Polimorfismos dos genes do citocromo P450, da glutationa S-transferase e do supressor de tumor *TP53* em populações sul-americanas e em pacientes com doença pulmonar obstrutiva crônica e câncer de pulmão

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Sumário

Introdução

I.1 – Variabilidade genética no metabolismo de xenobióticos

Diariamente os organismos entram em contato com grande quantidade de substâncias químicas de diversas fontes ambientais – os xenobióticos (Hasler *et al.* 1999, Lang & Pelkonen 1999). Muitos destes compostos são lipofílicos e podem se acumular no organismo atingindo concentrações tóxicas ou mesmo letais (Lang & Pelkonen 1999). O acúmulo e a toxicidade são evitados através de enzimas que os reconhecem e que os metabolizam a formas hidrofílicas que são facilmente eliminadas do organismo (Hasler *et al.* 1999, Wilkinson & Clapper 1997).

Duas classes de enzimas participam deste processo, as de fase I (as enzimas de ativação) e as de fase II (as enzimas de detoxificação). As de fase I, representadas principalmente pela superfamília citocromo P450 (CYP450), realizam o metabolismo oxidativo através da inserção de um átomo de oxigênio num xenobiótico, tornando-o altamente eletrofilico. As de fase II, como a família glutationa S-transferase (GST), por exemplo, conjugam os reativos intermediários eletrofílicos formados pelo metabolismo oxidativo da fase I com glutationa, tornando-os mais solúveis em água e mais facilmente elimináveis do organismo. Dependendo da estrutura do composto inicial, a reação de fase I pode ser suficiente para torná-lo solúvel em água e eliminá-lo do organismo sem a necessidade da reação de fase II (Guengerich & Shimada 1998, Nebert 1991, Nebert & Roe 2001, Puga *et al.* 1997, Venitt 1994). Em algumas situações a toxicidade da molécula é reduzida durante a fase I, em outras são gerados metabólitos secundários capazes de induzir dano ao ADN (Venitt 1994, Wilkinson & Clapper 1997). Estas substâncias apresentam a capacidade de formar ligações com o ADN, produzindo produtos quimicamente estáveis conhecidos como adutos (*adducts*). A formação de adutos é característico de substâncias carcinogênicas, podendo levar a deleções, adições e substituições de bases (Nebert 1991, Venitt 1994). Estas alterações genéticas são, no entanto, evitadas por proteínas codificadas pelos genes supressores de tumor, entre estes o *TP53*, cuja função é manter a estabilidade genômica através da interrupção do ciclo celular permitindo que o ADN seja reparado por enzimas específicas, além de induzir a transcrição de genes que regulam a apotose quando não for possível corrigir o erro genético (Agarwal *et al.* 1998, Müllauer *et al.* 2001).

O *TP53* e alguns dos genes envolvidos no metabolismo de xenobióticos apresentam uma grande diversidade de alelos e fenótipos com ampla variação interpopulacional, cujos produtos podem ser enzimas inativas ou com atividade reduzida (Aynacioglu *et al.* 1998, Ingelman-Sundberg 2001, Rebbeck 1997, Själander *et al.* 1996).

Lang & Pelkonen (1999), consideraram surpreendente que enzimas cuja função é proteger o organismo da ação tóxica de xenobióticos aumentem a toxicidade dos mesmos em algumas situações. Os autores sugeriram que, sob o ponto de vista evolutivo, estes sistemas enzimáticos devem ter fornecido uma vantagem adaptiva aos organismos protegendo-os da ação de substâncias externas e potencialmente prejudiciais. A geração de metabólitos tóxicos seria uma conseqüência secundária deste processo. A função primária das enzimas seria a de solucionar o problema agudo de acúmulo de compostos potencialmente letais ao organismo.

Quanto à diversidade de alelos e fenótipos observada em vários grupos étnicos, Lewis *et al.* (1998) e Nebert (1997) sugeriram que seria resultante da seleção que teria atuado sobre diferentes tipos de dieta experimentadas pelas populações humanas ao longo de sua história.

I.2 – Genes de fase I: a superfamília citocromo P450

A super-família citocromo P450 (CYP) é a principal representante do sistema de metabolização de fase I (Omura 1999). Além de atuarem sobre os xenobióticos, as CYPs também participam da metabolização de substratos endógenos como os esteróides, ácidos graxos e vitaminas lipossolúveis (Anzenbacher & Anzebacherová 2001, Omura 1999).

As primeiras formas de CYP provavelmente surgiram antes da divergência de procariotos e eucariotos (Lewis *et al.* 1998) sendo a sua função, possivelmente, de metabolizar compostos endógenos e não xenobióticos. As primeiras CYPs capazes de metabolizar xenobióticos possivelmente surgiram aproximadamente há 400–500 milhões de anos (Lang & Pelkonen 1999). Sugeriu-se que elas apareceram devido à coevolução entre animais e plantas: o consumo de plantas pelos animais atuou como pressão seletiva a favor de enzimas capazes de metabolizar as toxinas produzidas pelos vegetais (Gonzalez & Nebert 1990, Nebert 1997).

No genoma humano existem aproximadamente 58 genes *CYP*, subdivididos em 10 famílias gênicas (Autrup 2000, Ingelman-Sundberg 2001). Porém, a maioria dos xenobióticos são metabolizados pelos genes das famílias *CYP1*, *CYP2* e *CYP3* e destes, os mais

importantes na geração de metabólitos secundários capazes de induzir dano ao ADN são *CYP1A1*, *CYP1A2*, *CYP1B1*, *CYP2E1* e *CYP3A4* (Guengerich & Shimada 1998, Ingelman-Sundberg 2001, Lucas *et al*. 2001).

I.2.1 – *CYP1A1*

O *CYP1A1* está localizado no cromossomo 15q22-q24 e contém sete exons. A enzima CYP1A1 metaboliza vários xenobióticos como as aminas aromáticas e os hidrocarbonetos policíclicos aromáticos gerando outras substâncias tóxicas (Corchero *et al.* 2001, Guengerich & Shimada 1998, Jaiswal & Nebert 1986, Nebert 1991).

Foram descritos 12 polimorfismos distribuídos ao longo do gene (http://www.imm.ki.se/CYPalleles). Entre eles, a mutação Ile→Val, no exon 7, ocorre na região correspondente ao centro catalítico da enzima. Segundo Kawajiri *et al.* (1993) e Kiyohara *et al.* (1996), o alelo *CYP1A1*2C* (Val) codifica uma enzima com aumento de atividade. No entanto, outras investigações indicaram não haver diferença de atividade entre as duas formas da proteína (Persson *et al.* 1997, Zhang *et al.* 1996), provavelmente devido ao tipo de substrato que foi utilizado nos diferentes estudos de expressão (Schwarz *et al.* 2000). Uma mutação na região 3', que cria um sítio para *Msp*I (alelo *CYP1A1*2A*), foi associada à maior indução da transcrição (Landi *et al.* 1994). Mais detalhes destas duas alterações são apresentadas na tabela 2.

Tem sido descrito desequilíbrio de ligação completo entre as mutações *CYP1A1*2A* e *CYP1A1*2C* em europeus (Cascorbi *et al.* 1996) mas não em afro-americanos (Garte *et al.* 1996). Os dois alelos apresentam ampla variação étnica, sendo que as maiores freqüências de ambos foram detectadas em populações ameríndias (Kvitko *et al.* 2000, Muñoz *et al.* 1998). Nas africanas ou delas derivadas, CY*P1A1*2C* foi observado somente em uma amostra de afro-americanos, em baixa freqüência (3%), que os autores atribuíram ser provavelmente devido a mistura interétnica (Garte *et al.* 1996) e numa de afro-brasileiros (8%) do Rio de Janeiro (Hamada *et al.* 1995; Tabela 3).

Tabela 2. Dois polimorfismos do *CYP1A1* e suas nomenclaturas.

Região	Alelo	Posição do nucleotídeo	Nomenclaturas utilizadas	Nomenclatura recomendada[1]
	**1A*	A4889; T6235	*wt*, **1*	*CYP1A1*1A*
Exon 7, codon 462	**2C*	A4889G [Ile→Val]	*m2*, **2B*	*CYP1A1*2C*
Região 3'	**2A*	T6235C [RFLP (*Msp*I)]	*m1*, **2A*	*CYP1A1*2A*
Exon 7 + região 3'	**2B*	A4889 e T6235	*m1* + *m2*	*CYP1A1*2B*

1 Fonte: http://www.imm.ki.se/CYPalleles.

Tabela 3. Freqüências alélicas (%) de *CYP1A1*2A* e de *CYP1A1*2C* em várias populações.

Populações	*CYP1A1*2A*		*CYP1A1*2C*		Referência
	No. de amostras	Freqüência	No. de amostras	Freqüência	
Ameríndias	6	72–96	6	59–97	**1**
Asiáticas	5	31–38	9	18–35	**2**
Afro-brasileiras	–	–	1	8	**3**
Afro-americanas	1	22	1	3	**4**
Africanas	1	25	2	0	**5**
Euro-brasileiras	–	–	1	9	**6**
Euro-americanas	1	13	2	8–9	**7**
Européias	5	7–11	4	2–6	**8**

1 Ameríndias do Brasil: cinco tribos (Kvitko *et al.* 2000), do Chile: uma população (Muñoz *et al.* 1998). **2** Duzhak *et al.* (2000), Hayashi *et al.* (1991), Inoue *et al.* (2000), Kim *et al.* (1999), Morita *et al.* (1997), Murata *et al.* (2001), Oyama *et al.* (1995), Persson *et al.* (1999), Song *et al.* (2001). **3** Rio de janeiro (Hamada *et al.* 1995). **4** Garte *et al.* (1996). **5** Garte *et al.*(1996), Masimirembwa *et al.* (1998). **6** Rio de Janeiro (Hamada *et al.* 1995). **7** Garte *et al.* (1996), Park *et al.* (1997). **8** Aynacioglu *et al.* (1998), Esteller *et al.* (1997).

I.2.2 – *CYP2E1*

A enzima CYP2E1 é toxicologicamente importante devido à capacidade de metabolizar vários xenobióticos de baixo peso molecular tais como as N-nitrosaminas, benzeno e butadieno (Guengerich & Shimada 1998).

O gene está localizado no cromossomo 10q24.3-ter, abrange uma seqüência de ADN de 11,413 pb e contém nove exons (Umeno *et al.* 1988). Vários polimorfismos foram descritos distribuídos ao longo do gene (http://www.imm.ki.se/CYPalleles). Na região promotora, a mutação – 1053 (C→T, *Rsa*I) ocorre em desequilíbrio de ligação com a substituição – 1293 (G→C, *Pst*I) em algumas populações, mas não em outras (Kato *et al.* 1992, Liu *et al.* 2001). Onde é detectado o desequilíbrio completo, são descritos os haplótipos *CYP2E1*1A* e *CYP2E1*5B*, que geralmente são denominados de alelos. Estudos de expressão demonstraram que a forma mutante, *CYP2E1*5B*, resultou em aumento de transcrição do RNAm (Watanable *et al.* 1994). Outras informações sobres estas duas mutações são apresentadas na tabela 4. Ambos os marcadores foram estudados em diversas populações, com a freqüência de *CYP2E1*5B* variando de 0,2% em aborígenes australianos a 30% na Ásia (Tabela 5).

Tabela 4. Dois polimorfismos na região promotora do *CYP2E1* que apresentam alteração de expressão.

Localização	Alelos	Nomenclaturas utilizadas	Mutação	Nomenclatura recomendada [1]
Região 5’				
	**1A*	*Pst* – *Rsa*I +	– 1293 G; – 1053 C	*CYP2E1*1A*
	**5B*	*Pst* + *Rsa*I –	– 1293 G→C; – 1053 C→T	*CYP2E1*5B*

[1] Fonte: http://www.imm.ki.se/CYPalleles. Os sinais mais (+) e menos (–) correspondem a presença e à ausência dos sítios de restrição.

Tabela 5. Freqüência (%) do haplótipo *CYP2E1*5B* em diversas populações.

Populações	No. de amostras	*CYP2E1*5B*	Referência
Ameríndias	1	25	**1**
Asiáticas	2	19–30	**2**
Euro-brasileiras	1	5	**3**
Euro-americanas	3	2–8	**4**
Européias	3	3–5	**5**
Afro-americanas	3	1–2	**6**
Aborígenes australianos	1	0,2	**7**

1 Muñoz *et al.* (1998). **2** Tan *et al.* (2000), Watanable *et al.* (1995). **3** Rio de Janeiro (Hamada *et al.* 1995). **4** Kato *et al.* (1992), London *et al.* (1996), Stephens *et al.* (1994). **5** González *et al.* (1998), Lucas *et al.* (1995), Persson *et al.* (1993). **6** Kato *et al.* (1992), London *et al.* (1996), Stephens *et al.* (1994). **7** Griese *et al.* (2001).

I.3 – Genes de fase II: a família glutationa S-transferase

I.3.1 – *GSTM1*, *GSTT1* e *GSTP1*

A glutationa S-transferase (GST) corresponde a uma super-família de enzimas envolvidas na detoxificação de vários xenobióticos não carcinógenos e carcinógenos (Eaton & Bammler 1999). As GSTs são, geralmente, reconhecidas como enzimas de detoxificação, ou de fase II, devido à capacidade de catalisar a conjugação destes compostos com a glutationa (Ketter 1988, Miller *et al.* 1997, Wilce & Parker 1994).

No homem foram identificadas os locos *GSTA*, *GSTM*, *GSTT*, *GSTP* e *GSTZ* (Miller *et al.* 1997, Strange *et al.* 1998) sendo os mais analisados, em estudos de populações e de suscetibilidade a doenças ambientais, os genes *GSTM1*, *GSTT1* e *GSTP1*.

No loco *GSTM1*, localizado no cromossomo 1p13.1, foi descrita com, freqüências polimórficas, uma deleção do gene, *GSTM1 (–)*, que resulta em ausência completa de atividade enzimática (Pearson *et al.* 1993, Xu *et al.* 1998). A deleção ocorre devido a um evento de recombinação entre duas seqüências homólogas localizadas acima (*upstream*) e abaixo (*downstream*) do *GSTM1* (Xu *et al.* 1998). Estes autores sugeriram que a deleção resultaria de um evento de recombinação antigo, ou de eventos recombinacionais independentes porque a região seria um *hot spot* para recombinação. Outro interessante fenômeno biológico observado neste loco foi a presença da duplicação do gene, resultando em maior atividade da GSTM1 em populações da Arábia Saudita (McLellan *et al.* 1997).

A freqüência da deleção apresenta variação interétnica. Alguns grupos da Polinésia e da Micronésia são monomórficos para a deleção, asiáticos europeus e euro-derivados apresentam freqüências intermediárias enquanto que as menores foram detectados em aborígenes australianos (Tabela 6).

No loco *GSTT1*, localizado no cromossomo 22q11.2, também foi descrita uma deleção do gene, G*STT1 (–)*, que resulta em completa ausência de atividade enzimática (Pemble *et al.* 1994, Sprenger *et al.* 2000). A deleção ocorre também por recombinação entre duas seqüências homólogas localizadas *upstream* e *downstream* do *GSTT1* (Sprenger *et al.* 2000). As populações humanas apresentam uma grande variação da freqüência da deleção *GSTT1* (Tabela 6), com a maior freqüência na Ásia (64%) e a menor (11%) em índios Parakanã da Amazônia brasileira.

A enzima GSTP1 é codificada pelo gene *GSTP1*, localizado no cromossomo 11q13 (Smith *et al.* 1995). Até a presente data, dois polimorfismos foram descritos, um no codon 105 do exon 5 que resulta na mudança do aminoácido Ile→Val e outro, no codon 114 do exon 6, com alteração de Ala→Val (Ali-Osman *et al.* 1997, Zimniak *et al.* 1994). A mutação valina 105 (**V105*) ocorre na região correspondente ao centro catalítico e parece resultar em redução de atividade enzimática (Ali-osman *et al.* 1997, Zimniak *et al.* 1994, Watson *et al.* 1998). Outras investigações, no entanto, verificaram que a forma V105 está associada com aumento de atividade catalítica (Hu *et al.* 1997, Sundberg *et al.* 1998). Watson *et al.* (1998), avaliando estes resultados, sugeriram que estas diferenças devem ser devido ao substrato utilizado nos diferentes estudos de expressão. A freqüência do alelo **V105* varia de 11% em aborígenes australianos a 43% em afro-brasileiros (Tabela 7).

Tabela 6. Freqüência das deleções (%) de *GSTM1* e *GSTT1* em várias populações.

Populações	No. de amostras	*GSTM1*	No. de amostras	*GSTT1*	Referência
Ameríndias	1	20	1	11	**1**
Asiáticas	6	40–63	6	45–64	**2**
Aborígenes australianos	1	13	1	33	**3**
Ilhas do Pacífico	4	64–100	–	–	**4**
Européias	12	44–63	5	12–25	**5**
Euro-americanas	5	46–58	3	15–24	**6**
Euro-brasileiras	1	55	1	18	**7**
Afro-brasileiras	1	33	2	19–28	**8**
Afro-americanas	2	20–28	2	22–24	**9**
Africanas	3	20–30	2	24–37	**10**

1 Arruda *et al.* (1998). **2** Duzhak *et al.* (2000), Houlston (1999), Landi (2000), Miller *et al.* (1997). **3** Ilett *et al.* (2000). **4** Lin *et al.* (1994). **5** Houlston (1999), Landi (2000), Miller *et al.* (1997), Peluso *et al.* (1998), Rossi *et al.* (1999). **6** Houlston (1999), Miller *et al.* (1997). **7** São Paulo: Arruda *et al.* (1998). **8** São Paulo: Arruda *et al.* (1998), Rio Grande do Sul: Torres *et al.* (2001). **8** Chen *et al.* (1996), Ford *et al.* (2000), Nelson *et al.* (1995). **10** Masimirembwa *et al.* (1998), Mukanganyama *et al.* (1997), Wild *et al.* (2000).

Tabela 7. Freqüência do alelo **V105* do *GSTP1* em várias populações.

Populações	No. de amostras	**V105* (%)	Referência
Asiáticas	2	19-26	Harris *et al.* (1998), Watson *et al.* (1998)
Aborígenes australianos	1	11	Harris *et al.* (1998)
Afro-americanas	1	42	Watson *et al.* (1998)
Afro-brasileiras (RS)	1	43	Torres *et al.* (2001)
Européias	2	28-34	Harries *et al.* (1997), Matthias *et al.* (1998)
Euro-brasileiras (RS)	1	28	Torres *et al.* (2001)

RS: Rio Grande do Sul.

I.4 – Gene supressor de tumor *TP53*

O *TP53*, localizado no cromossomo 17p13.1, é um gene supressor de tumor envolvido na regulação da transcrição gênica, no controle do ciclo celular, no reparo do ADN e na apoptose (Agarwal *et al.* 1998, Kubbutat & Vousden 1998, Mcbride *et al.* 1986, Müllauer *et al.* 2001).

Vários polimorfismos foram descritos neste gene, sendo três os mais estudados: uma duplicação de 16-pb no intron 3, com o alelo *A1* correspondendo à ausência da duplicação; um RFLP para *Msp*I no intron 6, em que o alelo *A1* corresponde à ausência do sítio de restrição; e um RFLP para *Bst*UI no codon 72 do exon 4 (Själander *et al.* 1996, Weston *et al.* 1997). Neste caso a substituição da segunda base da seqüência CCC codificadora do aminoácido prolina (alelo *A1* ou **Pro*), resulta no codon CGC, codificador do aminoácido arginina (alelo *A2*) e gerando um sítio de restrição para *Bst*UI. Thomas *et al.* (1999), demonstraram que as duas formas proteicas possuem várias diferenças bioquímicas funcionais. Por exemplo, a $TP53_{Pro}$ ativa a transcrição de genes relacionados com o controle do ciclo celular de forma mais eficiente que a $TP53_{Arg}$, enquanto a $TP53_{Arg}$ induz a apoptose de forma mais eficaz que a $TP53_{Pro}$.

Beckman *et al.* (1994) verificaram que o alelo *A1* do codon 72 (*Bst*UI) apresentou variação étnica norte-sul e sugeriram que o polimorfismo seria balanceado e mantido por seleção natural. Para Själander *et al.* (1996) a variação interpopulacional deste alelo resultaria da ação da seleção frente a diferentes condições climáticas. A análise simultânea dos três marcadores em diversas populações mundiais revelou a ocorrência de grande variação interétnica (Tabela 8). A investigação combinada dos três marcadores demonstrou que os haplótipos *1-2-2* e *1-1-2* (16bp-*Bst*UI-*Msp*I) foram os mais freqüentes em populações da África, Ásia e Europa (Khaliq *et al.* 2000, Själander *et al.* 1996).

