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Variants in genes in the hormone-related pathway and risk of biliary tract cancer and stones: A population-based study

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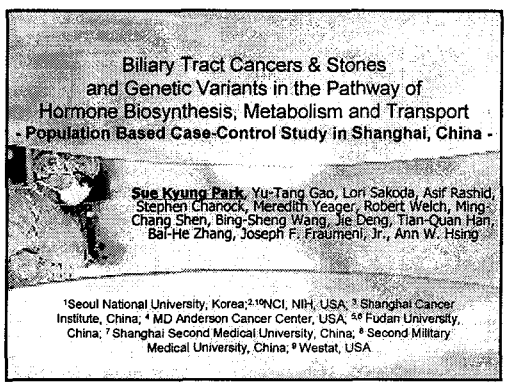

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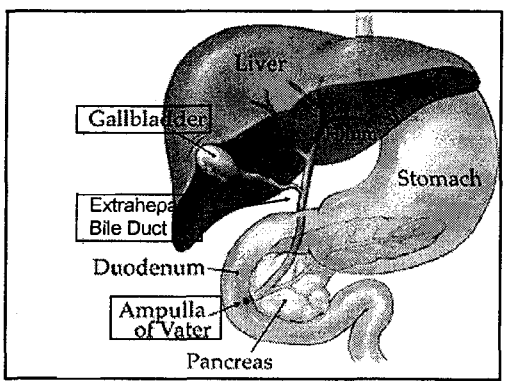


Backgrounds

- Uncommon tumors under 1%
- Encompassing cancer of the gallbladder, extrahepatic bile duct, and ampulla of Vater
- Origin: 46% Gallbladder, 27% Bile Duct, & 22% Ampulla of Vater

Incidence

- Incidence is rising rapidly in Shanghai, China

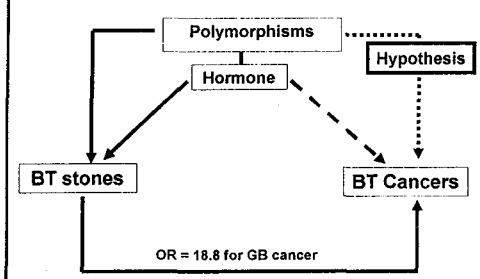
Previous studies

- GB ca. is that displaying a female preponderance, with incidence being 3-4 times more common among women *(Pandey 2003)*
- Primary risk factor of biliary tract cancer, as reported in all studies, is gallstone.
- Gallstones also occur more frequently in women than in men.

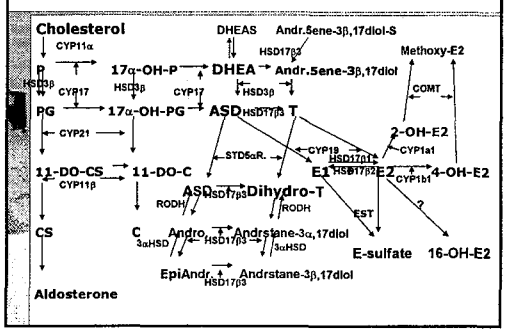
- Endogenous & exogenous estrogens and pregnancies
  → *increase the risk of BT stones including* gallstones *(Lambe 1993; Scragg 1984)*
- Early menarche, late menopause, multiple pregnancies and childbirth, and the use of HRT In women
  → increased the risk of BT cancers *(Pandey 2003; Tavani 1996; Moerman 1994; Scragg 1984; Everson 1982).*

- These results suggest that endogenous hormones can be involved in causal pathway of BT cancers and stones.

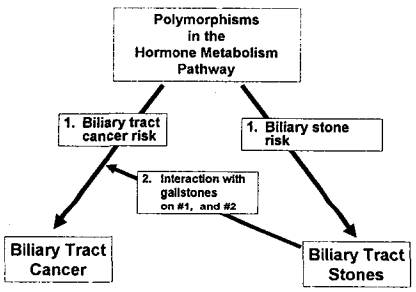
Rationale



Pathway of hormone biosynthesis & metabolism



Objectives



Materials and Methods

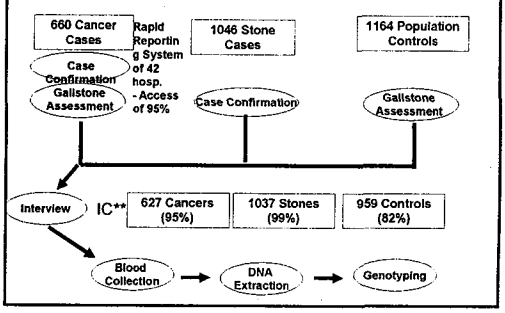
Shanghai Biliary Tract Cancer Study :

- Population-based case-control study conducted in Shanghai, China.
- designed to investigate the etiology of BTC
- conducted by the National Cancer Institute in collaboration with the Shanghai Cancer Institute from June, 1997 to May, 2001
- IRB of the U.S. NCI and the SCC approved the study protocol.

