ELECTRONIC LETTER

RNASEL mutations in hereditary prostate cancer

H Chen, A R Griffin, Y-Q Wu, L P Tomsho, K A Zuhlke, E M Lange, S B Gruber, K A Cooney

J Med Genet 2003;**40**:e21(http://www.jmedgenet.com/cgi/content/full/40/3/e21)

Prostate cancer linkage to a broad region on chromosome 1q24-25 was first described by Smith *et al*[1] in 1996. In this initial report, analysis of 91 multiplex prostate cancer families from North America and Sweden showed significant evidence for prostate cancer linkage surrounding marker D1S158 with a peak multipoint lod score assuming heterogeneity of 5.43 at marker D1S422. Although approximately one-third of prostate cancer families were estimated to be linked to this locus in this first set of families, additional studies suggest that the percentage of families with prostate cancer attributable to mutations in *HPC1* may be significantly less (<5%).[2]

Recently, Carpten *et al*[3] provided evidence that the *RNASEL* gene may be a candidate gene for *HPC1*. This gene is in the previously described 1q linkage peak, and deleterious mutations were identified in two of eight families with evidence for linkage to 1q24-25 markers. Additional functional data in support of the hypothesis that *RNASEL* mutations contribute to hereditary prostate cancer include: (1) evidence of loss of heterozygosity (LOH) of the wild type allele in prostate cancer from a patient harbouring a germline truncating mutation (E265X), and (2) absent *RNASEL* expression in prostate cancer tissue from a patient with the E265X mutation.

The University of Michigan Prostate Cancer Genetics Project (PCGP) was established in 1995 with the major goal of characterising the molecular basis of prostate cancer susceptibility. Fifty-nine families from the PCGP were used in the first confirmatory report of prostate cancer linkage to chromosome 1q24-25 markers.[4] In this present report, we set out to determine the contribution of *RNASEL* mutations to prostate cancer susceptibility in a set of PCGP families.

Key points

- Mutations in the *RNASEL* gene have been identified in some prostate cancer families leading to the hypothesis that *RNASEL* is the prostate cancer susceptibility gene *HPC1* on chromosome 1q.
- To test this hypothesis, we tested genomic DNA from 95 affected men in 75 multiplex prostate cancer families, including 19 families with evidence of linkage to 1q24-25 markers, from the University of Michigan Prostate Cancer Genetics Project for *RNASEL* mutations.
- Direct sequence analysis of the coding region showed four rare missense/nonsense mutations (G59S, I97L, V247M, and E265X) occurring in four unrelated families. None of four reported rare mutations (M1I, G59S, V247M, and E265X) were observed in a panel of 75 control DNA samples or in any of the 103 cases and 323 control samples from a population based study of prostate cancer in African Americans.
- Additional studies are needed to elucidate more clearly the role of *RNASEL* mutations in hereditary prostate cancer.

MATERIAL AND METHODS

Patient selection

Men with prostate cancer selected for this study are participants in the University of Michigan PCGP. Current enrolment criteria for this family based study of inherited prostate cancer susceptibility include families with two or more living members with prostate cancer. Confirmation of the diagnosis is obtained via review of medical records and/or death certificates. In some cases, the diagnosis of prostate cancer in dead subjects is achieved through verbal confirmation by two independent family members. Genomic DNA was isolated from whole blood using a commercial kit (Puregene, Gentra Systems, Inc, Plymouth, MN).

Ninety-five men with prostate cancer were selected from 75 PCGP families for direct sequence analysis of the *RNASEL* gene. Genotype data using two markers that surround the gene, D1S466 and D1S158, were available from affected men in 39 of the 75 families. Nineteen of the 39 families with genotype data were considered to have high evidence of linkage because the family non-parametric linkage (NPL) score was >1 (table 1). The 11 families with moderate evidence of linkage consisted of families with NPL scores ≤1 but ≥−1. The remaining nine families with low evidence of linkage consisted of families who had NPL scores <−1. In the 11 families with moderate evidence for linkage to 1q24-25 markers, genomic DNA samples from all affected men in these families were evaluated by direct sequencing (28 subjects). DNA samples from all affected men from three additional families without available linkage data were also sequenced (six men). In the remaining 61 families, which included 19 families with strong evidence and nine families with little evidence for linkage using markers D1S466 and D1S158, genomic DNA from only one affected man from each family was sequenced.