Tabela 8. Freqüência (%) do alelo *A1* dos três polimorfismos em várias populações mundiais.

Populações	Nº. de amostras	*TP53**			Referência
		Intron 3 (16-bp)	Exon 4 (*Bst*UI)	Intron 6 (*Msp*I)	
África	1	75	63	19	Själander *et al.* (1996)
Afro-americanos	1	68	63	33	Weston *et al.* (1997)
Europa	2	85–89	24–29	10–5	Själander *et al.* (1995)
Ásia	2	95–98	38–47	2–5	Själander *et al.* (1996)
Paquistão	9	67–90	35–60	20–56	Khaliq *et al.* (2000)

***** O alelo *A1* corresponde a ausência da duplicação e dos sítios de restrição.

I.5 – Doença pulmonar obstrutiva crônica

A doença pulmonar obstrutiva crônica (DPOC) é considerada como sendo um grave problema de saúde pública no Brasil. No ano de 1999, foi responsável por 14% das internações hospitalares decorrentes de alterações do aparelho respiratório (Menezes 2001).

A DPOC é caracterizada pela presença de obstrução ou limitação crônica ao fluxo aéreo com progressão lenta e irreversível, devido à diminuição da retração elástica pulmonar e

à obstrução das vias aéreas periféricas (Murray & Nadel 1994, Viegi *et al.* 2001). A doença é uma combinação de enfisema pulmonar e bronquite crônica (Murray & Nadel 1994, Kodavanti *et al.* 1998).

O enfisema se caracteriza pela dilatação dos espaços aéreos distais ao bronquíolo terminal, acompanhado de destruição de suas paredes alveolares (American Thoracic Society 1995, Kodavanti *et al.* 1998, Murray & Nadel 1994). A bronquite crônica é caracterizada, clinicamente, pela presença de tosse com catarro, na maioria dos dias, por três meses seguidos, durante pelo menos dois anos sucessivos, estando afastadas outras causas capazes de produzir expectoração crônica. A obstrução brônquica ocorre principalmente nas vias aéreas periféricas devido ao espessamento da parede bronquiolar, ao muco excessivo e à destruição bronquiolar (American Thoracic Society 1995, Murray & Nadel 1994).

A doença se manifesta geralmente entre os 40 e 60 anos de idade e resulta da ação de vários fatores de risco ambientais que atuam de forma independente ou sinergística, sendo o tabagismo o principal deles, contribuindo com aproximadamente 80 a 90% dos casos (Murray & Nadel 1994, Silverman & Speizer 1996). Entretanto, somente 10 a 15% das pessoas tabagistas desenvolvem a DPOC (Barnes 1999, Hanrahan *et al.* 1996). Uma das dificuldades encontradas no estudo dos fatores genéticos envolvidos na DPOC é a definição dos genes que são realmente importantes para a etiologia, uma vez que a mesma, provavelmente, é resultado da interação de diversos genes que atuam em rotas metabólicas diferentes, associados com agentes ambientais diversos (Barnes 1999, MacNee & Rahman 2001). A deficiência de alfa-1-antitripsina é a única alteração genética definida, seguramente, como sendo de risco para DPOC, porém o número de pessoas com esta deficiência é menor que 1% (Anto *et al.* 2001, Dahl *et al.* 2001, Feldmann *et al.* 2000, Murray & Nadel 1994). Outros marcadores investigados incluem locos do sistema antiprotease, do stress oxidativo e das citosinas, por exemplo (Sakao *et al.* 2001, Smith & Harrison 1997, Walter *et al.* 2000, Yamada *et al.* 2000), mas os resultados são inconclusivos.

Poucos estudos avaliaram os efeitos dos genes de metabolização de xenobióticos (*CYPs* ou *GSTs*) na predisposição à DPOC. Até o presente, investigaram-se as deleções dos genes *GSTM1* e *GSTT1*, a alteração polimórfica no exon 5 do *GSTP1* e duas mutações no loco da hidrolase do epóxido microssomal (*mEPHX*), uma no exon 3 e outra no exon 4 (Harrison *et al.* 1997, Ishii *et al.* 1999, Smith & Harrison 1997, Yim *et al.* 2000), tendo sido obtidos resultados contraditórios. Por exemplo, de acordo com Harrison *et al.* (1997), em ingleses, haveria associação entre a deleção do *GSTM1* e DPOC. No entanto, em coreanos, as deleções

de *GSTM1* e *GSTT1* não parecem influenciar o desenvolvimento desta doença (Yim *et al.* 2000). Por outro lado, a mutação no exon 5 do *GSTP1* foi considerado como fator de risco à DPOC em japoneses (Ishii *et al.* 1999).

Quanto ao gene *TP53*, somente foi analisado o polimorfismo no codon 72 (*Bst*UI) em pacientes afro e euro-americanos e não foi encontrada associação com a DPOC (Weston *et al*. 1994).

I.6 – Câncer de pulmão

O Instituto Nacional de Câncer (INCA) estimou para o Brasil, no ano de 2001, a ocorrência de cerca 305,000 novos casos de câncer, resultando em aproximadamente 177,000 óbitos, sendo o câncer de pulmão a principal causa de óbitos no sexo masculino e o segundo mais comum entre as mulheres (INCA).

O carcinoma brônquico ou câncer de pulmão, neoplasia epitelial primária do pulmão, representa cerca de 95% dos tumores que envolvem esse órgão. Os 5% restantes incluem tumores benignos, mesoteliomas e outros tumores que não apresentam como causa conhecida o tabagismo (Carbone 1997, Murray & Nadel 1994). Geralmente ocorre em pessoas com mais de 40 anos de idade e com pico de incidência em torno de 60 anos (Khuder 2001, Murray & Nadel 1994). O tabagismo é responsável por aproximadamente 90% dos casos de câncer de pulmão. Entretanto, somente 10–15% dos fumantes desenvolvem o tumor (Hecht 1999, Murray & Nadel 1994). Outras causas relacionadas são determinados agentes químicos (como o cromo, arsênico, amianto, berílio, radônio, níquel, cádmio e cloreto de vinila, encontrados principalmente no ambiente profissional), fatores dietéticos (baixo consumo de frutas e verduras), variabilidade genética e história familiar de câncer de pulmão (Ames *et al.* 1995, Ames & Gold 1997, Gauderman *et al.* 1997, Murray & Nadel 1994, Sellers *et al.* 1998).

É dividido em dois grandes grupos: o carcinoma de pequenas células (SCLC, *small-cell lung cancer*) que representa aproximadamente 10–20% das neoplasias de pulmão (Carbone 1997, Murray & Nadel 1994) e o carcinoma não de pequenas células (NSCLC, *non-small-cell lung cancer*). Este grupo pode ser subdividido em três tipos histológicos: carcinoma de células escamosas (ou carcinoma epidermóide), adenocarcinoma e carcinoma de grandes células (Carbone 1997, Khuder 2001, Murray & Nadel 1994). Na população do RS, o mais comum é o carcinoma escamoso com cerca de 47% dos casos, seguido do

adenocarcinoma (33%), do carcinoma de pequenas células (10%) e do carcinoma de grandes células (4%), sendo que nos 6% restantes não foi possível determinar o tipo histológico (Moreira *et al.* 2001).

O papel de polimorfismos de genes *CYPs* e *GSTs* no desenvolvimento de carcinoma brônquico é uma questão amplamente discutida na literatura científica (Autrup 2000, Bouchardy *et al.* 2001, Quiñones *et al*. 1999, Song *et al.* 2001). A hipótese básica sugere que um indivíduo que produza uma enzima CYP de fase I mais ativa e/ou uma GST de fase II menos ativa, poderá ter maior risco de desenvolver câncer devido à exposição e/ou acúmulo de produtos que induzem a formação de adutos de ADN (Nebert & Roe 2001, Rannug *et al.* 1995, Rebbeck 1997). Diversos estudos de associações entre os polimorfismos dos genes *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1, GSTP1* e *TP53* e câncer de pulmão foram realizados em populações européias, euro-derivadas e asiáticas (Bartsch *et al.* 2000, Birgander *et al.* 1995, Biros *et al.* 2001, Fan *et al.* 2000, Gsur *et al.* 2001, Houlston 1999, Indulski & Lutz 2000, Murata *et al.* 1996, 1998, Persson *et al.* 1999, Pierce *et al.* 2000, Quiñones *et al.* 1999, 2001, Song *et al.* 2001, To-Figueras *et al.* 2001, Wang *et al.* 1999), mas os resultados não são conclusivos. Algumas das possíveis explicações para as diferenças seriam devido as interações gene/gene, gene/ambiente e a não separação das amostras por grupo étnico (Bartsch *et al.* 2000, Perera 1996, Rebbeck 1997).

II. Justificativa e objetivos

As populações neo-brasileiras formadas por diferentes grupos étnicos, já em si altamente diversificados e os grupos ameríndios, bastante heterogêneos, muitos deles vivendo em condições de semi-isolamento constituem-se em excelente material de pesquisa para os estudos microevolutivos. A alta incidência de doenças pulmonares ambientais (doença pulmonar obstrutiva crônica e câncer de pulmão não de pequenas células) na população do Rio Grande do Sul (RS), torna instigante a avaliação dos efeitos de genes envolvidos com a metabolização de substâncias tóxicas e controle da divisão celular na predisposição a estas patologias. Considerando ainda que muitos dos genes envolvidos no metabolismo de xenobióticos e no controle do ciclo celular apresentam diferentes alelos polimórficos com ampla variação interpopulacional, o presente trabalho se propôs a analisar a variabilidade dos genes *CYP1A1, CYP2E1*, *GSTM1*, *GSTT1, GSTP1* e do supressor de tumor *TP53* em sete populações indígenas sul-americanas, em três grupos afro-brasileiros e em euro-brasileiros do RS, com os seguintes objetivos:

1. Estimar a diversidade genética e as relações intertribais de populações ameríndias, através de polimorfismos de *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1* e *TP53*;
2. Avaliar a distribuição do alelo *CYP1A1*2C* em afro-brasileiros;
3. Estudar a variabilidade genética de grupos euro-brasileiros através destes marcadores;
4. Analisar os possíveis efeitos destes marcadores na predisposição à doença pulmonar obstrutiva crônica e ao câncer de pulmão não de pequenas células.

III. – Artigos

III.1 - Gaspar PA, Hutz MH, Salzano FM and Weimer TA. 2001. *TP53* polymorphisms in South Amerindians and neo-Brazilians. Ann Hum Biol 28: 184-194.

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TP53 polymorphisms and haplotypes in South Amerindians and neo-Brazilians

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Summary. To evaluate the genetic diversity of Brazilian populations and contribute to the knowledge of their evolutionary history this study investigated three *TP53* polymorphisms (*Bst*UI and *Msp*I RFLPs in exon 4 and intron 6, respectively, and a 16 bp duplication in intron 3). The populations studied were: 114 Amerindians from five Brazilian Indian tribes (Gaviões, Surui, Zoró, Wai-Wai and Xavante), 95 Euro-Brazilians and 70 Afro-Brazilians. The polymorphisms were all analysed using PCR amplifications. Gene frequencies and haplotype prevalences were calculated using the ARLEQUIN software. The genetic affinities of these groups with other world populations were estimated by the D_A distance and neighbour joining method, using the NJBAFD computer program. Neo-Brazilians (immigrants from Europe and Africa) generally presented more variability than Amerindians, Afro-Brazilians being the most variable population. Among Amerindians, Gavião is the only group polymorphic for the three markers. Wai-Wai showed variability in *Bst*UI and *Msp*I RFLPs, while the other tribes were monomorphic for the 16 bp *A1* and *Msp*I *A2* alleles. A rare haplotype (*1-2-1*) was verified among the Wai-Wai. This haplotype was previously described in a Chinese sample only, but with low frequency. Therefore, either this combination was lost in the other tribes by genetic drift, recombination, or other factor, or it occurs in the Wai-Wai and Chinese by independent events. The Gavião also presented a haplotype (*2-1-1*) not observed in the other Amerindians; but since it is present in Euro- and Afro-Brazilians, its occurrence there is probably due to interethnic admixture. The relationships of several world populations obtained using *TP53* indicates that this marker is very efficient in clustering populations of the same ethnic group.

1. Introduction

The *TP53* gene, located on chromosome 17, is the tumour suppressor gene that most frequently mutates in human cancers (Hollstein, Sidransky, Vogelstein *et al.* 1991). At least 10 different polymorphisms of it have been described (Weston, Pan, Ksieski *et al.* 1997) that can be useful to characterize populations (Själander, Birgander, Kivelä *et al.* 1995, Själander, Birgander, Saha *et al.* 1996b). To date, several studies based on nuclear DNA analysis have been conducted on Brazilian populations (Santos, Ribeiro-dos-Santos, Guerreiro *et al.* 1998, Hutz, Callegari-Jacques, Bortolini *et al.* 1999, Andrade, Coimbra, Santos *et al.* 2000), but the progressive accumulation of more data can help to better understand their evolutionary history. For this purpose *TP53* polymorphisms can be very useful, due to their high inter-population heterogeneity. The ethnic background of Brazil is highly heterogeneous, being composed by Amerindians and neo-Brazilians (immigrants from Europe and Africa). Amerindians descend from populations who migrated mainly from Asia through the Bering Strait, some 20 000–40 000 years ago (Salzano and Callegari-Jacques 1988). Portuguese are the main European ancestors of the Brazilian population, but other nationalities (Italians, Germans and Spaniards) have also contributed to their gene pool (Salzano 1987). Africans were forced to come to Brazil to work as slaves, mainly from Africa's West Coast, but also from Mozambique (Salzano 1987).

This paper describes the genetic variability of three *TP53* polymorphisms in three ethnic groups: five Amerindian tribes from Amazonia and Central Brazil, as well as a Euro- and an Afro-derived sample living in Porto Alegre, in southern Brazil.

2. Subjects and methods

A total of 114 individuals from five Brazilian Indian tribes were investigated, and detailed information about them are presented in table 1. The Gavião, Surui and Zoró populations are closely related both culturally and geographically. They live in the southwestern region of the Brazilian Amazonia. The Wai-Wai Indians live further to the north, and the Xavante in Central Brazil. Blood samples were obtained by venipuncture, from individuals who volunteered in the several villages, refrigerated shortly after collection, and sent by air to Porto Alegre under refrigeration, where DNA extraction was performed.

The Afro- and Euro-Brazilian samples were obtained in Porto Alegre, the capital of Brazil's southernmost state. The Euro-Brazilians consisted of 95 adults who came to our laboratory for paternity testing. Bloods from the 70 Afro-Brazilians were collected from ambulatory patients who went for routine blood examination to the Santa Casa de Misericórdia Hospital.

Genomic DNA was isolated from whole blood by the salting out method of Miller, Dykes and Polesky (1988) for most samples, but the procedure described by Lahiri and Nurnberger (1991) was employed in the Wai-Wai specimens.

The *TP53* polymorphisms investigated in the present research were: (a) an intron 3 variation characterized by the absence (*A1* allele) or presence (*A2*) of a 16-base pair (bp) duplication (Lazar, Hazard, Bertin *et al.* 1993); (b) the absence/presence (*A1*/*A2* alleles) of a *Bst*UI restriction site in exon 4, codon 72 (Harris, Brill, Shohat *et al.* 1986), which results in two alternative proteins, proline (*A1*) or arginine (*A2*). This substitution seems to predispose *A1* homozygotes to lung or breast cancer (Själander, Birgander, Hallmans *et al.* 1996a, Wang, Chen, Chen *et al.* 1999); and (c) a *Msp*I restriction site in intron 6. The absence of the site is defined as the *A1* allele (McDaniel, Carbone, Takahashi *et al.* 1991). All polymorphisms were analysed by PCR amplifications according to Själander *et al.* (1995) as described in table 2. The amplified fragments were separated on agarose gel electrophoresis and stained with ethidium bromide.

Allele and haplotype frequencies were computed using an expectation-maximization (EM) algorithm (Excoffier and Slatkin 1995). Hardy–Weinberg equilibrium was evaluated by exact tests using the Markov chain (Guo and Thompson 1992); all these

Table 1. Characterization of the Brazilian Indian tribes investigated.

Tribes	Geographic location	Linguistic group*	Present population†	No. of individuals investigated
Gavião	61°8′W, 10°10′S	Tupi-Mondé	360	26
Surui	61°10′W, 10°50′S	Tupi-Mondé	586	20
Zoró	60°20′W, 10°20′S	Tupi-Mondé	257	22
Wai-Wai	57°55′W, 0°40′S	Carib	1366	21
Xavante	51°40′W, 13°20′S	Ge	7100	25

* Rodrigues (1986), Greenberg (1987).
† Santos (1991), Callegari-Jacques, Salzano, Weimer *et al.* (1996), Salzano, Franco, Weimer *et al.* (1997).

Table 2. PCR primers and conditions employed.

Polymorphism	Primers	Reaction conditions (for all sites)	Amplification conditions (for all sites)	Product size (bp)
Intron 3	1, 2	250 µg DNA 10 mM Tris HCl 1.5 mM $MgCl_2$ 50 mM KCl 0.1% triton 100 mM dNTP 12.5 pmol primers 0.5 U Taq	94°C – 5 min Touchdown (70.5°C → 67°C) 94°C 1 min, 67°C 2 min, for 35 cycles 72°C – 5 min	401/417
Exon 4	1, 2			401/417
Intron 6	3, 4			240

1: 5GCAGAGACCTGTGGGAAGCGA3'; 2: 5'ACCGTAGCTGCCCTGGTAGGT3'; 3: 5'TATGAG CCGCCTGAGGTCTGG 3'; 4: 5'TACAGGCATGAGCCACTGCGC3'

calculations were performed using the 1.1 version of the ARLEQUIN computer program (Schneider, Kueffer, Roessli *et al.* 1997). Pairwise linkage disequilibrium (D) and Drel (D/D_{max}) were calculated as suggested by Lewontin (1988). Phenotype differences among populations were evaluated by means of Fisher's exact test using the PEPI computer program (Gahlinger and Abramson 1995). Haplotype frequency differences among populations were estimated using χ^2 (for the comparison between Euro- and Afro-Brazilians) or the Roff and Bentzen (1989) test (for the differences found among the Amerindians), also using the PEPI software. Average heterozygosity (H; Nei 1987), genetic affinities among populations using the D_A distance (Nei 1987), and dendrograms based on the neighbour joining method (Saitou and Nei 1987), were estimated using the NJBAFD computer program (Takezaki 1999). No bootstrap test was made, since genetic distances and the dendrograms were based on one set of systems (haplotype frequencies) only.

3. Results

The genotype and allele frequency distributions of the three *TP53* polymorphisms are presented in table 3. Euro- and Afro-Brazilians generally presented more variability than Amerindians. Among the latter, Gavião was the only population polymorphic for the three markers: the Wai-Wai showed variability in both *Bst*UI and *Msp*I RFLPs, while the other tribes were monomorphic for the 16 bp *A1* and *Msp*I *A2* alleles. All distributions showed good agreement with Hardy–Weinberg expectations. The differences among the Amerindians were significant for the *Bst*UI RFLP only, in the comparisons Zoró vs Surui and Zoró vs Xavante (both $p < 0.05$). Afro-Brazilians presented significant differences from Euro-Brazilians in the three polymorphisms ($p < 0.01$), showing a somewhat higher value of 16 bp *A2*, and about two times more *Bst*UI *A1* and *Msp*I *A1* alleles.

Table 4 presents the estimated pairwise haplotype frequencies, as well as D and D_{rel} values obtained for these populations. Some significant differences were verified: the Zoró were different from the Surui and Xavante in relation to 16 bp/*Bst*UI and *Bst*UI/*Msp*I haplotypes ($p < 0.05$); Wai-Wai and Xavante *Bst*UI/*Msp*I haplotype distributions were also significantly different ($p < 0.05$). Euro- and Afro-Brazilians showed distinct haplotype frequencies in all pairwise combinations ($p < 0.001$).

Table 3. *TP53* genotype and allele frequencies in seven Brazilian populations.

