The *UGT1* locus is a determinant of prostate cancer recurrence after prostatectomy

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Abstract

The prognostic significance of common deletions in uridine diphospho-glucuronosyl-transferase 2B (*UGT2B*) genes encoding sex steroid metabolic enzymes has been recently recognized in localized prostate cancer (PCa) after radical prostatectomy (RP). However, the role of germline variations at the *UGT1* locus, encoding half of all human UGTs and primarily involved in estrogen metabolism, remains unexplored. We investigated whether variants of *UGT1* are potential prognostic markers. We studied 526 Caucasian men who underwent RP for clinically localized PCa. Genotypes of patients for 34 haplotype-tagged single-nucleotide polymorphisms (htSNPs) and 11 additional SNPs across the *UGT1* locus previously reported to mark common variants including functional polymorphisms were determined. The risk of biochemical recurrence (BCR) was estimated using adjusted Cox proportional hazards regression and Kaplan–Meier analysis. We further investigated whether variants are associated with plasma hormone levels by mass spectrometry. In multivariable models, seven htSNPs were found to be significantly associated with BCR. A greater risk was revealed for four *UGT1* intronic variants with hazard ratios (HRs) of 1.59–1.88 ($P < 0.002$) for htSNPs in *UGT1A10*, *UGT1A9*, and *UGT1A6*. Conversely, decreased BCR was associated with three htSNPs in introns of *UGT1A10* and *UGT1A9* (HR = 0.56–0.58; $P \leq 0.01$). An unfavorable *UGT1* haplotype comprising all risk alleles, with a frequency of 14%, had a HR of 1.68 (95% CI = 1.13–2.50; $P = 0.011$). Significant alteration in circulating androsterone levels was associated with this haplotype, consistent with changes in hormonal exposure. This study provides the first evidence, to our knowledge, that germline polymorphisms of *UGT1* are potential predictors of recurrence of PCa after prostatectomy.

Key Words

- biochemical recurrence
- germline polymorphisms
- glucuronidation
- prostate cancer
- UGT

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Introduction

Sex steroids play a central role in the development and progression of prostate cancer (PCa) (Mohler *et al.* 2012, Ryan *et al.* 2013). Recent evidence supports the notion that patient's genetic background influences the outcome (Audet-Walsh *et al.* 2011, 2012, Nadeau *et al.* 2011, Boyd

et al. 2012, Reese *et al.* 2012, Levesque *et al.* 2013, 2014a,b, Wyatt *et al.* 2013). In particular, common deletions of the uridine diphospho-glucuronosyltransferase (UGT) genes *UGT2B17* and *UGT2B28* involved in the catabolism of androgenic sex steroids have been recognized as

prognostic markers for localized PCa after radical prostatectomy (RP) (Nadeau *et al.* 2011). The UGT-mediated metabolic pathways, governed by the two gene families *UGT1* and *UGT2*, are among key regulators of sex steroid exposure responsible for their inactivation via glucuronic acid conjugation, which prevents binding to their respective nuclear receptors and facilitates their excretion. In support of the idea that inherited genetic variations can alter the hormonal environment to which cancer cells are exposed, common gene deletions of *UGT2B17* and *UGT2B28* have also been associated with altered levels of circulating androgen steroids (Nadeau *et al.* 2011).

Half of the human UGTs are encoded by the *UGT1* gene located on chromosome 2q37, which encodes nine functional enzymes. In contrast to *UGT2B* enzymes, *UGT1*As are mainly regulators of tissue exposure to estrogens, because most of them are involved in the glucuronidation of parental estrogen (estradiol (E_2) and estrone (E_1)), and inactivation of their hydroxyl moieties at position 2 or 4 as well as methoxy catechol estrogens (Gall *et al.* 1999, Lepine *et al.* 2004, Murai *et al.* 2006, Starlard-Davenport *et al.* 2007, Zhou *et al.* 2010, Sneitz *et al.* 2013). Conversely, there is little evidence to support them having a role in the inactivation of androgens, e.g., the potent androgen, dihydrotestosterone (DHT; Gall *et al.* 1999, Zhou *et al.* 2010). *UGT1A1*, *UGT1A3*, *UGT1A8*, *UGT1A9*, and *UGT1A10* conjugate E_1 and E_2 most efficiently, whereas *UGT1A4* is less efficient. (Lepine *et al.* 2004, Starlard-Davenport *et al.* 2007, Sneitz *et al.* 2013). Several groups have reported that estrogens and their metabolites contribute to both the development and progression of PCa (Cavalieri & Rogan 2011, Nelles *et al.* 2011, Hu *et al.* 2012). Genetic polymorphisms in factors that govern estrogen biosynthetic and metabolic pathways have been linked to altered exposure to sex steroid hormones and risk of PCa (Gu *et al.* 2014, Kanda *et al.* 2015). It has been reported that low-activity promoter alleles of the E_2 -conjugating enzyme *UGT1A1* (rs3064744; (TA)_n repeat) are associated with a risk of low-grade PCa and a slight elevation of estrogen levels but have no gene-dosage effect (Tang *et al.* 2011).

