and this locus is often referred to as HP1C1. In particular, a truncating variant, E265X, was identified in a single kindred with several cases of prostate cancer and functional analysis and the presence of loss of heterozygosity at markers close to RNASEL in one tumour supported a putative pathogenic role for this variant. However, subsequent publications have questioned the biological significance of truncating and missense variants in this gene and its true importance in determining prostate cancer risk remains uncertain.

Rentner et al. recently identified a variant in RNASEL, known as 471delAAAG, in an Ashkenazi Jewish (AJ) man who was diagnosed with prostate cancer at the age of 65. Notably, his mother was also affected with prostate cancer at the age of 57 and was found to be homozygous for this variant. The authors then looked for this variant in 119 Israeli men with prostate cancer, among whom 87 were of AJ origin. They identified six men who carried this variant, all among the AJ males (6.9%, 95% confidence interval CI 2.6 to 14.4%). Lower frequencies were found among elderly AJ without prostate cancer (2/83, 2.4%, 95% CI 0.29 to 8.4%) and among young AJ females (6/150, 4.0%, 95% CI 1.5 to 8.5%). No variants were identified in non-AJ subjects with or without prostate cancer.

Limited haplotype studies indicate that the variant identified is a founder within the AJ population.

MATERIAL AND METHODS

Using hospital based tumour registries, we have identified 437 self-reported AJ men with prevalent prostate cancer, diagnosed between 1991 and 2002, who were known to be alive in 2002. All men were diagnosed and/or treated in one of three large: McGill University affiliated hospitals in metropolitan Montreal, Canada. Most Montreal AJ men with prostate cancer are treated by physicians affiliated with one of these hospitals. The diagnosis of invasive prostate carcinoma came to the attention of the authors by examining the pathology reports present in the medical charts of all the eligible participants. At the time of writing, 227 have been contacted and 210 remain to be contacted (reasons for lack of contact: physician approval pending, returned unopened letters, address unknown, left town). Of the 227 contacted, 137 have responded to our letter. Of 157 affected males who were eligible to take part and have been contacted as of September 2002, 122 agreed to participate in the study and were genotyped for the RNASEL variant. Thirty-five affected males chose not to participate in this study. None of these men cited ill health as the reason for their refusal. Thirteen probands had one first degree relative with prostate cancer (10.7%) and two had more than one first degree relative with prostate cancer (1.6%). The median age at diagnosis of participants was 67.2 years (range 48.6-85.7 years) and the participants were tested at a median of five years since diagnosis (range 0.3-11.5 years). Of the 122 participants, 54 (27.9%) were found to have a Gleason score of 6/10 or lower (median number of months since diagnosis = 80) and 58 (47.5%) had a Gleason score of 6/10 or greater (median number of months since diagnosis = 63). This difference in the time interval between diagnosis and blood drawing, dichotomising at a Gleason score of 6, is statistically significant (p=0.046, Mann-Whitney U test). However, when we regressed the Gleason score against the time interval in months between the date of diagnosis and the blood draw there was less evidence for an effect across all scores. The correlation coefficient (r) was -0.106 (p=0.01, n=0.32). Thus we have some evidence that our sample is, perhaps not surprisingly, slightly biased towards longer surviving affected men with lower Gleason scores. We do not have the final Gleason score for 29 men (23.8%, median number of months since diagnosis = 72). After informed consent, a pedigree was drawn for all consenting subjects and a blood sample was taken for DNA extraction and genetic studies. Of the 122

Key points

- The gene RNASEL has been implicated in prostate cancer.
- A mutation, referred to as 471delAAAG, was identified in RNASEL in an Ashkenazi Jewish (AJ) man with a family history of prostate cancer. Subsequently, it was found in 6/87 unselected AJ men with prostate cancer and in 2/83 elderly AJ men without prostate cancer (odds ratio 3.0, p=0.28).
- We tested 111 AJ probands affected with prostate cancer for the 471delAAAG mutation; only one carrier was identified. In 105 AJ controls, two carriers were identified (odds ratio 0.47, p=0.61).
- The results do not suggest that this variant is associated with greatly increased risk of prostate cancer, which might be expected to occur if RNASEL is a candidate prostate cancer susceptibility gene within the linked HPIC region.
- These observations are preliminary. Very large sample sizes are required to exclude small relative risks when the allele frequency is low. This is a significant impediment to robust replication studies.

