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Association between polymorphisms in dopamine metabolic enzymes and tobacco consumption in smokers

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Central dopaminergic reward pathways give rise to dependence and are activated by nicotine. Allelic variants in genes involved in dopamine metabolism may therefore influence the amount of tobacco consumed by smokers. We developed assays for polymorphisms in dopamine-hydroxylase (*DBH*), monoamine oxidase (*MAO*) and catechol O-methyl transferase (*COMT*) using the polymerase chain reaction with sequence specific primers (PCR-SSP). We then typed 225 cigarette smokers to assess whether genotype was related to the number of cigarettes smoked a day. Smokers with DBH 1368 GG genotype smoked fewer cigarettes than those with GA/AA [mean difference −2.9 cigarettes, 95% confidence interval (CI) −5.5, −0.4; $P = 0.022$]. The effect reached statistical significance in women (−3.8, 95% CI −6.4, −1.0, $P = 0.007$) but not in men (−1.5, 95% CI −6.0, 3.0, $P = 0.498$). Overall, the effect was greater when analysis was confined to Caucasians (−3.8, 95% CI −6.6, −1.1, $P = 0.007$). Smokers with *MAO*-A 1460 TT/TO smoked more cigarettes than those with CC/CT/CO (2.9, 95% CI 0.6, 5.1, $P = 0.013$). Within each sex group, the trend was similar but not statistically significant (difference for men 2.9, 95% CI −1.0, 6.7; for women 2.0, 95% CI −0.7, 4.8). The effect of the allele was greater in smokers with a high body mass index (> 26) (difference 5.1, 95% CI 1.4, 8.8, $P = 0.008$). More heavy smokers (> 20 a day) had the *DBH* 1368A allele when compared to light smokers (< 10 a day). (Relative risk 2.3, 95% CI 1.1, 5.0, $P = 0.024$.) The trend for increasing prevalence of the *DBH* A allele in heavy smokers was greater when analysis was restricted to Caucasians (relative risk 3.2, 95% CI 1.3, 8.2, $P = 0.004$). Conversely, heavy smokers were less likely to have the *MAO*-A 1460C allele (relative risk 0.3, 95% CI 0.1, 0.7, $P = 0.012$). Variations in *DBH* and *MAO* predict whether a person is a heavy smoker and how many cigarettes they consume. Our results support the view that these enzymes help to determine a smoker's requirement for nicotine and may explain why some people are predisposed to tobacco addiction and why some find it very difficult to stop smoking. This finding has important implications for smoking prevention and offers potential for developing patient-specific therapy for smoking cessation. Pharmacogenetics 10:1–9 © 2000 Lippincott Williams & Wilkins

Keywords: tobacco, smoking, monoamine oxidase, dopamine-hydroxylase, catechol O-methyl transferase

Introduction

Smoking is one of the most important public health issues facing health professionals and governments. In the UK alone, there are 120 000 deaths attributed to tobacco use each year (SCOTH, 1998). Smoking cessation rates of approximately 20% are the best that can be achieved with the therapies currently available (ICRF, 1993; Schneider *et al.*, 1995). Deeper understanding of the molecular basis for tobacco addiction could lead to more effective strategies for prevention and for helping people to stop smoking.

Twin studies show a major genetic element in tobacco addiction (Heath *et al.*, 1995) and there may be a related predisposition to use other substances

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(Swan *et al.*, 1996). Data from 3997 twin pairs show that starting to smoke and continuing with the habit both have a substantial genetic component accounting for 50% of the variance in risk of smoking initiation and 70% of the risk of persistent smoking (True *et al.*, 1997). Taking up smoking and continuing with the habit come under separate genetic influences (Heath *et al.*, 1993) and it seems likely that there is a genetic component to the level of tobacco consumption in smokers.

