

***Interaction between cytochrome P450 1A2  
genetic polymorphism and cigarette smoking  
on the risk of hepatocellular carcinoma  
: a case-control study in Japan***

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## <Background>

The major causative factor of hepatocellular carcinoma (HCC) is chronic infection with hepatitis C virus and hepatitis B virus.

**Cigarette smoking** also has lately been recognized as a risk factor for HCC.

**Environmental  
chemicals**

**Phase I enzyme  
: CYP**

**Active  
carcinogens**

**Phase II enzyme  
: GST, NAT**

**Non-active  
carcinogens**

Mounting epidemiologic evidence suggests that genetic polymorphisms of drug metabolizing enzymes such as cytochrome P450 (**CYP**), glutathione S-transferase (**GST**) and N-acetyltransferase (**NAT**) may be involved in smoking-related hepatocarcinogenesis.

## <Objective>

To examine whether genetic polymorphisms of *CYP1A1*, *CYP1A2*, *CYP2A6*, *CYP2E1*, *GSTM1* and *NAT2*, all of which represent typical candidate genes in the development of smoking-related cancers, are related to the risk of HCC with any interaction with **cigarette smoking**.

## < Materials and Methods >

### Case group

**209 cases with HCC**, who were admitted or outpatients of 2 main hospitals in Saga City (Saga Medical School Hospital and Saga Prefectural Hospital) between April 2001 and March 2004.

### Control group 1

**275 hospital controls**, who were first time visitors at the general outpatient clinic of Saga Medical School Hospital between May 2001 and April 2003.

### Control group 2

**381 patients with chronic liver disease (CLD) without HCC**, who were out- or inpatients of the two hospitals between September 2001 and March 2004.

## Interview

Research nurses interviewed study subjects using a questionnaire on lifestyle factors (drinking & smoking)

## Hepatitis virus markers

Plasma HBsAg and HCVAb

## Genotyping

*CYP1A1* (PCR-RFLP with *MspI*, 3'-flanking region)

*CYP1A2* (PCR-RFLP with *DdeI*, G-2964A)

*CYP2A6* (PCR-RFLP with *AccII/Eco81I*, deletion)

*CYP2E1* (PCR-RFLP with *RsaI*, C-1019T)

*NAT2* (PCR-RFLP with *Asp718/TaqI/BamHI*,  
C481T/G590A/G857A)

*GSTM1* (PCR, deletion)

## Statistical analysis

Unconditional logistic regression models to estimate adjusted odds ratios and their 95% confidence intervals

## Characteristics of HCC cases, hospital controls (control group 1) and CLD patients (control group 2)

	HCC cases	Hospital controls	P value <sup>a</sup>	CLD patients	P value <sup>b</sup>
<b>Number</b>	<b>209</b>	<b>275</b>		<b>381</b>	
<b>Mean age (year)</b>	<b>67.0</b>	<b>60.6</b>	<b>&lt; 0.01</b>	<b>60.4</b>	<b>&lt; 0.01</b>
<b>Male (%)</b>	<b>67%</b>	<b>65%</b>	<b>0.64</b>	<b>54%</b>	<b>&lt; 0.01</b>
<b>Current smoker (%)</b>	<b>33%</b>	<b>25%</b>	<b>0.06</b>	<b>23%</b>	<b>&lt; 0.01</b>
<b>Heavy-drinking history<sup>c</sup> (%)</b>	<b>23%</b>	<b>8%</b>	<b>&lt; 0.01</b>	<b>10%</b>	<b>&lt; 0.01</b>
<b>HBsAg-positive (%)</b>	<b>9%</b>	<b>2%</b>	<b>&lt; 0.01</b>	<b>9%</b>	<b>0.97</b>
<b>HCVAb-positive</b>	<b>86%</b>	<b>8%</b>	<b>&lt; 0.01</b>	<b>86%</b>	<b>0.95</b>