Control samples

To estimate allele frequencies in both African Americans and whites, control samples were obtained from two sources. The Flint Men's Health Study (FMHS) is a continuing population based case-control study of prostate cancer in African American men conducted in Genesee County, Michigan.[5] For this report, DNA samples from 103 men with prostate cancer and 323 male controls were used. The average age at diagnosis for cases was 62.8 ± 8.3 years. All control men were screened for prostate cancer with serum PSA and digital rectal exam per protocol. The average age of the controls at the time of consent was 56.0 ± 10.2 years. Genomic DNA samples from 27 men and 48 women were also obtained from a continuing study called Genes, Environment and Melanoma (GEM) at the University of Michigan. The 75 DNA samples were obtained from spouses of study participants with melanoma. These subjects are white and range between 20 and 85 years of age.

For participants in all three studies (PCGP, FMHS, and GEM), written consent was obtained and all protocols were

Table 1 Characteristics of the 75 PCGP families analysed for *RNASEL* mutations. Criteria used to describe evidence for prostate cancer linkage to 1q24-25 markers are described in the Material and methods

	No of families	No of family members with prostate cancer	No of African American families
Families with high evidence for linkage to 1q24-25	19	3.1 ± 0.6	4
Families with moderate evidence for linkage to 1q24-25 markers	11	5.6 ± 3.8	8
Families with low evidence for linkage to 1q24-25 markers	9	4.4 ± 1.6	4
Families with no linkage data*	36	5.3 ± 2.3	0
Total	75	4.7 ± 2.4	16

*All affected family members from three of these families were sequenced for *RNASEL* mutations.

Table 2 Clinical features of 95 men with prostate cancer from 75 PCGP families

		Percent
Average age at diagnosis	58.3 ± 8.1 years	
Race/ethnicity		
White	69	72.6
African American	21	22.1
Median serum PSA at diagnosis	6.3 ng/ml	
Histological grade*		
Well differentiated (Gleason grade <6)	53	56.4
Moderately differentiated (Gleason 7)	33	35.1
Poorly differentiated (Gleason 8–10)	8	8.5
Treatment		
Radical prostatectomy	72	75.8
External beam radiotherapy	8	8.4
Hormones	7	7.4
Other	8	8.4

*Histological grade was not available from one participant.

approved by the Institutional Review Board at the University of Michigan Medical School.

DNA sequencing

PCR was used to amplify 20-40 ng of genomic DNA using primer sequences from Carpten *et al*.[3] PCR primers were M13 tailed and purchased from GIBCO BRL (Invitrogen, Carlsbad, CA). Each reaction contained 2.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 0.55 µmol/l for both forward and reverse primers, 1 unit of Platinum® *Taq* (Invitrogen, Carlsbad, CA), and 1 unit of Ampli*Taq* Gold® (Applied Biosystems, Foster City, CA) DNA polymerases. Amplification was carried out as follows: denaturing (95°C/14 minutes), annealing (35 cycles of 95°C/30 seconds, 56°C/30 seconds, and 72°C/45 seconds), and final extension (72°C/10 minutes). PCR products were purified using Microcon® PCR centrifugal filter devices (Millipore Corporation, Bedford, MA). Sequencing of PCR products was performed by the University of Michigan DNA Sequencing Core using ABI PRISM® 3700 Genetic Analyzers (Applied Biosystems, Foster City, CA). M13 forward and reverse primers were used to obtain both strand sequences. Sample sequences were aligned with *RNASEL* DNA sequence using the NCBI BLAST program to identify changes. Only changes clearly present in both strands were further evaluated.

Allele specific oligonucleotide (ASO) hybridisation and restriction fragment length polymorphism (RFLP) analysis

ASO experiments were performed as previously described.[6] Control DNA samples for the M1I substitution were kindly provided by W Issacs. For RFLP analysis, PCR products were generated using the appropriate RNASEL primers, incubated at 36°C in the presence of BseRI or 65°C in the presence of BsrDI for one hour, and analysed on 2% agarose gels.

