X Chromosomal short tandem repeat polymorphisms near the phosphoglycerate kinase gene in men with chronic prostatitis

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Abstract

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) causes substantial morbidity afflicting approximately 10% of adult males. Treatment is often empirical and ineffective since the etiology is unknown. Other prostate and genitourinary diseases have genetic components suggesting that CP/CPPS may also be influenced by genetic predisposition. We recently reported a highly polymorphic short tandem repeat (STR) locus near the phosphoglycerate kinase gene within Xq11–13. Because this STR is in a region known to predispose towards other prostate diseases, we compared STR polymorphisms in 120 CP/CPPS patients and 300 control blood donors. Nine distinct allele sizes were detected, ranging from 8 to 15 repeats of the tetrameric STR plus a mutant allele (9.5) with a six base deletion in the flanking DNA sequence. The overall allele size distribution in the CP/CPPS patients differed from controls (Chi-square = 19.252, df = 8, \( P = 0.0231 \)). Frequencies of two specific alleles, 9.5 and 15, differed significantly in CP/CPPS vs. control subjects and allele 10 differed with marginal significance. Alleles 9.5 and 10 were both more common in CP/CPPS patients than controls while allele 15 was less common. These observations suggest that Xq11–13 may contain one or more genetic loci that predispose toward CP/CPPS. Further investigations involving family studies, larger patient populations, and other control groups may help elucidate this potential genetic predisposition in CP/CPPS. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

‘Prostatitis’ is the diagnosis given to many men who present with pain referable to the lower genitourinary tract and perineum [1–3]. In 1978–79, prostatitis accounted for approximately 25% of outpatient visits for genitourinary complaints [4]. Of 12 760 000 visits to urologists in 1991, 5.3% were for prostatitis. Prostatitis ranked fourth of 20 diagnoses given for visits to urologists [4]. Prostatitis was a diagnosis in 2–7 million visits annually from 1990–96, including 8% of visits to urologists and 1% of all primary care visits [5,6]. A survey of male residents found that the overall prevalence rate of a physician-assigned diagnosis of prostatitis was 9%, similar to rates of ischemic heart disease and diabetes based on a random sampling survey of residents in Olmsted

Abbreviations: AR, androgen receptor gene; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome; dHT, dihydrotestosterone; hCG, human chorionic gonadotropin; PGK, phosphoglycerate kinase; STR, short tandem repeat

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County, MN, USA [7]. Depending on their age, men with a previous diagnosis of prostatitis had a 20–50% risk for recurrent episodes.

The current consensus classification of prostatitis syndromes includes four categories: acute bacterial prostatitis, chronic bacterial prostatitis, asymptomatic inflammatory prostatitis, and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) [8,9]. Acute bacterial prostatitis presents with acute symptoms of bacterial urinary tract infection caused by the standard uropathogens. Patients may also have systemic symptoms of infection, but most respond promptly to appropriate antibacterial therapy. Recurrent episodes of bacteriuria caused by the same uropathogen constitute a hallmark of chronic bacterial prostatitis. Between episodes of bacteriuria, careful culture methods can prove that the focus of infection is the prostate gland. Patients with chronic bacterial prostatitis are unusual, constituting less than 7% of patients attending our chronic prostatitis clinic [10]. Asymptomatic inflammatory prostatitis is diagnosed during evaluation of other conditions, for example based on finding inflammatory infiltrates in a prostate biopsy obtained for evaluation of possible prostate cancer. CP/CPPS is the most common type of chronic prostatitis accounting for more than 90% of patients with symptoms. Patients suffer debilitating symptoms, but they do not have bacteriuria or clear evidence of infection with the possible exception of recent PCR-based findings [11]. Because we have limited understanding of the causes of this syndrome, therapy is entirely empirical and often unsatisfactory.