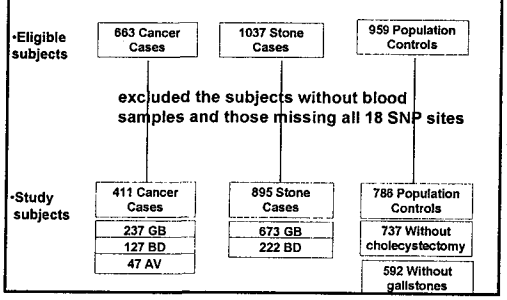
- Cancer case ascertainment: >95% of all cancer cases.
- Histological diagnoses of cancers: 70% of cancer cases (others - clinically confirmed)
- Nearly 100% of cancer cases and controls were assessed for gallstones

Shanghai Biliary Tract Cancer Study

Urban Shanghai residents, 35-74 years



In 1997-2001



Data collection

▪Interviews

- **demographic factors;**
- **consumption of cigarettes, alcohol, and tea;**
- **medical history;**
- **family history of cancers;**
- **reproductive history and exogenous hormone use (females only);**
- **diet;**
- **occupation.**
- **physical development and activity;**

Data collection

For checking accuracy and reliability,

- were tape recorded
- reviewed to ensure that they were conducted uniformly among participants and that the data were recorded accurately
- re-interview: 5% of the study subjects, who were randomly selected within 3 months after the enrollment.

→ Concordance rate: 90%.

Data collection

- **To obtain information on disease characteristics**
  → medical records of cases were abstracted

Gallstone detection

- **For cancer or stone cases – questionnaires, medical record review & clinical diagnostic examinations (abdominal ultrasound, CT, MRI, and/or ERCP)**
- **For controls – questionnaires and abdominal ultrasonography (85%).**

Blood collection

- Genomic DNA for genotype analyses was isolated from buffy coat or whole blood samples.
- 80% of subjects that gave consent gave overnight fasting blood samples.

Selection of Genes and SNPs

Gene selection

- Pathway of hormone metabolism related to gallstone and/or biliary tract cancer pathogenesis
- Assays were available at the time of analysis at the NCI Core Genotyping Facility (CGF).

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SNP selection

- With functional information at the time of analysis and known possible functional significances
- Rare allele frequency in Asian >5%
- Validated assay techniques

9 Genes: 18 SNPs

- **Hormone biosynthesis and metabolism**
  - Cyp1A1: EX7+131A>G; IVS1+606T>G
  - CYP1B1: EX2+143C>G; EX3+251G>C
  - COMT: EX3-104C>T; EX4-76C>G>T
  - HSB3B2: EX4-133C>T; EX4-88C>G
  - HSB17B3: EX11+43G>A
  - HSB17B1: Ex1-486G>A
  - SRD5A2: Ex1-17G>C
  - CYP19: Ex4-57A>G; IVS4-76A>G
    IVS7-106T>G; Ex8+47C>T
    IVS9-53A>G>T; Ex11+410G>T
- **Hormone transport**
  - SHBG: EX8+6G>A

Genotyping

Genotyping

- Taqman Assays

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Hardy-Weinberg equilibrium

- Population controls - no deviation from HWE ($p>0.05$).

Reliability of Genotyping

- Laboratory personnel - blinded to all cases & controls
- Selected total 80 samples (4 repeat samples from 20 individuals)
- 97% concordance
- Success rates of each genotyping - >96%

Statistical Analysis

SNP Effect

- **Age & Sex-adjusted, OR (95% CI): unconditional logistic regression**
- **Referent category – the most frequent genotype with the more frequent allele**
- **Comparisons**

GB cancer cases - control subjects without a history of cholecystectomy
BD and AV cancers - all controls.
Stones - all controls without stones and cholecystectomy

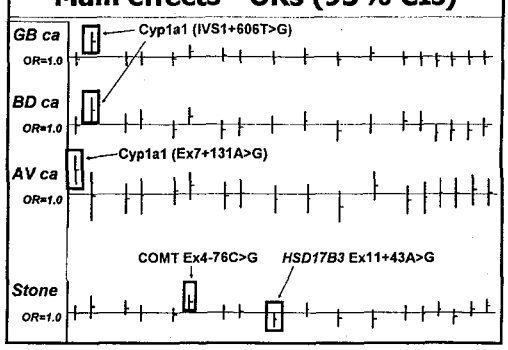
- Multiple SNP comparisons - a summary p-value of the overall association using Simes' global test
- Stratified analyses by putative confounding factors