Polymorphic sites and populations	No. of individuals tested	*TP53* type (%)			Allele frequencies ± SE *A1*
		1	1–2	2	
16 bp†					
Gavião	26	96	4	0	0.981 ± 0.017
Surui	20	100	0	0	1.000 ± 0.000
Zorò	22	100	0	0	1.000 ± 0.000
Wai-Wai	21	100	0	0	1.000 ± 0.000
Xavante	25	100	0	0	1.000 ± 0.000
Euro-Brazilians	95	77	22	1	0.879 ± 0.022
Afro-Brazilians	70	54	43	3	0.757 ± 0.043
*Bst*UI†‡					
Gavião	26	4	31	65	0.192 ± 0.061
Surui	20	5	40	55	0.250 ± 0.077
Zorò	22	0	14	86	0.068 ± 0.038
Wai-Wai	21	0	24	76	0.119 ± 0.046
Xavante	25	8	40	52	0.280 ± 0.067
Euro-Brazilians	95	6	50	44	0.310 ± 0.038
Afro-Brazilians	70	49	40	11	0.686 ± 0.048
*Msp*I†					
Gavião	26	0	4	96	0.019 ± 0.016
Surui	20	0	0	100	0.000 ± 0.000
Zorò	22	0	0	100	0.000 ± 0.000
Wai-Wai	21	0	14	86	0.071 ± 0.040
Xavante	25	0	0	100	0.000 ± 0.000
Euro-Brazilians	95	1	18	81	0.100 ± 0.021
Afro-Brazilians	70	4	37	59	0.229 ± 0.039

†All the Euro-Brazilian vs Afro-Brazilian comparisons yielded differences significant at the $p < 0.01$ level.
‡The Zorò vs Surui and Zorò vs Xavante comparisons yielded differences significant at the $p < 0.05$ level.

Among Amerindians, only the 16 bp/*Msp*I haplotype was found to be in linkage disequilibrium among the Gavião, but Euro- and Afro-Brazilians presented highly significant linkage disequilibrium values in all combinations.

Extended haplotype frequencies are presented in table 5. Among the eight possible haplotypes, only two were verified in most Amerindians, the exceptions being Gavião and Wai-Wai. This last population presented a haplotype (*1-2-1*) that was not present in any of the other samples, while the Gavião showed a combination (*2-1-1*) which was also present in Euro- and Afro-Brazilians. The latter showed five common haplotypes and a sixth exclusive for each one (*2-2-1* for Euro- and *2-2-2* for Afro-Brazilians). Statistical differences in haplotype distributions were verified between Zorò and Surui ($p < 0.05$), Zorò and Xavante ($p < 0.05$), Wai-Wai and Xavante ($p < 0.05$), as well as between Euro- and Afro-Brazilians ($p < 0.001$).

The haplotype frequencies observed here were compared with those found in other populations (Själander *et al.* 1996b) in figure 1. Only two arrangements occurred in all populations, and despite little differences there is good agreement among populations of the same ethnic group. The most frequent haplotypes, in all populations, were *1-2-2* and *1-1-2*.

Average heterozygosities and D_A genetic distances based on haplotype frequencies were estimated for these same world populations and are reported in table 6. Amerindians have low levels of variability, the H values ranging from 13% in Zoró to 41% in Xavante. Intrapopulation variability is most marked among the groups of African origin ($H = 71$–72%).

Table 4. Pairwise linkage disequilibrium between *TP53* polymorphisms in Brazilians.

Haplotypes and populations	Estimated pairwise frequencies ± SE				No. of alleles	D	D_{rel}[a]
	1–1	*1–2*	*2–1*	*2–2*			
16 bp–*Bst*UI [b, c]							
Gavião	0.173 ± 0.057	0.808 ± 0.058	0.019 ± 0.017	–	52	−0.015	−1.00[NS]
Surui	0.250 ± 0.074	0.750 ± 0.074	–	–	40	0.000	0.00
Zoró	0.068 ± 0.035	0.932 ± 0.035	–	–	44	0.000	0.00
Wai-Wai	0.119 ± 0.059	0.881 ± 0.059	–	–	42	0.000	0.00
Xavante	0.280 ± 0.069	0.720 ± 0.069	–	–	50	0.000	0.00
Euro-Brazilians	0.203 ± 0.033	0.676 ± 0.035	0.108 ± 0.022	0.013 ± 0.007	190	−0.073	−0.87**
Afro-Brazilians	0.461 ± 0.049	0.296 ± 0.047	0.224 ± 0.041	0.019 ± 0.018	140	−0.057	−0.75*
16 bp–*Msp*I [b]							
Gavião	–	0.981 ± 0.017	0.019 ± 0.017	–	52	−0.019	−1.00*
Surui	–	1.000 ± 0.000	–	–	40	0.000	0.00
Zoró	–	1.000 ± 0.000	–	–	44	0.000	0.00
Wai-Wai	0.071 ± 0.043	0.929 ± 0.043	–	–	42	0.000	0.00
Xavante	–	1.000 ± 0.000	–	–	50	0.000	0.00
Euro-Brazilians	0.011 ± 0.008	0.868 ± 0.027	0.089 ± 0.024	0.032 ± 0.011	190	−0.077	−0.88**
Afro-Brazilians	0.055 ± 0.019	0.702 ± 0.049	0.174 ± 0.036	0.069 ± 0.023	140	−0.118	0.68**
*Bst*UI–*Msp*I [b, c, d]							
Gavião	0.019 ± 0.019	0.173 ± 0.059	–	0.808 ± 0.058	52	0.015	1.00[NS]
Surui	–	0.250 ± 0.081	–	0.750 ± 0.081	40	0.000	0.00
Zoró	–	0.068 ± 0.040	–	0.932 ± 0.040	44	0.000	0.00
Wai-Wai	–	0.119 ± 0.050	0.071 ± 0.038	0.810 ± 0.059	42	−0.008	−1.00[NS]
Xavante	–	0.280 ± 0.068	–	0.720 ± 0.068	50	0.000	0.00
Euro-Brazilians	0.086 ± 0.021	0.224 ± 0.036	0.014 ± 0.024	0.676 ± 0.011	190	0.058	0.85**
Afro-Brazilians	0.229 ± 0.039	0.457 ± 0.044	–	0.314 ± 0.042	140	0.071	1.00*

[a] $D_{rel} = D/D_{max}$; Significance of the maximal linkage disequilibrium: * $p < 0.05$; ** $p < 0.001$; NS: non-significant.
[b] Significance of the differences in the Euro-Brazilian vs Afro-Brazilian comparisons: all $p < 0.01$.
[c] Significance of the Zoró vs Surui and Zoró vs Xavante comparisons: $p < 0.05$.
[d] Significance of the Xavante vs Wai-Wai comparisons: $p < 0.05$.

Table 5. *TP53* extended haplotype frequencies and standard errors (%) in three Brazilian ethnic groups. Numbers in parentheses are numbers of chromosomes tested.

16 bp–*Bst*UI–*Msp*I	*1-1-1*	*1-1-2*	*1-2-2*	*1-2-1*	*2-1-1*	*2-1-2*	*2-2-1*	*2-2-2*
Gaviões (52)	–	17.3 ± 6.1	80.8 ± 6.0	–	1.9 ± 1.5	–	–	–
Surui (40)	–	25.0 ± 7.6	75.0 ± 7.6	–	–	–	–	–
Zoró (44)	–	6.8 ± 4.0	93.2 ± 4.0	–	–	–	–	–
Wai-Wai (42)	–	11.9 ± 5.2	81.0 ± 6.4	7.1 ± 4.0	–	–	–	–
Xavante (50)	–	28.0 ± 6.9	72.0 ± 6.9	–	–	–	–	–
Euro-Brazilians (190)	1.1 ± 0.7	19.1 ± 3.5	67.7 ± 4.0	–	7.6 ± 2.2	3.2 ± 1.0	1.3 ± 0.8	–
Afro-Brazilians (140)	5.4 ± 1.7	40.2 ± 5.0	30.1 ± 4.5	–	17.5 ± 3.0	5.5 ± 2.0	–	1.3 ± 1.2

Significance of the differences in haplotype distributions: Zoró vs Surui, Zoró vs Xavante and Wai-Wai vs Xavante, $p < 0.05$; Euro-Brazilians vs Afro-Brazilians, $p < 0.001$.

Table 6. *TP53* average heterozygosities in the diagonal, and D_A distances based on haplotype frequencies, for 11 world populations (×100).

	GAV	SUR	ZOR	WWAI	XAV	CHIN1	CHIN2	EUROB	SWE	AFROB	NIG
GAV	**32.3**										
SUR	1.36	**38.4**									
ZOR	2.38	3.36	**12.9**								
WWAI	4.75	4.81	4.12	**33.3**							
XAV	1.72	0.06	4.28	5.38	**41.1**						
CHIN1	5.00	4.62	11.47	7.59	4.19	**54.6**					
CHIN2	4.86	3.52	12.46	11.52	2.83	2.59	**51.9**				
EUROB	4.06	6.89	9.17	10.87	7.06	7.30	7.00	**50.0**			
SWE	4.80	9.35	10.05	12.33	9.72	7.20	9.47	2.12	**48.8**		
AFROB	18.55	20.79	30.50	28.75	19.9	12.12	11.56	8.98	9.03	**71.6**	
NIG	16.21	17.94	26.81	25.49	17.18	11.07	10.45	5.95	8.37	1.58	**71.1**

GAV: Gaviões, SUR: Surui, ZOR: Zoró, WWAI: Wai-Wai, XAV: Xavante, CHIN1: Chinese from Singapore, CHIN2: Chinese from Guizhou, EUROB: Euro-Brazilians, SWE: Swedes, AFROB: Afro-Brazilians, NIG: Nigerians.

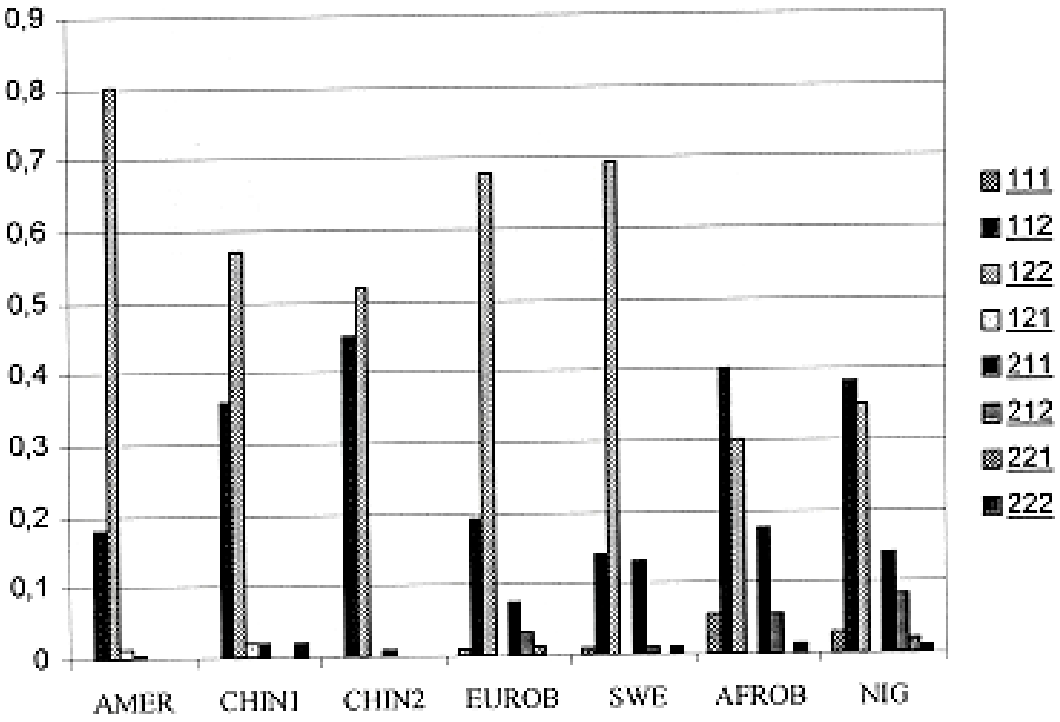

Figure 1. Comparison of *TP53* haplotype frequencies in several world populations. AMER: Gavião, Surui, Zoró, Wai-Wai, Xavante; CHIN1: Chinese from Singapore; CHIN2: Chinese from Guizhou; EUROB: Euro-Brazilians; SWE: Swedes, AFROB: Afro-Brazilians; NIG: Nigerians.

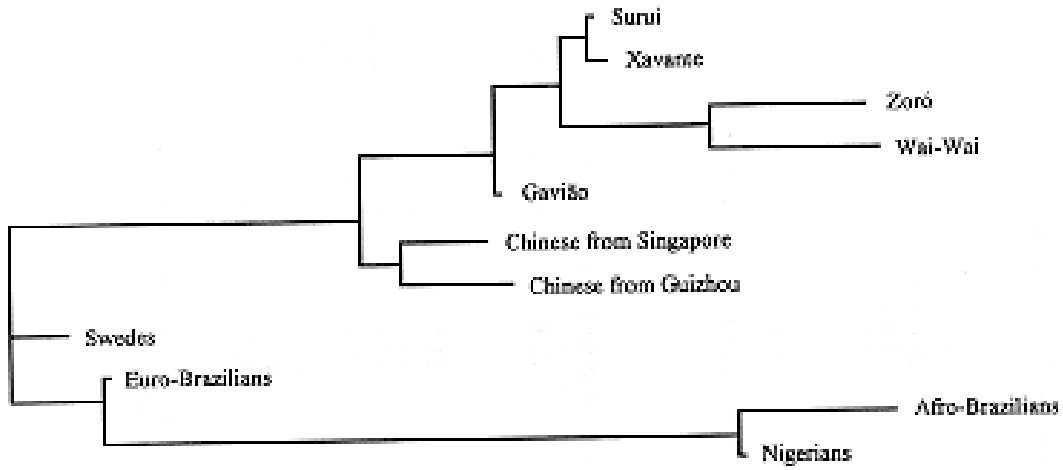

Figure 2. Neighbour joining tree based on haplotype D_A distances for 11 world populations.

The relationships among these 11 populations were evaluated through the neighbour joining method, using the D_A distances based on haplotype frequencies (figure 2). There is a good correlation between the clustering pattern and the ethnic groups. Amerindians clustered with Asian populations, Africans formed the most divergent cluster, and Europeans occur in an intermediate position. Essentially the same relationships were obtained when gene, instead of haplotype frequencies, have been used in the comparisons (data not shown).

4. Discussion

Only two investigations have been published so far considering these three polymorphisms simultaneously (Själander *et al*. 1995, 1996b). Although some differences occur in phenotypic frequencies, the haplotypes found in Amerindians are the same as those described for the Chinese (Själander *et al*. 1996b). These data provide an additional evidence for the widely held hypothesis of an Asian origin for Amerindians (Salzano and Callegari-Jacques 1988).

The rare (*1-2-1*) haplotype found in the Wai-Wai has to date only been observed in one other population (Chinese from Singapore, Själander *et al*. 1996b), but with a lower frequency (0.02). Two explanations can be given for these results: (a) this haplotype was present in the ancestral Amerindian population, but reached a relatively high frequency in the Wai-Wai and was lost in the other tribes due to genetic drift; or (b) the *1-2-1* haplotype occurs only in these two populations due to independent mutation or recombination events, originated from the most frequent arrangements. Only further investigations into a larger number of Asian and Amerindian populations can indicate which of the two alternatives is the correct one.

The Gavião presented haplotype *2-1-1* that was not observed in the other Amerindians. This arrangement has been already described, in low frequency, in Chinese (Själander *et al*. 1996b) and thus could be an Amerindian haplotype that was lost in the other tribes. But this haplotype was also observed in Euro- and Afro-Brazilians, and could be present in the Gavião due to interethnic admixture. According to Santos, Hutz, Coimbra *et al*. (1995) this tribe shows indications of about 4% of admixture with neo-Brazilians.

In relation to the other ethnic groups, although the Afro-Brazilians investigated here may have a high degree of European admixture (59% according to Bortolini, Weimer, Salzano *et al*. 1997, who based their assessment in protein markers), the heterogeneity observed between Afro- and Euro-Brazilians is of the same order of magnitude as that detected comparing European and African populations (Själander *et al*. 1996b).

The haplotype comparison involving all populations investigated so far indicated that two haplotypes are common to all populations (*1-1-2* and *1-2-2*). According to Watterson and Guess (1977), haplotypes common to all populations may be older than those present in some groups only. Therefore, the *1-1-2* and *1-2-2* arrangements probably predate the geographical dispersal of human beings. Among all populations, Nigerians and Afro-Brazilians are the most variable. They present high number of haplotypes and high frequencies of the probably derived arrangements. These data corroborate the hypothesis of an African origin for humankind (Seielstad, Bekele, Ibrahim *et al*. 1999, Quintana-Murci, Semino, Bandelt *et al*. 2000).

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References

Andrade, F. M., Coimbra, C. E. A., Jr, Santos, R. V., Goicoechea, A., Cranese, F. R., Salzano, F. M., and Hutz, M. H., 2000, High heterogeneity of apolipoprotein E gene frequencies in South American Indians. *Annals of Human Biology*, **27**, 29–34.

Bortolini, M. C., Weimer, T. A., Salzano, F. M., and Moura, L. B., 1997, Genetic structure of two urban Afro-Brazilian populations. *International Journal of Anthropology*, **12**, 5–16.

Callegari-Jacques, S. M., Salzano, F. M., Weimer, T. A., Franco, M. H. L. P., Mestriner, M. A., Hutz, M. H., and Schüler, L., 1996, The Wai Wai Indians of South America: history and genetics. *Annals of Human Biology*, **23**, 189–201.

Excoffier, L., and Slatkin, M., 1995, Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Molecular Biology and Evolution*, **12**, 921–927.

Gaiblinger, P. M., and Abramson, J. H., 1995, *Computer Programs for Epidemiologic Analysis: PEPI* (Stone Mountain: USD).

Greenberg, J. H., 1987, *Language in the Americas* (Stanford: Stanford University Press).

Guo, S., and Thompson, E., 1992, Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Harris, N., Brill, E., Shohat, O., Prokocimer, M., Wolf, D., Arai, N., and Rotter, V., 1986, Molecular basis for heterogeneity of the human p53 protein. *Molecular and Cellular Biology*, **6**, 4650–4656.

Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C., 1991, p53 mutations in human cancers. *Science*, **253**, 49–53.

Hutz, M. H., Callegari-Jacques, S. M., Bortolini, M. C., and Salzano, F. M., 1999, Variability in nDNA, mtDNA, and proteins—a test case. In *Genomic Diversity: Applications in Human Population Genetics*, edited by S. S. Papiha and R. Deka (New York: Plenum), pp. 23–32.

Lahiri, D. K., and Nurnberger, J. I., 1991, A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research*, **19**, 5444.

Lazar, V., Hazard, F., Bertin, F., Janin, N., Bellet, D., and Bressac, B., 1993, Simple sequence repeat polymorphism within the p53 gene. *Oncogene*, **8**, 1703–1705.

Lewontin, R. C., 1988, On measures of gametic disequilibrium. *Genetics*, **120**, 849–852.

McDaniel, T., Carbone, D., Takahashi, T., Chumakov, P., Chang, E. H., Pirollo, K. F., Yin, J., Huang, Y., and Meltzer, S. J., 1991, The MspI polymorphism in intron 6 of p53 (TP53) detected by digestion of PCR products. *Nucleic Acids Research*, **19**, 4796.

Miller, A. S., Dykes, D. D., and Polesky, H. F., 1988, A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, **16**, 1215.

Nei, M., 1987, *Molecular Evolutionary Genetics* (New York: Columbia University Press).

Quintana-Murci, L., Semino, O., Bandelt, H. J., Passarino, G., McElreavey, K., Santachiara-Benerecetti, A. S., 2000, Genetic evidence of an early exit of *Homo sapiens* from Africa through eastern Africa. *Nature Genetics*, **23**, 437–441.

Rodrigues, A. D., 1986, *Línguas Brasileiras. Para o Conhecimento das Línguas Indígenas* (São Paulo: Loiola).

Roff, D. A., and Bentzen, P., 1989, The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*, **6**, 539–545.

Saitou, N., and Nei, M., 1987, The neighbour joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Salzano, F. M., 1987, Brazil. In *Rassengeschichte der Menschheit, 12. Lieferung. America II: Mittel- und Südamerika*, edited by I. Schwidetzky (Munich: R. Oldenbourg), pp. 137–175.

Salzano, F. M., and Callegari-Jacques, S. M., 1988, *South American Indians. A Case Study in Evolution* (Oxford: Clarendon Press).

Salzano, F. M., Franco, M. H. L. P., Weimer, T. A., Callegari-Jacques, S. M., Mestriner, M. A., Hutz, M. H., Flowers, N. M., Santos, R. V., and Coimbra, C. E. A., Jr, 1997, The Brazilian Xavante Indians revisited: new protein genetic studies. *American Journal of Physical Anthropology*, **104**, 23–34.

