Background: N-acetyltransferase 2 is phase II metabolizing enzyme that participates in the bioconversion of heterocyclic arylamines into electrophilic nitrenium ions, which are important ultimate carcinogens that are directly implicated in tumor initiation process. This study was conducted to examine: (1) whether the N-acetyltransferase 2 (NAT2) genotype is a risk factor for prostate cancer, (2) to study effect of NAT2 genotype on modifying prostate cancer risk from tobacco use.

Methods: The case control study was undertaken over a period of 28 months and included 130 prostate cancer patients (CaP) and 140 controls. The NAT2 genotypes were identified by PCR-RFLP method in DNA extracted from peripheral blood. Genotype frequencies and the association of genotypes with patients and control groups were assessed by logistic regression model.

Results: We observed non-significant association of rapid acetylator genotype NAT2 (OR = 1.452, 95% CI: 0.54–1.87, P = 0.136) in prostate cancer patients. However significant association was observed between rapid acetylator genotype NAT2 and CaP tobacco users (OR = 3.43, 95% CI: 1.68–7.02, P-value < 0.001) when compared with controls.

Conclusion: The data suggests that the NAT2 rapid acetylator genotypes may play an important role in determining the risk of developing prostate cancer particularly in the tobacco users of north Indian population.
Some studies indicated that genetically variable NATs, CYP P450 and GSTs are involved in the metabolism of drugs, carcinogens and natural products; and may be responsible for cancer susceptibility [7,8]. It has been reported that rapid acetylators genotypes of NAT2 may be at increased risk of liver and colon cancer [9], hepatocellular carcinoma [10] and colorectal cancer [11] when exposed to environmental arylamines carcinogens, due to NAT2 rapid acylator mediated O-acetylation. Recent molecular epidemiological studies have analyzed the relationship between various metabolic enzymes, such as N-acetyltransferases (NATs) and cytochrome P450 (CYP) in etiology of prostate cancer [12,13].

It is known that human express two forms of N-acetyltransferases: NAT1 and NAT2; both genes are polymorphic. A recent review reported the nucleotide and amino-acid changes associated with various alleles and deduced phenotype from genotype. It also summarized results of molecular epidemiologic studies assessing the association of NAT1 and NAT2 genotypes with cancer risk of bladder, colon, breast, lung, head and neck and prostate [14]. A review by Chen, (2001) in prostate cancer suggests that the frequencies of some polymorphisms in certain genes differ among different racial and ethnic groups [15]. Whether these genetic variants can help explain part of the large differences in prostate cancer risk in various populations await further clarification.

The present study was undertaken to study the following objectives, i) To observe the frequencies of rapid and slow acetylators (NAT2) in CaP and control individuals ii) to study effect of NAT2 genotype on modifying prostate cancer risk from tobacco use.

**Methods**

**Patient selection**

The study group consisted of 130 prostate cancer patients mean age (63.3 ± 9.9) and 140 normal healthy controls mean age (56.7 ± 13.9). The criteria for the patient selection was based on clinical proforma, pathological, and histo-pathological records from the outpatient department of Sanjay Gandhi postgraduate institute of medical science, Lucknow. This study was approved by ethical committee of the Institute. Only histologically confirmed prostate cancer patients were included in the study. All cancer patients had higher Gleason scores (6–9) and was detected at advance stage due to lack of structured screening program under any health scheme in our country. Informed consent was obtained from each participant. The inclusion criteria for the controls were absence of any prior history of cancer or pre-cancerous lesions. Serological (prostate serum antigen, < 4 ng/dl), physical (digital rectal examination) and radiological examinations were performed in all control individuals in order to exclude the possibility of malignancy. The consumption of tobacco in any form (cigarette/ bidi smoking, chewing tobacco in beetle leaf, pan-masala/ gutka etc.) in both groups (cases and controls) was noted through a detailed questionnaire. The criteria of non-users are persons who never use tobacco related material like cigarette/ bidi smoking, pan-masala/ gutka, or chewing tobacco in beetle leaf whereas tobacco user were those who used all these carcinogenic material. These questionnaires were published in our other studies [24-26].