RESULTS

Sixteen of the 75 families (17%) included in this report were African American and the remainder of the families were white (table 1). The average number of confirmed affected men in the families was 4.7 ± 2.4. The clinical features of the 95 men with prostate cancer who were sequenced for *RNASEL* mutations are shown in table 2.

Sequence analysis of the *RNASEL* gene in 95 men with prostate cancer selected from 75 PCGP families showed three rare missense substitutions and one nonsense mutation (table 3). One of the missense substitutions, I97L, was identified in two unrelated affected men. Sequence analysis of the other affected men in each family from which DNA was available (family 345: brother and maternal cousin; family 392: second cousin) failed to identify this mutation. These results were also confirmed using RFLP analysis, since this mutation creates a novel *Bse*RI site in the PCR product created using primer RNASEL2 (data not shown). This I97L substitution has been identified in men with prostate cancer in two separate reports with a frequency of ≤1%.[3 7]

A novel missense mutation was identified in subject 168-01, which results in the substitution of methionine for valine at

Table 3 Summary of missense and nonsense mutations identified through sequence analysis of 95 men with prostate cancer from 75 PCGP families. All mutations were identified through sequence analysis, and five of the substitutions were previously reported by Carpten *et al*[3]

Exon	Primer pair	Amino acid change	Nucleotide substitution	No of affecteds identified with mutation
2	RNASEL2	G59S	G175A	1 (PCGP 1115-02)
2	RNASEL2	I97L	A289C	2 (PCGP 345-02 and 392-02)
2	RNASEL3	V247M	G739A	1 (PCGP 168-01)*
2	RNASEL4	E265X	G793T	1 (PCGP 1115-02)
2	RNASEL5	R462Q	G1385A	Common
4	RNASEL7	D541E	T1623G	Common

*Novel mutation in this report.

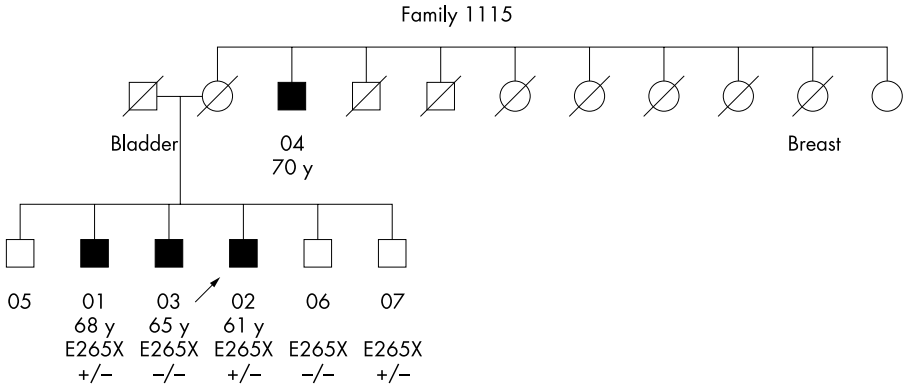

Figure 1 PCGP family with *RNASEL* E265X mutation. The pedigree structure is provided for family 1115 in which men with prostate cancer are illustrated by filled boxes, and the age at diagnosis is listed below each subject. The arrow indicates the participant who was selected first for sequence analysis. Mutation status for E265X is also indicated for family members that have provided a DNA sample for laboratory analysis. The same family members that are heterozygous for the E265X mutation are also heterozygous for the G59S mutation.

amino acid position 247 (table 3). The presence of this mutation in the proband was confirmed by RFLP analysis (data not shown). We also identified the E265X nonsense mutation previously described by Carpten *et al*[3] in one (subject 1115-02) of the 95 men with prostate cancer who were selected for the initial mutation screen (table 3). Sequence analysis confirmed the presence of the stop codon in one of two affected brothers but also in one of two unaffected brothers in this same family (fig 1C). All three men who were heterozygous for the E265X substitution were also heterozygous for the G59S substitution.