Germline variations in genes involved in the biosynthesis and metabolism of androgens and estrogens have been associated with progression of PCa (Audet-Walsh *et al.* 2011, 2012, Nadeau *et al.* 2011, Levesque *et al.* 2013, 2014a,b). The relevance of studying steroidogenic pathways in the context of progression of PCa arises from observations that PCa tumor cells can use multiple biotransforming pathways to *de novo* synthesize more potent hormones (Montgomery *et al.* 2008, Mitsiades *et al.* 2012). In support

of these results, we have recently reported that single-nucleotide polymorphisms (SNPs) in genes associated with E_2 -related metabolic pathways, either individually or in combination, are predictors of progression of PCa. These variants are located in genes encoding cytochrome P450 1B1, which is involved in the production of 4-hydroxy metabolites of E_2 and E_1 (4-OH), catechol-O-methyltransferase, which forms 2-methoxy catechol estrogens, sulfotransferase 2B1, which catalyzes the formation of less-reactive sulfate metabolites, and 17 β -hydroxysteroid dehydrogenase type 2, which is involved in the conversion of E_2 to E_1 (Levesque *et al.* 2014a). However, it remains unclear whether the outcome of PCa is influenced by common germline polymorphisms in the estrogen catabolic pathway mediated by *UGT1* gene products.

The objective of this study was to determine whether common variations across the *UGT1* locus, which encodes multiple estrogen-metabolizing *UGT1A* enzymes, are associated with a modified risk of biochemical recurrence (BCR) after prostatectomy in patients with localized PCa and to ascertain their relationships with levels of endogenous circulating hormones. After adjustments for known clinico-pathological variables, seven *UGT1* SNPs were found to be significantly associated with time to BCR, three SNPs were protective and four were associated with an increased risk. Additional analyses identified an unfavorable *UGT1* haplotype, characterized by the latter four risk markers, that is also linked with changes in exposure to circulating hormones.

Materials and methods

Clinical data and outcome collections

The cohort included 526 Caucasian patients with localized PCa. All patients underwent RP at l'Hôtel-Dieu de Québec Hospital (QC, Canada) between February 1999 and December 2002 (Audet-Walsh *et al.* 2011, Nadeau *et al.* 2011). All participants provided written informed consent for genetic analysis, and the Local Research Ethics Committee of the CHU de Québec approved the research protocol. The clinical characteristics of the studied cohort are given in Table 1.

Genetic analysis and steroid measures

Polymorphisms of the *UGT1* locus were assessed by PCR amplification by Sequenom iPLEX matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. With the objective of covering most of the haplotype

Table 1 Clinical and pathological characteristics of prostate cancer patients.

Characteristics	n (%)
Localized PCa	526 (100)
Mean age at diagnosis (years)	63.3
S.D. age at diagnosis (years)	6.8
Range age at diagnosis	43.5–80.7
Median follow-up (months)	88.8
Biochemical recurrence risk	130 (24.7)
PSA at diagnosis (ng/ml)	
≤ 10	362 (69)
> 10–20	103 (20)
> 20	56 (11)
Pathological Gleason score	
≤ 6	158 (31)
7	244 (48)
≥ 8	107 (21)
Pathological T stage	
pT = T2	313 (60)
pT = T3a	131 (25)
pT ≥ T3b	77 (15)
Nodal invasion	
N0	481 (92)
N+	44 (8)
Neoadjuvant hormone therapy	
Yes	31 (6)
No	495 (94)
Adjuvant hormone therapy	
Yes	30 (6)
No	496 (94)
Margin status	
Negative	368 (70)
Positive	154 (30)
Smoking status	
Non/ex-smoker	438 (83)
Smoker	85 (16)
D'Amico risk classification	
Low	187 (36)
Intermediate	208 (40)
High	122 (24)

PCa, prostate cancer; PSA, prostate-specific antigen; T, tumor; N, node.

