

Age-Specific Association of Steroid Hormone Pathway Gene Polymorphisms with Breast Cancer Risk

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Condensed abstract: This investigation of steroid hormone pathway genes in a large case-control study has identified and confirmed age-specific genetic associations with breast cancer risk for SNPs in four genes. Age-related associations with risk could have profound implications for the use of SNP genotyping to accurately predict breast cancer risk in women.

Running Title: ASGAs in Breast Cancer

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ABSTRACT

BACKGROUND. Breast cancer (BC) is a complex disease with incidence rates increasing with age. Both environmental factors and genetics impact BC risk. While the effects of environmental factors may vary with age, the penetrance of single nucleotide polymorphisms (SNPs) has generally been assumed to be constant throughout life. The current study demonstrates that certain SNPs exhibit BC risk associations that vary considerably with age. **METHODS.** SNPs in 12 steroid hormone (SH) pathway genes were investigated for associations with BC risk in Caucasian women enrolled in an age-matched case-control (1:2, respectively) study consisting of a discovery set (n = 5000) and an independent validation set (n = 1583). **RESULTS.** Significant age-related trends were identified and confirmed for SNPs in four genes associated with BC risk. The C/C genotype of cytochrome P450 XIB2 (*CYP11B2*) was associated with decreased risk at younger ages (30-44 years) but increased risk at older ages (55-69 years). The CG/CG genotype of UDP glycosyltransferase 1A7 (*UGT1A7*) was associated with increased risk at younger ages but decreased risk at older ages. Associations in cytochrome P450 19 (*CYP19*) and progesterone receptor (*PGR*) were confined to middle-age (45-54 years). **CONCLUSIONS:** This discovery of age-specific genetic associations (ASGAs) could have profound implications for future etiological studies of BC, as well as the use of SNP genotyping to accurately predict BC risk in women.

KEY WORDS: Age-Specific Genetic Associations, Steroid Hormone Pathway, SNPs, Breast Cancer

INTRODUCTION

Worldwide BC is the most commonly diagnosed cancer affecting 1 in 8 women in the US.¹ For decades, studies of hormonally-related risk factors contributed substantially toward understanding the origins of BC.^{2,3} The modern era of BC risk assessment began with the identification of highly penetrant mutations in the *BRCA1* and *BRCA2* genes that explain the etiology of BC in some families with strong histories.^{4,5} However, the majority of BC cases occur sporadically in individuals with little or no family history and no clear role for the BRCA genes in sporadically occurring BC has emerged. Predisposition to sporadic BC is likely associated with relatively common but weakly penetrant genetic variants in multiple genes.^{6,7} Advances in genotyping technologies have enabled the identification of such weakly penetrant polymorphisms.^{8,9} The relationship of SH levels to BC risk led to the investigation of SNPs in genes that influence endogenous estrogen levels or the bioavailability and detoxification of reactive estrogen metabolites and many have been associated with BC risk.¹⁰⁻¹¹

The influence of sex SH levels, especially estrogens, on BC risk is known to vary depending on a woman's age or menopausal status¹²⁻¹⁷ This supports the hypothesis that SNPs in functional regions of genes involved in SH synthesis, signaling and metabolism may differentially impact BC risk depending on age or menopausal status. Indeed, some studies of SNPs in sporadic BC suggest that their influence on risk is related to menopausal status¹⁸⁻²⁰ or age of onset.^{21,22} However, in most studies the risks associated with SNPs have been evaluated without respect to age, partly because statistical methods (e.g., linkage analysis) assume age-invariant ORs or because study sizes are too small to identify age-stratified associations.

Thus, while the contribution of SH related factors to BC risk vary with age, age-specific genetic contributions to risk have not been extensively investigated. In this study, the potential

association of 18 candidate SNPs in 12 genes from pathways related to SH synthesis, signaling or metabolism with BC risk was investigated. These SNPs have been previously studied for association with risk of breast or other cancers. To detect potential ASGAs, in Caucasian women we analyzed a large discovery set of 1,667 cases and 3,333 age-matched cancer-free controls and stratified them into three age groups (30-44, 45-54 and 55-69 years). Our findings were validated using an independent set of 526 cases and 1057 controls. Four gene polymorphisms that exhibit ASGAs with BC were identified and validated.

MATERIALS AND METHODS

Study Description

Women were enrolled in six geographically distinct regions of the U.S. Approximately half were enrolled in the greater Oklahoma City area (1996-2006) with the remaining recruited from Seattle, Southern California, Kansas City, Florida and South Carolina (2003-2006). Patients were approached consecutively without prior knowledge of disease status as they presented for appointments at mammography centers. For cases enrolled in oncology clinics, the controls were obtained in general practice clinics in the same medical complex. Some cases and controls were enrolled at Komen Races or other community-based events. At all collection sites, the majority of individuals approached enrolled in the study. The characteristics of cases and controls were similar for epidemiological factors related to lifetime SH exposure but variations in hormone replacement therapy (HRT) use were observed (see web supplement at www.intergenetics.com, Table A).