Santos, R. V., 1991, Coping with change in native Amazonia: a bioanthropological study of Gavião, Surui, and Zoró, Tupi-Mondé speaking societies from Brazil. Ph.D. Thesis, Indiana University, Bloomington.

Santos, F. R., Hutz, M. H., Coimbra, C. E. A., Jr, Santos, R. V., Salzano, F. M., and Pena, S. D. J., 1995, Further evidence for the existence of a major founder Y-chromosome haplotype in Amerindians. *Brazilian Journal of Genetics*, **18**, 669–672.

Santos, S. E. B., Ribeiro-dos-Santos, A. K. C., Guerreiro, J. F., Santos, E. J. M., Weimer, T. A., Callegari-Jacques, S. M., Mestriner, M. A., Franco, M. H. L. P., Hutz, M. H., and Salzano, F. M., 1998, New protein genetic studies in six Amazonian Indian populations. *Annals of Human Biology*, **25**, 505–522.

Schneider, S., Kueffer, J. M., Roessli, D., and Excoffier, L., 1997, *Arlequin ver. 1.1: A Software for Population Genetic Data Analysis* (Geneva: Genetics and Biometry Laboratory, University of Geneva).

Seielstad, M., Bekele, E., Ibrahim, M., Toure, A., and Traore, M., 1999, A view of modern human origins from Y chromosome microsatellite variation. *Genome Research*, **9**, 558–567.

Själander, A., Birgander, R., Kivelä, A., and Beckman, G., 1995, p53 polymorphisms and haplotypes in different ethnic groups. *Human Heredity*, **45**, 144–149.

Själander, A., Birgander, R., Hallmans, G., Cajander, S., Lenner, P., Athlin, L., Beckman, G., and Beckman, L., 1996a, p53 polymorphisms and haplotypes in breast cancer. *Carcinogenesis*, **17**, 1313–1316.

Själander, A., Birgander, R., Saha, M., Beckman, L., and Beckman, G., 1996b, p53 polymorphisms and haplotypes show distinct differences between major ethnic groups. *Human Heredity*, **46**, 41–48.

Takezaki, N., 1999, NJBAFD: Neighbour joining Tree Construction from Allele Frequency Data. ftp://ftp.nig.ac.jp/pub/Bio/njbafd/dos

Wang, Y. C., Chen, C. Y., Chen, S. K., Chang, Y. Y., and Lin, P., 1999, p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. *Clinical Cancer Research*, **5**, 129–134.

Watterson, G. A., and Guess, H. A., 1977, Is the most frequent allele the oldest? *Theoretical Population Biology*, **11**, 141–160.

Weston, A., Pan, C. F., Ksieski, H. B., Wallenstein, S., Berkowitz, G. S., Tartter, P. I., Bleiweiss, I. J., Brower, S. T., Senie, R. T., and Wolff, M. S., 1997, p53 haplotype determination in breast cancer. *Cancer Epidemiology, Biomarkers & Prevention*, **6**, 105–112.

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Zusammenfassung. Diese Studie untersucht drei *TP53* Polymorphismen (*Bst*UI und *Msp*I RFLPs im Exon 4 und Intron 6 und eine 16 bp Verdopplung im Intron 3), um die genetische Verschiedenartigkeit der brasilianischen Bevölkerungen zu beurteilen und ihre Entwicklungsgeschichte zu erforschen. Zu den untersuchten Bevölkerungen: 114 Amerindianer von fünf brasilianisch-indianischen Stämmen (Gavião, Surui, Zoró, Wai-Wai und Xavante), 95 Euro-Brasilianer und 70 Afro-Brasilianer. Alle Polymorphismen wurden mit PCR-Amplification analysiert. Genfrequenzen und Haplotyp-Prävalenzen wurden mit der ARLEQUIN-Software errechnet. Die genetischen Affinitäten dieser Gruppen zu anderen Populationen aus verschiedenen Teilen der Welt wurden durch den D_A-Abstand und die 'neighbour joining' Methode mit dem NJBAFD-Computerprogramm geschätzt. Bei Neo-Brasilianern (Immigranten aus Europa und aus Afrika) war im allgemeinen eine größere Variabilität als bei Amerindianern zu finden, wobei Afro-Brasilianer die variabelste Bevölkerung darstellen. Bei den Amerindianern ist Gavião die einzige Gruppe, die für die drei Marker polymorph ist. Die Wai-Wai zeigten eine Variabilität bei *Bst*UI und in *Msp*I RFLPs, während die anderen Stämme für 16 bp *A1* und *Msp*I *A2* Allele monomorph waren. Ein seltener Haplotyp (*1-2-1*) wurde bei den Wai-Wai nachgewiesen. Dieser Haplotyp wurde vorher nur in einer chinesischen Stichprobe beschrieben, aber mit geringer Frequenz. Entweder ist diese Kombination in den anderen Stämmen durch genetische Drift, Rekombination oder andere Faktoren verloren gegangen, oder sie tritt unabhängig bei den Wai-Wai und den Chinesen auf. Die Gavião haben auch einen Haplotyp (*2-1-1*), der bei anderen Amerindianern nicht zu finden ist; da dieser aber bei Euro- und Afro-Brasilianern vorhanden ist, beruht sein Auftreten dort vermutlich auf einer interethnischen Vermischung. Die mit *TP53* untersuchten Beziehungen einiger Populationen aus verschiedenen Teilen der Welt zeigen, daß dieser Marker für die Clusterung ethnischer Gruppen sehr effizient ist.

Résumé. Afin d'étudier la diversité génétique des populations brésiliennes et de contribuer à la connaissance de leur évolution historique, ce travail examine trois polymorphismes *TP53* (respectivement les RFLP *Bst*UI et *Msp*I dans l'exon 4 et l'intron 6, et une duplication de 16 bp dans l'intron 3). Les populations étudiées sont: 114 amérindiens appartenant à cinq tribus du Brésil (Gavião, Surui, Zoró, Wai-Wai et Xavante), 95 euro-brésiliens et 70 afro-brésiliens. Les polymorphismes sont analysés par amplification PCR. Les fréquences génique et les prévalences haplotypiques sont calculées au moyen du programme ARLEQUIN. Les affinités génétiques de ces groupes avec d'autres populations sont estimées par distance D_A et par méthode d'agglomération de voisinage au moyen du programme NJBAFD. Les néo-brésiliens (immigrants d'Afrique et d'Europe) présentent généralement plus de variabilité que les amérindiens, les afro-brésiliens étant la population la plus variable. Parmi les amérindiens le groupe Gavião seul est polymorphique pour les trois marqueurs. Les Wai-Wai présentent de la variabilité en RFLP de *Bst*UI et *Msp*I alors que les autres tribus sont monomorphiques pour les allèles de 16bp A1 et *Msp*I *A2*. Un haplotype rare (*1-2-1*) est certifié chez les Wai-Wai. Cet haplotype n'a auparavant été décrit

que dans un échantillon chinois, mais avec une fréquence basse. Cette combinaison pourrait donc avoir été perdue par dérive génétique, recombinaison ou par un autre facteur dans les autres tribus, ou bien il est apparu indépendamment chez les chinois et les Wai-Wai. Les Gavião présentent également un haplotype (*2-1-1*) non observé chez les autres amérindiens, mais dans la mesure où il est présent chez les euro- et afro-brésiliens, sa présence est probablement le produit de mélanges interethniques. Les associations obtenues par *TPOX* de quelques populations dans le monde, indiquent que ce marqueur est très efficace pour agglomérer des populations de même groupe ethnique.

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Gene Polymorphisms of CYP1A1, CYP2E1, GSTM1, GSTT1, and TP53 Genes in Amerindians

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ABSTRACT

Polymorphisms at the TP53, cytochrome P-450 (CYP) and glutathione S-transferase (GST) genes are related to cancer susceptibility and present high diversity in allele frequencies among ethnic groups. This study informs about CYP2E1, GSTM1, and GSTT1 polymorphisms in seven Amerindian populations (Xavante, Guarani, Aché, Wai Wai, Zoró, Surui, and Gavião). Polymorphic sites at CYP1A1, and TP53 were also studied in the Aché and Guarani tribes and compared with previous results about these systems already obtained in the other populations. The CYP2E1*5B haplotype showed respectively the highest and the lowest frequencies already observed in human groups. High frequencies of CYP1A1*2A and CYP1A1*2C alleles and low values of GSTM1*0/*0 and GSTT1*0/*0 genotypes were observed. These data may be interpreted as being due to genetic drift or selection for these high-frequency CYP1A1 alleles and against GST null genotypes during America's colonization. Average heterozygosity varied from 0.19 (Guarani) to 0.38 (Surui), and 90% of the total diversity was due to the variability within populations. The relationships between these Amerindians and with other ethnic groups were evaluated based on D_A distances and the neighbor-joining method. No correlation could be observed between genetic relationships and geographic distances or linguistic groups. In the TP53 comparison with other ethnic groups, Amerindians clustered together and then joined Chinese populations. The cluster analysis seems to indicate that the Aché tribe might descend from a Gê group that could have first colonized that Paraguayan region, but had also assimilated some amount of the Guarani gene pool, maybe through intertribal admixture.

KEY WORDS: genetic diversity; South American Indians; molecular markers

The cancer process is usually a multistep phenomenon, during which consecutive somatic cell mutations occur. Genes involved in cell-cycle control, genetic repair systems, or codifying enzymes for the biotransformation of environmental carcinogens have important roles in it (Indulski and Lutz, 2000).

TP53 is a tumor suppressor gene with a critical role in cell-cycle control and is frequently mutated in many human cancers (Sansom and Clarke, 2000). At least three TP53 polymorphisms have been reported as involved in cancer, showing also a high inter-population heterogeneity in allele and haplotype frequencies: a 16-bp duplication in intron 3, an amino acid change in exon 4 (72 Arg→Pro), and a MspI RFLP in intron 6 (Själander et al., 1996; Khaliq et al., 2000; Gaspar et al., 2001).

Cytochrome P-450 (CYP) comprises a superfamily of enzymes that acts on phase I of xenobiotic metabolic transformation. During these reactions, toxic metabolites are generated which might be processed by phase II enzymes (Indulski and Lutz, 2000). The CYP1A1 gene encodes for the CYP1A1 enzyme that catalyzes the bioactivation of polyciclic aromatic hydrocarbons (Indulski and Lutz, 2000). Two CYP1A1 gene polymorphisms have been extensively studied in relation to cancer susceptibility: a 462 Ile→Val substitution at exon 7 (CYP1A1*2C allele) and an associated 6235 T→C mutation at the 3' non-coding region (CYP1A1*2A allele; Hayashi et al., 1991b). The frequencies of these mutations also exhibit significant interethnic differences (Aynacioglu et al., 1998; Kvitko et al., 2000). CYP2E1 metabolizes several occupational and environmental carcinogens (Indulski and Lutz, 2000). Two RFLPs in linkage disequilibrium at the regulatory region of the CYP2E1 gene have been described (Hayashi et al., 1991a). Due to linkage disequilibrium two main arrangements are usually found: CYP2E1*5B and CYP2E1*1A. Although rare in many populations (Hamada et al., 1995; Griese et al., 2001), the CYP2E1*5B haplotype is common in Asians (19% to 30%; Morita et al., 1997; Tan et al., 2000), and Amerindians (25%; Munõz et al., 1998).

Glutatione S-transferases (GST) are a group of phase II enzymes that detoxify endogenous and exogenous electrophiles, determined by a gene family. Genetic polymorphisms at these loci seem to be related to a higher risk of cancer development (Indulski and Lutz, 2000). Two deletions of the GSTM1 or GSTT1 loci which result in no enzymatic activity have been described; both GSTM1*0/*0 and GSTT1*0/*0 genotype frequencies present inter-ethnic variability (Rebbeck, 1997).

This study furnishes data on CYP2E1, GSTM1, and GSTT1 polymorphisms in seven South Amerindian populations. Polymorphic sites at CYP1A1, and TP53 were also studied in the Aché and Guarani tribes. Previous results about these systems from other populations were compiled to allow genetic relationship analyses. A comparison of these Amerindian populations with other ethnic groups was also made using TP53 haplotypes. The specific questions posed by us were as follows: 1. Would the population relationships and genetic variability obtained with these markers, which may be influenced by selection, present the same pattern as those found with polymorphisms in which this process may not have acted as strongly? 2. Could we confirm some unusual frequencies that had been previously obtained in Amerindians for the CYP1A1, GSTM1, and GSTT1 systems? and 3. Since the origin of the Aché, a recently contacted tribe of Paraguay, is still obscure, would these systems provide a clue about it?

SUBJECTS AND METHODS

Samples of 257 individuals were obtained from seven South American Indian tribes living in Brazil and Paraguay (Table 1 and figure 1). More details about these populations can be found in Hill and Hurtado (1996) and Hutz et al. (1999).

Genomic DNA was isolated from whole blood by the salting out method of Miller et al. (1988) or using the procedure described by Lahiri and Nurnberger (1991). This latter methodology was employed for the Aché and Wai Wai samples.

Table 2 presents the characterization of the CYP1A1, CYP2E1, GSTM1, GSTT1, and TP53 polymorphisms investigated here. CYP1A1 was analyzed according to Hayashi et al. (1991b) and Cascorbi et al. (1996), and TP53 following Själander et al. (1995). For GSTM1, GSTT1 and CYP2E1 typing a multiplex protocol was developed which consisted briefly of the following procedures: an initial denaturation at 94 °C for 5 min, 6 touchdown cycles including 1 min at 94 °C, 2 min at 59-54 °C with a decrease of 1° in each cycle, and 1 min at 72 °C, followed by 30 cycles at 94 °C for 1 min, 1 min at 55 °C, and 1 min at 72 °C; plus a final extension of 5 min at 72 °C. The reaction mixture consisted of 100 ng of genomic DNA, 15 pmol of GSTM1 and GSTT1 primers, 7.5 pmol of CYP2E1 primers, 10 mM Tris HCl, 4 mM $MgCl_2$, 50 mM KCl, 150 mM dNTPs, and 1.0 U of Taq DNA polymerase. Primer sequences were those reported by Kato et al. (1992), Bell et al. (1993), and Pemble et al.(1994).

Allele frequencies were estimated by gene counting. Haplotypes were derived using the Multiple Locus Haplotype Analysis program (Long, 1999), which uses the E-M algorithm (Long et al., 1995; Peterson et al., 1999). Hardy-Weinberg equilibrium fit was evaluated by exact tests using the Markov Chain (Guo and Thompson, 1992) through the Arlequin software ver. 2.0 (Schneider et al., 2000). Phenotype differences among populations were tested by

means of the Roff and Bentzen (1989) χ^2 test using the PEPI computer program (Gahlinger and Abramson, 1995). Linkage disequilibrium was calculated on basis of the estimated haplotype frequencies and using the Arlequin software. The D' value (D/D_{max}) was obtained as suggested by Lewontin (1988).

Genetic affinities among populations were evaluated through D_A distances (Nei, 1987), and the neighbor-joining clustering method (Saitou and Nei, 1987), using the NJBAFD computer program (Takezaki, 1999). This latter software was also employed to estimate the genetic diversity values. The reliability of the trees was tested by 2,000 bootstrap replications (Hedges, 1992). The dendrogram comparing Amerindians with the other ethnic groups was based on TP53 haplotype frequencies only, since data for the other markers were not available for the other populations.

RESULTS

Table 3 presents GSTM1*0/*0 and GSTT1*0/*0 genotype frequencies, allele frequencies for the other genetic systems, as well as the genetic diversity values.

Genotype distribution was consistent with Hardy-Weinberg expectations for all loci and populations. For the majority of the markers, genotype and allele distributions were highly heterogeneous among populations, regardless if they were of the same geographic region or linguistic group. The only exception was TP53, in which 16bp*A1 and MspI*A2 alleles were fixed in almost all samples. The average heterozygosity varied from 0.19 (Guarani) to 0.38 (Surui); most of the total diversity (90%) was due to heterogeneity within populations.

As for the CYP2E1 gene, as previously described (Hayashi et al., 1991a) a complete linkage disequilibrium between the PstI and RsaI sites was observed and resulted in only two haplotypes: CYP2E1*1A (PstI -, RsaI +) and CYP2E1*5B (PstI +, RsaI -). The

prevalence of CYP2E1*5B (and of its complementary arrangement) also shows wide variation among populations – from 2% in the Wai Wai to 42% in the Aché. But these extreme values are restricted to three populations only, the two above-indicated and the Xavante (3%), while the other values (19%-33%) are more in accordance with previous Asian or Asian-derived groups.

Table 4 presents the estimated haplotype frequencies for the CYP1A1 polymorphisms and the linkage disequilibrium (D') values. The Aché population was monomorphic for CYP1A1*2B, the most frequent haplotype in the six other populations. Highly significant linkage disequilibrium was observed in five of these groups.

The estimated TP53 haplotypes are given in Table 5. Only two haplotypes were observed in the Aché and Guarani, as was found for three of the five other Amerindian tribes previously considered by Gaspar et al. (2001).

The relationships among the seven populations obtained with the D_A distances and the neighbor-joining method are shown in Figure 2. The extremes of this unrooted tree are occupied on one hand by the Wai Wai (a Carib group from northern Brazil) and Surui (a Tupi-Mondé tribe from southwestern Amazonia), while on the other the Xavante (a Gê speaking population from Central Brazil) and Aché (a Tupi-Guarani speaking group from the Paraguayan forest) occurs. No clear correlation could be observed between the genetic relationships and geographic distances or linguistic group.

Figure 3 shows the TP53 relationships obtained considering the Amerindians and other ethnic groups. Three clusters can be observed, one involving Asian or Asian-derived (Amerindian) groups, another composed by the two European or European-derived populations, and a third including Africans, African Brazilians, and Pakistani.

DISCUSSION

Polymorphisms at the TP53, CYP and GST loci have been described as related to cancer susceptibility. For example, the GSTM1*0/*0 and GSTT1*0/*0 genotypes, CYP1A1*2A, CYP1A1*2C, and TP53 BstUI*A1 alleles, as well as the CYP2E1*5B haplotype, have been associated to different tumors in several human populations (Weston et al., 1997; Indulski and Lutz, 2000). These polymorphisms also present high diversity among ethnic groups. Trying to explain the differences of allele frequencies observed in genes which influence the phase I and phase II detoxifying process Nebert (1997) proposed an effect of natural selection in response to tribal differences in diet. Själander et al. (1996) had already suggested that population differences on TP53 allele frequencies might result from selective effects on ecological adaptation to climatic conditions.

Despite extensive changes that most Amerindians have suffered due to contact with other groups, most of the populations studied here retain many of their previous ecological conditions, such as nomadism, hunting, gathering, and horticulture (Hill and Hurtado, 1996; Santos et al., 1996), and are not continuously exposed to environmental chemicals. All the tribes presently investigated presented high levels of diversity in most of the systems. Therefore, although they live in small groups, evolutionary factors such as selection or drift seem not to have significantly decreased their genetic diversity. These data confirm the degree of variability previously verified in the same populations for other systems (Hutz et al., 1999; Bogdawa et al., 2000; Battilana et al., 2001).

This investigation confirmed that most Amerindians are monomorphic for both the 16bp*A1 allele in intron 3, and for the presence of the MspI*A2 allele in intron 6 of the TP53 gene. Beckman et al. (1994) and Själander et al. (1996) found a correlation between the variability at codon 72 of the TP53 gene and latitude, suggesting a possible role for natural selection involving climatic adaptation. The Guarani and Aché live in the southern part of South

America and speak a Tupi-Guarani language, but show TP53 BstUI*A1 gene frequencies of respectively 9% to 37%. On the other hand, the Zoró, Gavião, and Surui, who live in the north of South America in about the same area and speak languages classified as a Tupi sub-group, presented frequencies of the same allele varying from 7% to 25%. Therefore our data do not show any correlation between the frequencies of this allele and latitude.

As was previously indicated, the CYP2E1*5B distribution presented remarkable (2%-42%) differences. These are the most extreme values reported so far for any other ethnic group, except the 0.02% detected among Australian Aborigines (Griese et al. 2001). They are however restricted to the Wai Wai, Xavante and Aché. When an inspection is made of the prevalences observed in the other systems studied here, the peculiarities of the Aché stand out clearly. The figures displayed in Tables 3-5 are not completely independent, but in most cases the most extreme values were provided by the Aché. The Xavante also presented some extreme, but less numerous differences, while the Wai Wai conform more to the average. Similar results were obtained considering blood group plus protein and other nDNA and mtDNA systems (Callegari-Jacques et al., 1996; Battilana et al., 2001; Coimbra et al., 2001).