diversity, polymorphisms in *UGT1* were chosen on the basis of results from our previous study of Caucasian volunteers recruited in the same geographical region (Menard *et al.* 2009) and as described by the genetics of the CEU (Utah residents with Northern and Western European ancestry) HapMap population. A region covering all exons, introns, and 5 kb upstream and downstream of each gene was screened using a haplotype-tagging SNP (htSNP) strategy (Audet-Walsh *et al.* 2011, 2012). Overall, we genotyped 34 htSNPs (Fig. 1) with an additional 11 associated SNPs across the *UGT1A* locus previously reported to mark common variants in regulatory domains, exons, and intron–exon boundaries, including some functional polymorphisms (Supplementary Table 1, see section on supplementary data given at the end of

this article). This included genotyping of the *UGT1A1* promoter variant (rs3064744; c.-54/-53insTA) genotyped by direct sequencing of PCR-amplified products, as described previously (Girard *et al.* 2008). Negative controls were included for every run of analysis, and quality controls consisting of random samples were successfully performed on 5% of the study cohort. SNPs that had 5% missing genotypes were excluded from the analysis. None of the studied SNPs deviated from the Hardy–Weinberg equilibrium. A total of 495 plasma samples were available and collected the morning of the surgical procedure. Levels of circulating sex steroids for this cohort of patients have been reported (Nadeau *et al.* 2011). Data are expressed as the mean ± the S.E.M. To ensure the validity of each analysis, quality controls that included known steroid concentrations with deuterated isotopes were included in each run of analysis.

Statistical analysis

To analyze associations between polymorphisms and BCR, each htSNP was first categorized according to homozygosity/heterozygosity (i.e. major allele homozygote, heterozygote, and minor allele homozygote), because the functions of most of these htSNPs are unknown. Rare homozygotes (frequency <2%) were combined with heterozygotes. Cox regression analysis was performed for each SNP with adjustment for clinico-pathological variables. Multivariable models included prostate-specific antigen (PSA) at diagnosis, Gleason score, pathological T stage, age at diagnosis, surgical margins, nodal invasion status, smoking status, neoadjuvant therapy, adjuvant therapy. All covariables were treated as categorical, and ≤4% of all covariables had missing values. The censoring variable was BCR, as reported previously (Nadeau *et al.* 2011). Haplotypes were inferred using Phase version 2.1.1 (Stephens *et al.* 2001, Stephens & Donnelly 2003). Pairwise linkage disequilibrium was determined with HAPLOVIEW 3.32 (www.broad.mit.edu/mpg/haploview). Kaplan–Meier analyses (log-rank) and univariate Cox regression analyses were performed for each SNP. As we tested multiple polymorphisms ($n=45$), false-discovery rates (q values) were calculated to determine the degree to which the tests were prone to false positives using the R QVALUE package (<http://genomics.princeton.edu/storeylab/qvalue/>). Analysis of covariance was used to compare means of each natural log-transformed hormonal variable between haplotype groups adjusted for age. Patients with missing genotype or hormone level data were excluded. For comparisons among haplotype groups, any significant

Figure 1

UGT1 common germline variations genotyped in the cohort of 526 Caucasian men with clinically localized prostate cancer and treated by radical prostatectomy. We genotyped 34 htSNPs and 11 additional associated SNPs across the *UGT1* locus previously reported to mark

common variants in regulatory domains, exons, and intron–exon boundaries, including some functional polymorphisms. Pairwise linkage disequilibrium (r^2 values) is graphically represented by different shades of gray.

differences revealed by analysis of covariance were further investigated using Fisher's least significant difference *post-hoc* pairwise comparison. Patients who had previously received androgen deprivation therapy were excluded from our analyses. Statistical analyses were performed using PASW Statistics version 17 (SPSS, Inc.).

Results

Overall, 24.7% of patients experienced recurrence after prostatectomy (median follow-up time, 7.4 years). None of the germline variations tested was associated with the main predictors of BCR (PSA at diagnosis, Gleason score, pathological T stage, and surgical margin status; [Supplementary Table 2](#), see section on [supplementary data](#) given at the end of this article). After adjustments for known prognostic factors had been made, seven variants located in the intronic or regulatory regions of *UGT1* were associated with an altered risk of BCR. The relative frequency of each htSNP and its corresponding hazard

ratio (HR; 95% CI) are presented in [Table 2](#). One variation in the *UGT1A10* (rs1823803) and two variations in the *UGT1A9* intronic region (rs2741048 and rs2602381) were associated with a significantly decreased risk of BCR with HR values between 0.56 and 0.58 ($P < 0.01$). Conversely, htSNPs located in the *UGT1A10* intronic region (rs17862847 and rs7608713) and in the *UGT1A9* intronic region (rs4663871) were associated with an increased risk for BCR (HR=1.59–1.88; $P < 0.02$). In addition, one variation rs6751673 in *UGT1A6* was positively associated with BCR and displayed an increased risk of events (HR=1.68; $P = 0.008$). The Kaplan–Meier curves showing the BCR-free survival for the *UGT1* markers significantly associated with BCR and their log-rank P values are available in the supplementary material ([Supplementary Fig. 1](#), see section on [supplementary data](#) given at the end of this article). No significant association was observed for any of the known functional *UGT1* variants evaluated, e.g., the TATA box promoter variant *UGT1A1**28 (rs34815109; c.-54/-53insTA), *UGT1A3* V47A