**PCR-RFLP and alleles genotyping**

Genomic DNA was isolated from peripheral leucocytes by Proteinase -K digestion and phenol/chloroform method [16]. The NAT2 genotypes were determined using the PCR-RFLP as described previously [17]. PCR product of 1093 bp was generated by polymerase chain reaction using the following primer:

Forward 5'-TCTAGCATGAATCACTCTGC-3'

Reverse 5'- GGAACA AAITGG AC TTGG -3'.

Genomic DNA 200 ng was added to a PCR mixture, comprising 18.5 pmol of each primer, 200 µmol dNTP, 1.5 unit of Taq polymerase, and 5 µl, 10X PCR buffer (10 mmol/ml Tris HCl pH = 8.4, 50 mmol/ml KCl and 2.5 mmol/ml MgCl₂) in a total volume of 50 µl. PTC-100 thermocycler (MJ Research, U.S.A.) for polymerase chain reaction was employed. The reaction mixture was subjected to initial denaturation at 94°C for 5 min, followed by 35 cycles (94°C, 1 min), annealing (58.5°C, 1 min) and extension (72°C, 1 min). The final extension was done at 72°C for 10 min. Following PCR, 7 µl of PCR products were digested with four separate enzymes including Kpn1 for NAT2*5 allele, at 37°C for 2 hrs; Taq1 for NAT2*6 allele, at 56°C for 4 hrs; BamH1 for NAT2*7 allele at 37°C for 2 hrs; and Alu1 for NAT2*14 allele at 37°C for 2 hrs. Digested product was run on 2% agarose gel for NAT2*5, NAT2*7, NAT2*14 alleles and 3% agarose gels for NAT2*6 allele.

NAT2 have many alleles but more common alleles studied frequently are NAT2*5, NAT2*7, NAT2*14 and NAT2*6 allele as described in the present study also.
However, we could not genotypes many other additional alleles of NAT2, which could be the limitation in our study.

**Estimating the frequency of rapid and slow acetylator**

The variant and non-variant NAT2 alleles were recorded and rapid or slow acetylator phenotype assignments were deduced on the basis of NAT2 genotype [4]. Genotypes possessing two variant alleles (NAT2*5, NAT2*6, NAT2*7, or NAT2*14) were assigned as slow acetylator phenotype whereas others were assigned as rapid acetylator phenotype.

**Statistical analysis**

Statistical analysis was done with SPSS 11.5 software program. Differences in genotype prevalence and association between case and control groups were assessed by binary logistic regression model. Odds ratios (OR) and its 95% confidence interval (CI) were obtained by summarizing data over two habit strata (tobacco users/ non-users). We evaluated age adjusted (confounder OR) and age unadjusted odds ratios, and 95% CI using logistic regression models. Univariate analysis, odds ratios, and 95% CI were used to describe the strength of association.

**Results**

Comparative details of the observed frequency of different alleles are presented in (Table 1); which indicates significant interethnic variation in NAT2 genotypes in different populations. The NAT2*5 and NAT2*6 allele is most commonly present in our population and also in South Indian and Caucasian- American population but is rare in Japanese population, whereas the NAT2*14 allele is only found in African – American population.

The distribution of genotypes of NAT2 in control and cancer patients is shown in (Table 2). Higher frequency of NAT2 rapid acetylator was observed (64.6%) among the patient groups as compared to the controls (55.7%). However, this was statistically non significant (OR = 1.452, 95% CI: 0.54–1.87, \(P = 0.136\)).

The association between tobacco users and NAT2 genotypes are summarized in (Table 3). The OR for the rapid acetylator genotypes verses slow acetylator genotypes was 3.43 fold higher for the susceptibility of prostate cancer as compared to the controls (OR = 3.43, 95% CI: 1.68–7.02, \(P\)-value = 0.001) (Table 3).

We categorized prostate serum antigen value (PSA) into three group (PSA = <10 ng/ml, >10 ng/ml,>20 ng/ml) and Gleason score into two group (GS = 6–7 and 8–9). And we observed that NAT2 genotypes were non-significant with PSA (\(P = 0.090\)) or Gleason score (= 0.678) for risk of prostate cancer in our population (Fig 1 and Fig 2).