There is substantial evidence to suggest that dopaminergic neurones arising in the ventral tegmental area of the thalamus and projecting to the nucleus accumbens are the final common pathway for addiction to a wide variety of substances (Clarke, 1998; Wickelgreen, 1998). Enzymes involved in dopamine metabolism may therefore be important in determining susceptibility to substance abuse. Monoamine oxidase is involved in the oxidative deamination of dopamine and noradrenaline. Levels of this enzyme are lower in platelets of patients with substance abuse (Faraj *et al.*, 1994) and smokers have lower monoamine oxidase activity in the brain than non-smokers (Fowler *et al.*, 1998). It has been suggested that inhibitors of monoamine oxidase present in tobacco smoke contribute to the development of addiction (Fowler *et al.*, 1998). Similarly, lower plasma levels of dopamine-hydroxylase are related to drug dependence (Gabel *et al.*, 1995) although no link has yet been established with smoking. Patients with high activities of catechol O-methyl transferase (COMT) are more susceptible to developing polysubstance abuse (Vandenbergh *et al.*, 1997) but again there is no known association with smoking.

We investigated the relationship between common polymorphisms in enzymes involved in dopamine metabolism and level of cigarette consumption in smokers.

Materials and methods

PARTICIPANTS

Patients were randomly selected from a population-based cohort of people who responded to an invitation from their general practitioner for a health check in the OXCHECK study (ICRF, 1994, 1995). From the 11 090 patients invited 8109 attended for the check, 7692 agreed to give a blood sample, and of these 1773 reported that they smoked cigarettes at the time of their health check. Blood was collected in ethylenediaminetetraacetic acid and buffy coat lymphocytes were separated and stored at −80 °C. This study is based on a sample of 226 smokers selected using computer-generated random numbers. The current analysis and the original OXCHECK trial were both approved by the Central Oxfordshire Research Ethics Committee.

GENOTYPING

We selected polymorphisms in monoamine oxidase A (Craddock *et al.*, 1995), monoamine oxidase B, COMT (Vandenbergh *et al.*, 1997) and dopamine-hydroxylase (Cubells *et al.*, 1997) that could be typed using the polymerase chain reaction (PCR) with sequence specific primers, using methods previously described for human leukocyte antigens (HLA) (Bunce *et al.*, 1995). The polymorphisms are described in Table 1. PCR-sequence specific primers (SSP) assays for the monoamine oxidase A T1460C and monoamine oxidase B G644A polymorphisms have been previously described (Marshall *et al.*, 1999). Reactions were developed to detect both the common and variant alleles. Each reaction mixture included control primers to detect a conserved sequence to eliminate the possibility of false negative results. Genomic DNA was isolated from samples and genotyped using the primers shown below.

Monoamine oxidase A, Xp11.3, exon 8 G941T (M68850)

Sense primer: 5′-CGTAATTAATGCGATCCCTCC-3′;
antisense: 5′-GACAGCTCCCATTGGAAGC-3′,
5′-GACAGCTCCCATTGGAAGA–3′.

Monoamine oxidase A, Xp11.3, exon 14 T1460C (M68856)

Sense primers: 5′-GGAAGGTGACCGAGAAAGAC-3′
5′-GGAAGGTGACCGAGAAAGAT-3′;
antisense: 5′-TGGCCCAATGACACAGCCT–3′.

Monoamine oxidase B, Xp11.3, intron 13 G644A (Z29071)

Sense primer: 5′-CTGACAGTTCCTCTGATGTC-3′;
antisense: 5′-CACACTGGCAAATAGCAAAAGC-3′,
5′-CACACTGGCAAATAGCAAAAGT–3′.

Catechol O-methyl transferase, 22q11.2, exon 3, G1947A (Z26491), $Val^{108}Met$

Sense primers: 5′-ATGGTGGATTTCGCTGGCG-3′,
5′-ATGGTGGATTTCGCTGGCA-3′;
antisense: 5′-GATGTCCTGGACGCTCC–3′.

Dopamine-hydroxylase, 9q34, exon 4, G910T (X13260), $Ala^{304}Ser$

Sense primer: 5′-CCTGGGCCCTGGGTGCCA-3′;
antisense primers: 5′-CCTGGACCCCCGAAGGC-3′,
5′-CCTGGACCCCCGAAGGA–3′.