Table 2 Significant associations of *UGT1* polymorphisms with BCR

	<i>UGT1</i> htSNPs ^a	MAF	Genotype frequency ^b			L-R P value	HR ^c	95% CI	P value	q value
			BCR	no BCR	% BCR					
SNPs associated with a reduced risk of BCR	1A10 c.-6058	0.46	23/64/42	78/205/97	23/24/30	0.058	0.56	0.37–0.83	0.004	0.019
	rs1823803									
	1A9 IVS + 313	0.38	18/53/57	47/210/131	28/20/30	0.016	0.56	0.38–0.82	0.003	0.018
	rs2741048									
SNPs associated with an increased risk of BCR	1A9 IVS + 2889	0.47	25/61/43	82/199/99	23/23/30	0.062	0.58	0.39–0.88	0.010	0.032
	rs2602381									
	1A10 IVS + 2988	0.14	1/45/83	4/84/290	20/35/20	0.004	1.88	1.26–2.80	0.002	0.018
	rs17862847									
	1A10 IVS + 21028	0.22	8/50/71	12/130/237	40/28/23	0.118	1.62	1.11–2.38	0.013	0.040
	rs7608713									
	1A9 IVS + 152	0.20	6/50/74	15/121/256	29/29/22	0.067	1.59	1.09–2.32	0.016	0.044
	rs4663871									
	1A6 IVS + 2392	0.29	15/57/57	26/161/193	37/26/23	0.180	1.68	1.15–2.45	0.008	0.029
	rs6751673									

BCR, biochemical recurrence risk; HR, hazard ratio; MAF, minor allele frequency; L-R, log-rank test P value.

^aFor each SNP, the major allele > minor allele is indicated.^bThe numbers represent minor allele homozygotes, heterozygotes, and major allele homozygotes respectively.^cMultivariate models included PSA at diagnosis, Gleason score, pathological T stage, age at diagnosis, neoadjuvant therapy, smoking status, adjuvant therapy, surgical margin status, and nodal invasion status. The reference genotype was set at 1.00. Dominant models are presented.

(rs6431625; c.140T>C), or other non-synonymous common variations located in exons *UGT1A3* and *UGT1A4*, and in the common exons 2–5 or the 3' UTR.

Five haplotypes inferred using the seven positive *UGT1* markers are significantly associated with the occurrence of BCR at an allelic frequency of $\geq 5\%$ in our cohort. The occurrence rates of BCR were 23, 22, 33, 21, and 19%, respectively, for haplotypes 1–5 (H1–H5; Fig. 2A). After adjustments for other variables, we found that H3, characterized by the presence of the four unfavorable alleles and no protective allele, displayed a greater risk of BCR (Table 3). H3 carriers display a HR of 1.68 ($P=0.011$) compared with the reference H1 that includes none of the increased risk and/or the protective alleles described earlier. We also observed that individuals who carry both H3 and two or more deleted copies of *UGT2B17* and *UGT2B28* tend to have decreased BCR-free survival as shown by the Kaplan–Meier curves for BCR. The BCR-free stratifications are 71 and 55% respectively, when H3 is sub-classified according to the number of *UGT2B* deletions (0–1 *UGT2B* deletion ($n=112$) versus ≥ 2 *UGT2B* deletions ($n=33$); Fig. 2B).

We also compared the mean levels of plasma unconjugated sex steroids and androgen glucuronides for H3 carriers with only zero or one *UGT2B* deletion, because individuals with more than two *UGT2B* deletions had been previously reported to present reduced circulating levels of DHT metabolites, namely androsterone glucuronide (ADT-G) and 3α -androstenediol-17G (Nadeau et al. 2011). Compared with non-carriers of *UGT1* risk alleles (i.e. H1), carriers of H3 presented a 15% lower level of ADT (206.30 ± 9.83 pg/ml ($n=112$) versus 176.21 ± 11.93 pg/ml ($n=50$); $P=0.01$), whereas no significant changes in androgen glucuronides, E_2 , and E_1 could be detected (data not shown).

Discussion

We provide evidence for the prognostic value of *UGT1* for localized PCa. As has been observed *UGT2B* deletions, we found that several *UGT1* htSNPs are associated with an increased risk of recurrence. The markers positively linked with a greater risk of BCR are located in intronic regions of exons 1A6, 1A9, and 1A10. This finding supports our hypothesis that genetic variants of *UGT1* may modify the progression of PCa – probably by altering sex steroid metabolism – although direct biological evidence for this hypothesis is lacking.