In the report of Carpten *et al*,[3] the E265X mutation was identified in 0.54% of unaffected white men (n=186) from a prostate cancer screening study and 0.52% of CEPH parents (n=96). Over 50% of the participants in the PCGP receive their care at the University of Michigan Medical Center. To determine the frequency of this mutation in the patient population at our medical centre, we analysed DNA from 75 spouses of patients with melanoma (GEM study) for the E265X mutation as well as the G59S and V247M substitutions (table 4). No heterozygous or homozygous carriers of any of these mutations were identified in this panel of controls. We also tested genomic DNA samples from a population based prostate cancer case-control study that focuses solely on African American men for the M1I mutation. This substitution of isoleucine for methionine at codon 1 was one of the two deleterious mutations described in the report of Carpten *et al*[3] and it was identified in a single African American family. None of the case or control men was shown to be a carrier of the M1I mutation or the G59S, V247M, or E265X mutations.

Table 4 Results of ASO hybridisation experiments to determine frequency of M1I, G59S, G247S, and E265X mutations. DNA samples from 75 controls from the GEM study were studied for the presence of each of four previously reported *RNASEL* mutations. 103 men with prostate cancer and 323 unaffected controls from the FMHS, which is a population based case-control study of prostate cancer in African American men, were also analysed. In each assay, several samples were not amplified sufficiently for this analysis and were removed from the total number of samples assayed

Mutation	GEM	FMHS prostate cancer cases	FMHS controls
M1I	0/74	0/92	0/311
G59S	0/75	0/103	0/317
V247M	0/75	0/102	0/310
E265X	0/72	0/102	0/320

DISCUSSION

There are several factors that make *RNASEL* a plausible candidate for the prostate cancer susceptibility gene *HPC1*. First, although the candidate region remains large, linkage peaks and recombinant data often indicate that the most likely region for *HPC1* is near marker D1S158.[3] The gene encodes an endoribonuclease which is a member of the interferon regulated 2-5A system. In vitro experiments suggest that the *RNASEL* gene may function as a tumour suppressor gene.[8] RNase L -/- murine thymocytes have suppressed apoptosis,[9] although similar studies have not been completed with prostate cells in which the *RNASEL* gene has been inactivated. Finally, the identification of deleterious mutations in two of eight multiplex prostate cancer families linked to 1q24-25 suggest that *RNASEL* requires further study as a prostate cancer susceptibility gene.

Our data provide limited support for the hypothesis that the *RNASEL* gene is *HPC1*. Analysis of one or more probands from 75 large (average >4 affected men/family) prostate cancer families has shown four rare missense/nonsense mutations among four unrelated families. Only two of 19 families with strong evidence of *HPC1* linkage were found to have *RNASEL* mutations (families 168 and 392). In family 168, DNA samples from two affected brothers are not available for *RNASEL* mutation testing. In family 392, however, only one of two affected family members harboured the I97L mutation (see below). The other *RNASEL* mutations detected in our study (G59S, I97L, and E265X) did not completely cosegregate with affected status in three families.

Prostate cancer genetic studies are complicated by several factors including the variable participation in early detection or screening tests and the occasional diagnosis of latent or clinically insignificant prostate cancer within a family. The E265X nonsense mutation was first identified in a family with four affected brothers and each brother was heterozygous for the allele with the stop codon.[3] Clinical information was available from three of the four brothers; all three men had clinically significant prostate cancer with Gleason scores in each case ⩾7. However, in our report, only two of three affected brothers in family 1115 were discovered to be heterozygous carriers of the E265X mutation (fig 1). Review of clinical records shows no significant differences in clinical stage at presentation between the brothers with and the one without the E265X mutation. There are also two unaffected brothers in family 1115 who are participating in the PCGP research project; both brothers have had serum PSA tests with values

<2.5 ng/ml within the last 12 months. Thus, it is unlikely that the unaffected brother harbouring the E265X mutation (1115-07), who was 57 at the time of study enrolment, has undetected prostate cancer. Rokman *et al*[10] recently reported the identification of the *RNASEL* E265X mutation in five index patients from 116 Finnish hereditary prostate cancer families. Similar to our results, segregation of the E265X mutation with disease status was observed in only one of the five families. In our study, the E265X mutation was found to be in complete linkage disequilibrium with the G59S mutation. This observation is consistent with the findings of others[10] and suggests the occurrence of a rare (allele frequency <1%) founder allele in the US white population.