Also extremely interesting results are the very high values of the CYP1A1*2A and CYP1A1*2C alleles and the mostly low frequencies of the GSTM1*0/*0 and GSTT1*0/*0 genotypes. The highest frequency so far described in non-Amerindians for the first two markers are 33% (Japan; Aynacioglu et al., 1998) and 35% (Siberia; Duzhak et al., 2000), respectively; which are much lower than the 81%-100% and 54%-100% observed here. Our values, however, are similar to those observed in the Mapuche of Chile (respectively 83% and 77%; Muñoz et al., 1998). The GSTM1*0/*0 frequencies vary from 22% in Africa to 100% in Oceania, most of populations presenting values above 30% (review in Rebbeck, 1997). The values observed here (4%-43%) are in some cases lower, in agreement with a 20% frequency obtained previously among the Amazonian Parakanã Indians (Arruda et al., 1998). The

GSTT1*0/*0 frequency, on the other hand, is about 16% in Caucasians and above 38% in the other ethnic groups (Rebbeck, 1997), while the interval observed here was of 0%-30%. The Parakanã studied by Arruda et al. (1998) showed a prevalence of 11% of this genotype.

The enzymes codified by CYP1A1*2A and CYP1A1*2C alleles have higher catalytic activities than the products of the wild alleles, producing a larger amount of toxic metabolites which are mainly detoxified by both the GSTM1 and GSTT1 proteins (Indulski and Lutz, 2000). As the GSTM1 and GSTT1 null genotypes have no enzyme activity, toxic products induced by the action of the CYP enzymes could accumulate in these individuals.

It is possible therefore that CYP1A1*2A and CYP1A1*2C allele frequencies had increased during America's colonization just by genetic drift (Cavalli-Sforza et al., 1994), or by selection in response to new environmental challenges (Nebert, 1997). These high CYP values could have acted as selective factors reducing GSTM1*0/*0 and GSTT1*0/*0 frequencies, since the ratio between CYP and GST activities is critical to avoid the accumulation of toxic reactive intermediates (Rebbeck, 1997).

The origin of Aché population has been controversial, some authors considering them as a differentiated Guarani group, while others claim that they descend from a Gê group that preceded the Guarani colonization of Paraguay (Hill and Hurtado, 1996). The cluster analysis made here seems to support the second hypothesis, since the Aché showed a closer relationship with the Xavante, a Gê speaking population than with the four Tupi-Guarani groups included in the analysis. But they clearly differentiated from all the other populations studied, showing their genetic distinctiveness. Battilana et al. (2001), who examined the variability of 20 blood group plus protein systems and 12 Alu insertions in the same Aché, Xavante, and Guarani samples studied here, obtained also indications of the Aché distinctiveness, although in their data the closest group to cluster with the Aché was the Guarani. It is therefore possible that the Aché might have descended from a Gê group that had

first colonized Paraguay, but had also assimilated some amount of the Guarani gene pool, maybe through intertribal admixture. New data are needed to clarify this point.

The answers to the questions posed in the introduction, therefore, are: 1; Yes, the patterns of population relationships obtained here show distinctive features (but not the levels of genetic variability) which may in some way be due to selective process; 2. The unusual Amerindian CYP1A1, GSTM1 and GSTT1 frequencies have been confirmed; and 3. Due to the Aché genetic distinctiveness, their origin still remains an open question.

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LITERATURE CITED

Arruda VR, Grignolli CE, Gonçalves MS, Soares MC, Menezes R, Saad STO, Costa FF. 1998. Prevalence of homozygosity for the deleted alleles of glutahione S-transferase mu (GSTM1) and theta (GSTT1) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet 54:210-214.

Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Roots I. 1998. High frequency of CYP1A1 mutations in a Turkish population. Arch Toxicol 72:215-218.

Battilana J, Bonatto SL, Freitas LB, Hutz MH, Weimer TA, Callegari-Jacques SM, Batzer MA, Hill K, Hurtado AM, Tsuneto LT, Petzl-Erler LT, Salzano FM. 2001. Alu insertion vs blood group plus protein genetic variability in four Amerindian populations. Ann Hum Biol (in press).

Beckman G, Birgander R, Själander A, Saha N, Holmberg PA, Kivelä A, Bekman L. 1994. Is p53 polymorphism maintained by natural selection? Hum Hered 44:266-270.

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. 1993. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J Natl Cancer Inst 85:1159-1164.

Bogdawa HM, Hutz MH, Salzano FM, Weimer TA. 2000. Diversity of two short tandem repeat loci (CD4 and F13A1) in three Brazilian ethnic groups. Hum Biol 72:1045-1053.

Callegari-Jacques SM, Salzano FM, Weimer TA, Franco MHLP, Mestriner MA, Hutz MH, Schüler L. 1996. The Wai Wai Indians of South America: history and genetics. Ann Hum Biol 23:189-201.

Cascorbi I, Brockmöller J, Roots I. 1996. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res 56:4965-4969.

Cavalli-Sforza LL, Menozzi P, Piazza A. 1994. The history and geography of human genes. Princeton: Princeton University Press.

Coimbra CEAJr, Flowers NM, Salzano FM, Santos RV. 2001. The Xavante in transition: health, ecology and bioanthropology in Central Brazil. Ann Arbor: Michigan University Press (in press).

Duzhak T, Mitrofanov D, Ostashevskii V, Gutkina N, Chasovnikova O, Posukh O, Osipova L, Lyakhovich VV. 2000. Genetic polymorphisms of CYP2D6, CYP1A1, GSTM1 and p53 genes in a unique Siberian population of tundra. Pharmacogenetics 10:1-7.

Gahlinger PM, Abramson JH. 1995. Computer programs for epidemiologic analysis: PEPI. Stone Mountain: USD.

Gaspar PA, Hutz MH, Salzano FM, Weimer TA. 2001. TP53 polymorphisms and haplotypes in South Amerindians and neo-Brazilians. Ann Hum Biol 28:184-194.

Greenberg JH. 1987. Language in the Americas. Stanford: Stanford University Press.

Griese EU, Ilett KF, Kitteringham NR, Eichelbaum M, Powell H, Spargo RM, LeSouef PN, Musk AW, Minchin RF. 2001. Allele and genotype frequencies of polymorphic cytochromes P4502D6, 2C19 and 2E1 in Aborigines from Western Australia. Pharmacogenetics 11:69-76.

Guo S, Thompson E. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Hamada GS, Sugimura H, Suzuki I, Nagura K, Kiyokawa E, Iwase T, Tanaka M, Takahashi T, Watanable S, Kino I, Tsugane S. 1995. The heme-binding region polymorphism of cytochrome P450IA1 (CypIA1), rather than the RsaI polymorphism of IIE1 (CypIIE1), is associated with lung cancer in Rio de Janeiro. Cancer Epidemiol Biomarkers Prev 4:63-67.

Hayashi SI, Watanabe J, Kawajiri K. 1991a. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J Biochem 110:559-565.

Hayashi SI, Watanabe J, Nakachi K, Kawajiri K. 1991b. Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IAI. J Biochem 110:407-411.

Hedges SB. 1992. The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. Mol Biol Evol 9:366-369.

Hill K, Hurtado AM. 1996. Ache life history. The ecology and demography of a foraging people. New York: Aldine de Gruyter.

Hutz MH, Callegari-Jacques SM, Bortolini MC, Salzano FM. 1999. Variability in nDNA, mtDNA, and proteins: a test case. In: Papiha SS, Deka R, Chakraborty R, editors. Genomic diversity: applications in human population genetics. New York: Kluwer Academic / Plenum. p 23-32.

Indulski JA, Lutz W. 2000. Metabolic genotype in relation to individual susceptibility to environmental carcinogenesis. Int Arch Occup Environ Health 73:71-85.

Kato S, Shields PG, Caporaso NE, Hoover RN, Trump BF, Sugimura H, Weston A, Harris CC. 1992. Cytochrome P45IIEI genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res 57:6712-6715.

Khaliq S, Hameed A, Khaliq T, Aybur Q, Qamar R, Mohyuddin A, Mazhar K, Mehdi Q. 2000. p53 mutations, polymorphisms, and haplotypes in Pakistani ethnic groups and breast cancer patients. Genet Test 4:23-29.

Kvitko K, Nunes JCB, Weimer TA, Salzano FM, Hutz MH. 2000. Cytochrome P4501A1 polymorphisms in South American Indians. Hum Biol 72:1039-1043.

Lahiri DK, Nurnberger JI. 1991. A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444.

Lewontin RC. 1988. On measures of gametic disequilibrium. Genetics 120:849-852.

Long JC. 1999. Multiple locus haplotype analysis, version 2.0. Software and documentation distributed by the author. Bethesda, MD: Section on Population Genetics and Linkage, Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health.

Long JC, Williams RC, Urbanek M. 1995. An E-M algorithm and testing strategy for multiple locus haplotypes. Am J Hum Genet 56:799-810.

Miller AS, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Morita S, Yano M, Shiozaki H, Tsujinaka T, Ebisuti C, Morimoto T, Kishibuti M, Fujita J, Ogawa A, Taniguchi M, Inoue M, Tamura S, Yamazaki K, Kikkawa N, Mizunoya S, Monden M. 1997. CYP1A1, CYP2E1 and GSTM1 polymorphisms are not associated with susceptibility to squamous-cell carcinoma of the esophagus. Int J Cancer 71:192-195.

Munõz S, Vollrath V, Vallejos MP, Miquel JF, Covarrubias C, Raddatz A, Chianale J. 1998. Genetic polymorphisms of CYP2D6, CYP1A1 and CYP2E1 in the South-Amerindian population of Chile. Pharmacogenetics 8:343-351.

Nebert WN. 1997. Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? Am J Hum Genet 60:265-271.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Pemble S, Schroeder R, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. 1994. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300:271-276.

Peterson RJ, Goldman D, Long JC. 1999. Nucleotide sequence diversity in non-coding regions of ALDH2 as reveled by restriction enzyme and SSCP analysis. Hum Genet 104:177-187.

Rebbeck TR. 1997. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 6:733-743.

Rodrigues AD. 1986. Linguas brasileiras. Para o conhecimento das línguas indígenas. São Paulo: Loiola.

Roff DA, Bentzen P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Mol Biol Evol 6:539-545.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 6:539-545.

Sansom OJ, Clarke AR. 2000. P53 null mice: damaging the hypothesis? Mut Res 452:149-162.

Santos RV, Flowers NM, Coimbra Jr CEA, Gugelmin SA. 1996. Human ecology and health in the context of change: the Xavante Indians of Mato Grosso, Brazil. In: Follér ML, Hansson L, editors. Human ecology and health adaptation to a changing world. Göteborg University, Göteborg. p 94-117.

Schneider S, Kueffer JM, Roessli D, Excoffier L. 2000. Arlequin ver 2.000: A Software for Population Genetic Data Analysis. Geneva: Genetics and Biometry Laboratory, University of Geneva.

Själander A, Birgander R, Kivelä A, Beckman G. 1995. p53 polymorphisms and haplotypes in different ethnic groups. Hum Hered 45:144-149.

Själander A, Birgander R, Saha N, Beckman L, Beckman G. 1996. p53 polymorphisms and haplotype show distinct difference between major ethnic groups. Hum Hered 46:41-48.

Takezaki N. 1999. NJBAFD: neighbor-joining tree constructions from allele frequency data. ftp://ftp.nig.ac.jp/pub/Bio/njbafd/dos/

Tan W, Song N, Wang G, Liu Q, Tang H, Kadlubar FF, Lin D. 2000. Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferase M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. Cancer Epidemiol Biomarkers Prev 9:551-556.

Weston A, Pan C, Ksieski B, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, Brower S.T., Senie RT, Wolff MS. 1997. p53 haplotype determination on breast cancer. Cancer Epidemiol Biomarkers Prev 6:105-112.

TABLE 1. Amerindian groups investigated in the present study, their location and languages

Population	Country	Localities	Geographical coordinates	Language [1]	Linguistic group [1]	No. of individuals investigated
Xavante	Brazil	Etéñitépa	51°40'W, 13°20'S	Chavante	Gê-Kaingang	33
Guarani	Brazil	Amambai	55°12'W, 23° 6'S	Guarani	Tupi	51
		Limão Verde	55°6' W, 23°12' S			
		Porto Lindo	54°30'W, 23°48'S			
Aché	Paraguay	Arroyo Bandera	55°50'W, 23°30'S	Guayaki	Tupi	67
		Chupa-pou	56°30'W, 24°10'S			
Wai Wai	Brazil	Mapuera village	57°55'W, 0°40'S	Parukoto-Charumã	Carib	26
Zoró	Brazil	Aripuanã Park	60°20'W, 10°20'S	Mondé	Tupi	28
Surui	Brazil	Sete de Setembro	61°10'W, 10°50'S	Mondé	Tupi	21
Gavião	Brazil	Igarapé Lourdes	61°8'W, 10°10'S	Mondé	Tupi	31

[1] According to Rodrigues (1986), Greenberg (1987).

TABLE 2. Characterization of the CYP1A1, CYP2E1, GSTM1, GSTT1, and TP53 polymorphisms

Loci	Gene location	Allele or genotype	Phenotypes [1]	Mutation	Haplotypes [2]
CYP1A1 [3]	3' flanking	CYP1A1*2A	MspI +	6325T→C	1A: *1A-*1A
	Exon 7	CYP1A1*2C	BsrDI –	462A→G (Ile →Val)	2A: *2A-*1A
					2C: *1A-*2C
					2B: *2A-*2C
CYP2E1 [3]	5' flanking	PstI –; RsaI +	PstI –; RsaI +	- 1293 G; - 1053 C	CYP2E1*1A
		PstI +; RsaI –	PstI +; RsaI –	- 1293 G→C; - 1053 C→T	CYP2E1*5B
GSTM1	Whole gene	GSTM1*0/*0	No amplification	**Gene deletion**	-
GSTT1	Whole gene	GSTT1*0/*0	No amplification	**Gene deletion**	-
TP53	Intron 3	*A2	Duplication	16bp duplication	1-1-2: *A1-*A1-*A2
	Exon 4 (codon 72)	*A1	BstUI –	72C→G (Arg→Pro)	1-2-2: *A1-*A2-*A2
					1-2-1: *A1-*A2-*A1
	Intron 6	*A1	MspI –	A→G	2-1-1: *A2-*A1-*A1

[1] Plus and minus signs indicate the presence or absence of the indicated restriction site. [2] CYP1A1 haplotypes: 3' flanking-exon7; TP53 haplotypes: intron 3-exon 4-intron 6; only TP53 haplotypes observed in this study were indicated. [3] Allele nomenclature as recommended in http://www.imm.ki.se/CYPalleles.

TABLE 3. Genotype frequencies (in %) for the GST loci, allele frequencies (in %) for CYP and TP53, and genetic diversity values (x 100)

Characteristic	Populations						
	Xavante	Guarani	Aché	Wai Wai	Zoró	Surui	Gavião
Genotype or allele							
GSTM1*0/*0	18.2	3.9	35.8	26.9	14.3	43.0	12.9
GSTT1*0/*0	30.3	11.8	17.9	0	14.3	0	6.5
CYP1A1*2A [1]	95.0	96.1	100	81.0	87.0	96.0	72.0
CYP1A1*2C [1]	97.0	90.2	100	81.0	76.0	54.0	59.0
CYP2E1*5B	3.0	18.6	42.5	1.9	32.1	33.3	29.0
TP53 16bp*A1 [1]	100	100	100	100	100	100	98.1
TP53 BstUI*A1 [1]	28.0	8.8	36.6	11.9	6.8	25.0	19.2
TP53 MspI*A1 [1]	0	0	0	7.1	0	0	1.9
Sample size	33	51	67	26	28	21	31
Genetic diversity							
Average heterozygosity	24.4	18.8	34.7	24.4	29.8	38.2	32.4
Interpopulation variability	G_{ST} ± SE: 10.3 ± 1.2						

[1] Data from Kvitko et al. (2000) and Gaspar et al. (2001), except for the Aché and Guarani.

TABLE 4. Estimated CYP1A1 haplotype frequencies (%) and linkage disequilibrium (D') values

Populations [1]	Haplotypes				D'	p	2n
	1A	2A	2C	2B			
Xavante	3.0	3.0	-	94.0	1.00	*	42
Guarani	2.9	6.9	1.1	89.1	.72	*	102
Aché	-	-	-	100	-	-	134
Wai Wai	16.0	16.0	5.0	63.0	.60	*	52
Zoró	8.0	16.0	5.0	71.0	.50	*	60
Surui	4.0	42.0	-	54.0	1.00	NS	48
Gavião	27.0	14.0	-	59.0	1.00	*	60

[1] With the exception of the Aché and Guarani, the haplotype frequencies have been previously published by Kvitko et al. (2000); * = $p < 0.001$; NS = $p > 0.05$.

TABLE 5. Estimated TP53 haplotype frequencies (%) in seven South Amerindian populations

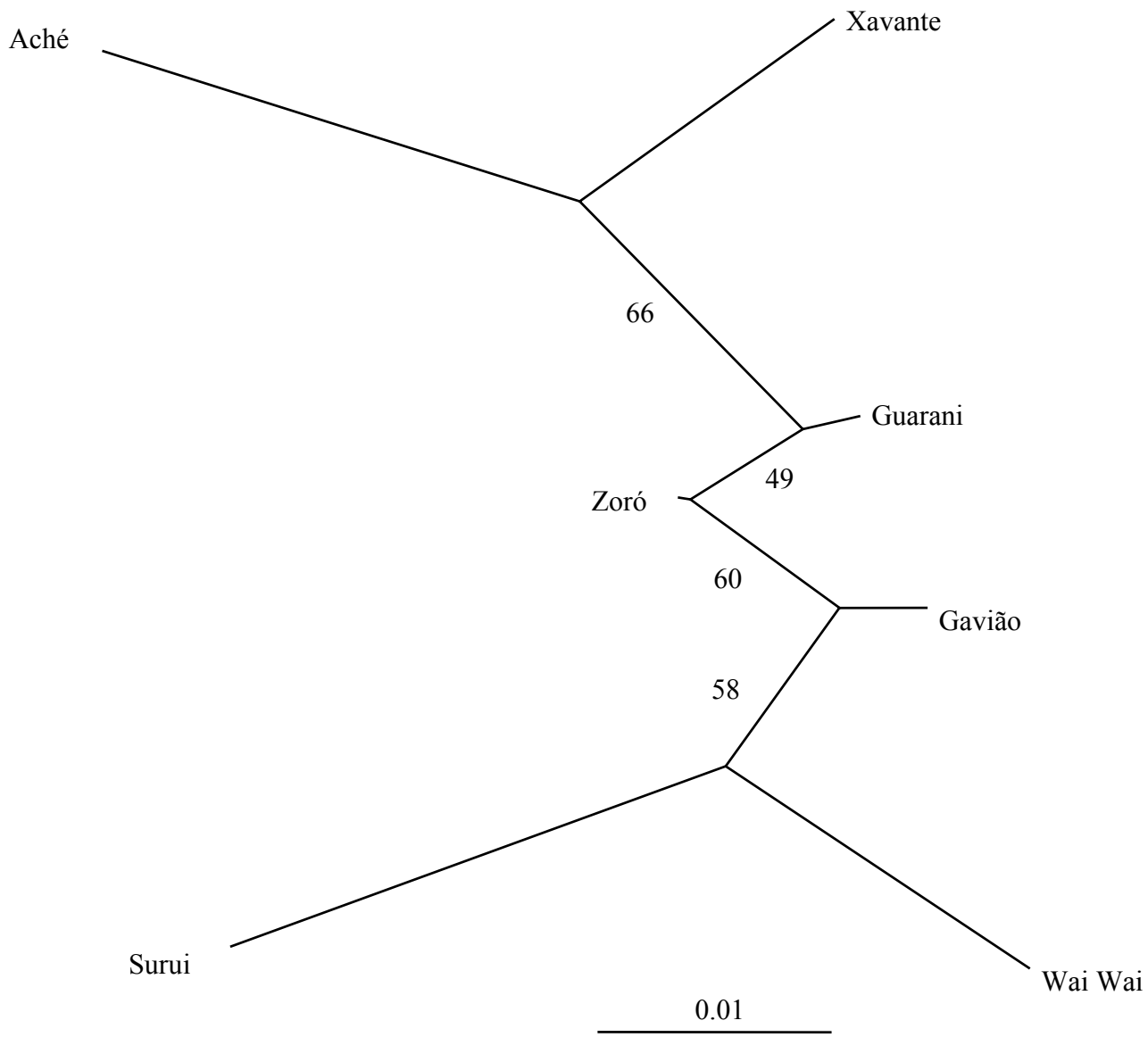
Populations	Haplotypes (16bp-BstUI-MspI) [1]				2n
	1-1-2	1-2-2	1-2-1	2-1-1	
Xavante	28.0	72.0	-	-	50
Guarani	7.8	92.2	-	-	102
Aché	38.1	61.9	-	-	134
Wai Wai	11.9	81.0	7.1	-	42
Zoró	6.8	93.2	-	-	44
Surui	25.0	75.0	-	-	40
Gavião	17.3	80.8	-	1.9	52