We had previously reported a significant decrease in circulating androgen metabolites, namely ADT-G and

Figure 2

Kaplan-Meier curves for (A) the five most frequent *UGT1A* haplotypes associated with BCR in the cohort of Caucasian men with localized PCa ($n=526$) and (B) the high-risk haplotype 3 combined with *UGT2B17* and *UGT2B28* deletions. Log-rank (LR) P values are reported in each frame.

3 α -diol-17G, in PCa patients with two or more deletions in the androgen-conjugating *UGT2B17* and *UGT2B28* (Nadeau *et al.* 2011). On the basis of the substrate-specificity of the nine functional *UGT1A*s involved in estrogen catabolism, we expected to find little if any influence on formation of androgen glucuronides associated with htSNPs. In fact, few reported results support the ability of recombinant *UGT1A*s to conjugate androgens,

with the exception of *UGT1A3* and *UGT1A4*, which have weak activity toward DHT and its metabolite ADT compared with *UGT2B* enzymes (Gall *et al.* 1999, Zhou *et al.* 2010). This is consistent with our observation that no significant changes in circulating androgen glucuronides are observed for carriers of *UGT1*-related risk haplotypes. *UGT1A* enzymes are well known to be involved in the metabolism of estrogens, including the parental estrogens E_2 and E_1 , and catechol estrogens and their methoxy derivatives; however, no significant changes in circulating levels of unconjugated E_1 and E_2 could be detected in our study. It remains to be ascertained if levels of estrogen metabolites, including their glucuronide derivatives, are modified by variants of *UGT1*. We found positive markers mostly in regions related to *UGT1A9* and *UGT1A10*, with their gene products being mainly involved in the metabolism of 2-OH- E_2 , 4-OH- E_2 , and 2-methoxy- E_2 (Lepine *et al.* 2004). We are currently developing a mass spectrometry assay to address whether the identified *UGT* markers could be involved in recurrence of PCa by promoting unbalanced glucuronidating metabolism for these specific estrogen metabolites or by their relative abundance compared with that of androgens, thereby favoring progression of cancer. The carcinogenicity of catechol oxidative metabolites has been recognized for various cancers, including PCa (Bosland & Mahmoud 2011, Cavalieri & Rogan 2011). Results of a pilot study by Kosti *et al.* (2011) indicated that some catechol estrogens are present in greater concentrations in the urine of PCa patients compared with that of healthy men, indicating an association between estrogen metabolites and risk of PCa. However, based on a method developed by Xu *et al.* (2007), they did not directly measure the levels of estrogen glucuronide derivatives but reported total estrogen concentrations (glucuronidates + sulfates + unconjugated sex steroids following hydrolysis with sulfatase and β -glucuronidase).

Very few studies have documented the expression of *UGT1* gene products in human prostate. Court *et al.* (2012) detected nine *UGT1A* transcripts in a pool of human prostates and detected all *UGT1A*s but at significantly lower levels compared with those found in liver samples. During normal prostate differentiation, downregulation of *UGT1A* transcripts was observed in normal prostatic stem cells compared with more differentiated cells (Williamson *et al.* 2013). Furthermore, assessment of individual *UGT1A* transcripts revealed that the expression of *UGT1A8* was elevated in progenitor cells, with no change in amounts of *UGT2B* expressed. *UGT1A8* is the major *UGT1A* involved in inactivation of the carcinogenic metabolites

Table 3 Identification of an unfavorable *UGT1* haplotype associated with the risk of biochemical recurrence (BCR) in prostate cancer patients

Haplotype	No. of alleles (%)	Effect of SNP on risk of BCR							HR ^d	95% CI	P value
		rs1823803, C > T	rs17862847, T > A	rs7608713, G > A	rs4663871, G > A	rs2741048, A > C	rs2602381, C > T	rs6751673, G > A			
H1	299 (28.4)	C	T	G	G	A	C	G	1.00 (reference)	0.66–1.28	0.614
H2	375 (35.6)	T ^a	T	G	G	C ^a	T ^a	G	0.92	1.13–2.50	0.011
H3	145 (13.8)	C	A ^b	A ^b	A ^b	A	C	A ^b	1.68	0.60–2.00	0.771
H4	62 (5.9)	T ^a	T	G	A	A	C	A ^b	1.09	0.58–2.10	0.774
H5	58 (5.6)	C	T	A ^b	G	A	T ^a	A ^b	1.10	0.87–2.09	0.179
Others ^c	113 (10.7)	–	–	–	–	–	–	–	1.35		

^aIndividual SNP associated with a reduced risk (↓) of BCR.^bIndividual SNP associated with an increased risk (↑) of BCR.^cOthers haplotypes arising at a frequency of <5% were combined.^dBold type indicates statistically significant results ($P < 0.05$).