Many patients ask if their family history is an important determinant of their own risk for developing prostate disease. Genetic background is important in prostate cancer that has been associated with a number of genes on the X chromosome [12–16,18,27–29]. Although literature searches revealed no reports of genetic predisposition to CP/CPPS, 23% of patients attending our clinic reported a history of first-degree male relatives with prostatitis compared to a rate of 9% described in the overall male population [7]. Those histories did not exclude father/son relationships. The high rate of prostatitis among relatives of patients was similar to the experience of the Prostatitis Foundation, where a 20% rate was reported among respondents to an internet survey (http://prostatitis.org/aboutpf.html). Patient interviews suggested influence from the maternal lineage (http://prostatitis.freeservers.com). We identified a polymorphic short tandem repeat (STR) on the X chromosome that allowed us to evaluate if there is a potential genetic predisposition to CP/CPPS.

Phosphoglycerate kinase (PGK; ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) is a metabolic housekeeping enzyme located within Xq11–Xq13. The PGK gene is closely linked [30] to AR within a region implicated in a number of X-linked urologic disorders. These disorders include: familial predisposition to prostate cancer [14–16], androgen insensitivity [17,31] and perineal hypospadias [32,33]. Initially, we identified a highly polymorphic STR DNA sequence near the PGK gene [34,35]. In over 500 people tested, 10 distinct alleles were detected with frequencies ranging from 2% to 38%. Since the locus is X-linked, heterozygosities must be measured in women, and heterozygosity is generally > 80%. Herein, we report our findings comparing the distributions of STR alleles in patients with CP/CPPS to a control population. Our findings are consistent with the clinical suggestion that there may indeed be an inherited predisposition for some men to develop CP/CPPS.
2. Materials and methods

2.1. CP/CPPS patient population and clinical evaluation

Subjects with CP/CPPS were recruited from patients attending the University of Washington Prostatitis Clinic following a protocol approved by the University of Washington Human Subjects Committee. No subject had taken antimicrobial agents for 6 weeks prior to enrollment and patients with documentation of bacteriuria were excluded. Following a standardized history and physical examination, we obtained specimens for urethral Gram stain and cultures for *Neisseria gonorrhoea*, *Chlamydia trachomatis*, genital mycoplasmas, and *Trichomonas vaginalis* [36,37]. Each patient had a uroflow study and residual urine determination by ultrasound to exclude significant structural or functional abnormalities of the lower urinary tract. We also excluded potential subjects who had uropathogens localized to the prostate by the four-glass urine test (chronic bacterial prostatitis), urethritis (\(>5\) leukocytes/400× field on the Gram-stained urethral smear), or positive urethral cultures for *C. trachomatis* or *T. vaginalis*.

2.2. CP/CPPS patient specimens

Each CP/CPPS patient underwent prostatic biopsy using a perineal, rather than a transrectal, approach to prevent microbial contamination. Biopsies were obtained by the double-needle biopsy technique described previously [11]. Control samples were obtained of the perineal skin surface and of peri-prostatic tissues. Prostate biopsies were sent to the PCR laboratory in sterile containers within 30 min of collection.

2.3. Control population

Patients attending the general hospital clinics were sampled on three consecutive days in batches of approximately 100. Patient data were screened to determine ethnic information, sex, diagnoses, and clinics attended. Male samples in the control group were not used if there was a history of prostate disease in their medical records. While representative of our general clinical population, we cannot exclude unreported histories or predispositions toward prostate disease among members of the control group. Female blood samples were included in the control group since, for the X-linked PGK STR, female samples have twice the information content of male samples. Also, female samples would have arrived at the clinic for reasons other than prostate disease making these a useful sampling of the gene pool within our clinical patient population. Since men inherit their X chromosomes from their mothers, there is no reason a priori to expect population differences comparing male and female PGK STR distributions and no differences were found in the present study.

Stoppered tubes containing ethylenediaminetetraacetic acid and disposed blood drawn from 300 men and women were obtained from the University of Washington Medical Center Central Processing facility. Blood was drawn between 8 and 48 h prior to DNA isolation. DNA was extracted from 300 control blood samples (132 male, 168 female) and prostatic biopsy specimens of 120 prostatitis patients.