**Discussion**

The present NAT2 genotyping study based on molecular methods in discriminating the acetylator genotypes both in controls and prostate cancer patients is the first of its kind in north Indian population. Rapid acetylator genotypes were comparatively predominant (55.7% and

### Table 1: Frequency of NAT2 alleles in of north Indian control and other ethnic population.

| Population                  | Allelic frequency of NAT2 | Reference |
|-----------------------------|---------------------------|-----------|
|                             | NAT2*5 | NAT2*6 | NAT2*7 | NAT2*14 |
| North Indians (n = 140)     | 0.50   | 0.30   | 0.25   | 0.0     | Present study |
| South Indians (n = 166)     | 0.22   | 0.37   | 0.25   | 0.0     | [20]         |
| Caucasian-American (n = 372)| 0.45   | 0.28   | 0.02   | 0.0     | [22]         |
| African – American (n = 128)| 0.30   | 0.22   | 0.02   | 0.09    | [22]         |
| Japanese (n = 173)          | 0.01   | 0.20   | 0.13   |         | [1]          |

### Table 2: Frequency distribution of NAT2 genotypes in prostate cancer patients and controls.

| Patients                    | NAT2 genotype   | P-value | Unadjusted OR (95% CI) | Adjusted OR |
|-----------------------------|-----------------|---------|------------------------|-------------|
|                             | Slow – acetylators | Rapid-acetylators |                   |             |
| Controls (n = 140)          | 62 (44.29%)      | 78 (55.71%)  | 1.0 (Reference)        | 1.0         |
| Prostate cancer (n = 130)   | 46 (35.38%)      | 84 (64.62%)  | 0.136                  | 1.452 (0.54–1.87) | 1.348 (0.39–2.28) |

Adjusted OR = Age adjusted odds ratio.
64.6%) as compared to the slow acetylator genotypes (44.3% and 35.4%).

The results observed in the present study suggest, that NAT2 genotype has a trend of association for prostate cancer risk when considered alone (OR = 1.452, 95% CI: 0.54–1.87, P = 0.136) but is statistically non-significant (Table 2). However, no association could be established between NAT2 genotype and PSA and/or Gleason score (Fig 1 and Fig 2). Our findings agree with previous studies that showed significant association of prostate cancer for NAT2 rapid acetylator genotype in American study (23) whereas non significant association was observed in Swedish, Danish [12]) and Spanish population [13]. On the contrary, there was significant association reported with slow acetylator genotypes of NAT2 in Japanese population [1]. The discrimination in association study from our observation could be related to ethnic variation.

However, in the present study we observed significant association between the NAT2 genotypes in tobacco users as compared to the controls (Table 3). The synergistic interaction between the rapid acetylators genotype with tobacco users obtained in our study implies that exposure to tobacco, activates heterocyclic amines that are substrates for NAT2 which may increase the risk for prostate cancer. These observations are in agreement with the reports published by the investigators in liver and colon. In the liver heterocyclic amines may be N-hydroxylated by the hepatic CYP1A2, and in turn O-acetylated by NAT enzymes to an active form that can develop DNA adducts [5,9]. NAT2 genotypes studied in hepatocellular carcinoma [10] and colorectal cancer [11] have indicated the
prevalence of rapid acetylator among patient population. Thus, the present study suggests that rapid acetylator genotype could be associated with the susceptibility to prostate cancer especially in tobacco users. The mechanisms behind this association indicate that the slow acetylator genotypes should decrease the generation of critical intracellular concentration of such ultimate carcinogens, and thus reduce tumorigenesis upon environmental exposure (tobacco users). However, rapid acetylator genotypes should increase the generation of such ultimate carcinogens and enhance tumorigenesis by the pathway of O-acetylation. The results found in the present study reveal a markedly increased frequency of allele encoding the active genotype, among the patients cohort that entirely fit the above model, and are consistent with genotype / phenotype based studied that demonstrate an excess of rapid acetylator among the prostate cancer patients.