Previous studies by us and others have shown rare cases of allelic loss in prostate cancer tumour tissue using markers that map to the *HPC1* candidate interval. The frequency of 1q24-25 LOH appears to be ~10% in both sporadic (C Bettis, L Huang, K A Cooney, unpublished data)[11 12] and hereditary[12–14] prostate cancer. Previous investigations in our laboratory using tumour tissue from subject 392-01 showed evidence of allelic loss using two chromosome 1q markers (D1S158 and D1S518, data not shown). Examination of the linkage data from subject 392-01 and his affected second degree cousin (392-02) showed that the non-shared, presumably normal, allele was deleted consistent with the hypothesis that *HPC1* may be acting as a tumour suppressor gene in this family. This observation led us to include both 392-01 and his affected second cousin 392-02 in our sequence analysis of the *RNASEL* gene. However, the I97L *RNASEL* missense mutation was identified only in subject 392-02 and presumably on the non-shared allele. Taken together, the findings do not provide evidence to support the hypothesis that the *RNASEL* gene is a tumour suppressor gene causing hereditary prostate cancer in this family.

Review of published linkage studies provides reasonable evidence to support the existence of a prostate cancer susceptibility gene at 1q24-25. Mutations in this *HPC1* gene are likely to be relatively rare and may preferentially lead to aggressive prostate cancer.[15] *RNASEL* mutations may contribute to prostate cancer in some families, as suggested by our results, as well as those contained in two recent publications.[7 16] Wang *et al*[7] screened 326 men with prostate cancer from 163 unrelated families for *RNASEL* mutations. These investigators failed to identify any clearly deleterious mutations (including E265X); however, a common polymorphism, namely Arg462Gln, was associated with familial prostate cancer compared to either sporadic prostate cancer or compared to control men. Rennert *et al*[16] identified a founder frameshift mutation in *RNASEL* (471delAAAG) in Ashkenazi Jews that was found in higher frequency among men with prostate cancer (6.9%) compared to either elderly male or young women controls (2.4% and 4.0%, respectively). Additional studies, including a meta-analysis, are needed to elucidate more clearly the role of *RNASEL* mutations in hereditary prostate cancer.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grants P50-CA69568 (SPORE) and R01-CA79596. We gratefully acknowledge Caroline Mohai, Gina Claeys, Kay Doerr, and the Flint Men's Health Study Research Team for assistance with patient recruitment and data collection.

Authors' affiliations

H Chen, A R Griffin, Y-Q Wu, L P Tomsho, K A Zuhlke, S B Gruber, K A Cooney, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA
K A Cooney Department of Urology, University of Michigan Medical School, Ann Arbor, MI, USA
S B Gruber, Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI, USA
K A Cooney, Ann Arbor Department of Veteran's Affairs, Ann Arbor, MI, USA
E M Lange, Section of Biostatistics, Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Correspondence to: Dr K A Cooney, 7310 CCGC, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0946, USA; kcooney@umich.edu