Abbreviations: AJ, Ashkenazi Jewish; CI, confidence interval. RNASEL, -ribonuclease T
Table 1 Prevalence of the 471delAAAG variant in RNASEL in Ashkenazi Jewish men with prostate cancer and in Ashkenazi Jewish controls

<table>
<thead>
<tr>
<th>Cancer Status</th>
<th>Carriers (no. of cases)</th>
<th>No. of carriers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate ca diagnosed at ≥65 years</td>
<td>1/38 (2.63)</td>
<td>1/11 (0.90)</td>
</tr>
<tr>
<td>Prostate ca diagnosed at ≥65 years (95% CI)</td>
<td>(0.02 to 4.9)</td>
<td></td>
</tr>
<tr>
<td>All controls</td>
<td>2/105 (1.9)</td>
<td>(0.23 to 6.7)</td>
</tr>
<tr>
<td>Comparison of cases and controls (95% CI)</td>
<td>Odds ratio = 0.47, p=0.61*</td>
<td></td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.08 to 9.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Fisher’s exact test, two sided p value.

We used the previously published primer sequences to amplify the relevant fragments from RNASEL. PCR was performed using 100 ng of genomic DNA in the presence of 300 µM of each dNTP, 20 µM of each primer, 1 x QIAGEN standard PCR buffer (Includes 1.5 mM MgCl2), and 5 U of HotStar Taq DNA polymerase (QIAGEN Inc, Mississauga, Ontario, Canada). A forward primer labelled with Cy5.5 was used. After an initial denaturing step of 15 minutes at 95°C, amplification was performed over 35 cycles in a Perkin Elmer 9600 thermocycler at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. PCR products were mixed with formamide loading dye and separated on 5% acrylamide denaturing gels for 3½ hours at 70 W. Fragments were visualised using the STORM 860 phosphorimager (Molecular Dynamics, Amersham Biosciences, Sunnyvale, CA). The variant was visible as a band shift below the normal product on the gel. We included a PCR product of DNA from LNCaP cells on all screening runs, as this cell line has also been shown to carry this variant. The presence and copy number of the variant was confirmed in all carriers by direct sequencing. DNA samples were sequenced using the Visible Genetics OpenGene® system (Visible Genetics, Toronto, Ontario, Canada) and the Cy5.5 dye terminator cycle sequencing kit (Amer sham Pharmacia Biotech, Piscataway, NJ, USA). Data acquisition and base calling was achieved with the GeneObjects™ software (Visible Genetics).

RESULTS

We identified a single 471delAAAG carrier among the 111 probands (0.90%, 95% CI 0.02 to 4.9, table 1). Among 105 anonymised but unaffected AJ controls, we found two carriers of this variant (0.4%, 95% CI 0.06 to 6.7%, table 1). This difference is not statistically significant (OR 0.47, p=0.61, table 1).

The variants seen were confirmed by direct sequencing, including LNCaP as a positive control (data not shown). The single affected carrier was diagnosed with a Gleason grade 9 prostate cancer in 1994. He was treated by hormonal therapy followed by prostatectomy and is currently free of disease. His blood was drawn for this study in 2001. His sister was diagnosed with brain cancer at the age of 47, but there are no reported cases of prostate cancer in his family (fig 1).

We confirmed that 471delAAAG is likely a founder variant by examining the genotypes of all three carriers at five variable loci surrounding the RNASEL region (D15S2883, D15S2619, D15S2623, D15S2172, and D15S240; see the Genome Database Website, www.gdb.org, for details regarding PCR amplification conditions). All carriers shared a common allele for each locus, while LNCaP was observed to diverge at two loci, D15S2619 and D15S240, located 168 kb downstream and 2.5 Mbp upstream of RNASEL, respectively (marker positions according to the June 2002 freeze of the Human Genome Browser at GoldenPath). A graphic representation of the region is provided in fig 2.

DISCUSSION

It is clear that in our study, as in the original report, there is no significant difference in the frequency of the RNASEL 471delAAAG allele in those with or without prostate cancer. We compared the allele frequencies in the two series of cases and controls. Although the allele frequencies in the two groups of controls do not differ (X²=3.13, df=1, p=0.08, two sided), when comparing the cases there were significantly fewer 471delAAAG carriers in Montreal (1/111) than in Israel (6/87) (p=0.02, two sided). It is important to recognise that given the low prevalence of the founder variant (~3%), when combined, these sample sizes provide an...
hence, RNASEL does not appear to be such an allele. As such, it would be premature to consider offering RNASEL genetic testing for prostate cancer risk on the basis of the current data.

ACKNOWLEDGMENTS
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REFERENCES