Cases were defined as women with a self-reported diagnosis of BC while controls had never been diagnosed with any cancer. No exclusions were in effect for enrollment in the study. All participants were enrolled under informed consent, completed a questionnaire on personal medical history and family history of cancer and provided a buccal cell sample collected in commercial mouthwash. All study protocols were IRB approved, monitored, and performed as previously described.²³

This report focuses only on the genetic analysis of Caucasian women. The age range for women in the study was 30-69 years with age at diagnosis used for cases and age at enrollment used for controls. The primary discovery set consisted of 5,000 women (1,667 BC cases/ 3,333 cancer-free controls) age-matched to the cases within one year. Age matching was done to adjust for potential confounding effects due to age-related risk factors when assessing ORs across different

ages. An independent validation set consisting of 526 cases and 1,057 controls was used to confirm the discovered associations.

SNPs

Samples were genotyped for the following 18 SNPs in 12 SH pathway candidate genes: *COMT* (rs4680), *CYP1A1* (rs4646903, rs1048943), *CYP11B2* (rs1799998), *CYP1B1* (rs10012, rs1056836), *CYP17* (rs743572), *CYP19* (rs10046, rs700519), *EPHX1* (rs1051740), *ERA* (rs2077647), *PGR* (rs1042838, rs10895068), *SHBG* (rs6529, rs1799941), *SOD2* (rs1799725) and *UGT1A7* (rs17868324, rs11692021). Information concerning the six SNPs most relevant to this report is shown in Table 1.^{18,19,24-36} This information is available in the web supplement for all 18 SNPs (Table B).

Genotyping

Genomic DNA was isolated and the majority of the samples were genotyped by microbead-based allele-specific primer extension as previously described.²³ Primer sequences and genotyping conditions are available from the authors upon request. ASPE assays had reproducibility rates >99.4%. RFLP assays were done on ~3% of samples and had reproducibility rates >98%. Genotyping was done blinded to the case-controls status and internal reproducibility was confirmed by examining 5% or more of the specimens in duplicate.

Statistical Methods

SNP associations and their age interactions were evaluated using both descriptive and analytic statistics. Genotype frequencies were summarized and a χ^2 test was used to evaluate Hardy-

Weinberg equilibrium (HWE) for individual SNP genotypes in the controls.³⁷ SNP associations with case and control status were assessed using χ^2 test statistics with two degrees of freedom in 3x2 contingency table analyses which are equivalent to unconditional logistic regression.³⁸ This analysis was also used to compute ORs and related statistics using the most common homozygote as the reference genotype.³⁹ Analyses were first performed without age stratification then stratification of the entire sample set into three age groups (30-44, 45-54 and 55-69 years) was done to identify ASGAs. The mean age of menopause for women in the U.S. is approximately 50.5 years,⁴⁰ but the endocrine hormonal transition exhibits approximately a ± 5 year window.⁴¹ Thus, we chose age categories which are likely representative of pre-, peri- and post-menopausal life stages to minimize the effect of differing ages of menopause in individuals. Raw P-values without correcting for multiple comparisons are reported. For SNPs demonstrating ASGAs adjusted analyses were performed to control for HRT use. Analysis of the discovery set was followed by validation in the independent set of cases and controls. Finally to fully quantify ASGAs as a function of SNP penetrance, a sliding 10-year window strategy was used to estimate genotypic ORs and related statistics for one-year incremental age groups (30-39, 31-40, ..., 59-68, and 60-69). Because precise age associations may vary from individual to individual, this 10-year sliding window strategy was utilized to increase power and reduce the noise of random variation between single year differences in each individual.

RESULTS

Overall Associations with BC Risk. Overall, age-independent analyses of associations were performed on the discovery set. All SNPs conformed to HWE ($p > 0.05$) in the control population as would be expected in a general population at steady state and were used in subsequent association analyses. Table 2 shows the results for the SNPs relevant to this paper while the results for all SNPs are presented in the web supplement (Table C). The only significant association ($p < 0.05$) with BC risk was for the C/C genotype of the *SOD2* gene (OR=1.2, $p=0.02$). SNP genotypes for *COMT*, *CYP11B* (Table 2) and the 3'UTR of *CYP1A1* (web supplement, Table C) exhibited suggestive associations ($0.05 < p < 0.1$). In the validation set the *SOD2* association was not replicated (OR=1.0) suggesting a possible false discovery.