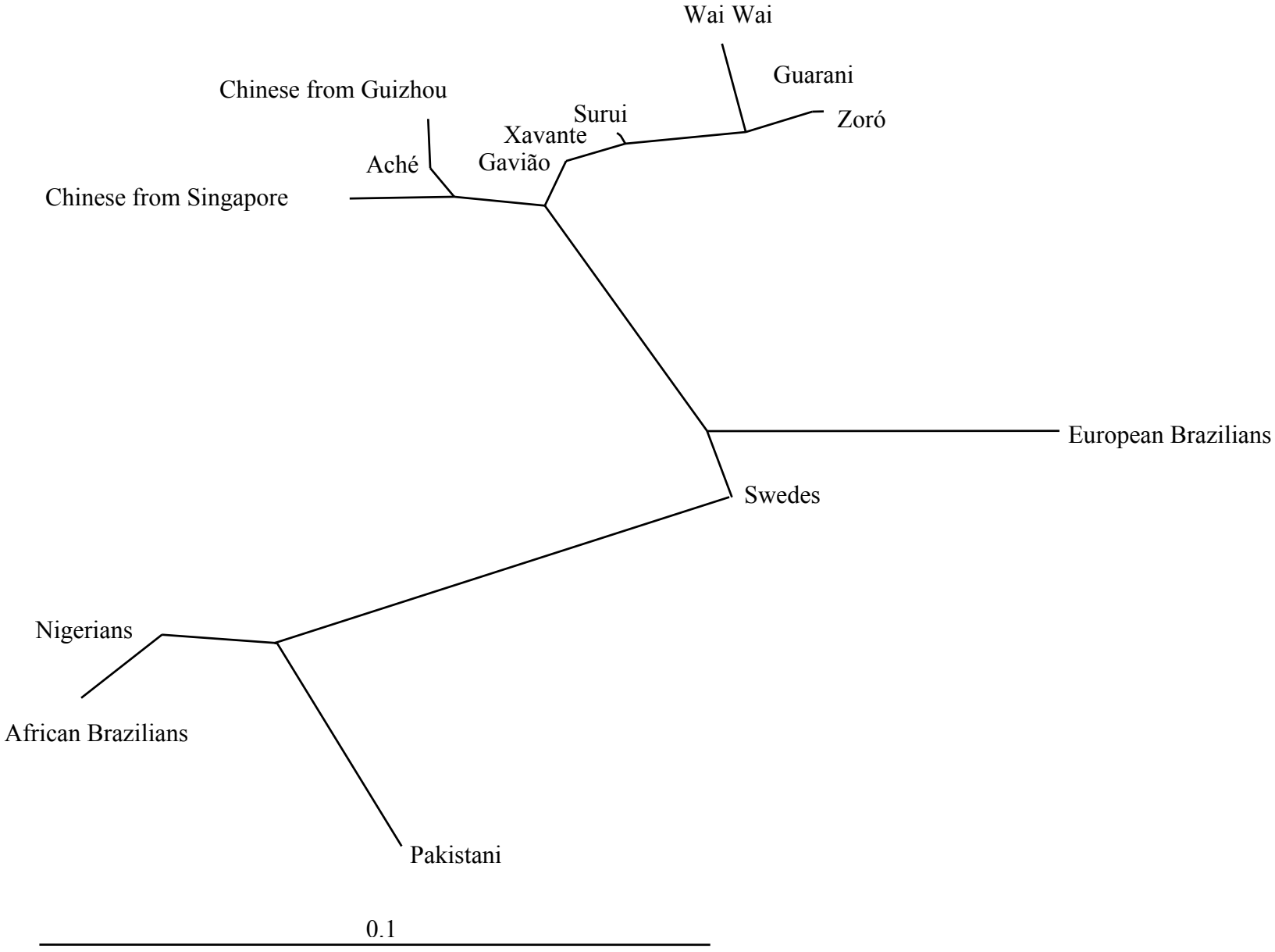
[1] Data from Gaspar et al. (2001), except for the Aché and Guarani.

Fig. 1. Geographic location of the four groups for which genetic data are reported here. Key to the population names: ACH: Aché; GAV: Gavião; GUA: Guarani; SUR: Surui; WWA: Wai Wai; XAV: Xavante; ZOR: Zoró.

Fig. 2. Dendrogram obtained using the neighbor-joining method and the D_A distance, considering the CYP1A1, CYP2E1 and TP53 haplotypes, as well as the GSTM1 and GSTT1 genotype frequencies. Numbers indicate bootstrap values.

Fig. 3. Dendrogram obtained using the neighbor-joining method and D_A distances, based on TP53 haplotype frequencies. The data from Nigerians, Chinese and Swedes were obtained from Själander et al. (1996); Pakistani, from Khaliq et al. (2000); African and European Brazilians, from Gaspar et al. (2001).


Aché
Xavante
66
Guarani
49
Zoró
60
Gavião
58
Surui
Wai Wai
0.01


Wai Wai
Guarani
Chinese from Guizhou
Surui
Zoró
Xavante
Aché
Gavião
Chinese from Singapore
European Brazilians
Swedes
Nigerians
African Brazilians
Pakistani
0.1

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*High CYP1A1*2C^{allele} frequency in Brazilian populations*

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Abstract

The genetic variability of the *CYP1A1 I462V* polymorphism was investigated in four Brazilian populations: three samples of African ancestry and one group of European descent. *CYP1A1* polymorphism was analyzed by two different procedures, i.e. the PCR-RFLP/*Bsr*DI method and the allele-specific PCR method. The frequency of *CYP1A1*2C* was 11% in Brazilians of European descent, which is slightly higher, but not statistically different from the values observed in European populations. In Brazilians of African ancestry this value was very high (12 to 15%). This allele was not observed in the only two African populations investigated thus far. Interethnic admixture with populations of European descent and/or Amerindian populations and genetic drift although possibly occurring, cannot explain by themselves only the high values observed here. Our findings suggested that the *CYP1A1*2C* allele may possibly be present in Africa, although restricted to some ethnic groups, not yet investigated. Environment factors of South American might also have acted as selective factors increasing *CYP1A1*2C* gene frequency. Our data also suggested that the *CYP1A1*2C* allele might possibly have originated in Africa.

Most human cancers are related to environmental exposure to genotoxic agents. The carcinogen biotransformation system contains two main classes of enzymes: phase I, which mediates oxidative metabolism, and phase II, which conjugates electrophilic substrates with glutathione (review in Indulski and Lutz 2000).

The *CYP1A1* gene is a component of the phase I cytochrome P450 superfamily. This family encodes aromatic hydrocarbon hydroxylase, an enzyme playing a role in the metabolism of polycyclic aromatic hydrocarbons (Nebert 1991), which are regarded as important environmental carcinogens (Šrám 1998).

Several polymorphisms have been described for this gene and have been associated with an elevated risk of cancer in some populations (Park et al. 1997; Ishibe et al. 1998). Among them, a polymorphic site at exon 7, codon 462, alters the protein structure by replacing an isoleucine with a valine (462V; Hayashi et al. 1991). The 462 Val allele has been reported to be associated with a higher risk for certain types of cancer (Cascorbi et al. 1996; Esteller et al. 1997; Garte 1998). However, in other populations no direct evidence supporting association with cancer susceptibility of this polymorphism was found (Morita et al. 1997; Marchand et al. 1998).

Such contradictory results may be due to, at least in part, ethnic differences in allele distributions (Garte 1998; Inoue et al. 2000). The *CYP1A1*Val* allele is present at higher frequency in Asians (from 14 to 35%; Zhao et al. 1995; Duzhak et al. 2000) and Amerindians (from 54 to 97%; Muñoz et al. 1998; Kvitko et al. 2000) and at low frequency in Europeans (from 2.8 to 5.8%; Cascorbi et al. 1996; Esteller et al. 1997). In the only two African populations so far investigated this allele was not detected (Garte et al. 1996; Garte 1998; Masimirembwa et al. 1998).

The determination of the distribution of this polymorphism in a larger number of populations can help us to understand the effect of this gene in cancer predisposition.

This investigation describes the distribution of the *CYP1A1*Val* allele [or *CYP1A1*2C*, according to the recommended nomenclature for the genetic polymorphisms in human P450 genes (http://www.imm.ki.se/CYPalleles)] in four Brazilian populations of African and European origin.

Subjects and Methods

Blood samples were obtained from three populations of African ancestry and one population of European descent, as follows:

1) The African-Brazilians come from:

a) Porto Alegre, the capital of the southernmost Brazilian state (30^{o} 5' S; 51^{o} 10' W) whose population is about 1,300,000 inhabitants 15% of them of African ancestry and 0.09% of Amerindian origin (www.ibge.gov.br). The samples were collected from ambulatory patients seen at two General Public Hospitals for prenatal or presurgical examinations (mean age, 38 years; 68% males).

b) Rio de Janeiro, the capital of the eastern state of Rio de Janeiro (22° 53' S, 43° 17' W) whose population is about 5,800,000 inhabitants 39% of them of African ancestry and 0.08% of Amerindian origin (www.ibge.gov.br). The samples were obtained from blood donors of the Hemoterapy Service at a Public University Hospital (mean age, 34 years; 100% males).

c) Salvador, the capital of the Brazilian northeastern state of Bahia (12^{o} 55' S; 38^{o} 29' W) whose population is about 2,400,000 inhabitants 79% of them of African ancestry and 0.16% of Amerindian origin (www.ibge.gov.br). The samples were obtained from newborn children at a Public Maternity Hospital and from blood donors at a Public University Hospital (mean age, 29 years; 86% males).

The distance between Porto Alegre and Salvador is about 3,000 Km, and Rio de Janeiro is situated between these cities (about 1,500 Km from each one).

2) The European-Brazilians are from Porto Alegre and the samples were obtained from unrelated adults who came to our laboratory for paternity investigations (mean age, 31 years; 51% males).

All of the subjects in this study, their relatives or legal tutors were adequately informed about the aims of this investigation and gave their approval to participate.

Genomic DNA was isolated from blood samples by a standard salting out procedure (Miller et al. 1988).

CYP1A1 polymorphism was analyzed by two methods: the samples of European and African descent from Porto Alegre were investigated (a) by the allele-specific methodology using the primers and PCR conditions indicated by Hayashi et al. (1991); and (b) reanalyzed by PCR before digestion with *Bsr*DI, using the primers and reaction conditions described by Cascorbi et al. (1996). All the other samples were tested only by the Cascorbi et al. (1996) methodology; according these authors, this is a more appropriate technique for the analysis of this *CYP1A1* polymorphism.

Allele frequencies were obtained by gene counting. Hardy-Weinberg equilibrium was evaluated for each sample by exact tests using the Markov Chain (Guo and Thompson 1992) and the Arlequin software, ver 2.0 (Schneider et al. 2000). Heterogeneity among populations was estimated by the Roff and Bentzen (1989) χ^2 test using the PEPI software (Gahlinger and Abramson 1995).

Results and Discussion

Table 1 presents the *CYP1A1*2C* distribution detected in Brazilian samples compared with data for other world populations. All Brazilian sample distributions were found to be in accordance to Hardy-Weinberg expectations.

The frequency of this allele in European-Brazilians was closely similar to that of another Brazilian population of European descent (Hamada et al. 1995), and slightly higher than those observed in Europeans (Hirvonen et al. 1992; Esteller et al. 1997), but the differences were not statistically significant.

All the three African-Brazilian samples here analyzed presented very high values of the *CYP1A1*2C* allele (12-15.7%). This was an unexpected result since this mutation had not been found in the only two African populations (Mali and Zimbabwe) so far investigated (Garte et al. 1996; Garte 1998; Masimirembwa et al. 1998). As already indicated in the Material and Methods section, the sample from Porto Alegre was analyzed by two methodologies and the same results were obtained. Therefore we conclude that methodological errors are not the reason for such high values.

One of the factors that might explain these results is interethnic admixture with European-Brazilians and/or Amerindians. However, although possibly present, it cannot explain by itself only the high frequency observed for the *CYP1A1*2C* allele for several reasons. The allele frequency detected in African-Brazilians was higher than that observed in European-Brazilians or in European samples. Although the frequency of this allele was high in Brazilian Indians (54-97%; Kvitko et al. 2000), admixture estimates indicated a low Amerindian contribution to the gene pool of African-Brazilians living in the cities here investigated [Rio de Janeiro (7%; Palatnik et al. 2000), Porto Alegre (0-7%; Bortolini et al. 1999), and Salvador (4-11%; Bortolini et al. 1999)]. Demography data also indicated low values of Amerindian descents in these populations (www.ibge.gov.br).

A second explanation could be genetic drift acting during the formation of Brazilian populations. However, we can not expected similar effects of drift in the three groups here analyzed, because they have been formed by different ethnic origins and through several migrations in different times between the XVIth and IXth centuries (Salzano 1986).

A third explanation is the possible occurrence of this allele in African populations who gave origin to Brazilian groups, but that have not been investigated for this gene. In fact, only two African populations have been investigated so far (Mali and Zimbabwe) and African groups are of highly heterogeneous ethnic origin. The Mali population is derived from five ethnic groups (Minianka, Malinke, Senoufe, Bambara and Peul; Garte et al. 1996; Garte 1998) whereas the Zimbabwe population is derived from Shona, Ndebele and Venda groups (Masimirembwa et al. 1998). The African-Brazilians descend from Africans who were forced to come to Brazil to work as slaves and have a highly heterogeneous ethnic background. Those from Salvador came mainly from Guinea's gulf whose population today is mainly composed of Yoruba, Jege, Hausa and Bantu groups (Souza Andrade 1988). Those from Rio de Janeiro and Porto Alegre are mainly descendants from Angola and Congo, areas of Bantu influence (Weimer 1991; Florentino 1997). Therefore, based on these data, we suggest that the *CYP1A1*2C* allele may have occurred in Africa before the slave traffic to South America, being, however, restricted to some ethnic groups.

It is important to point out that the frequency of *CYP1A1*2C* is also high in native South American populations (Muñoz et al. 1998; Kvitko et al. 2000). It is possible then that environmental factors of South America such as diet, parasitic diseases or exposure to different chemicals may also act as selective factors, increasing *CYP1A1*2C* frequencies in South American groups. If selection had been the main microevolutionary force acting in African Brazilian populations it would have had a very high value to change the gene frequencies in about 500 years only.

Garte et al. (1996) and Garte (1998), analyzing the world distribution of *CYP1A1*2C*, suggested that this allele may have originated after the split between Africans and non-Africans, and Duzhak et al (2000) found evidences for the origin of this mutation before the split of Caucasian and Oriental races. Our data suggested, however, an African origin for the

*CYP1A1*2C* allele. Therefore more analyses of this polymorphism in several populations are needed to clarify this point.

In addition to the differences in population frequencies, the *CYP1A1*2C* allele has been reported to be associated with colorectal, lung, endometrial, oral, and breast cancer in some populations, including those of Southeast Brazil (Sivaraman et al. 1994; Hamada et al. 1995; Esteller et al. 1997; Park et al. 1997; Ishibe et al. 1998). Cancer rates in Brazil are among the highest in the world (Parkin and Muir 1992). The high incidence of this disease in Brazil may therefore result from the high frequency of the *CYP1A1*2C* allele, although this possibility needs further investigation.

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Literature Cited

Bortolini, M.C., W.A. Silva Junior, D.C. Guerra et al. 1999. African-derived South American populations: a history of symmetrical and asymmetrical matings according to sex revealed by bi- and uni-parental genetic markers. *Am. J. Hum. Biol.* 11:551-563.

Cascorbi, I., J. Brockmöller, and I. Roots. 1996. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res.* 56:4965-4969.

Duzhak, T., D. Mitrofanov, V. Ostashevskii et al. 2000. Genetic polymorphisms of CYP2D6, CYP1A1, GSTM1 and p53 genes in a unique Siberian population of Tundra Nentsi. *Pharmacogenetics* 10:1-7.

Esteller, M., A. Garcia, J.M. Martínez-Palones et al. 1997. Susceptibility to endometrial cancer: influence of allelism at p53, glutathione S-transferase (GSTM1 and GSTT1) and cytochrome P-450 (CYP1A1) loci. *Br. J. Cancer* 75:1385-1388.

Florentino, M. 1997. *Em costas negras: uma história do tráfico de escravos entre a África e o Rio de Janeiro*. São Paulo, Brazil: Companhia das Letras.

Gahlinger, P.M., and J.H. Abramson. 1995. *Computer Programs for Epidemiologic Analysis: PEPI* (Stone Mountain: USD).

Garte, S. 1998. The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. *Carcinogenesis* 19:1329-1332.

Garte, S., J. Trachman, F. Crofts et al. 1996. Distribution of composite CYP1A1 genotypes in Africans, African-Americans and Caucasians. *Hum. Hered*. 46:121-127.

Guo, S., and E. Thompson. 1992. Performing the exact test of Hardy-Weimberg proportions for multiple alleles. *Biometrics* 48:361-372.

Hamada, G.S., H. Sugimura, I. Suzuki et al. 1995. The heme-binding region polymorphism of cytochrome P450IA1 (CYPIA1), rather than the RsaI polymorphism of IIE1 (CYPIIE1), is associated with lung cancer in Rio de Janeiro. *Cancer Epidemiol. Biomarkers Prev*. 4:63-67.

Hayashi, S., J. Watanabe, and K. Kawajiri. 1992. High susceptibility to lung cancer analyzed in terms of combined genotypes of P450IA1 and Mu-class Glutathione S-transferase genes. *Jpn. J. Cancer Res*. 83:866-870.

Hayashi, S., J. Watanabe, K. Nakachi et al. 1991. Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P4501A1 gene. *J. Biochem*. 110:407-411.

Hirvonen, A., K. Husgafvel-Pursiainen, A. Karjalainen et al. 1992. Point-mutational MspI and Ile-Val polymorphisms closely linked in the CYP1A1 gene: lack of association with susceptibility to lung cancer in a Finnish study population. *Cancer Epidemiol. Biomarkers Prev*. 1:485-489.

Indulski, J.A., and W. Lutz. 2000. Metabolic genotype in relation to individual susceptibility to environmental carcinogens. *Int. Arch. Occup. Environ. Health* 73:71-85.

Inoue, K., T. Asao, and T. Shimada. 2000. Ethnic-related differences in the frequency distribution of genetic polymorphisms in the CYP1A1 and CYP1B1 genes in Japanese and Caucasian populations. *Xenobiotica* 30:285-295.

Ishibe, N., S.E. Hankinson, G.A. Colditz et al. 1998. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer risk in the Nurses' health study. *Cancer Res.* 58:667-671.

Kim, K.S., S.W. Ryu, Y.J. Kim et al. 1999. Polymorphism analysis of the CYP1A1 locus in Koreans: presence of the solitary m2 allele. *Mol. Cells* 9:78-83.

Kvitko, K., J.C.D. Nunes, T.A. Weimer et al. 2000. Cytochrome P4501A1 polymorphisms in South American Indians. *Hum. Biol.* 72:1039-1043.

Marchand, L.L., L. Sivaraman, L. Pierce et al. 1998. Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. *Cancer Res.* 58:4858-4863.

Masimirembwa, C.M., C. Dandara, D.K. Sommers et al. 1998. Genetic polymorphism of cytochrome P4501A1, microsomal epoxide hydrolase, and glutathione S-transferase M1 and T1 in Zimbabweans and Venda of Southern Africa. *Pharmacogenetics* 8:83-85.

Miller, S.A., D.D. Dyres, and H.F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16:1215.

Morita, S., M. Yano, H. Shiozaki et al. 1997. CYP1A1, CYP2E1 and GSTM1 polymorphisms are not associated with susceptibility to squamous-cell carcinoma of the esophagus. *Int. J. Cancer* 71:192-195.

Muñoz, S., V. Vollrath, M.P. Vallejos et al. 1998. Genetic polymorphisms of CYP2D6, CYP1A1 and CYP2E1 in the South-Amerindian population of Chile. *Pharmacogenetics* 8:343-351.

Nebert, W.N. 1991. Role of genetics and drug metabolism in human cancer risk. *Mutat. Res.* 247:267-281.