In the controls, slow allele of NAT2 is present up to 90% in Arab population [21], 40–60% in Caucasians including Indian [18] and 5–25% in East Asian [19]. We observed 44% of slow acetylator genotypes; however, another study from South Indian population indicated 74% of slow acetylator genotype [20]. Thus it indicates that frequency of slow allele observed in our population matched with studies in Caucasians population [18]. Differences of distribution of slow allele of NAT2 between our and South Indian population is due to the different ethnic /or geographical environment.

Conclusion

In conclusion, this study indicates that NAT2 rapid acetylator genotype exhibit a trend of association with the risk of developing prostate cancer, and more so in case of patients who are tobacco users.

Competing interests

The author(s) declare that they have no competing interests.

Authors’ contributions

DSI Srivastava carried out the molecular genetic studies, participated in analyzing the data & drafted the manuscript. RD Mittal participated in designing of the study & manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank Dr. A. MANDHANI for providing the clinical samples and Dr. B. MITTAL for helpful suggestions. One of the authors (D.S.I. Srivastava) is thankful to Council of Scientific and Industrial Research, New Delhi, for awarding senior research fellowship and financial aid.

References

1. Hamsaki T, Inatomi H, Katoh T, Aono H, Ikuyama T, Muratani T, Matsumoto T: NAT2 gene polymorphism as a possible biomarker for susceptibility to prostate cancer in Japanese. Int J Urol 2003, 10:167-173.
2. Inatomi H, Katoh K, Kawamoto T, Matsumoto T. NAT2 gene polymorphism as a possible biomarker for susceptibility to bladder cancer in Japanese. Int J Urol 1999, 6:464-56.
3. Hein DW, Doll MA, Gray K, Rustan TD, Ferguson RJ: Metabolic activation of N-hydroxy-2-acetylaminofluorene by NAT1 and NAT2 in colon cytosols of Syrian hamster congenic at the NAT2 locus. Cancer Res 1993, 53:509-514.
4. Vatsis KP, Weber WW, Bell DA: Nomenclature for N-acetyltransferase. Pharmacogenetics 1995, 5:1-17.
5. Hein DW, Doll MA, Rustan TD, Gray K, Fung Y, Ferguson RJ, Grant DM: Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 1993, 14:1633-1638.
6. Cescorbi I, Brockmoller J, Mrziolekiewicz PM, Muller A, Roots I: Arylamines N-acetyltransferase activity in man. Drug Metab Dispos 1999, 27:1361-1367.
7. Caporaso N, Goldstein A: Cancer genes: single and susceptibility: exposing the difference. Pharmacogenetics 1995, 5:59-63.
8. Guengerich FP: Influence of nutrients and dietary materials on cytochrome P-450 enzymes. Am J Clin Nutr 1995, 61:651S-658S.
9. Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF: Metabolic activation of carcinogenic heterocyclic amines by human liver and colon. Carcinogenesis 1991, 12:1839-845.
10. Huang YS, Chern HD, Wu JC, Chao Y, Huang YH, Chang FY, Lee SD: Polymorphism of the N-acetyltransferase 2 gene, red meat intake, and the susceptibility of hepatocellular carcinoma. Am J Gastroenterol 2003, 98:1417-1422.
11. Gill JG, Lechner MC: Increased frequency of wild type arylamines-N-acetyltransferase allele NAT2*4 homozygotes in Portuguese patients with colorectal cancer. Carcinogenesis 1999, 19:37-41.
12. Wadelius M, Atrup JL, Stubbins MJ, Johansson JE, Atrup ML, Stubbins MJ, Andersson SO, Johansson JE, Wadelius M, Wolf CR, Atrup H, Rane A: Polymorphism in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association in prostate cancer. Pharmacogenetics 1999, 9:333-340.
13. Agundez JA, Martinez C, Olivera M, Gallardo L, Ladero JM, Rosado C, Prados J, Rodriguez-Molina J, Resel L, Benitez J: Expression in human prostate of drug and carcinogen metabolizing enzymes: association with prostate cancer risk. Br J Cancer 1998, 78:1361-1367.
14. Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina US, Nangju NA, Feng Y: Molecular genetics of NAT1 and NAT2 acetylation polymorphisms. Cancer Epidemiol Biomarkers Prev 2000, 9:29-42.
15. Chen C: Risk of prostate cancer in relation to polymorphism of metabolic genes. Epidemiol Rev 2001, 23:30-35.
16. Sambrook J, Fritsch E, Maniatis T: Molecular Cloning-A Laboratory Manual 2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
17. Hsieh FL, Pu YS, Chern HD, Hsu JJ, Chiu HY, Chen CJ: Genetic polymorphisms of N-acetyltransferase 1 and 2 and risk of cigarette smoking-related bladder cancer. Br J Cancer 1999, 81:537-541.
18. Xie HG, Xu ZH, Ou-Yang DS, Shu Y, Yang DL, Wang JS, Yan XD, Huang SL, Wang W, Zhou HH: Meta-analysis of phenotype and genotype of NAT2 deficiency in Chinese population. Pharmacogenetics 1997, 7:503-514.
19. Waddell M, Atrup JL, Stubbins MJ, Andersson SO, Johansson JE, Wadelius M, Wolf CR, Atrup H, Rane A: Polymorphism in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association in prostate cancer. Pharmacogenetics 1999, 9:333-340.
20. Chen C: Risk of prostate cancer in relation to polymorphism of metabolic genes. Epidemiol Rev 2001, 23:30-35.
21. Sambrook J, Fritsch E, Maniatis T: Molecular Cloning-A Laboratory Manual 2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
22. Hsieh FL, Pu YS, Chern HD, Hsu JJ, Chiu HY, Chen CJ: Genetic polymorphisms of N-acetyltransferase 1 and 2 and risk of cigarette smoking-related bladder cancer. Br J Cancer 1999, 81:537-541.
23. Xie HG, Xu ZH, Ou-Yang DS, Shu Y, Yang DL, Wang JS, Yan XD, Huang SL, Wang W, Zhou HH: Meta-analysis of phenotype and genotype of NAT2 deficiency in Chinese population. Pharmacogenetics 1997, 7:503-514.
24. Waddell M, Atrup JL, Stubbins MJ, Andersson SO, Johansson JE, Wadelius M, Wolf CR, Atrup H, Rane A: Polymorphism in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association in prostate cancer. Pharmacogenetics 1999, 9:333-340.
25. Chen C: Risk of prostate cancer in relation to polymorphism of metabolic genes. Epidemiol Rev 2001, 23:30-35.
26. Sambrook J, Fritsch E, Maniatis T: Molecular Cloning-A Laboratory Manual 2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989.
27. Hsieh FL, Pu YS, Chern HD, Hsu JJ, Chiu HY, Chen CJ: Genetic polymorphisms of N-acetyltransferase 1 and 2 and risk of cigarette smoking-related bladder cancer. Br J Cancer 1999, 81:537-541.
28. Xie HG, Xu ZH, Ou-Yang DS, Shu Y, Yang DL, Wang JS, Yan XD, Huang SL, Wang W, Zhou HH: Meta-analysis of phenotype and genotype of NAT2 deficiency in Chinese population. Pharmacogenetics 1997, 7:503-514.
29. Waddell M, Atrup JL, Stubbins MJ, Andersson SO, Johansson JE, Wadelius M, Wolf CR, Atrup H, Rane A: Polymorphism in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association in prostate cancer. Pharmacogenetics 1999, 9:333-340.
double bladder and prostate cancers in a case-comparison study. Anticancer Res 2002, 22:3529-35.
24. Mittal RD, Srivastava DS, Kumar A, Mittal B: Polymorphism of GSTM1 and GSTT1 genes in prostate cancer: A Study from North India. Indian J Cancer 2004, 41:115-119.
25. Srivastava DS, Kumar A, Mittal B, Mittal RD: Polymorphism of GSTM1 and GSTT1 genes in bladder cancer: a study from North India. Arch Toxicol 2004, 78:430-434.
26. Srivastava DS, Mandhani A, Mittal B, Mittal RD: Genetic polymorphism of glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1) and susceptibility to prostate cancer in Northern India. BJU Int 2005, 95:170-173.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2490/5/12/prepub