Age-Stratified Associations with BC Risk

Due to our focus on potential ASGAs for these SNPs, we had matched cases and controls by age within one year. To investigate potential age-specific SNP associations, we computed ORs for each SNP within three age groups: young (30-44), middle (45-54) and old (55-69). Table 3 shows the ORs with 95% CIs and genotype frequencies determined in the discovery set for five (*COMT*, *CYP11B2*, *CYP19*, *PGR*, and *UGT1A7*) genes with SNP genotypes exhibiting significant associations with BC risk in one or more age groups. Risk associations for SNPs in three genes (*COMT*, *CYP19*, *PGR*) were limited to only one age group. For *COMT*, both homozygous G/G (OR=1.5, $p=0.02$) and heterozygous G/A (OR=1.4, $p=0.01$) were associated with elevated BC risk in the young group. For *CYP19*, the homozygous C/C genotype was associated with reduced risk (OR=0.7, $p=0.02$) only in the middle-aged group. The homozygous T/T genotype in *PGR* was

associated with significantly increased BC risk confined to the middle-aged group (OR=2.0, p=0.04).

The SNPs in the other two genes (*CYP11B2*, *UGT1A7*) exhibited an unexpected pattern of ASGAs. For both genes, risk associations reversed between the younger and older groups such that genotypes associated with increased risk in the younger group became protective in the older group, or vice versa. In *CYP11B2*, the homozygous C/C genotype was significantly associated with reduced risk (OR=0.6, p=0.008) in the young group. In contrast, the homozygous C/C (OR=1.7, p=0.002) and heterozygous C/T (OR=1.5, p=0.004) genotypes were both associated with increased risk in the older group. Similarly, the homozygous CG/CG genotype at *UGT1A7* was associated with a gradual decline in risk from young, to middle to old age (OR=1.5, 1.0 and 0.7, respectively). In addition, the heterozygous AA/CG genotype was associated with reduced risk confined to the older group. Four additional genes with ASGAs that approached significance ($0.05 < p < 0.1$) are shown in the web supplement (Table D). To determine if use of HRT might be a confounding factor affecting the observed ASGAs, analyses were repeated adjusting for HRT use. The results were similar to the unadjusted analyses for the majority of the ASGAs (data not shown). The only exception was for the T/T genotype of PGR which was suggestive of an increased risk in the young; however, this result was far from being statistically significant (p=0.22). Thus, we conclude that HRT use had little or no impact these ASGAs.

Validation of Discovered Associations

To validate these five potential ASGAs, their ORs, 95% CIs and pertinent genotype frequencies were computed for the independent validation set (Table 4). Although none of the ORs reach statistical significance, for *CYP11B2* the age-specific pattern of the C/C and C/T genotypes were

consistent with that observed in the discovery set. For the C/C genotype of *CYP19* a decreased OR (0.8) in the middle group was similar to the 0.7 in the discovery set. The result for the homozygous T/T genotype for *PGR* also replicated with an estimated OR of 1.5 in the middle group. The estimated ORs for the homozygous CG/CG genotype of *UGT1A7* followed the same pattern in the validation set as in the original discovery data set, gradually decreasing from 1.1, to 0.7 and to 0.5 ($p=0.05$) over the young, middle and old age groups, respectively. Although the absolute magnitudes of the OR values differed, this age-specific pattern was consistent with that observed in the discovery set (1.5, to 1.0 and to 0.7). Finally, the results for *COMT* failed to replicate in the validation set.

Nonparametric Evaluation of ASGAs

The ASGAs were further delineated using a sliding window strategy employing decade increments to analyze ORs non-parametrically. Figure 1 shows the relationship between OR and age for SNP genotypes from the four validated associations (*CYP11B2*, *UGT1A7*, *CYP19* and *PGR*). The homozygous C/C genotype of *CYP11B2* is associated with a gradual increase in OR from 0.5 around 35 years of age to 1.7 around 65 years of age (Figure 1A). The trend appears linear with $R^2=0.95$. In contrast, the OR for the heterozygous C/T genotype does not appear to vary with age until 50 years, and then exhibits a local increase from 1.0 to 1.7 from ages 50 to 69 (local $R^2=0.95$). The ORs associated with the CG/CG and AA/CG genotypes of *UGT1A7* exhibit a similar gradual decline over age from 35 to 65 with $R^2=0.88$ (Figure 1B). For *CYP19*, the ORs of both the T/T and C/T genotypes increase linearly beginning at age 50 and continuing until age 69 with $R^2=0.95$ for C/T and 0.69 for C/C (Figure 1C). Individuals with the T/T genotype at the *PGR* locus exhibit an elevated BC risk only in middle-age (Figure 1D).