2.4. PCR assays

Based on the reported PGK flanking DNA sequence [35] PCR primers were constructed to amplify the PGK 3′ STR. The primer sequences used in the current study were: PGK11: 5′-TGGGCAACAGTGAGACTCT-3′; PGK12: 5′-CAACTACAGACAGCAGGTA-3′.

Thermal cycles were: 94°C for 5 min, then 35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 1 min followed by extension at 72°C for 7 min. PCR buffer consisted of 10 mM Tris–HCl (pH 8.3) 50 mM KCl, and 1.0 mM MgCl₂. Initially, MgCl₂ concentrations were varied over the range 0.5–5 mM, with 1.0 mM proving optimal. Annealing temperatures were initially varied from 60°C to 70°C, with 62°C proving optimal. *Taq* polymerase (Perkin-Elmer; Norwalk, CT, USA, 5 U/100 μl of reaction) was used as the thermostable enzyme.

2.5. Allele size determination

We synthesized fluorescent PCR primers complementary to an STR in the 3′ flank of the PGK1 gene. The amplified products were electrophoresed, sized and sequenced using a LiCor automated sequencer.
Allelic sizing at the PGK STR locus, coupled with high-throughput fluorescent PCR product detection, facilitated rapid typing of samples. Approximately 50 PCR products could be electrophoresed and detected in a little over 30 min. Electrophoretic gels in this system have been reloaded up to five times with little deterioration in the signal-to-noise ratio. Fluorescent detection of PCR products was made possible by incorporating 1–2 pmol IRD41 (absorbance maximum 795 nm, fluorescence maximum 833 nm; Eastman Kodak, Rochester, NY, USA)-labeled PGK12 primer synthesized at LiCor (Lincoln, NE, USA).

3. Results

3.1. Polymorphism and hemizygosity

Nine distinct alleles were detected including eight...
based on variations in the number of TATC repeats (Figs. 1 and 2). Since the PGK1 STR is located on the X chromosome, males exhibit one allele each, while women each exhibit two alleles. Alleles were named for the number of STR repeats they contained, or for their size. The allele frequency distributions are shown in the Table 1.

None of the male tissue or blood samples demonstrated more than a single PGK STR allele. A single allele per male sample was consistent with the X chromosomal location of the PGK STR and also suggested that cross-contamination of these samples was infrequent since cross-contamination would lead to multiple allele sizes appearing in male samples.

3.2. Cases vs. control distributions

The median allele size among 120 CP/CPPS cases was 11 (range 8–14). This was lower than the median allele size of 12 (range 8–15) among the 300 control subjects (Mann–Whitney U = 14912, P = 0.006). The overall distribution of alleles (Fig. 1) was also different comparing CP/CPPS patients and controls (Chi-square = 19.252, df = 8, P = 0.0231).

3.3. Significance tests of individual alleles

Analysis of individual alleles showed that the difference between CP/CPPS patients and controls was largely accounted for by three alleles, 9.5, 10 and 15. Based on previously reported DNA sequence data, the 9.5 allele is actually a mutant, 11 allele (11 repeats of 4 bp each) with a 6 bp deletion in sequence flanking the repeat [34]. Only 6 (1.4%) of 420 total subjects exhibited the 9.5 allele. Of these six patients, five were in the CP/CPPS group. Thus, 4.2% of the 120 CP/CPPS patients had the 9.5 allele compared to only 0.3% of the 300 controls (Chi-square = 8.944, df = 1, P = 0.0028). Of the 420 total subjects who had distinct alleles, 123 (29%) exhibited the 10 allele. These 123 subjects with the 10 allele included 35.8% of 120 CP/CPPS patients compared to 26.7% of 300 controls (Chi-square = 3.478, df = 1, P = 0.062). Of the 420 total subjects who exhibited distinct alleles, 10 had the 15 allele. All 10 were in the control group. Thus, the 15 allele was present in 3.3% of the 300 controls but in none of the 120 CP/CPPS cases (Chi-square = 4.098, df = 1, P = 0.0429). There were no significant differences in the frequencies of the 8, 9, 11, 12, 13 and 14 alleles between cases and controls. Of these, alleles 8 and 9 were relatively rare (<5%) in the overall population of 420 subjects.