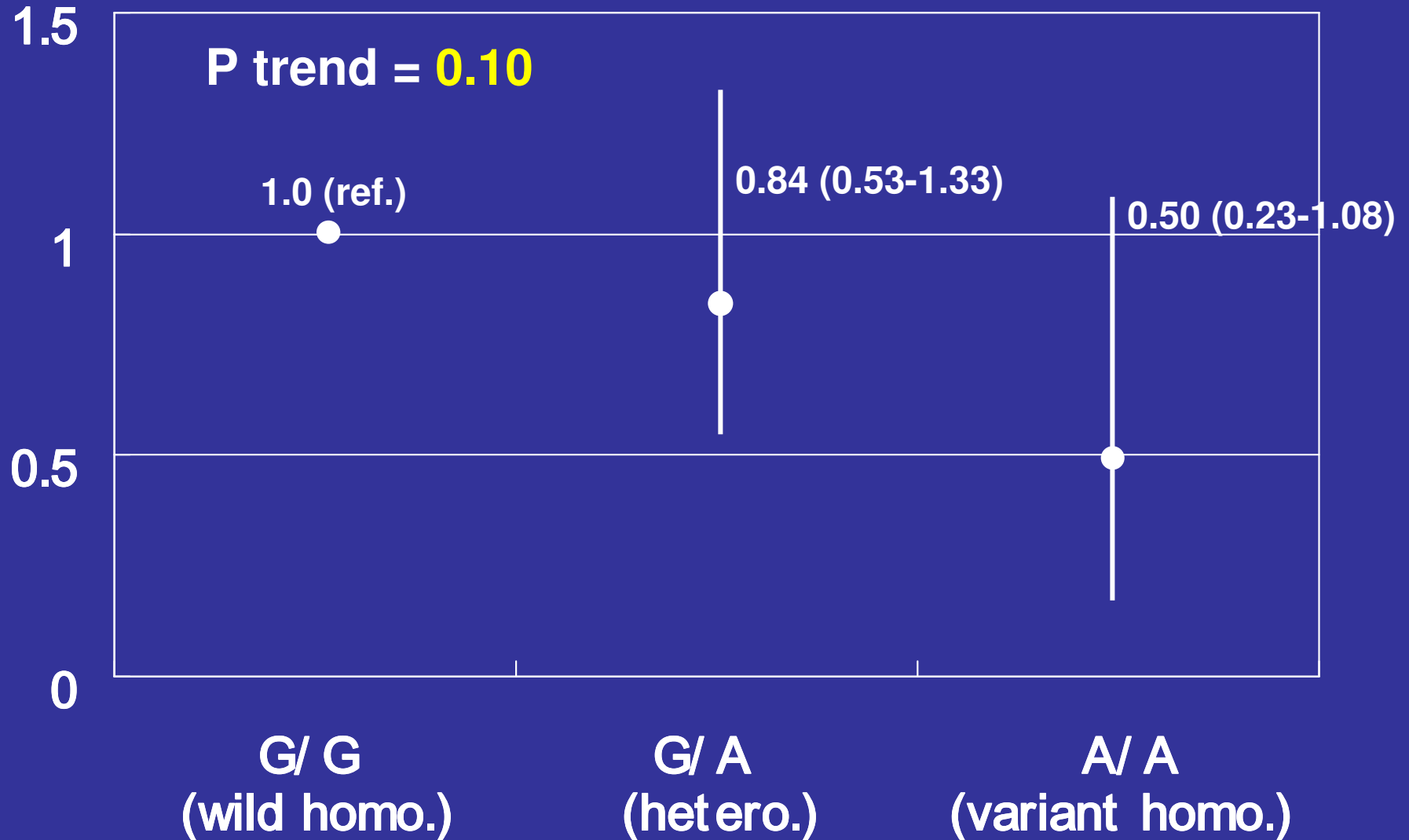
(%) <sup>a</sup> P values for HCC cases vs. hospital controls.

<sup>b</sup> P values for HCC cases vs. CLD patients.