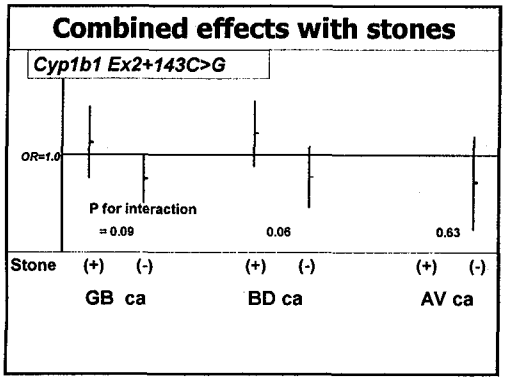
(biliary stones, obesity, diabetes, consumption of cigarette and alcohol, and female history of reproduction and use of oral contraceptives)

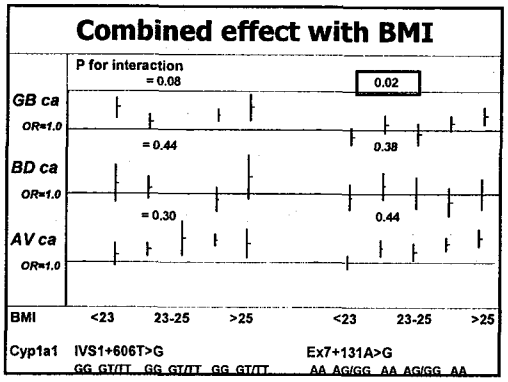
Haplotype Association for CYP19 genetic polymorphisms

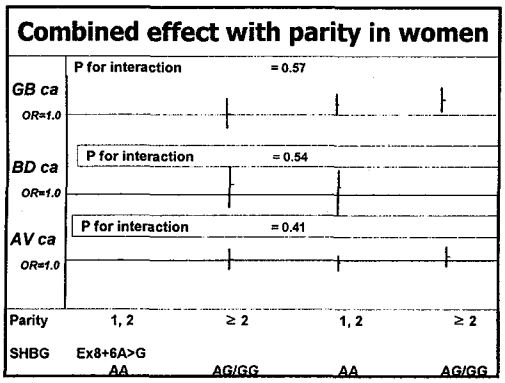
- Linkage disequilibrium (LD) between loci exhibiting genetic variation among controls – pairwise Lewontin's D´ and r2 values using Haploview (v 3.11)
- Risks of haplotypes – age and sex adjusted OR (95% CI) using Haplostats (R-v 2.0.1; expectation-maximization algorithm)
- To assess overall differences in haplotype frequencies – global score test

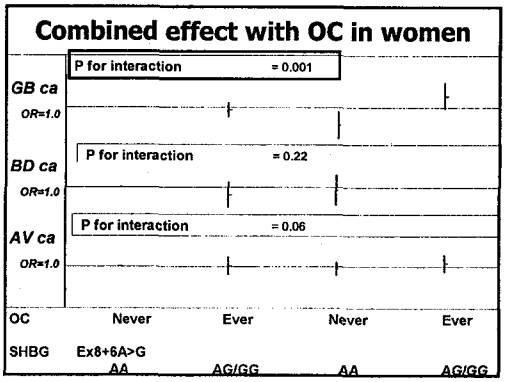
Main effects - ORs (95% CIs)











Haplotype analysis for Cyp19

	GB ca	BD ca	AV ca	Stone
CYP19	OR (95% CI)[1]	OR (95% CI)[1]	OR (95% CI)[1]	OR (95% CI)[1]
AAGCTT GGGCTT GGTCG G GGTTGT	1.0 (reference) 1.1 (0.7-1.6) 1.0 (0.8-1.3) 1.0 (0.7-1.3)	1.0 (reference) 1.1 (0.7-1.6) 1.0 (0.8-1.3) 1.0 (0.7-1.3)	1.0 (reference) 1.4 (0.7-2.9) 1.1 (0.6-1.8) 1.1 (0.6-2.0)	1.0 (reference) 1.1 (0.8-1.5) 1.2 (1.0-1.4) 1.0 (0.8-1.2)
p-global test	*0.99*	*0.53*	*0.79*	*0.44*

Multiple comparison

(summary *p*-values)

	GB ca	BD ca	AV ca	Stone
	Summary p	Summary p	Summary p	Summary p
E-biosynthesis pathway	**0.012**	ns	ns	ns
E-metabolism pathway	**0.032**	ns	**0.036**	ns
A-biosynthesis pathway	ns	ns	ns	**0.056**

Strengths and Limitations

Strengths

- Largest interdisciplinary study of biliary tract cancers
- High case ascertainment
- High response rate
- Minimizing misclassification bias
- Complete gallstone assessment
- First study to investigate multiple genes and SNPs in lipid metabolism pathway

Limitations

- Small number of ampulla of Vater cancers
- May limit formal test for interactions
- Results may not be generalizable to non-Asian ethnic groups

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