Table 1. Polymorphisms included in the study

Gene	*Location*	*Nucleotide change*	*Amino acid change*	*Functional significance*	*Disease associations*
Monoamine oxidase A	Xp 11.3	Exon 8 G91T		Reduced level of enzyme activity in fibroblasts with T allele (Hotamisligil & Breakefield, 1991)	
		Exon 14 T1460C		Reduced level of enzyme activity in fibroblasts with T allele (Hotamisligil & Breakefield, 1991)	
Monoamine oxidase B	Xp 11.3	Intron 13 G644A		Not known	Reduced Parkinson's disease risk in smokers with G allele (Checkoway *et al.*, 1998)
Catechol O-methyl transferase	22q 11.2	Exon 3 G1497A	$Val^{108}Met$	Reduced enzyme activity with A allele (Lachman *et al.*, 1996)	Association of A allele with alcoholism (Tiihonen *et al.*, 1999) and substance abuse (Vandenbergh *et al.*, 1997)
Dopamine β-hydroxylase	9q 34	Exon 4 G910T	$Ala^{304}Ser$	Reduced enzyme activity with T allele suggested (Ishii *et al.*, 1991), but not confirmed (Cubells *et al.*, 1997; Li *et al.*, 1996)	
		Exon 8 G136A			

*Note that in our population the *MAO* A 941T, *MAO* A 1460 T and *MAO* B 644G were in linkage disequilibrium as were *DBH* 910G and *DBH* 1368A.

Dopamine -hydroxylase, 9q34, exon 8, G1368A (X13264)

Sense primer: 5′-AGAAGGTCGTGTCGGTCCAT-3′;
antisense primers: 5′-CCAGCTCCCGGTCTTCC-3′,
5′-CCAGCTCCCGGTCTTCT–3′.

Concentrations of the primers in the reaction mixture were adjusted so that all reactions were optimized for the same conditions. Buffers, PCR and gel electrophoresis conditions were as previously described (Bunce *et al.*, 1995).

SAMPLE SIZE AND STATISTICAL ANALYSIS

A previous study on smokers in the OXCHECK cohort showed a mean (SD) of 14.9 (7.4) cigarettes smoked a day (Haldar *et al.*, submitted for publication). To detect a mean difference of three cigarettes a day, assuming an SD of 8.0 in each of two equal groups (with and without variant alleles), a study needs 226 patients (80% power, two-sided = 0.05).

Mean differences in numbers of cigarettes smoked each day for patients with each genotype are presented with 95% confidence intervals (95% CI) and *P*-values from Student's *t*-test. Because the distribution of number of cigarettes smoked each day deviated from normal, these values were checked using non-parametric tests and similar results were obtained. Differences in proportions of smokers with variant alleles across quartiles of cigarette consumption were compared using the chi-squared test for linear trend.

Forward stepwise multiple linear regression was used to adjust for potential confounders (age sex, marital status, social class, body mass index, and alcohol consumption). The *P*-values for entry and removal were 0.05 and 0.10, respectively. To improve fit, number of cigarettes was normalized using a square root transformation. Monoamine oxidase is X-linked, thus sex was always included in the models. In all analyses, we grouped together smokers who had one or more variant alleles and compared them to those who were homozygous for the common allele. This dominant mode of action has been suggested by previous studies (Checkoway *et al.*, 1998; Cubells *et al.*, 1998).

Nominal *P*-values are presented throughout and $P < 0.05$ has been reported as statistically signifi-

cant. In interpreting these *P*-values it should be noted that since all allelic variants at the *MAO* and *DBH* loci were in linkage disequilibrium and we tested one polymorphism in *COMT*, only three independent loci were examined. However, at each locus, we performed six tests of significance on different subgroups. If the Bonferroni correction method were used to allow for these subgroup tests (Bland & Altman, 1995), $P < 0.008$ would be considered significant at the 0.05 level.

Results

Clinical characteristics of the smokers are shown in Table 2. We were able to extract DNA of sufficiently high quality for genotyping from 225 of the 226 samples taken from the randomly selected patients.