<sup>c</sup> Having drunk  $\geq 69$  g of alcohol per day for  $\geq 10$  years

# Adjusted odds ratios\* and 95% confidence intervals of HCC for *CYP1A2* G-2964A genotype

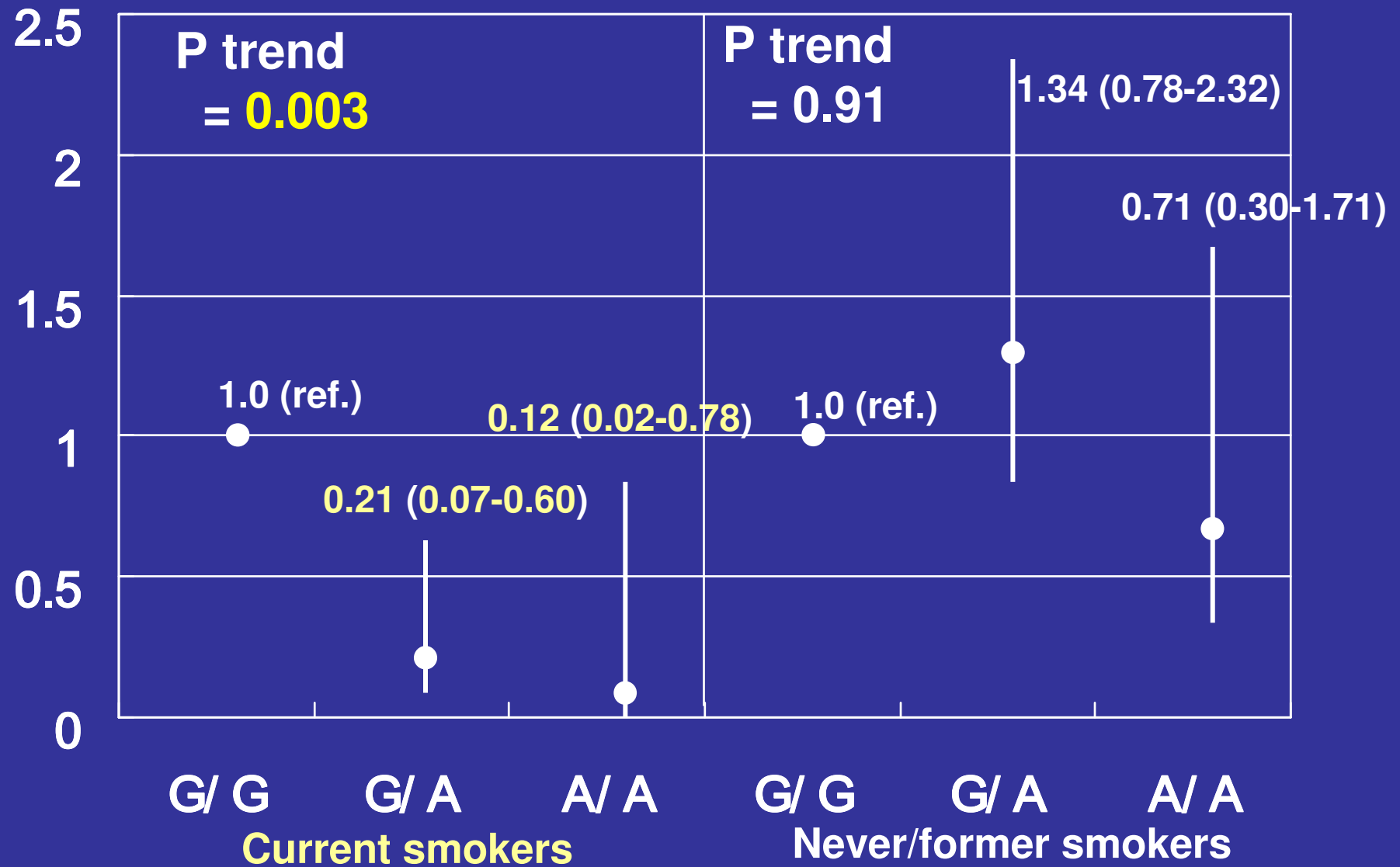
209 HCC cases vs. 381 CLD patients (control group 2)