REFERENCES

1 **Smith JR**, Freije D, Carpten JD, Gronberg H, Xu J, Isaacs SD, Brownstein MJ, Bova GS, Guo H, Bujnovszky P, Nusskern DR, Damber JE, Bergh A, Emanuelsson M, Kallioniemi OP, Walker-Daniels J, Bailey-Wilson JE, Beaty TH, Meyers DA, Walsh PC, Collins FS, Trent JM, Isaacs WB. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. *Science* 1996; **274**:1371-4.
2 **Xu J**. Combined analysis of hereditary prostate cancer linkage to 1q24-25: results from 772 hereditary prostate cancer families from the International Consortium for Prostate Cancer Genetics. *Am J Hum Genet* 2000;**66**:945-57.
3 **Carpten J**, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. *Nat Genet* 2002;**30**:181-4.
4 **Cooney KA**, McCarthy JD, Lange E, Huang L, Miesfeldt S, Montie JE, Oesterling JE, Sandler HM, Lange K. Prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. *J Natl Cancer Inst* 1997;**89**:955-9.
5 **Cooney KA**, Strawderman MS, Wojno KJ, Doerr KM, Taylor A, Alcser KH, Heeringa SG, Taylor JM, Wei JT, Montie JE, Schottenfeld D. Age-specific distribution of serum prostate-specific antigen in a community-based study of African-American men. *Urology* 2001;**57**:91-6.
6 **Cooney KA**, Nichols WC, Bruck ME, Bahou WF, Shapiro AD, Bowie EJ, Gralnick HR, Ginsburg D. The molecular defect in type IIB von Willebrand disease. Identification of four potential missense mutations within the putative GpIb binding domain. *J Clin Invest* 1991;**87**:1227-33.
7 **Wang L**, McDonnell SK, Elkins DA, Slager SL, Christensen E, Marks AF, Cunningham JM, Peterson BJ, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN. Analysis of the RNASEL gene in familial and sporadic prostate cancer. *Am J Hum Genet* 2002;**71**:116-23.
8 **Lengyel P**. Tumor-suppressor genes: news about the interferon connection. *Proc Natl Acad Sci USA* 1993;**90**:5893-5.
9 **Zhou A**, Paranjape J, Brown TL, Nie H, Naik S, Dong B, Chang A, Trapp B, Fairchild R, Colmenares C, Silverman RH. Interferon action and apoptosis are defective in mice devoid of 2′,5′-oligoadenylate-dependent RNase L. *EMBO J* 1997;**16**:6355-63.
10 **Rokman A**, Ikonen T, Seppala EH, Nupponen N, Autio V, Mononen N, Bailey-Wilson J, Trent J, Carpten J, Matikainen MP, Koivisto PA, Tammela TLJ, Kallioniemi OP, Schleutker J. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. *Am J Hum Genet* 2002;**70**:1299-304.
11 **Latini JM**, Rieger-Christ KM, Wang DS, Silverman ML, Libertino JA, Summerhayes IC. Loss of heterozygosity and microsatellite instability at chromosomal sites 1q and 10q in morphologically distinct regions of late stage prostate lesions. *J Urol* 2001;**166**:1931-6.
12 **Dunsmuir WD**, Edwards SM, Lakhani SR, Young M, Corbishley C, Kirby RS, Dearnaley DP, Dowe A, Ardern-Jones A, Kelly J, The CRC/BPG UK Familial Prostate Cancer Study Collaborators, Eeles RA. Allelic imbalance in familial and sporadic prostate cancer at the putative human prostate cancer susceptibility locus, *HPC1*. *Br J Cancer* 1998;**78**:1430-3.
13 **Ahman AK**, Jonsson BA, Damber JE, Bergh A, Emanuelsson M, Gronberg H. Low frequency of allelic imbalance at the prostate cancer susceptibility loci HPC1 and 1p36 in Swedish men with hereditary prostate cancer. *Genes Chrom Cancer* 2000;**29**:292-6.
14 **Bergthorsson JT**, Johannesdottir G, Arason A, Benediktsdottir KR, Agnarsson BA, Bailey-Wilson JE, Gillanders E, Smith J, Trent J, Barkardottir RB. Analysis of HPC1, HPCX, and PCaP in Icelandic hereditary prostate cancer. *Hum Genet* 2000;**107**:372-5.
15 **Gronberg H**, Isaacs SD, Smith JR, Carpten JD, Bova GS, Freije D, Xu J, Meyers DA, Collins FS, Trent JM, Walsh PC, Isaacs WB. Characteristics of prostate cancer in families potentially linked to the hereditary prostate cancer 1 (*HPC1*) locus. *JAMA* 1997;**278**:1251-5.
16 **Rennert H**, Bercovich D, Hubert A, Abeliovich D, Rozovsky U, Bar-Shira A, Soloviov S, Schreiber L, Matzkin H, Rennert G, Kadouri L, Peretz T, Yaron Y, Orr-Urtreger A. A novel founder mutation in the RNASEL gene, 471delAAAG, is associated with prostate cancer in Ashkenazi Jews. *Am J Hum Genet* 2002;**71**:981–4.