4-OH-E₁/E₂ (Lepine *et al.* 2004). In support of a role for UGT1As in progression of PCa, transient repression of UGT1A expression in the PCa cell line LNCaP has been shown to contribute to activation of androgen receptors leading to upregulation of PSA along with increased cell survival in the standard steroid-containing medium (Williamson *et al.* 2013). This study also provided additional evidence that reduced expression of UGT1A is a feature of clinical PCa associated with a poorer prognosis and may be explained by the fact that upon depletion of UGT1A, a higher level of steroids is expected. However, Williamson and colleagues did not address whether levels of expression of UGT1A affect levels of steroids and whether estrogens rather than androgens are altered upon partial repression of UGT1A. In fact, their results obtained *in vitro* with LNCaP cells might be caused by the action of androgens and estrogens because both types of steroids are present in a complete medium and because androgens are converted to estrogens by the action of aromatase. A role for estrogens in this context is thus plausible because UGT1A more efficiently inactivates estrogens compared with androgens such as DHT, and it is known that estrogens are proficient in activating the mutated androgen receptor in the LNCaP cell line (Tan *et al.* 1997). In addition, expression of aromatase is upregulated by approximately 30-fold ($P < 0.001$) in metastatic PCa tumors compared with primary tumors (Montgomery *et al.* 2008), indicating a greater production of estrogens during progression of PCa. Changes in UGT1A expression have also been reported in clinical specimens. In castration-resistant patients, low levels of expression of UGT1A have been found compared with that observed in early PCa (Varambally *et al.* 2005, Williamson *et al.* 2013), as well as decreased UGT1A immunostaining in aggressive disease (Williamson *et al.* 2013). Altogether, these findings provide evidence supporting the suggestion that reduced expression of UGT1A is linked to poor prognosis and may represent a mechanism for accumulation of sex steroids, probably estrogens, within PCa cells. In this study linking several genetic markers of the *UGT1* gene to BCR, the decreased levels of the DHT metabolite ADT in carriers of the *UGT1*-risk haplotype may reflect enhanced production of estrogens from androgens or a modification of androgen metabolism, although additional investigations are required to validate this hypothesis.

In conclusion, our study provides the first evidence, to our knowledge, that germline polymorphisms in the *UGT1* locus modify the likelihood of BCR after prostatectomy. Our findings also highlight the need to better

characterize the levels of E₂ and estrogen metabolites, the main known steroid hormone substrates of UGT1A enzymes, in PCa patients. Further studies are needed to define the underlying mechanisms through which UGT1As contribute to the biology of PCa and patient outcomes.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-14-0423>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

É Lévesque and C Guillemette conceived the study concept, and designed and supervised the study. L Lacombe and Y Fradet were responsible for patient recruitment and clinical data. I Laverdière, C Flageole, É Audet-Walsh, É Lévesque, and C Guillemette were involved in acquisition of data and statistical analysis. P Caron performed the experiments. I Laverdière, C Flageole, É Audet-Walsh, É Lévesque, and C Guillemette wrote the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. É Lévesque, C Guillemette, Y Fradet, and L Lacombe obtained funding for this work.