\*Adjusted for sex, age, smoking, drinking, HBsAg and HCVAb

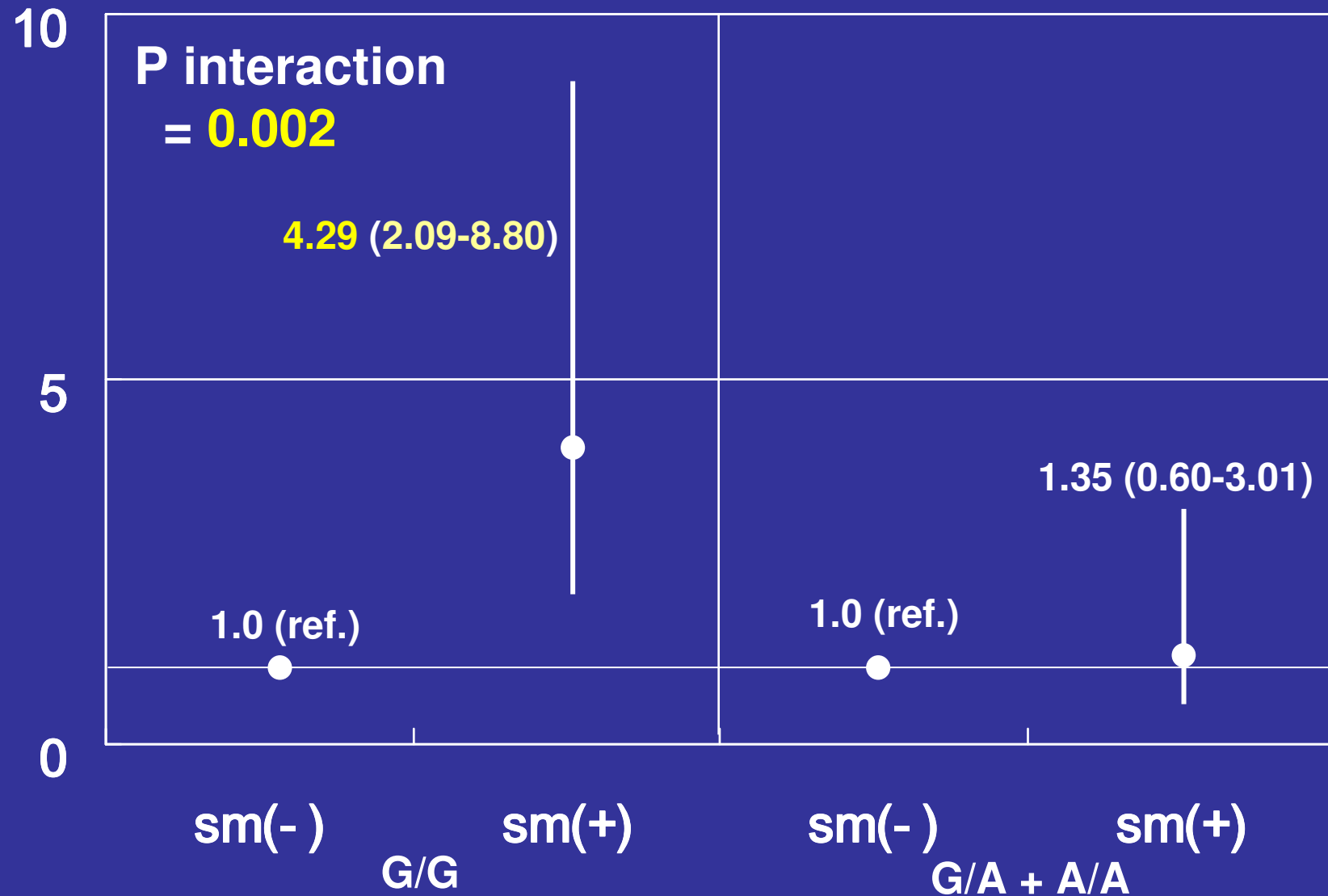
# Adjusted odds ratios and 95% confidence intervals of HCC for *CYP1A2* G-2964A genotype by smoking status