DISCUSSION

SH exposure, especially to estrogens, has long been recognized to contribute significantly to BC risk in a manner that is dependent on menopausal status. The levels of specific hormones associated with pre-menopausal risk differ from those associated with post-menopausal risk.⁴²⁻⁴⁶ Epidemiological factors that are surrogate markers for lifetime exposure to estrogen also impact BC risk differentially in relationship to age and menopausal status.^{12-15,47} For some SH related factors the risk associated with BC reverses depending on menopausal status. Both nulli-parity and obesity have been associated with lower BC risk in pre-menopausal women and increased risk later in life.⁴⁸⁻⁵² Because SH related factors affect BC risk differentially with age or menopausal status, perhaps it is not surprising that association of certain SNPs in SH pathway genes with BC risk were similarly influenced. Our most striking finding was that for SNP genotypes in *CYP11B2* and *UGT1A7* not only does the magnitude of BC risk associations for some SH pathway gene SNPs vary with age, but the direction of risk changed with age. Additionally, associations confined to the middle-aged group were observed for SNP genotypes in *CYP19* and *PGR*. The validity of these unanticipated ASGAs required careful scrutiny because the discovery set findings were based on statistical assessment of p-values without correcting for multiple comparisons. To minimize the possibility of false-positive discoveries, an independent data set was analyzed, and the ASGAs discovered for *CYP11B*, *UGT1A7*, *CYP19* and *PGR* were confirmed.

The observed patterns of ASGAs only become evident by analysis of a study cohort large enough to permit age-stratification. Clearly, these significant ASGAs were not apparent in overall analyses because they were either balanced by opposite risk associations occurring in the young vs. old groups (*CYP11B2*, *UGT1A7*) or diluted by lack of association in the entire sample set (*CYP19*, *PGR*). The size of our study enabled us to further characterize ASGAs by utilizing non-parametric

analyses to evaluate ORs continuously over all ages ranging from 35 to 65. These results clearly supported the conclusion that ORs associated with these SNPs vary significantly with age.

Our findings concerning ASGAs with SNPs in *CYP11B2*, *UGT1A7*, *CYP19* and *PGR* may indicate an informative new way to evaluate genetic associations with BC risk. Prior studies of polymorphisms in SH pathway genes suggested some were differentially associated with BC risk depending on menopausal status.¹⁸⁻²⁰ While menopausal status is certainly correlated with age, examining age-specific penetrance such as we describe may be more informative. Considering the variation in a woman's hormonal status with age, perhaps our findings are not surprising, however; there are no clear indications in current literature why the phenomenon occurs in these particular genes. *CYP11B2* is a key enzyme that ultimately converts 11-deoxycorticosterone to aldosterone. Earlier studies had reported association of the C/C genotype with increased risk of type II diabetes which, in turn, has been associated with increased risk of BC in postmenopausal women.⁵³⁻⁵⁵ Our finding of an increased risk of BC associated with the C/C genotype in the older age group is consistent with these earlier studies. Although, the function of *UGT1A7* in conjugating a wide variety of substrates including steroids, environmental mutagens and pharmaceuticals suggests its potential to influence BC risk,⁵⁶ this gene has not been previously investigated in BC but the low activity allele has been associated with increased colon and orolaryngeal cancer risk.³⁴⁻³⁶ The *CYP19* gene encodes the terminal enzyme in the estrogen biosynthetic pathway and one study has reported an overall protective effect of the C/C polymorphism in BC.²⁹ Finally, the missense polymorphism in *PGR* is in complete linkage disequilibrium with several other polymorphisms in the gene including *PROGINS*.^{30,31} and has been associated with decreased BC risk, especially in young pre-menopausal women in some studies³¹ but not in others.⁵⁷

Hopefully, these intriguing results will provide an impetus for other investigators to search for ASGAs in breast and other cancers. This study focused on Caucasian women and further studies will be necessary to determine the relevance of ASGAs in other ethnicities. If ASGAs are validated in independent studies, they could have significant impact on the application of SNP technologies for use in BC risk prediction and in cancer screening and prevention. ASGAs could explain the lack of reproducibility of genetic associations across different studies,⁵⁸ either because of variability in the mean age of the sample sets being studied or because overall analyses in a study average the risk across all ages. Thus, consideration of the age distribution of participants could have significant implications in the design of future SNP association studies. Certainly, when designing studies to develop risk predictive models, there is a clear need to recruit clinical populations large enough to permit examination of potential ASGAs and replicate the studies in a target population with a similar age distribution. Finally, when developing a genetic test for BC risk one must have a suitable age distribution in the target population to be sure that ASGAs are not missed in the analysis. Indeed, we hope that our discovery brings us one step closer to the implementation of personalized medicine and accurately assessing BC risk in all women.