4. Discussion

Our data suggest that certain men may have a

<table>
<thead>
<tr>
<th>Number of repeats</th>
<th>8</th>
<th>9</th>
<th>9.5</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (%)</td>
<td>2.0</td>
<td>2.7</td>
<td>0.3</td>
<td>26.7</td>
<td>22.3</td>
<td>26.0</td>
<td>10.7</td>
<td>6.0</td>
<td>3.3</td>
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<td>n = 300</td>
<td>(6)</td>
<td>(8)</td>
<td>(1)</td>
<td>(80)</td>
<td>(67)</td>
<td>(78)</td>
<td>(32)</td>
<td>(18)</td>
<td>(10)</td>
</tr>
<tr>
<td>CP/CPPS (%)</td>
<td>2.5</td>
<td>2.5</td>
<td>4.2</td>
<td>35.8</td>
<td>21.7</td>
<td>20.0</td>
<td>7.5</td>
<td>5.8</td>
<td>0</td>
</tr>
<tr>
<td>n = 120</td>
<td>(3)</td>
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<td>(5)</td>
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<td>(26)</td>
<td>(24)</td>
<td>(9)</td>
<td>(7)</td>
<td>(0)</td>
</tr>
<tr>
<td>Significance*</td>
<td>0.75</td>
<td>0.92</td>
<td>&lt;0.01</td>
<td>0.06</td>
<td>0.88</td>
<td>0.2</td>
<td>0.32</td>
<td>0.95</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Chi-square for individual alleles. For the overall distribution, Chi-square = 19.252, df = 8, P = 0.0231.
genetic predisposition to develop CP/CPPS. Significant associations of certain alleles at the X-linked PGK1 STR fit with the clinical observation that many CP/CPPS patients have blood relatives with prostatitis, particularly in their maternal pedigrees (http://prostatitis.freeservers.com). This potential predisposition may be located in a specific region of the X chromosome near other sites that appear to be critical for prostate growth and development, as well as for development of some familial prostate cancers.

The PGK1 gene is located within the DXS983–DXS995 interval of GeneMap'99 at 94.4–97.4 cM. AR is in the adjacent interval, DXS991–DXS983 at 86.9–94.4 cM. Ordering of loci is still uncertain, but the distance between PGK1 and AR may be relatively short. We examined cross-over frequencies at the GenLink, Human Meiotic Map (http://www.genlink.wustl.edu/). Using the marker DXS1275 as a reference present in overlapping intervals, we calculated that there were two cross-over events between AR and PGK1 out of 227 jointly informative meioses. AR is the only obvious candidate for genitourinary tract effects in this region of the chromosome, but there are almost certainly uncharacterized genes in the vicinity making it premature to assign our results to any known gene.

Literature searches revealed no previous reports suggesting a genetic predisposition to develop CP/CPPS. However, patient interviews frequently suggest affected relatives on the maternal side. Of 100 patients responding to a survey, 18% reported an afflicted maternal grandfather compared with 9% who had paternal grandfathers with CP/CPPS. Of the same 100 patients, 14% reported maternal uncles with CP/CPPS while 0% reported CP/CPPS afflicted paternal uncles. These observations are consistent with an X-linked predisposing factor(s) although much further work will be needed to confirm or deny this. Since the X chromosome has only a small region of homology with the Y chromosome, most X-linked genes lack compensatory homologues if there is a deficient or otherwise marginally active gene.