209 HCC cases vs. 381 CLD patients (control group 2)



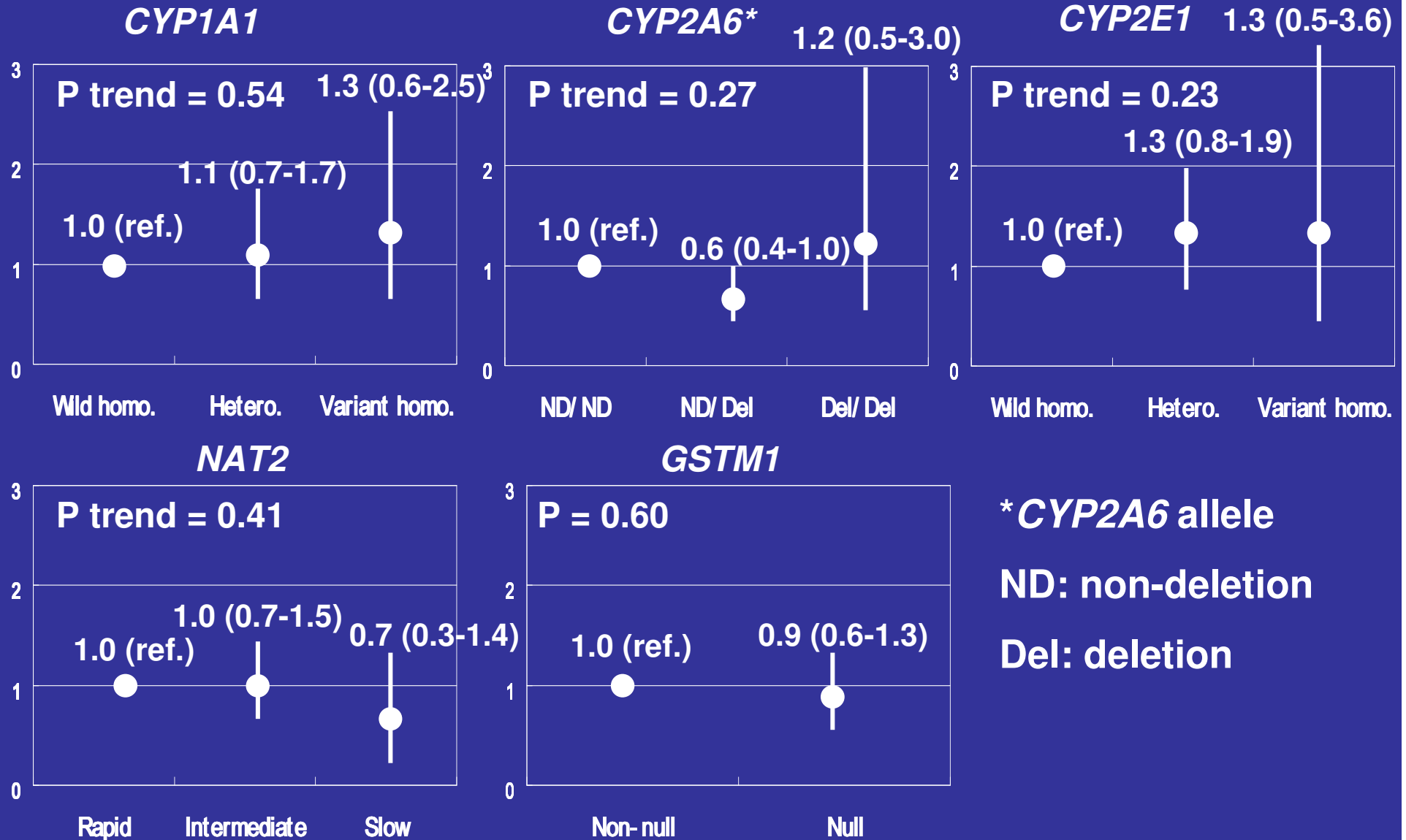
# Adjusted odds ratios and 95% confidence intervals of HCC for current smoking (sm) by *CYP1A2* G-2964A genotype

209 HCC cases vs. 381 CLD patients (control group 2)



# Adjusted odds ratios and 95% confidence intervals of HCC for *CYP1A1*, *CYP2A6*, *CYP2E1*, *NAT2* & *GSTM1* genotypes

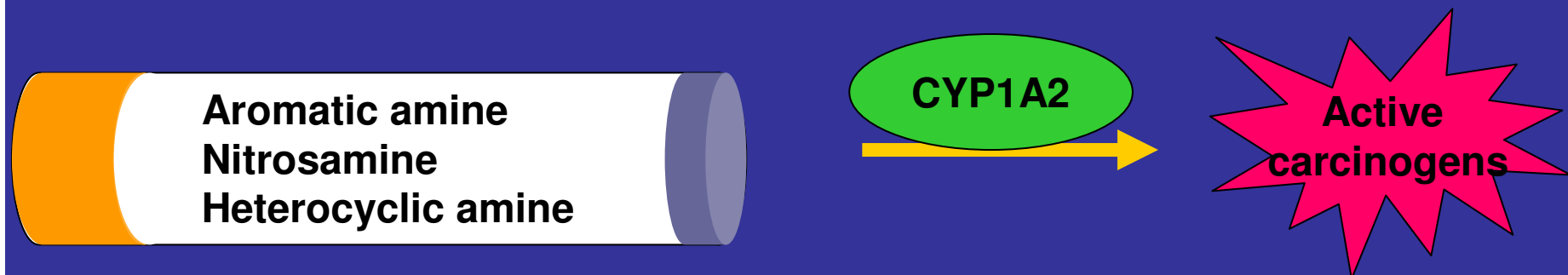
209 HCC cases vs. 381 CLD patients (control group 2)