TABLE 1
SNPs in SH Pathway Genes

Gene	Name	Function	dbSNP ID	Polymorphism	Functional effect	References	
						Functional	Epidemiological
<i>COMT</i>	Catechol-O-methyltransferase	Inactivates catechol estrogens by methylation.	rs4680	G→A Val158Met	↓ methylation activity	24	18,19,25
<i>CYP11B2</i>	Cytochrome P450 Family XIB, polypeptide 2	Synthesis of aldosterone in renin-angiotensin system.	rs1799998	C→T Promoter -344	↑ aldosterone secretion	26,27	28
<i>CYP19</i>	Cytochrome P450 , family 19, Subfamily A, polypeptide 1	Terminal enzyme in estrogen synthesis that catalyzes formation of C18 estrogens from C19 androgens.	rs10046	C→T 3'UTR	↑ activity phenotype	29	29
<i>PGR</i>	Progesterone Receptor	Mediates the effects of progesterone during breast development. Two isoforms: PGR-A (opposes the effects of PGR-B) and PGR-B (promotes breast cell proliferation).	rs1042838	G→T Val660Leu	↑ half life of PR mRNA	30	31
<i>SOD2</i>	Manganese superoxide dismutase	Intra-mitochondrial, manganese-dependent, free radical scavenger that metabolizes reactive oxygen species to hydrogen peroxide.	rs1799725	C→T Val16Ala	may affect protein transport	32	33
<i>UGT1A7</i>	UDP glycosyltransferase 1 family, polypeptide A7	Detoxification of lipophilic xenobiotics, hormones and drugs by glucuronidation.	rs17868324	AA→CG Lys131Arg	↑ enzyme activity	34	35,36

TABLE 2
Overall associations with BC risk in the discovery set

SNP	Genotype	Case n (%)	Control n (%)	RR(95%CI)^a	HWE p-value^b
COMT	A/A	405(25)	900(27)	1.0(ref) ^c	0.8
	G/A	825(51)	1631(50)	1.1(0.9-1.3)*	
	G/G	396(24)	755(23)	1.2(0.9-1.4)*	
CYP11B2	T/T	486(29)	1044(32)	1.0	0.5
	C/T	842(51)	1613(48)	1.1(0.9-1.3)*	
	C/C	323(20)	651(20)	1.1(0.9-1.3)	
CYP19	T/T	461(28)	883(27)	1	0.6
	C/T	830(51)	1650(50)	1.0(0.8-1.1)	
	C/C	349(21)	758(23)	0.9(0.7-1.0)	
PGR	G/G	1140(69)	2344(71)	1.0	0.3
	T/G	454(28)	879(27)	1.1(0.9-1.2)	
	T/T	47(3)	71(2)	1.4(0.9-2.0)	
SOD2	T/T	392(24)	861(26)	1.0	0.7
	C/T	816(49)	1667(50)	1.1(0.9-1.2)	
	C/C	440(27)	786(24)	1.2(1.0-1.5)**	
UGT1A7	AA/AA	645(41)	1335(42)	1.0	0.2
	AA/CG	727(46)	1446(45)	1.0(0.9-1.2)	
	CG/CG	211(13)	430(13)	1.0(0.8-1.2)	