The two monoamine oxidase A polymorphisms were in linkage disequilibrium (chi-squared = 430.2, 4 d.f., $P < 0.0001$) in the study group. Monoamine oxidase A 1460 genotype was also strongly related to monoamine oxidase B 644 genotype (chi-squaerd = 21.8, 4 d.f., $P < 0.0001$). Alleles at the monoamine oxidase A 941 and monoamine oxidase B 644 loci were therefore omitted from the analysis. The dopamine-hydroxylase 910 and 1368 alleles were significantly correlated although less strongly related (chi-squared = 9.5, 2 d.f., $P < 0.009$). Data on associations with dopamine-hydroxylase 910 alleles are therefore not presented.

The monoamine oxidase 1460 polymorphism was in approximate Hardy–Weinberg equilibrium (chi-squared (men) = 0.28; $P > 0.2$, chi-squared (women) = 3.5; $P > 0.05$). The dopamine-hydroxylase 1368 polymorphism was also in equilibrium (chi-squared = 1.3, $P > 0.1$). However, the COMT alleles showed significant deviation from equilibrium (chi-squared = 10.8, $P = 0.001$).

The mean numbers of cigarettes smoked for the genotypes at each locus are shown in Tables 3 and 4. Smokers homozygous for the dopamine-hydroxylase 1368G allele consumed fewer cigarettes and this effect was particularly marked in women. When analysis was confined to Caucasians, greater differences were seen in cigarette consumption between dopamine-hydroxylase genotypes. The overall effects of the dopamine-hydroxylase 1368 allele remained significant ($P = 0.011$) when adjusted for alcohol consumption and sex in multivariate linear regression models. Smokers homozygous or hemizygous for the monoamine oxidase 1460T allele consumed a significantly greater number of cigarettes. The effect remained significant when adjusted by linear regression for sex and alcohol consumption ($P = 0.046$). The effects of the allele were particularly marked in those with a high body mass index irrespective of whether the analysis was stratified by sex. Because monoamine oxidase is sex-linked, the observed mean difference in cigarette consumption between groups with different alleles is confounded. The overall difference in tobacco consumption would have been reduced from 2.9 to 2.3 cigarettes ($P = 0.043$) if there had been equal numbers of men and women with each allele.

The proportion of patients with one or more of the

Table 2. Characteristics of the smokers included in the study

Characteristic	*Study group*
Age (Years)	
Mean (SD)	50.1 (8.3)
Median (range)	49.0 (36–67)
Sex	
Male *n* (%)	100 (44%)
Female *n* (%)	125 (56%)
Number of cigarettes smoked each day	
Mean (SD)	16.7 (8.8)
Median (range)	15.0 (1–50)
Age at initiation of smoking (years)	
Mean (SD)	19.8 (6.5)
Median (range)	18.0 (5–53)
Alcohol consumed (units a day)	
Mean (SD)	11.5 (20.3)
Median (range)	3.5 (0–145)
Socio-economic class	
n (%)	
Professional (I)	6 (3%)
Managerial (II)	30 (13%)
Clerical (IIIN)	44 (19%)
Skilled manual (IIM)	54 (24%)
Semi skilled manual (IV)	32 (14%)
Unkilled (V)	8 (4%)
Housewife	25 (11%)
Unclassified	4 (2%)
No response	22 (10%)
Marital status	
Married/living as married	172 (76%)
Divorced/separated	23 (13%)
Widowed	14 (8%)
Single, never married	13 (6%)
Unknown	3 (1%)
Ethnicity	
Caucasian	201 (89%)
Asian	4 (2%)
Afro Caribbean	1 (< 1%)
Other	6 (3%)
No response	13 (6%)

Table 3. Mean number of cigarettes smoked each day by dopamine beta hydroxylase genotype