## <Discussion>

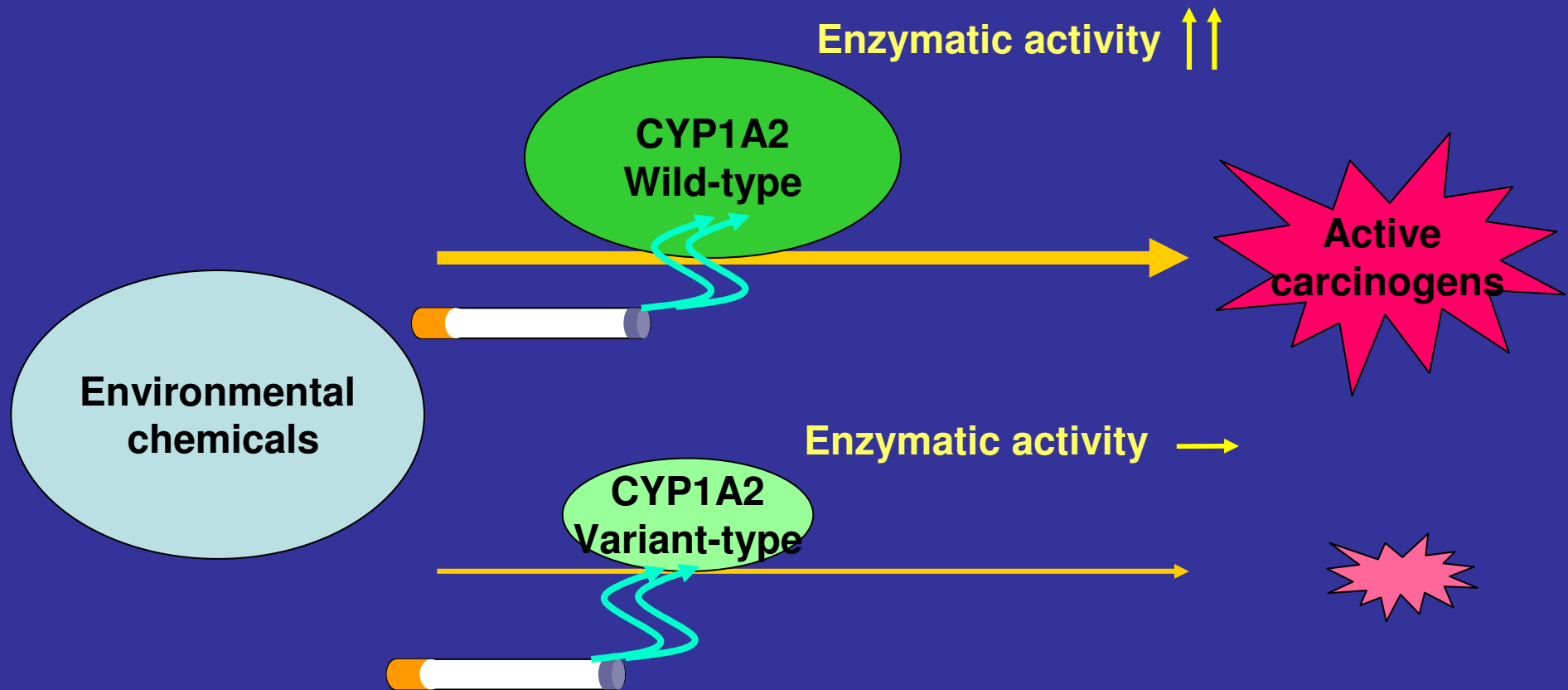
**CYP1A2** is associated with the metabolic activation of environmental chemicals such as aromatic amines, tobacco-specific nitrosamines, and heterocyclic amines.

⇒ **CYP1A2** polymorphism has been suspected to have an influence on carcinogenesis.



Nakajima et al. observed that the wild-type allele (-2964G) , but not variant allele (-2964A), of *CYP1A2* was associated with increased enzymatic activity by smoking.

Nakajima et al. : *J. Biochem*, 1999.



Chen et al. demonstrated that homozygous carriers of the major haplotype (-3860G/-3113G /5347C) of **CYP1A2** were associated with increased HCC susceptibility in **heavy smokers** (odds ratio 2.14; 95% confidence interval 1.21-3.80).

Chen et al. : *Pharmacogenetics and Genomics*, 2006

In this study, the comparison between HCC cases and **hospital controls** (control group 1) did not show any significant association with the genetic polymorphisms including the *CYP1A2* polymorphism.



This may be due to the low positivity of HBV and HCV infection among hospital controls; statistical adjustment for hepatitis virus infection made relevant odds ratios very unstable.

## <Conclusion>

The *CYP1A2* G-2964A polymorphism was associated with HCC risk among current smokers suffering from CLD, and it modified the risk associated with cigarette smoking among CLD patients.

Tobacco constituents metabolized by *CYP1A2* may play an important role in hepatocarcinogenesis.