^a RR, relative risk; CI, confidence interval

^b HWE, Hardy-Weinberg equilibrium

^c (ref), reference; **, p≤0.05 also shown in bold; *, 0.05<p<0.1

TABLE 3
ASGAs with BC risk in the discovery set

SNP	Genotype	Case	Control	RR(95%CI) ^a	Case	Control	RR(95%CI)	Case	Control	RR(95%CI)
		Young			Middle			Old		
		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
COMT	A/A	106(21)	282(27)	1.0(ref) ^b	160(27)	339(28)	1.0	130(28)	253(27)	1.0
	G/A	272(53)	512(50)	1.4(1.1-1.8)**	289(49)	593(50)	1.0(0.8-1.3)	236(50)	473(50)	0.9(0.7-1.3)
	G/G	131(26)	238(23)	1.5(1.1-2.0)**	145(24)	261(22)	1.2(0.9-1.6)	104(22)	226(23)	0.9(0.6-1.2)
CYP11B2	T/T	163(32)	306(30)	1.0	183(30)	372(31)	1.0	124(26)	332(35)	1.0
	C/T	272(53)	499(48)	1.0(0.8-1.3)	293(49)	599(50)	1.0(0.8-1.2)	251(52)	460(48)	1.5(1.1-1.9)**
	C/C	79(15)	228(22)	0.6(0.5-0.9)**	125(21)	232(19)	1.1(0.8-1.5)	106(22)	170(17)	1.7(1.2-2.3)**
CYP19(3'UTR)	T/T	142(28)	267(26)	1.0	181(30)	303(25)	1.0	127(26)	286(30)	1.0
	C/T	254(50)	538(53)	0.9(0.7-1.1)	297(50)	614(52)	0.8(0.6-1.0)*	251(53)	444(46)	1.3(1.0-1.6)*
	C/C	114(22)	220(21)	0.9(0.7-1.3)	119(20)	279(23)	0.7(0.5-0.9)**	101(21)	230(24)	1.0(0.7-1.4)
PGR(V660L)	G/G	368(71)	724(71)	1.0	419(70)	870(73)	1.0	319(67)	669(70)	1.0
	T/G	134(26)	275(27)	1.0(0.7-1.2)	159(27)	309(26)	1.1(0.8-1.3)	144(30)	267(28)	1.1(0.9-1.4)
	T/T	13(3)	26(2)	1.0(0.5-1.9)	19(3)	20(1)	2.0(1.0-3.7)**	13(3)	25(2)	1.1(0.5-2.2)
UGT1A7(K131R)	AA/AA	188(38)	439(44)	1.0	226(39)	488(42)	1.0	207(46)	366(39)	1.0
	AA/CG	229(46)	447(44)	1.2(0.9-1.5)	289(49)	524(45)	1.2(0.9-1.5)	188(42)	425(46)	0.8(0.6-1.0)**
	CG/CG	77(16)	122(12)	1.5(1.0-2.0)**	71(12)	155(13)	1.0(0.7-1.4)	56(12)	139(15)	0.7(0.5-1.0)*

a RR, relative risk; CI, confidence interval

b (ref), reference; **, p≤0.05 also shown in bold; *, 0.05<p<0.1

TABLE 4
ASGAs with BC risk in the validation set

SNP	Genotype	Case			Control			RR(95%CI) ^a		
		Case	Control	RR(95%CI) ^a	Case	Control	RR(95%CI)	Case	Control	RR(95%CI)
		Young			Middle			Old		
		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
COMT	A/A	48(27)	84(24)	1.0	57(31)	94(25)	1.0	35(26)	76(27)	1.0
	G/A	89(50)	173(49)	0.8(0.5-1.3)	95(51)	180(48)	0.9(0.6-1.3)	68(49)	140(50)	1.1(0.6-1.7)
	G/G	41(23)	95(27)	0.8(0.4-1.3)	33(18)	100(27)	0.5(0.3-0.9)**	34(25)	63(23)	1.2(0.7-2.1)
CYP11B2	T/T	59(33)	99(28)	1.0 (ref) ^b	60(32)	125(33)	1.0	40(29)	93(33)	1.0
	C/T	85(47)	183(51)	0.8(0.5-1.2)	91(49)	178(47)	1.1(0.7-1.6)	72(53)	141(50)	1.2(0.7-1.9)
	C/C	35(20)	75(21)	0.8(0.5-1.3)	36(19)	74(20)	1.0(0.6-1.7)	25(18)	47(17)	1.2(0.7-2.3)
CYP19(3'UTR)	T/T	49(28)	99(28)	1.0	57(31)	109(29)	1.0	36(26)	66(24)	1.0
	C/T	81(45)	176(50)	0.9(0.6-1.4)	90(48)	176(47)	0.9(0.6-1.5)	60(43)	151(54)	0.7(0.4-1.2)
	C/C	47(27)	78(22)	1.2(0.7-2.0)	39(21)	87(23)	0.9(0.5-1.4)	43(31)	62(22)	1.3(0.7-2.2)
PGR(V660L)	G/G	116(65)	248(70)	1.0	127(69)	266(72)	1.0	98(70)	202(72)	1.0
	T/G	58(33)	97(27)	1.3(0.9-1.9)	51(27)	94(25)	1.1(0.8-1.7)	38(27)	74(26)	1.1(0.7-1.7)
	T/T	4(2)	10(3)	0.8(0.3-2.8)	7(4)	10(3)	1.5(0.6-3.9)	4(3)	5(2)	1.7(0.4-6.3)
UGT1A7(K131R)	AA/AA	63(36)	138(40)	1.0	86(48)	138(38)	1.0	62(47)	117(43)	1.0
	AA/CG	84(48)	152(44)	1.2(0.8-1.8)	69(38)	169(46)	0.7(0.4-0.9)**	61(46)	121(44)	1.0(0.6-1.5)
	CG/CG	29(16)	57(16)	1.1(0.6-1.9)	25(14)	59(16)	0.7(0.4-1.2)	9(7)	37(13)	0.5(0.2-1.0)**