	Dopamine β-hydroxylase 1368 genotype		*Mean difference (95% confidence interval)*	*P value for difference*
	GG	*GA/AA*		
All participants ($n = 225$)				
n (%)	62 (28%)	163 (72%)		
Mean (SD) no. of cigarettes smoked	14.6 (8.3)	17.5 (9.0)	−2.9 (−5.5, −0.4)	0.022
Men ($n = 100$)				
n (%)	26 (26%)	74 (74%)		
Mean (SD) no. of cigarettes smoked	17.7 (9.9)	19.2 (9.5)	−1.5 (−6.0, 3.0)	0.498
Women ($n = 125$)				
n (%)	36 (29%)	89 (71%)		
Mean (SD) no. of cigarettes smoked	12.4 (6.1)	16.1 (8.3)	−3.8 (−6.4, −1.0)	0.007
Caucasian ($n = 201$)				
n (%)	56 (28%)	145 (72%)		
Mean (SD) number of cigarettes smoked	14.2 (7.7)	18.0 (9.1)	−3.7 (−6.6, −1.1)	0.007

Table 4. Mean number of cigarettes smoked each day by monoamine oxidase genotype

	Monoamine oxidase 1460 genotype		*Mean difference (95% confidence interval)*	*P value for difference*
	TT/TO	*CC/CT/CO*		
All participants ($n = 225$)				
n (%)	135 (60%)	90 (40%)		
Mean (SD) no. of cigarettes smoked	17.6 (9.3)	15.0 (7.8)	2.9 (0.6, 5.1)	0.013
Men ($n = 100$)				
n (%)	68 (68%)	32 (32%)		
Mean (SD) no. of cigarettes smoked	19.7 (10.1)	16.8 (8.3)	2.9 (−0.9, 6.7)	0.139
Women ($n = 125$)				
n (%)	67 (54%)	58 (46%)		
Mean (SD) no. of cigarettes smoked	15.8 (8.3)	14.0 (7.3)	2.0 (−0.7, 4.8)	0.150
Body mass index $\leqslant 26$ ($n = 139$)				
n (%)	83 (60%)	56 (40%)		
Mean (SD) no. of cigarettes smoked	17.7 (10.0)	16.0 (7.4)	1.8 (−1.14, 4.7)	0.232
Body mass index > 26 ($n = 83$)				
n (%)	49 (59%)	34 (41%)		
Mean (SD) no. of cigarettes smoked	18.5 (8.2)	13.4 (8.3)	5.1 (1.4, 8.8)	0.008
Caucasian ($n = 201$)				
n (%)	119 (59%)	82 (41%)		
Mean (SD) no. of cigarettes smoked	18.0 (9.4)	15.5 (7.9)	2.5 (0.0, 5.0)	0.048

variant alleles by cigarette consumption is shown in Table 5. There was a significant trend for those who smoked more heavily to have one or more variant alleles at the dopamine-hydroxylase 1368 locus. The relative risk for those smoking more than 20 cigarettes a day compared to those smoking less than 10 cigarettes a day was 2.32 (95% CI 1.1, 5.0, $P = 0.024$).

When analysis was restricted to Caucasians, the trend for heavy smokers to have one or more dopamine-hydroxylase A alleles was more pronounced. The relative risk for those smoking more than 20 cigarettes a day compared to those smoking

Table 5. Proportion of patients with one or more variant allele by cigarette consumption

Locus	*Genotype*	*Frequency of genotype by reported daily cigarette consumption n (%)*				*Significance*[a]
		0–9 cigarettes	*10–14 cigarettes*	*15–19 cigarettes*	*20+ cigarettes*	
All participants ($n = 225$)		70	52	63	40	
Dopamine β-hydroxylase 1368	AG or AA	44 (63%)	40 (77%)	45 (71%)	34 (85%)	0.028
Monoamine oxidase A 1460	CT or CC	34 (49%)	19 (37%)	28 (44%)	9 (23%)	0.033
Catechol O-methyl transferase	AG or GG	56 (80%)	39 (75%)	49 (78%)	33 (83%)	0.815
Caucasians ($n = 201$)		58	46	61	36	
Dopamine β-hydroxylase 1368	AG or AA	35 (60%)	34 (74%)	44 (77%)	32 (89%)	0.006
Monoamine oxidase A 1460	CT or CC	27 (47%)	19 (41%)	27 (44%)	9 (25%)	0.095
Catechol O-methyl transferase	AG or GG	45 (78%)	39 (74%)	31 (78%)	31 (86%)	0.337

[a]chi-squared for linear trend.

less than 10 cigarettes a day was 3.22 (95% CI 1.3, 8.2, $P = 0.006$).

