

# **Joint effects of Nat1, Nat2 genes and smoking on bladder carcinogenesis: a HuGE literature-based systematic review and evidence synthesis.**

*Protocol for a HuGE association review*

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Simon Sanderson  
Public Health Genetics Unit, Cambridge, UK

Georgia Salanti  
Public Health Genetics Unit and MRC Biostatistics Unit, Cambridge, UK

Julian PT Higgins  
Public Health Genetics Unit and MRC Biostatistics Unit, Cambridge, UK

## *Background*

Bladder cancer is becoming an increasingly important international public health problem. It is one of the commonest urological malignancies worldwide, with around 330,000 new cases occurring each year. In the United Kingdom, bladder cancer is already the fifth commonest cancer with 12,500 new cases and 5,000 deaths per year. Roughly 80 percent of bladder cancers occur in people aged 60 and over and as the population ages, the incidence of bladder cancer will increase. In developed countries, over 90 percent of bladder cancers are urothelial transitional cell carcinomas, whilst squamous cell carcinomas are more common in the rest of the world where schistosomiasis is the main cause. A number of other factors have also been associated with bladder carcinogenesis: increasing age, male gender, and exposure to carcinogenic aromatic and heterocyclic amines (such as benzidine), either through occupation or through cigarette smoke. Genetic variants involved in the metabolism of these chemicals have also been investigated, including GSTMI, NAT1 and NAT2.

## *Objective*

To quantify the magnitude of association of the individual effects of NAT1 and NAT2 and their joint effects with smoking on bladder carcinogenesis. We will employ a novel approach to synthesising evidence of joint effects [Salanti and Higgins; submitted to *Statistics in Medicine*], in which studies providing information on only one or two of the risk factors can still contribute relevant information on the three-way joint effects.

## *Methods*

### **Criteria for considering studies**

#### *Polymorphisms*

The NAT1 and NAT2 genes are both located on chromosome 8 (8p22 for NAT2 and 8p21.3-23.1 for both NAT1 and NAT2). NAT1\*4 and NAT2\*4 are the reference (wild-type) alleles. Genetic variants of NAT1 and NAT2 are known to alter the metabolic rate of compounds such as caffeine, isoniazid and dapsone. Individuals can be classified as possessing a slow or fast acetylator phenotype, by directly measuring the metabolism of a probe drug or indirectly by measuring the genotype. NAT1 and NAT2 variants are thought to modify human cancer risk by altering the rate at which potentially carcinogenic compounds are either neutralised or activated in different organs and tissues.

#### *Clinical outcomes*

Transitional cell carcinoma of the urinary bladder.

## *Types of studies*

All reports studying the association of NAT1 and/or NAT2 with bladder cancer, including both case-control and cohort designs, will be eligible. In case-control studies, cases must have a confirmed diagnosis of bladder cancer and controls must be individuals without bladder cancer selected from a suitable population. Cohort studies must be prospective and population-based. Studies without a disease-free control group will be excluded.

## **Search methods**

A comprehensive search of electronic databases will be conducted, using a pre-determined search strategy based on MeSH (or equivalent thesaurus) headings and text words; Entrez PubMed, BIOSIS, EMBASE, and the Science Citation Index will be searched from the earliest date of each database to December 2005. No language restrictions will be imposed. Additional references will be sought from published reviews and reference lists of identified primary studies. Titles and abstracts of identified reports will be screened to determine whether they meet the inclusion criteria. Two reviewers will extract data from the original articles using a pre-piloted proforma. A third reviewer is available for arbitration. The authors of primary studies will be contacted in an attempt to identify unpublished studies, to obtain additional data or to obtain clarification about study details.

## **Quality assessment**

The methodological quality of each study to be included will be assessed according to the considerations described by Little et al. (1).

## **Data extraction**

This will include:

- Demographic characteristics of participants, including ethnic origin, age, and gender
- Study design and conduct, including selection criteria for cases and controls
- Matching procedures
- Cross-classification by smoking status and how this data was collected
- Whether assessment of genotype and/or phenotype was blind to disease status
- Conformity to Hardy-Weinberg equilibrium
- Explicit consideration of population stratification
- Classification of acetylation status (based on genotype or phenotype)
- Linkage disequilibrium between NAT1 and NAT2 genes

## **Data synthesis**

We will conduct random-effects meta-analyses of associations between NAT1 and bladder cancer and NAT2 and bladder cancer using odds ratios as the effect measure. The presence of possible publication bias will be assessed graphically using funnel plots, and statistically by Begg's and Egger's tests. Joint effects of the three risk factors (NAT1, NAT2 and cigarette smoking) will be estimated using a novel approach to utilise the complete dataset. In brief, the goal of the analysis is to estimate simultaneously the odds ratios for each combination of NAT1, NAT2 and smoking status compared to a 'low-risk' reference group of NAT1 rapid, NAT2 slow and non-smoking. The model will be fitted using Markov chain Monte Carlo methods within a Bayesian framework using WinBUGS.

## *Potential conflict of interest*

None known

## **References:**

1. Little J et al. Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. *Am. J. Epidemiol.* 2002; 156: 300-10.