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References

- Audet-Walsh E, Bellemare J, Nadeau G, Lacombe L, Fradet Y, Fradet V, Huang SP, Bao BY, Douville P, Girard H *et al.* 2011 *SRD5A* polymorphisms and biochemical failure after radical prostatectomy. *European Urology* **60** 1226–1234. (doi:10.1016/j.eururo.2011.06.020)
- Audet-Walsh E, Bellemare J, Lacombe L, Fradet Y, Fradet V, Douville P, Guillemette C & Levesque E 2012 The impact of germline genetic variations in hydroxysteroid (17-β) dehydrogenases on prostate cancer outcomes after prostatectomy. *European Urology* **62** 88–96. (doi:10.1016/j.eururo.2011.12.021)
- Bosland MC & Mahmoud AM 2011 Hormones and prostate carcinogenesis: androgens and estrogens. *Journal of Carcinogenesis* **10** 33. (doi:10.4103/1477-3163.90678)
- Boyd LK, Mao X & Lu YJ 2012 The complexity of prostate cancer: genomic alterations and heterogeneity. *Nature Reviews. Urology* **9** 652–664. (doi:10.1038/nrurol.2012.185)
- Cavaliere EL & Rogan EG 2011 Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer. *Journal of Steroid Biochemistry and Molecular Biology* **125** 169–180. (doi:10.1016/j.jsbmb.2011.03.008)
- Court MH, Zhang X, Ding X, Yee KK, Hesse LM & Finel M 2012 Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica* **42** 266–277. (doi:10.3109/00498254.2011.618954)
- Gall WE, Zawada G, Mojarrabi B, Tephly TR, Green MD, Coffman BL, Mackenzie PI & Radominska-Pandya A 1999 Differential glucuronidation of bile acids, androgens and estrogens by human UGT1A3 and 2B7. *Journal of Steroid Biochemistry and Molecular Biology* **70** 101–108. (doi:10.1016/S0960-0760(99)00088-6)
- Girard H, Butler LM, Villeneuve L, Millikan RC, Sinha R, Sandler RS & Guillemette C 2008 *UGT1A1* and *UGT1A9* functional variants, meat intake, and colon cancer, among Caucasians and African-Americans. *Mutation Research* **644** 56–63. (doi:10.1016/j.mrfmmm.2008.07.002)
- Gu Z, Wang G & Chen W 2014 Estrogen receptor α gene polymorphisms and risk of prostate cancer: a meta-analysis involving 18 studies. *Tumour Biology* **35** 5921–5930. (doi:10.1007/s13277-014-1785-4)
- Hu WY, Shi GB, Hu DP, Nelles JL & Prins GS 2012 Actions of estrogens and endocrine disrupting chemicals on human prostate stem/progenitor cells and prostate cancer risk. *Molecular and Cellular Endocrinology* **354** 63–73. (doi:10.1016/j.mce.2011.08.032)
- Kanda S, Tsuchiya N, Narita S, Inoue T, Huang M, Chiba S, Akihama S, Saito M, Numakura K, Tsuruta H *et al.* 2015 Effects of functional genetic polymorphisms in the *CYP19A1* gene on prostate cancer risk and survival. *International Journal of Cancer* **136** 74–82. (doi:10.1002/ijc.28952)
- Kosti O, Xu X, Veenstra TD, Hsing AW, Chu LW, Goldman L, Bebu I, Collins S, Dritschilo A, Lynch JH *et al.* 2011 Urinary estrogen metabolites and prostate cancer risk: a pilot study. *Prostate* **71** 507–516. (doi:10.1002/pros.21262)
- Lepine J, Bernard O, Plante M, Tetu B, Pelletier G, Labrie F, Belanger A & Guillemette C 2004 Specificity and regioselectivity of the conjugation of estradiol, estrone, and their catecholestrogen and methoxyestrogen metabolites by human uridine diphospho-glucuronosyltransferases expressed in endometrium. *Journal of Clinical Endocrinology and Metabolism* **89** 5222–5232. (doi:10.1210/jc.2004-0331)
- Levesque E, Huang SP, Audet-Walsh E, Lacombe L, Bao BY, Fradet Y, Laverdiere I, Rouleau M, Huang CY, Yu CC *et al.* 2013 Molecular markers in key steroidogenic pathways, circulating steroid levels, and prostate cancer progression. *Clinical Cancer Research* **19** 699–709. (doi:10.1158/1078-0432.CCR-12-2812)
- Levesque E, Laverdiere I, Audet-Walsh E, Caron P, Rouleau M, Fradet Y, Lacombe L & Guillemette C 2014a Steroidogenic germline polymorphism predictors of prostate cancer progression in the estradiol pathway. *Clinical Cancer Research* **20** 2971–2983. (doi:10.1158/1078-0432.CCR-13-2567)
- Levesque E, Laverdiere I, Lacombe L, Caron P, Rouleau M, Turcotte V, Tetu B, Fradet Y & Guillemette C 2014b Importance of 5α-reductase gene polymorphisms on circulating and intraprostatic androgens in prostate cancer. *Clinical Cancer Research* **20** 576–584. (doi:10.1158/1078-0432.CCR-13-1100)
- Menard V, Girard H, Harvey M, Perusse L & Guillemette C 2009 Analysis of inherited genetic variations at the *UGT1* locus in the French-Canadian population. *Human Mutation* **30** 677–687. (doi:10.1002/humu.20946)

- Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, Fleisher M, Sander C, Sawyers CL & Scher HI 2012 Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Research* **72** 6142–6152. (doi:10.1158/0008-5472.CAN-12-1335)
- Mohler JL, Armstrong AJ, Bahnson RR, Boston B, Busby JE, D'Amico AV, Eastham JA, Enke CA, Farrington T, Higano CS *et al.* 2012 Prostate cancer, version 3.2012: featured updates to the NCCN guidelines. *Journal of the National Comprehensive Cancer Network* **10** 1081–1087.
- Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD & Nelson PS 2008 Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Research* **68** 4447–4454. (doi:10.1158/0008-5472.CAN-08-0249)
- Murai T, Samata N, Iwabuchi H & Ikeda T 2006 Human UDP-glucuronosyltransferase, UGT1A8, glucuronidates dihydrotestosterone to a monoglucuronide and further to a structurally novel diglucuronide. *Drug Metabolism and Disposition* **34** 1102–1108. (doi:10.1124/dmd.106.009621)
- Nadeau G, Bellemare J, Audet-Walsh E, Flageole C, Huang SP, Bao BY, Douville P, Caron P, Fradet Y, Lacombe L *et al.* 2011 Deletions of the androgen-metabolizing UGT2B genes have an effect on circulating steroid levels and biochemical recurrence after radical prostatectomy in localized prostate cancer. *Journal of Clinical Endocrinology and Metabolism* **96** E1550–E1557. (doi:10.1210/jc.2011-1049)
- Nelles JL, Hu WY & Prins GS 2011 Estrogen action and prostate cancer. *Expert Review of Endocrinology & Metabolism* **6** 437–451. (doi:10.1586/eem.11.20)
- Reese AC, Pierorazio PM, Han M & Partin AW 2012 Contemporary evaluation of the National Comprehensive Cancer Network prostate cancer risk classification system. *Urology* **80** 1075–1079. (doi:10.1016/j.urology.2012.07.040)
- Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S *et al.* 2013 Abiraterone in metastatic prostate cancer without previous chemotherapy. *New England Journal of Medicine* **368** 138–148. (doi:10.1056/NEJMoa1209096)
- Sneitz N, Vahermo M, Mosorin J, Laakkonen L, Poirier D & Finel M 2013 Regiospecificity and stereospecificity of human UDP-glucuronosyltransferases in the glucuronidation of estriol, 16-epiestriol, 17-epiestriol, and 13-epiestradiol. *Drug Metabolism and Disposition* **41** 582–591. (doi:10.1124/dmd.112.049072)
- Starlard-Davenport A, Xiong Y, Bratton S, Gallus-Zawada A, Finel M & Radominska-Pandya A 2007 Phenylalanine⁹⁰ and Phenylalanine⁹³ are crucial amino acids within the estrogen binding site of the human UDP-glucuronosyltransferase 1A10. *Steroids* **72** 85–94. (doi:10.1016/j.steroids.2006.11.016)
- Stephens M & Donnelly P 2003 A comparison of bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics* **73** 1162–1169. (doi:10.1086/379378)
- Stephens M, Smith NJ & Donnelly P 2001 A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* **68** 978–989. (doi:10.1086/319501)
- Tan J, Sharief Y, Hamil KG, Gregory CW, Zang DY, Sar M, Gumerlock PH, deVere White RW, Pretlow TG, Harris SE *et al.* 1997 Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. *Molecular Endocrinology* **11** 450–459. (doi:10.1210/mend.11.4.9906)
- Tang L, Yao S, Till C, Goodman PJ, Tangen CM, Wu Y, Kristal AR, Platz EA, Neuhauser ML, Stanczyk FZ *et al.* 2011 Repeat polymorphisms in estrogen metabolism genes and prostate cancer risk: results from the Prostate Cancer Prevention Trial. *Carcinogenesis* **32** 1500–1506. (doi:10.1093/carcin/bgr139)
- Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, Shah RB, Chandran U, Monzon FA, Becich MJ *et al.* 2005 Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* **8** 393–406. (doi:10.1016/j.ccr.2005.10.001)
- Williamson SC, Mitter R, Hepburn AC, Wilson L, Mantilla A, Leung HY, Robson CN & Heer R 2013 Characterisations of human prostate stem cells reveal deficiency in class I UGT enzymes as a novel mechanism for castration-resistant prostate cancer. *British Journal of Cancer* **109** 950–956. (doi:10.1038/bjc.2013.399)
- Wyatt AW, Mo F, Wang Y & Collins CC 2013 The diverse heterogeneity of molecular alterations in prostate cancer identified through next-generation sequencing. *Asian Journal of Andrology* **15** 301–308. (doi:10.1038/aja.2013.13)
- Xu X, Keefer LK, Ziegler RG & Veenstra TD 2007 A liquid chromatography-mass spectrometry method for the quantitative analysis of urinary endogenous estrogen metabolites. *Nature Protocols* **2** 1350–1355. (doi:10.1038/nprot.2007.176)
- Zhou J, Tracy TS & Remmel RP 2010 Glucuronidation of dihydrotestosterone and *trans*-androsterone by recombinant UDP-glucuronosyltransferase (UGT) 1A4: evidence for multiple UGT1A4 aglycone binding sites. *Drug Metabolism and Disposition* **38** 431–440. (doi:10.1124/dmd.109.028712)

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