A significant inverse trend was seen at the monoamine oxidase A 1460 locus with those smoking more than 20 cigarettes a day being less likely to have variant alleles (genotype CT or CC or CO), relative risk 0.31 (95% CI 0.13, 0.74, $P = 0.012$). This effect was less marked when analysis was restricted to Caucasians (relative risk 0.5, 95% CI 0.3, 1.0).

Discussion

We have shown that genetic variations in enzymes that metabolize dopamine are important in determining the amount of tobacco that smokers consume. This study is the first to demonstrate this link.

Our results are likely to be generally applicable because we studied randomly selected smokers from a cohort of patients who responded to an invitation from their general practitioner to attend a health check. The overall response rate to the invitation was high at 73% (ICRF, 1995) implying that study participants are likely to be representative of the population who attend health centres in the UK. This is an advance on previous studies where recruitment often depended on response to a media advertisement (Lerman *et al.*, 1997). Such studies may be biased by including substantial numbers of people who are not representative of the general population and whose tobacco dependence behaviour is atypical.

The role of dopamine metabolic enzymes

Ours is the first study linking dopamine metabolic enzymes to tobacco consumption and the magnitude of the effects that we saw (differences of 3–4 cigarettes a day) are consistent with those seen in studies of other candidate genes. It seems likely that the enzymes we studied exert their effects on smoking behaviour by altering dopamine breakdown; however, it is also possible that they have more wide ranging effects since they form key parts of the metabolic pathways of other monoamines.

Whilst dopamine metabolism may be an important factor in tobacco addiction there is also evidence linking polymorphisms affecting dopamine receptors (Noble *et al.*, 1994; Comings *et al.*, 1996; Spitz *et al.*, 1998) the dopamine transporter (Caporaso *et al.*, 1997; Lerman *et al.*, 1999; Sabol *et al.*, 1999), cytochrome P450 enzymes (Turgeon *et al.*, 1995; Pianezza *et al.*, 1998), and a wide variety of neurotransmitters (Wickelgreen, 1998) to smoking behaviour.

Dopamine-hydroxylase

The polymorphisms in dopamine -hydroxylase that we studied have not previously been linked to smoking phenotype or to human disease. The 910 polymorphism causes an alanine to serine amino acid substitution in the enzyme but the 1368 variant is 'silent'. Associations have been found however, between serum dopamine-hydroxylase levels and two other polymorphisms in linkage disequlibrium with each other and close to the 910 and 1368 loci (Cubells *et al.*, 1998). It seems highly likely therefore that *DBH* gene is a major determinant of dopamine-hydroxylase activity and it may be that the polymorphisms we studied are in linkage disequilibrium with an allele that controls this activity.

Monoamine oxidase

Both polymorphisms in the monoamine oxidase A gene (positions 941 and 1460) are conservative substitutions and are unlikely to be themselves

responsible for variations in phenotype. Again, it seems likely that these alleles are in linkage disequilibrium with alleles that cause functional changes in monoamine oxidase activity. Perhaps the most likely candidate for this is a variable number tandem repeat in the promoter region of the gene (Sabol *et al.*, 1998). This polymorphism is in linkage disequilibrium with a number of genetic markers in the monoamine oxidase A and monoamine oxidase B genes. Alleles with 3.5 or four copies of the repeat sequence are transcribed between two and 10 times more efficiently than those with three or five copies of the repeat. It seems likely that this increased transcription will result in increased enzyme activity.