Oyama, T., T. Mitsudomi, T. Kawamoto et al. 1995. Detection of CYP1A1 gene polymorphism using designed RFLP and distributions of CYP1A1 genotypes in Japanese. *Int. Arch. Occup. Environ. Health* 67:253-256.

Palatnik, M., W.A. Silva Jr, A.C. Estalote et al. 2000. Gene admixture and type 2 diabetes mellitus (type 2 DM) in Rio de Janeiro, Brazil. *Genet. Mol. Biol.* , 23, suppl 3:608.

Park, J.Y., J.E. Muscat, Q. Ren et al. 1997. CYP1A1 and GSTM1 polymorphisms and oral cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 6:791-797.

Parkin, D.M., and C.S. Muir. 1992. Cancer Incidence in Five Continents. Comparability and quality of data. *IARC Scient. Pub.* 120:45-173.

Roff, D.A., and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample. *Mol. Biol. Evol.* 6:539-545.

Salzano, F.M. 1986. Em busca das raizes. *Ciência Hoje* 5:48-53.

Schneider, S., D.S. Roessli, and L. Excoffier. 2000. *Arlequin: A software for population genetics data analysis*. Ver 2.000. Geneva: Genetics and Biometry Lab. University of Geneva.

Sivaraman, L., M.P. Leatham, J. Yee et al. 1994. CYP1A1 genetic polymorphisms and in situ colorectal cancer. *Cancer Res.* 54:3692-3695.

Souza Andrade, M.J.S. 1988. *A mão de obra escrava em Salvador, 1818/1860*. São Paulo, Brazil: Corrupio.

Šrám, R.J. 1998. Effect of glutathione S-transferase M1 polymorphisms on biomarkers of exposure and effects. *Environ. Health Perspect.* 106:231-239.

Sugimura, H., K. Wakai, K. Genka et al. 1998. Association of Ile462Val (Exon 7) polymorphism of cytochrome P450IAI with lung cancer in the Asian population: further evidence from a case-control study in Okinawa. *Cancer Epidemiol. Biomarkers Prev.* 7:413-417.

Taioli, E., J. Ford, J. Trachman et al. 1998. Lung cancer risk and CYP1A1 genotype in African Americans. *Carcinogenesis* 19:813-817.

Weimer, G. 1991. *O trabalho escravo no Rio Grande do Sul*. Porto Alegre, Brazil: UFRGS/Sagra.

Zhao, B., E.J. Lee, J.Y. Wong et al. 1995. Frequency of mutant CYP1A1, NAT2 and GSTM1 alleles in normal Indians and Malays. *Pharmacogenetics* 5:275-280.

Table 1. Distribution of *CYP1A1*2C* [1] in several populations (in percentage)

Populations	**2C*	Sample size	Method	Reference
Brazil (African-Brazilians)				
Porto Alegre	15.7	137	allele-specific/RFLP	This study
Rio de Janeiro	11.7	64	RFLP	This study
Rio de Janeiro	8.0	21	allele-specific	Hamada et al 1995
Salvador	15.0	30	RFLP	This study
Mali	0.0	116	allele-specific	Garte et al. 1996; Garte 1998
Zimbabwe	0.0	225	allele-specific	Masimirembwa et al. 1998
USA (African-Americans)	3.0	828	allele-specific	[2]
Brazil (European-Brazilians)				
Porto Alegre	11.6	86	allele-specific/RFLP	This study
Rio de Janeiro	8.8	87	allele-specific	Hamada et al. 1995
Europe	2.8-5.8	924	Allele-specific/RFLP	[3]

Table 1 (cont.)

Populations	**2C*	Sample size	Method	Reference
Australia	7.0	146	RFLP	Sugimura et al. 1998
USA (European-Americans)	6.7-9.0	532	Allele-specific/RFLP	[4]
Brazil (5 Amerindian tribes)	54.0-97.0	131	Allele-specific	Kvitko et al. 2000
Chile (Mapuche Indians)	77.0	84	Allele-specific	Muñoz et al. 1998
Siberia	35.0	102	Allele-specific	Duzhak et al. 2000
Japan	18.0-25.0	1013	Allele-specific/RFLP	[5]
China	14.0	39	RFLP	Sugimura et al. 1998
Korea	15.0	48	RFLP	Kim et al. 1999
Malaysia	31.0	146	Allele-specific	Zhao et al. 1995

[1] Allele nomenclature as recommended in http://www.imm.ki.se/CYPalleles; [2] Garte et al. (1996); Garte (1998); Taioli et al. (1998); [3] Hirvonen et al. (1992); Cascorbi et al. (1996); Esteller et al. (1997); [4] Garte et al. (1996); Inoue et al. (2000); [5] Hayashi et al. (1991, 1992); Oyama et al. (1995); Morita et al. (1997); Sugimura et al. (1998); Inoue et al. (2000).

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CYP1A1, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, and *TP53* polymorphisms: do they affect chronic obstructive pulmonary disease and non-small-cell lung cancer susceptibility?

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Keywords: CYPs, GSTs and TP53 polymorphisms; Chronic obstructive pulmonary disease; Non-small-cell lung cancer; Genetic susceptibility.

Abstract

Gene polymorphisms of phase I (*CYP1A1* and *CYP2E1*) and phase II (*GSTM1*, *GSTT1*, and *GSTP1*) enzymes, as well as the *TP53* tumour suppressor gene were studied in 258 Brazilian subjects of European descent, 97 patients with non-small-cell lung cancer (NSCLC), 75 patients with chronic obstructive pulmonary disease (COPD), and 86 controls. No effect of these markers was verified on NSCLC susceptibility. With respect to COPD, the distribution of *CYP1A1*, *GSTP1* and *TP53* genotypes was similar to that of the controls. However, heterozygous *CYP2E1*1A/*5B* was about 4 times more frequent in COPD than in controls [OR= 4.2 (1.1-24.8)] and the *GSTT1* null phenotype was twice more common in the COPD group [OR= 2.5 (1.2-4.9)]. The effect of the *GSTT1* null phenotype on COPD susceptibility seems to be increased about 4 times due to its interaction with *CYP1A1*1A/*2A* [(OR= 3.7 (1.1-14.6)], and *GSTP1Ile/Val* genotypes [OR= 4.0 (1.2-14.6)]. These results suggest that *GSTT1, GSTP, CYP2E1, CYP1A1* polymorphisms may be predictive of COPD susceptibility, at least in this population of European ancestry.

1. Introduction

Chronic obstructive pulmonary diseases (COPD) and non-small-cell lung cancer (NSCLC) are directly associated with cigarette smoking. However, only a small proportion of smokers develop these diseases [1]. Susceptibility to cigarette smoke might be associated with genetic variability of the genes involved in chemical carcinogen metabolism. A large fraction of these compounds is biotransformed to more toxic metabolites (by phase I activation enzymes) or to non-toxic compounds (by phase II detoxification enzymes). The rate of these competing metabolic pathways is an important determinant of DNA damage [2]. However, the cellular response to DNA damages is mediated by another group of genes, i.e., the tumour suppressor genes [3].

Many enzymes involved in either phase I or phase II carcinogen metabolism are polymorphically expressed, with the alleles presenting different enzymatic activities and some of them have been associated with cancer susceptibility [2].

Among the tumour suppressor genes, *TP53* has a key and potent role in the cellular response to DNA damage [4]. An unusual spectrum of *TP53* mutations resulting in loss or disruption of tumour suppressor function has been described in several human cancer tissues [4], and some *TP53* polymorphisms seem also to be related to cancer susceptibility [3].

The environmental exposure to cigarette carcinogens associated with genetic variants of tumour suppressor genes and/or with genes of phase I or phase II enzymes has been hypothesised to pose a differential risk of lung cancer or COPD development [2,3,5]. However the effect of these polymorphisms on lung diseases is far from consensual [6].

This study analysed seven genetic polymorphisms of phase I (*CYP1A1*, *CYP2E1)*, phase II (*GSTM1*, *GSTT1*, *GSTP1)*, and *TP53* tumour suppressor genes in COPD and NSCLC patients and in a control sample of European origin, in order to evaluate the role of these genetic markers in the prediction of susceptibility to these pulmonary pathologies.

2. Subjects and Methods

A total of 258 European Brazilian subjects were investigated: 97 patients with NSCLC, 75 individuals with COPD, and 86 controls. Only previously untreated NSCLC subjects with a cancer diagnosis confirmed by histology (according to WHO guidelines [7]) were analysed. The COPD group consisted of individuals, whose diagnosis was confirmed by pulmonary function tests and radiography according to the guidelines of the European Respiratory Society [8]. Patient blood samples were collected from August 1998 to July 2001 at a general hospital (Santa Casa de Misericórdia de Porto Alegre, RS, Brazil). Information about patient smoking habits was obtained and quantified as previously described [9]. The hospital ethics committee approved this investigation, and the subjects were previously informed about this research and signed an informed consent sheet.

The control group consisted of adults who came to our laboratory for paternity tests. This sample is representative of the Porto Alegre population (whose total size is 1.360.033 inhabitants) in terms of sex and age distribution (http://www.ibge.gov.br/). No data about smoking habits or health conditions were obtained for these individuals. Data about *TP53* polymorphism and *CYP1A1*2C* allele distribution in this sample have been previously described [10,11].

Genomic DNA was isolated from whole blood by the salting out method [12]. Seven polymorphic markers were analysed by PCR-based methods (Table 1). Genotyping of *CYP* and *TP53* polymorphisms was performed according to the references indicated in Table 1 and *GSTM1, GSTT1* and *GSTP1* markers were tested using the primers described in the references in Table 1 and the reaction conditions used by Gaspar et al. 2001 (A. J. Physical. Anthropol., submitted).

Gene frequencies were estimated by gene counting and Hardy-Weinberg equilibrium was evaluated by the χ^2 test for goodness of fit adjusted for small samples when appropriate.

Heterogeneity between groups was estimated by the Mann-Whitney or Fisher test and the odds ratio (OR) with 95% confidence interval (CI) was employed to verify possible effects of genetic markers on lung diseases. The tests were carried out for every independent locus and considering all combinations of two simultaneous loci. All statistical analyses were performed using the PEPI computer program, V. 2.0 [19].

3. Results

Table 2 presents data about age and gender of patient and control subjects as well as patient smoking status and cancer histology classification. No difference was observed between the NSCLC and COPD groups in relation to smoking status, mean age or sex distribution. However, the frequency of males was higher in NSCLC and COPD patients ($P<0.001$) and they were older than the controls ($P<0.001$). With respect to tumour histology, about 50% of NSCLC patients presented adenocarcinoma, while the others had squamous cell carcinoma.

The genotype distributions and allele frequencies are presented in Table 3. No deviation from Hardy-Weinberg expectations was verified for each polymorphism or sample group. Genotype and allele distributions were similar for males and females in all groups (controls, NSCLC and COPD patients).

The frequencies of *TP53*Pro*, *CYP1A1*2A*, *CYP1A1*2C*, *CYP2E1*5B,* and *GSTP1*Val* and of *GSTM1* null and *GSTT1* null phenotypes in the control sample were similar to those verified for European populations [13,18,20-22].

No significant differences in genotype distribution were detected between controls and NSCLC patients, considering the sample as a whole, the histological cell type groups, and smoking status.

Genotype distributions for *CYP1A1*, *GSTP1* and *TP53*, were similar among COPD patients, but the frequency of heterozygous *CYP2E1*1A/*5B* was about 4 times higher in

COPD patients than in controls [OR= 4.2 (1.1-24.8)] and the *GSTT1* null phenotype was twice more frequent in the COPD group [OR= 2.5 (1.2-4.9)].

The analyses considering two loci indicated two significant associations, both involving the *GSTT1* null phenotype (Table 4). Subjects who presented simultaneously the *GSTT1* null phenotype and *GSTP1 Ile/Val* genotype as well as the *GSTT1* null and *CYP1A1*1A/*2A* genotypes had a risk about four times higher of having COPD [OR= 4.0 (1.2-14.6) and OR= 3.7 (1.1-14.6), respectively].

5. Discussion

The differences observed in age and sex distribution between controls and patients were in fact expected since both lung cancer and COPD are generally associated with older ages [1]. Besides the control sample being a representative sample of Porto Alegre population in relation to sex and age distribution (http://www.ibge.gov.br/), no effect of age or sex was detected on the genotype distributions and no influence of ageing was verified for these markers [23-25]. In addition, the allele and genotype frequencies observed in the control sample for all markers were similar to those verified for populations of European origin [13,18,20-22].

Previous data about the effect of *TP53*, *CYP* or *GST* markers on lung cancer are conflicting. However, in a general way, no associations have been described for European populations, while positive results have been obtained for Asian groups. These differences are probably due to ethnic and/or environmental heterogeneity as well as to gene/environment and gene/gene interactions [3,5,6,20,29].

Data about COPD are scarce and as far as we know this is the first investigation reporting *CYP1A1*, *CYP2E1*, *GSTT1*, *GSTP1* and *TP53* gene polymorphisms in COPD

patients in populations of European origin. Studies analysing these genes and COPD risk have been previously performed in Asian populations [5,26].

Our data suggest that *CYP2E1*1A/*5B* genotype and *GSTT1* null phenotype could be predictive of COPD susceptibility, the effect of *GSTT1* null phenotype being increased by its interaction with the *CYP1A1-Msp*I or *GSTP1* loci. These associations are clearly explained by the biological role of these enzymes: phase I enzymes (CYP1A1, CYP2E1) activate procarcinogens to highly reactive intermediates, with the enzyme generated by *CYP1A1*2A* and *CYP2E1*5B* alleles having higher activity on some toxic compounds than the wild allele [27,28]. The oxygen reactive species generated by phase I enzymes are converted to inactive derivatives by phase II enzymes (GSTT1 and GSTP1*)*, with the *GSTT1* null phenotype and *GSTP1*Val* allele presenting absent and lower activity [2,20,29].

The interaction of two phase II deficient enzymes (GSTT1 null and GSTP1 Val) or a phase I hyperactive enzyme (CYP1A1 2A) and a phase II absent enzyme (GSTT1 null) results in a larger amount of toxic compounds that might have a role in the initiation or progression of COPD [20,29-31].

COPD is a complex disease in which multiple loci are involved and only the joint analysis of several markers could provide new clues for prediction of its occurrence. The associations verified here denote the importance of the genetic variation of phase I and phase II enzyme genes for susceptibility. However more data about other populations are needed to confirm these findings before these polymorphisms can be used as predictive factors of COPD risk.

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References

[1] J.F. Murray, J.A. Nadel, Textbook of respiratory medicine. 2nd ed. Philadelphia: Saunders Company, 1994 pp.1332-1535.

[2] H. Autrup, Genetic polymorphisms in human xenobiotica metabolising enzymes as susceptibility factors in toxic response. Mut. Res. 464 (2000) 65-76.

[3] M. Murata, M. Tagawa, K. Kimura, K. Kakisawa, H. Shirasawa, T. Fujisawa, Correlation of the mutation of p53 gene and the polymorphism at codon 72 in smoking-related non-small cell lung cancer patients. Int. J. Cancer 12 (1998) 577-581.

[4] W.P. Bennett, P. Hussain, K.H. Vahakangas, M.A. Khan, P.G. Shields, C.C. Harries, Molecular epidemiology of human cancer risk: gene-environment interactions and *p53* mutation spectrum in human lung cancer. J. Pathol. 187 (1999) 8-18.

[5] T. Ishii, T. Matsuse, S. Teramoto, H. Matsui, Miyao, M. H. Takahashi, Y. Fukuchi, Y. Ouchi, Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease. Thorax 54 (1999) 693-696.

[6] H. Bartsch, U. Nair, A. Risch, M. Rojas, H. Wilkman, K. Alexandrov, Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiol. Biomarkers Prev. 9 (2000) 3-28.

[7] WHO. Histological typing of lung tumors. Am. J. Clin. Pathol. 77 (1982) 123-126.

[8] N.M. Siafakas, P. Vermeire, N.B. Pride, P. Paoletti, J. Gibson, P. Howard, J.C. Yernault, M. Decramer, T. Higenbottam, D.S. Postma, et al, Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. Eur. Respir. J. 8 (1995) 1398-420

[9] H. Sugimura, G.S. Hamada, I. Suzuki, T. Iwase, E. Kiyokawa, I. Kino, S. Tsugane, CYP1A1 and CYP2E1 polymorphism and lung cancer: case-control study in Rio de Janeiro, Brazil. Pharmacogenetics 5 (1995) S145-S148.

[10] P.A. Gaspar, M.H. Hutz, F.M. Salzano, T.A. Weimer, TP53 polymorphisms and haplotypes in South Amerindians and neo-Brazilians. Ann. Hum. Biol. 28 (2001) 184-194.

[11] P.A. Gaspar, K. Kvitko, L.G. Papadópolis, M.H. Hutz, T.A. Weimer, High CYP1A1*2C^{allele} frequency in Brazilian populations. Hum Biol (2001, in press).

[11] A.S. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1995 16 (1995) 1215.

[13] I. Cascorbi, J. Brockmöller, I. Roots, A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res. 56 (1996) 4965-4969.

[14] S. Hayashi, J. Watanable, K. Nakachi, K. Kawajiri, Genetic linkage of lung cancer-associated MspI polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. J. Biochem.110 (1991) 407-411.

[15] S. Kato, P.G. Shields, N.E. Caporoso, R.N. Hoover, B.F. Trump, H. Sugimura, A. Weston, C.C. Harries, Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res. 52 (1992) 6712-6715.

[16] D.A. Bell, J.A. Taylor, D.F. Paulson, C.N. Robertson, J.L. Mohler, G.W. Lucier, Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene gluthatione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. J. Natl. Cancer Inst. 85 (1993) 1159-1164.

[17] S. Pemble, K.R. Schroeder, S.R. Spencer, D.J. Meyer, E. Hallier, H.M. Bolt, B. Ketterer, J.B. Taylor, Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem. J. 300 (1994) 271-276.

[18] L.W. Harries, M.J Stubbins, D. Forman, G.C.W. Howard, C.R. Wolf, Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 18 (1991) 641-644.

[19] P.M. Gahlinger, J.H. Abramson, Computer Programs for Epidemiologic Analysis: PEPI (Stone Mountain: USD).

[20] T.R. Rebbeck, Molecular epidemiology of the human glutahione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol. Biomarkers Prev. 6 (1997) 733-743.

[21] A. Rannug, A.K. Alexandrie, I. Persson, M. Ingelman-Sundberg, Genetic polymorphism of cytochromes P450 1A1, 2D6 and 2E1: regulation and toxicological significance. J. Occup. Environ. Med. 37 (1995) 25-36.

[22] S. Aynacioglu, I. Cascorbi, P.M. Mrozikiewicz, I. Roots, High frequency of CYP1A1 mutations in a Turkish population. Arch. Toxicol. 72 (1998) 215-218.

[23] M.L. Muiras, P. Verasdonck, F. Cottet, F. Schächter, Lack of association between human longevity and genetic polymorphism in drug-metabolising enzymes at the NAT2, GSTM1 and CYP2D6 loci. Hum. Genet. 102 (1998) 526-532.

[24] J.A. Agundez, I. Rodrigues, M. Oliveira, J.M. Ladero, M.A. Garcia, J.M. Ribera, J. Benitez, CYP2D6, NAT2 and CYP2E1 genetic polymorphisms in nonagenarians. Age Ageing 26 (1997) 147-151.

[25] M. Bonafè, F. Olivier, D. Mari, G. Baggio, R. Mattace, M. Berardelli, P. Sansoni, G. Benedictis, M. Luca, F. Marchegiani, L. Cavallone, M. Cardelli, S. Giovagnetti, L. Ferrucci, L. Amadio, R. Lisa, M.G. Tucci, L. Troiano, G. Pini, P. Gueresi, M. Morellini, S. Sorbi, G. Psaaeri, C. Barbi, S. Valensin, D. Monti, L. Deiana, G.M. Pes, C. Carru, C. Franceschi, p53 codon 72 polymorphism and longevity: additional data on centenarians from continental Italy and Sardinia. Am. J. Hum. Genet. 65 (1999) 1782-1785.

[26] J.J. Yim, G.Y. Park, C.T. Lee, Y.W. Kim, S.K. Han, Y.S. Shim, C.G. Yoo, Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. Thorax 55 (2000) 121-124.

[27] M.T. Landi, P.A. Bertazzi, P.G. Shields, G. Clark, G.W. Lucier, S.J. Garte, G. Cosma, N.E. Caporaso, Association between genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics 4 (1994) 242-246.

[28] J. Watanable, S. Hayashi, K. Kawajiri, Different regulation and expression of the human CYP2E1 gene due to the RsaI polymorphism in the 5' – flanking region. J. Biochem. 116 (1994) 321-326.