Males who are castrated or otherwise lose testicular function prior to puberty are termed eunuchs. Eunuchs have prostate glands but do not develop the major prostate diseases: benign prostatic hyperplasia, prostate cancer and CP/CPPS. These observations suggest that hormones produced by the testes, specifically testosterone and, if 5-α reductase is active, dihydrotestosterone (dHT), may determine vulnerability to prostate diseases.

Hormones and their receptors play important roles in many stages of prostate development. In the second month of embryo development, placental human chorionic gonadotropin (hCG) induces Leydig cells at the genital ridge to secrete testosterone [38]. Testosterone, if converted to dHT by an active 5-α reductase, promotes masculinization of the bi-potential gonads in the third month of gestation, as well as promoting prostate differentiation and masculinization of the external genital structures. Leydig cell testosterone secretion, while controlled by placental hCG during the critical stage of sexual differentiation, is later controlled by fetal pituitary luteinizing hormone. Assuming a normal AR, dHT continues to promote prostate growth and masculinization at puberty. Defective genes have been described at virtually every stage of this developmental cascade [38]. Deficiency in 5-α reductase or in the AR can lead to severe interruptions in genitourinary development early in life. Adult onset prostate diseases may reflect subtle variations in the activities of some of the same genes that are crucial in early development. For these reasons, we investigated polymorphic variation, using the PGK1 STR, in the region of the X chromosome where AR resides.

Since the majority of men may eventually be affected by prostate disease, it is extremely difficult to identify an ideal control group for comparison with CP/CPPS patients. We elected instead to compare CP/CPPS cases with general clinical, control blood samples. Lifetime incidences of prostate diseases are still in dispute, but susceptible individuals in the control group would tend to reduce differences between CP/CPPS and controls in this study. We previously demonstrated that the PGK1 STR allele size is somatically stable allowing comparisons of blood DNA in one population with prostate tissue DNA in another [34].

Mitochondrial DNA sequences suggest that humans likely evolved from a very limited population [39,40]. We reasoned that during this early bottle-
neck in evolution, there was likely only one, or perhaps a few variants of AR, or another gene affecting prostate development. Later, genetic drift would have led to lineages with slightly different susceptibilities to diseases such as CP/CPPS.

The results presented here are consistent with variations in the Xq11–Xq13 region, perhaps AR, or another gene, predisposing men to CP/CPPS. It seems unlikely that variants of the housekeeping enzyme, PGK1, contribute to CP/CPPS predisposition. Rather, the variant PGK1 STRs detected probably mark ancient human lineages that would, by chance, be expected to have subtle variations in AR or other gene activities.

While statistically significant, differences at the 9.5, 15 alleles among CP/CPPS patients vs. controls were relatively subtle. The difference for the 10 allele ($P = 0.06$) is marginal and technically only significant at 90% confidence. Currently, our CP/CPPS population was not stratified for differences such as family history of CP/CPPS. It is possible that populations selected for family history may exhibit more dramatic differences in PGK1 STR allele types. Such studies are underway.

In addition to Xq11–Xq13, other regions of the X chromosome and of autosomes have been implicated in genetic predisposition to urologic diseases, particularly prostate cancer. Hypothesizing that variant genes implicated in other urologic diseases may play a role in prostate embryogenesis and development, we also plan to search for additional STRs in those regions. It is possible that if more associations are found, the combined typing of multiple STRs may provide practical diagnosis of a patient’s vulnerability to CP/CPPS. This work is in progress.

In summary, potential genetic predispositions associated with the PGK gene, or another gene within Xq11–Xq13, such as AR, should be further investigated. This approach may provide important insights into the pathogenesis of CP/CPPS and other prostate diseases and open this field to powerful new genetic techniques. Work is in progress to scan the X chromosome with other polymorphic markers. Ultimately, it may prove possible to use such genetic information to predict a man’s susceptibility to CP/CPPS or his response to possible therapeutic interventions.

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**References**