Catechol O-methyl transferase

It is perhaps surprising that we saw no association between smoking and variants at the COMT, 1947 locus where the G allele results in a four fold increase in enzyme activity. Although there are no previous studies exploring its relationship with tobacco use or level of consumption, this high activity allele has been linked with abuse of other substances (Vandenbergh *et al.*, 1997). These researchers also showed that the *COMT*, 1947 alleles were in Hardy–Weinberg equilibrium in controls but not in substance abusers, which is consistent with our data. It may be that the allele contributes to the maintenance of nicotine dependence but is not an important influence on the amount of tobacco consumed. This hypothesis will need to be evaluated in further studies.

Clinical implications

Our results are consistent with the suggestion that dopaminergic reward pathways are important in determining tobacco consumption. Polymorphisms that reduce dopamine activity may contribute to a 'reward deficiency syndrome' where self-administration of nicotine restores dopaminergic transmission to 'normal' levels (Blum *et al.*, 1995). If this is so, we would expect to find that the polymorphisms we studied were associated with increased dopamine breakdown and impaired dopaminergic transmission.

A deeper understanding of why people smoke may be the key to developing more effective ways of helping them to stop. The genetic component to smoking behaviour is likely to be multifactorial with different molecular mechanisms contributing to the habit in different people. Identification of the mechanisms involved in causing disease may allow specific treatments to be used (Bell, 1998). Our study suggests that different genetic mechanisms operate in men and women. This information may be important in planning interventions to help people to stop smoking.

At present in the UK, nicotine replacement therapy is advised for all people wishing to give up smoking. Other effective treatments are likely to become available soon (Hurt *et al.*, 1997; Jorenby *et al.*, 1999). It may be that different patients will respond to different treatments or combinations of treatments. For example, people who metabolize nicotine quickly may respond best to a nicotine patch. Those who metabolize nicotine very rapidly may need a higher replacement dose than slower metabolizers. People with polymorphisms in the dopamine transporter may respond particularly well (or badly) to dopamine reuptake inhibitors such as buproprion for smoking cessation. Using genotyping to target the most appropriate treatment to the individual smoker could lead to more effective treatment for tobacco addiction.

Future research

Our study was limited to correlating genetic variations with reported tobacco consumption. Further studies should examine the effects of polymorphisms in dopamine metabolic enzymes on smoking initiation, persistent smoking behaviour and on ability to stop smoking. This will involve comparing genotypes of those who have ever taken up smoking, those who decided to continue and those who successfully stopped with genotypes of non-smoking controls. Genes influencing the activity of neurotransmitters other than dopamine, such as glutamate and acetylcholine, are also likely to be important in modifying smoking behaviour (Wickelgreen, 1998).

The groups of genes contributing to initiation and cessation of smoking are each likely to comprise several functionally related genes. Sufficiently powered studies will be needed in future to determine whether the effects of different polymorphisms on tobacco consumption are subtractive, additive or synergistic and whether there is a limit to the contribution that can be made by certain gene groups. Mathematical models of the genetic contribution to tobacco addiction will be very useful but are likely to be extremely complex. Clearly the data sets upon which they are based will need to be very large.

Tobacco consumption is rapidly increasing in developing countries where the prevalence of other risk factors for cardiovascular disease is high (van der Sande *et al.*, 1997). It has been suggested that seven million of the 10 million deaths worldwide from tobacco in 2025 will occur in the developing world (Mackay & Crofton, 1996). Whilst much of this increase in smoking may be due to the marketing activities of tobacco companies, there are consider-

able ethnic differences in the prevalence of genetic variations associated with smoking. For example, high activity alleles of CYP2A6, linked with increased tobacco use (Pianezza *et al.*, 1998) are more prevalent in people of African descent (Sellers, 1998) and African-Americans seem more susceptible to the effects of the polymorphism in the dopamine D_4 receptor on smoking behaviour (Shields *et al.*, 1998).

Our results show that genetic variations in enzymes involved in dopamine metabolism influence the amount of tobacco used by smokers. This important finding fits well with other work implicating dopaminergic reward pathways in the development of dependence. Further work is in progress on the molecular basis of tobacco dependence which may lead to new, more effective treatments for tobacco addiction.

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