^a RR, relative risk; CI, confidence interval

^b (ref), reference; **, p≤0.05 also shown in bold; *, 0.05<p<0.1

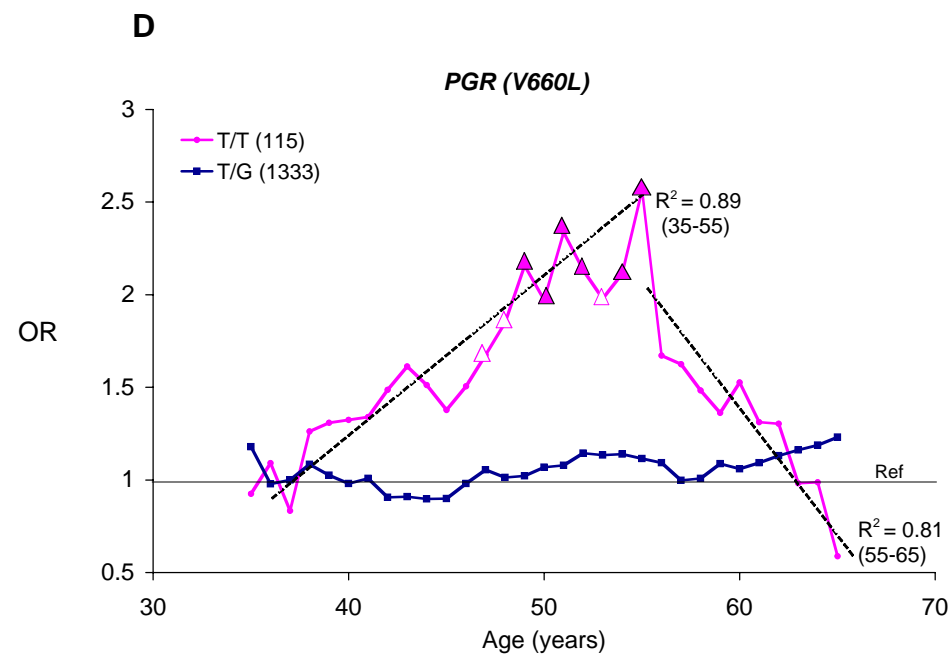
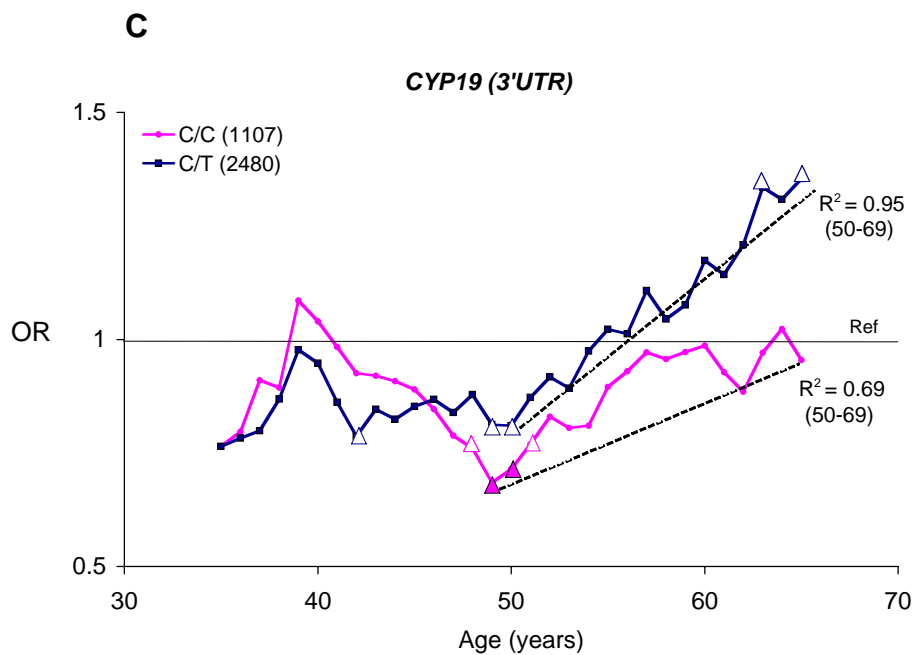
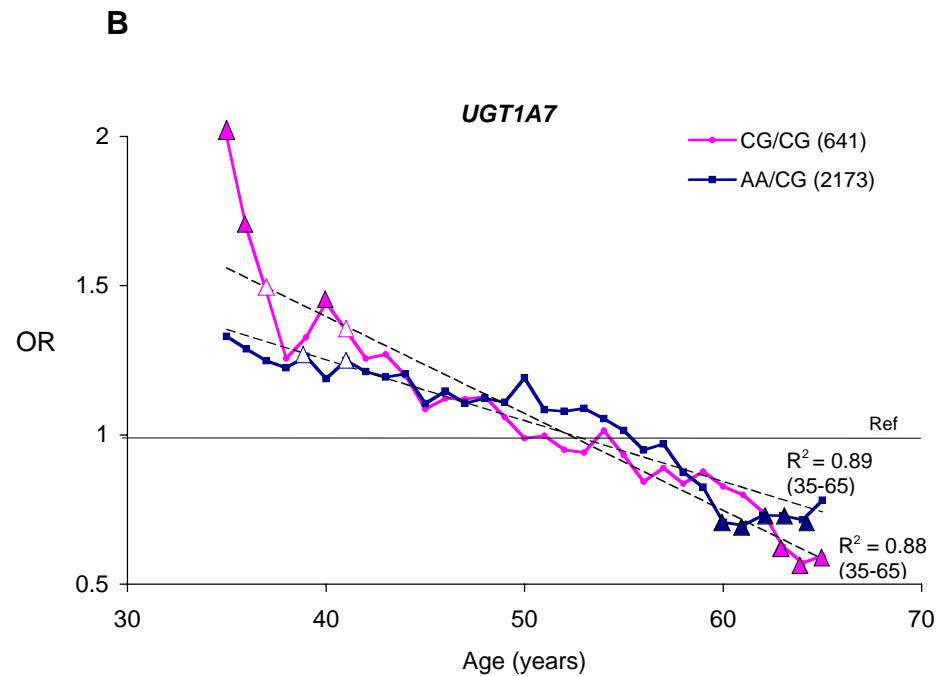
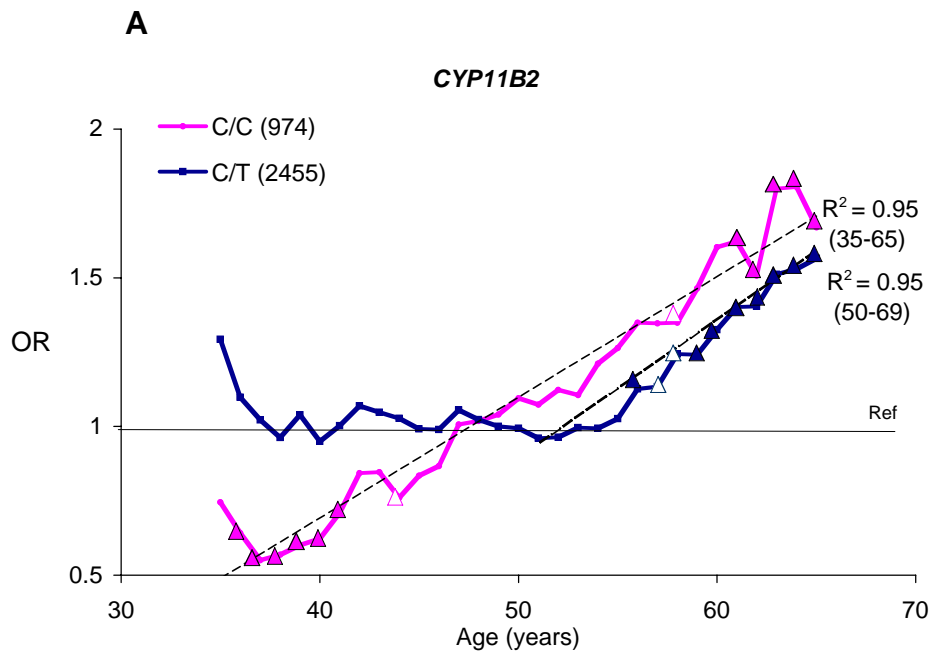


Figure 1. ASGA trends for SNP genotypes in SH pathway genes. ORs and p-values for 10-year sliding windows were calculated individually for the least common homozygous and heterozygous genotypes using the most common homozygous genotype as reference. The relationship between OR (y-axis) and age for each increment (x-axis) is shown for *CYP11B2* (Panel A), *UGT1A7* (Panel B), *CYP19* (Panel C) and *PGR* (Panel D). The genotypes and number of individuals analyzed are shown at the top of each panel and shaded to match their respective lines on the plots. Estimated ORs were plotted against the middle age point within each age interval and points are designated with ▲ if the corresponding unadjusted p-value ≤ 0.05 and Δ if $0.05 < p < 0.1$. The temporal pattern was examined for the trend and the R^2 and age interval for each trend line are shown. The solid horizontal line on each of the plots shows Reference (Ref) to OR=1.0.

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