[29] J.A. Indulski, W. Lutz, Metabolic genotype in relation to individual susceptibility to environmental carcinogens. Int. Arch. Occup. Environ. Health 73 (2000) 71-85.

[30] M.A Watson, R.K. Stewart, G.B.J. Smith, T.E. Massey, D.A. Bell, Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19 (1998) 275-280.

[31] R.Walter, D.J. Gottlieb, G.T. O'Connor, Environmental and genetic risk factors and gene-environmental interactions in the pathogenesis of chronic obstructive lung disease. Environ Health Perspect 108 (2000) (supll 4) 733-742.

Table 1. *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1* and *TP53* polymorphisms investigated in this study

Loci	*Gene location*	Allele or genotype	Phenotype [a]	Method	Reference
CYP1A1 [b] *(*MspI*)*	*3' flanking*	CYP1A1*2A	Msp*I* +	PCR-RFLP	[13,14]
*(*Ile/Val*)*	Exon 7	CYP1A1*2C	*Bsr*DI – (*Val*)	PCR-RFLP	
CYP2E1 [b]	5' flanking	*CYP2E1*1A*	*Pst*I –; *Rsa*I +	PCR-RFLP	[15]
		*CYP2E1*5B*	*Pst*I +; *Rsa*I –		
GSTM1 [c]	Whole gene	*GSTM1* (–)	No amplification	Multiplex-PCR	[16]
GSTT1 [c]	Whole gene	*GSTT1* (–)	No amplification	Multiplex-PCR	[17]
GSTP1	Exon 5	*Ile	*Bsma*I –	Multiplex-PCR	[18]
		*Val	*Bsma*I +		
TP53	Exon 4	**Arg*	*Bst*UI +	PCR-RFLP	[10]
		**Pro*	*Bst*UI –		

[a] Plus and minus signs indicate the presence or absence of the restriction site; [b] Allele nomenclature as recommended in http://www.imm.ki.se/CYPalleles; [c] *GSTM1* (–) and *GSTT1* (–) indicate gene deletion.

Table 2. Main characteristics of the sample

Variable	NSCLC (n = 97)	COPD (n = 75)	Controls (n = 86)
Age (years)			
Mean (SD) [1]	65.3 (8.6)	64.3 (11.0)	31.8 (9.5)
Range	43-81	41-87	16-59
Gender, n (%) [2]			
Male	67 (69.1)	61 (81.3)	47 (52.2)
Female	30 (30.9)	14 (18.7)	43 (47.8)
Tumour histology (%)			
Adenocarcinoma	49 (50.5)	–	–
Squamous cell carcinoma	48 (49.5)	–	–
Smoking status, n (%)			
≤ 40 pack-years [3]	49 (50.5)	30 (40.0)	–
> 40 pack-years [3]	48 (49.5)	45 (60.0)	–
Mean years smoked (SD) [4]	52.7 (36.9)	39.2 (12.6)	–
Mean pack-years (SD) [4]	57.7 (36.9)	60.8 (39.8)	–

[1] Mann-Whitney test, NSCLC x Controls (C) and COPD x C: $P<0.001$; NSCLC x COPD: not significant (ns). [2] Fisher's exact test, NSCLC x C and COPD x C: $P<0.05$; NSCLC x COPD: ns. [3] Fisher's exact test, NSCLC x COPD: ns. [4] Mann-Whitney test, NSCLC x COPD: ns.

Table 3. *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, and *TP53* genotype (%) and allele frequencies

Genotype and allele frequencies (in bold)	NSCLC n = 97	NSCLC vs C OR (95% CI)	Controls (C) n = 86	COPD vs C OR (95% CI)	COPD n = 75
CYP1A1 (MspI)					
**1A/*1A*	67.0		72.1		65.3
**1A/*2A*	28.9	1.4 (0.7-3.0)	22.1	1.6 (0.7-3.5)	33.3
**2A/*2A*	4.1	0.7 (0.02-3.8)	5.8	0.3 (0.05-2.5)	1.3
CYP1A1*2A	**.185**		**.169**		**.180**
CYP1A1 (Ile/Val)					
**1A/*1A*	72.2		80.2		78.7
**1A/*2C*	24.7	1.7 (0.8-4.0)	16.3	1.3 (0.5-3.2)	21.3
**2C/*2C*	3.1	1.0 (0.1-8.0)	3.5	–	–
CYP1A1*2C	**.155**		**.116**		**.107**
CYP2E1					
*1A/*1A	90.7		96.5		86.7
**1A/*5B*	9.3	2.1 (0.6-9.6)	3.5	4.2 (1.1-24.8) *	13.3
CYP2E1*5B	**.046**		**.017**		**.067**
GSTM1					
Null	51.5	1.1 (0.6-1.9)	50.0	0.6 (0.3-1.1)	37.3
GSTT1					
Null	22.7	1.1 (0.5-2.2)	22.1	2.5 (1.2-4.9) *	40.0
GSTP1					
Ile/Ile	42.3		52.3		46.7
Ile/Val	46.4	1.4 (0.7-2.7)	40.7	1.3 (0.6-2.6)	46.7
Val/Val	11.3	1.8 (0.6-6.0)	7.0	1.0 (0.2-4.6)	6.6
GSTP1*Val	**.345**		**.273**		**.300**
TP53					
Arg/Arg	48.5		43.0		57.3
Arg/Pro	39.2	0.7 (0.4-1.2)	51.2	0.6 (0.3-1.2)	38.7
Pro/Pro	12.4	1.9 (0.5-7.0)	5.8	0.5 (0.1-2.8)	4.0
TP53*Pro	**.320**		**.314**		**.233**

* Significant associations - CYP2E1*1A/*5B, COPD vs C, P = 0.044; GSTT1 null, COPD vs C, P = 0.022.

Table 4. Combined loci effects comparing COPD and controls (%)

Combined loci		COPD n = 75	*Controls* n = 86	OR (95% CI)
GSTP1	*GSTT1*[1]			
Ile/Ile	(+)	28.0	39.5	
	(–)	19.0	12.8	2.0 (0.7-6.0)
Ile/Val	(+)	27.0	33.7	1.1 (0.5-2.7)
	(–)	20.0	7.0	4.0 (1.2-14.6) *
Val/Val	(+)	5.0	4.7	1.6 (0.3-9.7)
	(–)	1.0	2.3	0.8 (0.01-16.5)
CYP1A1 *(*MspI*)*				
**1A/*1A*	(+)	41.3	55.8	
	(–)	24.0	16.3	2.1 (0.8-5.2)
**1A/*2A*	(+)	17.3	16.3	1.1 (0.4-3.1)
	(–)	16.1	5.8	3.7 (1.1-14.6) *
**2A/*2A*	(+)	1.3	5.8	0.3 (0.006-3.0)

[1] *GSTT1* (+): *GSTT1* wild type; *GSTT1* (–): *GSTT1* null phenotype.

- Significant associations: *GSTT1* (–) & *GSTP1Ile/Val*, P = 0.019; *GSTT1* (–) & *CYP1A1*1A/*2A*, P = 0.036.

Table 4. Combined loci effects comparing COPD and controls (%)

Combined loci		COPD n = 75	*Controls* n = 86	OR (95% CI)
GSTP1	*GSTT1*[1]			
Ile/Ile	(+)	28.0	39.5	
	(–)	19.0	12.8	2.0 (0.7-6.0)
Ile/Val	(+)	27.0	33.7	1.1 (0.5-2.7)
	(–)	20.0	7.0	4.0 (1.2-14.6) *
Val/Val	(+)	5.0	4.7	1.6 (0.3-9.7)
	(–)	1.0	2.3	0.8 (0.01-16.5)
CYP1A1 *(*MspI*)*				
**1A/*1A*	(+)	41.3	55.8	
	(–)	24.0	16.3	2.1 (0.8-5.2)
**1A/*2A*	(+)	17.3	16.3	1.1 (0.4-3.1)
	(–)	16.1	5.8	3.7 (1.1-14.6) *
**2A/*2A*	(+)	1.3	5.8	0.3 (0.006-3.0)

[1] *GSTT1* (+): *GSTT1* wild type; *GSTT1* (–): *GSTT1* null phenotype.

- Significant associations: *GSTT1* (–) & *GSTP1Ile*/*Val*, P = 0.019; *GSTT1* (–) & *CYP1A1*1A/*2A*, P = 0.036.

IV. Discussão

Os estudos envolvendo a distribuição dos polimorfismos de *CYPs* e *GSTs* em neo-brasileiros e ameríndios são poucos e recentes (Arruda *et al.* 1998, Gattas & Soares-Vieira 2000, Kvitko *et al.* 2000, Muñoz *et al.* 1998) e não há informações sobre marcadores do *TP53* nestas populações. Além disso, até a presente data, esta é a primeira investigação a analisar simultaneamente locos codificadores de enzimas detoxificadoras de fase I (CYP) e de fase II (GST) e da proteína supressora tumoral TP53 em uma mesma população.

Neste estudo verificaram-se, em afro-brasileiros, freqüências de *CYP1A1*2C* (12–16%) muito mais altas que as observadas em outras populações negras ou miscigenadas (0–8%; Garte *et al.* 1996, Hamada *et al.* 1995, Masimirembwa *et al.* 1998). O produto da enzima de fase I *CYP1A1*2C* apresenta maior atividade (Kawajiri *et al.* 1993, Kyohara *et al.* 1996), com a formação de maior quantidade de produtos tóxicos (Nebert & Roe 2001, Rebbeck 1997). Dados sobre a incidência de doenças pulmonares ambientais em populações afro-brasileiras são escassos, mas pode-se especular que devido as altas freqüências deste alelo tais populações poderiam ter maior predisposição à patologias ambientais.

Nos ameríndios observaram-se freqüências muito elevados de *CYP1A1*2A* (96–100%) e *CYP1A1*2C* (90–100%) e mais baixos das deleções de *GSTM1* (4–43%) e *GSTT1* (0–30%). Segundo Landi (2000), Lewis *et al.* (1998), Xu *et al.* (1998) estes marcadores seriam neutros, mas é possível que os diferentes produtos destes alelos tenham tido um papel adaptativo, possibilitando a metabolização diferencial de compostos ambientais de origem natural (Lang & Pelkonen 1999, Miller *et al.* 2001).

Dados sobre populações ameríndias indicam que elas vem sofrendo, gradativamente, um processo de aculturação que resulta em mudança das suas características socioeconômicas, culturais e ecológicas tradicionais (Coimbra Jr. *et al.* 1994, 2001, Ribas *et al.* 2001, Santos *et al.* 1996). Está aumentando o consumo de sal, de gorduras saturadas, de álcool e de tabagismo e diminuindo a intensidade de atividades físicas (Coimbra Jr. *et al.* 2001, Gugelmin & Santos 2001). Segundo Coimbra Jr. & Santos (2000), a escassez de dados sobre a relação saúde-doença das populações indígenas não possibilita uma compreensão mais abrangente das

conseqüências que este processo está exercendo sobre o bem-estar destas populações. Os poucos dados disponíveis demonstram um aumento da prevalência de hipertensão arterial, de obesidade, de infecção por *Paracoccidioides brasilienses*, de risco para doenças cardiovasculares e do surgimento de diabetes mellitus tipo II (Cardoso *et al.* 2001, Coimbra Jr *et al.* 1994, 2001, Gugelmin & Santos 2001, Vieira Filho 1994). Quanto à ocorrência de câncer, os dados são ainda mais restritos. No final da década de 1950 não se constatou nenhum caso da doença em populações da Amazônia (Campos 1961), mas três pacientes com câncer foram detectados num grupo de 306 indígenas da tribo Parkatêjê da Amazônia, em 1992: um de leucêmia linfoblástica, um de leiomiosarcoma e um de tumor cervical uterino (Vieira Filho 1994). Segundo Koifman *et al.* (1998), estes tumores poderiam, talvez, estar associados com exposição a pesticidas, a vírus, a campos eletromagnéticos de freqüência baixa ou a outros xenobióticos presentes no ambiente.

É importante salientar que algumas doenças ambientais se manifestam geralmente após a quarta década de vida. Esperar-se-ia então que as populações ameríndias não as desenvolvessem devido a, em geral, não atingirem a idade média em que as doenças se manifestam pois, como povos pré-industriais, sua expectativa de vida média foi estimada em 20-30 anos (Crawford 1998). Informações específicas sobre a sobrevida de indígenas sul-americanos são poucos, mas nos Yanomani é de aproximadamente 21 anos (Neel & Weiss 1975), nos Xavante é cerca de 45 anos (que é considerada como sendo alta devido as condições sociais e ambientais desta população; Callegari-Jacques *et al.* 2001), e entre os Ache é de 37 anos para os que vivem na floresta e de 46-50 para os que vivem nas reservas (Hill & Hurtado 1996). Salzano & Callegari-Jacques (1988) estudando cerca de 70 tribos sul-americanas verificaram que aproximadamente 42% da população tinha entre 0-14 anos de idade.

Porém estas caraterísticas e a exposição a fatores de risco estão mudando rapidamente e, pelo menos para parte das populações indígenas, isto têm resultado em aumento na incidência de doenças crônicas (Baruzzi *et al.* 2001, Cardoso *et al.* 2001, Coimbra Jr *et al.* 1994, 2001, Gugelmin & Santos 2001, Vieira Filho 1994). Estes achados demonstram a importância de se conduzir estudos genético-epidemiológico a fim de se avaliar a influência que os genes envolvidos no metabolismo de xenobióticos representam no contexto saúde-doença destes povos. Caso eles de fato aumentem, neste grupo, a suscetibilidade a determinadas doenças ambientais – como ocorre nos euro-

brasileiros do RS – então é importante conhecer a variabilidade de outros genes do metabolismo de xenobióticos que não foram incluídos neste estudo. Talvez esta soma de conhecimentos contribua para uma melhor compreensão dos riscos à saúde a que estas populações estarão expostas à medida que estão sendo alterados seus padrões de vida tradicional.

Os resultados deste trabalho sugerem os genes *CYP1A1, CYP2E1, GSTT1* e *GSTP1* como sendo fatores predisponentes a COPD mas não a NSCLC. É importante salientar que os pacientes com neoplasia pulmonar, nesta amostra, tinham idade entre 43 e 81 anos, não tendo sido possível avaliar a influência destes marcadores na manifestação precoce de tumores. A análise futura de pacientes pediátricos com alguma forma de câncer pode ajudar a esclarecer melhor o papel desses polimorfismos como fatores predisponentes na manifestação e/ou progressão de doenças ambientais.

V. Resumo e conclusões

As populações neo-brasileiras, devido a suas diferentes origens étnicas, e as tribos ameríndias, bastante heterogêneas, muitas vivendo em condições de semi-isolamento constituem-se em excelente material para os estudos microevolutivos. Na população do Rio Grande do Sul a incidência de doenças pulmonares ambientais (doença pulmonar obstrutiva crônica e câncer de pulmão não de pequenas células) é muito alta. Estes fatores tornam instigante a avaliação dos efeitos de genes envolvidos na metabolização de substâncias tóxicas e no controle da divisão celular na predisposição a estas patologias. Muitos dos genes envolvidos nestes processos apresentam ampla variação interpopulacional em freqüências alélicas. Assim, este trabalho se propõe a analisar a variabilidade dos genes *CYP1A1, CYP2E1*, *GSTM1*, *GSTT1, GSTP1* e do supressor de tumor *TP53* em sete tribos indígenas sul-americanas, em três populações afro-brasileiras e em euro-brasileiros do RS, com os seguintes objetivos:

1. Estimar a diversidade genética e as relações intertribais de populações ameríndias, através de polimorfismos de *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1* e *TP53*;

2. Avaliar a distribuição do alelo *CYP1A1*2C* em afro-brasileiros;

3. Estudar, em euro-brasileiros, a variabilidade genética destes locos;

4. Analisar os possíveis efeitos destes marcadores na predisposição à doença pulmonar obstrutiva crônica e ao câncer de pulmão não de pequenas células.

Foram investigados os seguintes polimorfismos:

1) No loco*CYP1A1,* uma mutação de ponto no exon 7 (alelos *CYP1A1*1A* e *CYP1A1*2C*) e outra na região 3' (alelos *CYP1A1*1A* e *CYP1A1*2A*);
2) No gene *CYP2E1*, duas mutações na região promotora, um RFLP para *Pst*UI e outro para *Rsa*I (haplótipos *CYP2E1*1A* e *CYP2E1*5B*);
3) A deleção dos genes *GSTM1* e *GSTT1*, correspondendo aos fenótipos *GSTM1 (–)* e *GSTT1 (–)*;

4) Uma mudança de base no exon 5 do *GSTP1* (A→G: **Ala105 → *Val105*);

5) Três polimorfismos *TP53*, um RFLP para *Bst*UI no exon 4 e um RFLP para *Msp*I no intron 6 e uma duplicação de 16pb no intron 3. O alelo *A1*, dos três marcadores, corresponde à ausência da duplicação de 16pb e à ausência dos sítios de restrição.

A pesquisa envolveu sete populações indígenas, três afro-brasileiras e três amostras da população euro-brasileira do RS:

a) Populações indígenas: seis grupos brasileiros, Xavante (n = 33), Guarani (n = 51), Wai Wai (n = 26), Zoró (n = 28), Surui (n = 21), Gavião (n = 31) e um paraguaio, os Aché (n = 67);

b) Afro-brasileiros de Porto Alegre, n = 137, Rio de Janeiro, n = 64 e Salvador, n = 30;

c) Euro-brasileiros da população geral de Porto Alegre, n = 95;

d) Pacientes euro-brasileiros do RS com doença pulmonar obstrutiva crônica, n = 75 e câncer de pulmão não de pequenas células, n = 97.

Os principais resultados e conclusões foram:

1) Nos 3 polimorfismos do *TP53*, os ameríndios apresentaram freqüências alélicas similares às observadas na Ásia, corroborando a hipótese de uma origem asiática para estas populações;

2) Entre os oito haplótipos possíveis para o *TP53*, somente quatro foram observados em ameríndios. A combinação *1-2-1*, previamente detectada em duas populações asiáticas, foi verificada apenas entre os Wai Wai. Ou este haplótipo teria sido perdido, pelas outras tribos, por deriva genética ou recombinação ou não estaria presente nas populações ameríndias primitivas que colonizaram a América do Sul, tendo surgido nos Wai Wai por mutação ou recombinação nova;

3) O haplótipo *2-1-1* foi observado somente entre os indivíduos da tribo Gavião. Esta combinação ocorre também nos neo-brasileiros estando, possivelmente presente nos Gavião por mistura interétnica;

4) Nos ameríndios observaram-se valores muito elevados de *CYP1A1*2A* (96–100%) e *CYP1A1*2C* (90–100%) e baixos das deleções de *GSTM1* (4–43%) e *GSTT1* (0–

30%). As altas freqüências de genes fase I (*CYP1A1*2A* e *CYP1A1*2C*) podem resultar de deriva genética ou de pressão seletiva em resposta a novos desafios ambientais durante a ocupação do continente sul-americano. Estes valores elevados de *CYPs* atuariam seletivamente reduzindo as freqüências das deleções de *GSTM1* e *GSTT1*.

5) Devido às freqüências elevadas de alguns destes marcadores em populações ameríndias e ao aumento de contato crescente e gradativo destas com a cultura ocidental é provável que, no futuro venha a ocorrer, nestas tribos, um risco aumentado de doenças ambientais;

6) As análises de agrupamento envolvendo todos os polimorfismos de *CYP1A1, CYP2E1, GSTM1, GSTT1* e *TP53* indicaram que os Aché possivelmente descendem de um grupo Gê, mas que também apresentam uma certa porção de genes de origem Guarani, através de mistura intertribal;

7) Na comparação entre tribos indígenas, 90% da diversidade foi devida à variabilidade intrapopulacional. O menor valor (0,19) foi observado nos Guarani e o maior (0,38) nos Surui;

8) Os afro-brasileiros apresentaram freqüências de *CYP1A1*2C* (12–16%) muito mais altas que as observadas em outras populações negras ou miscigenadas. Mistura interétnica e/ou deriva genética, embora possivelmente ocorrendo ,não podem explicar, por si só, estes valores. Nossos dados sugerem que é possível que o alelo *CYP1A1*2C* exista na África, embora restrito a algumas populações ainda não investigadas.

9) Não se encontrou evidências de efeito destes marcadores na suscetibilidade à câncer de pulmão na população de euro-brasileiros do RS;

10) Em relação a COPD, heterozigotos para *CYP2E1*1A/*5B* foram quatro vezes mais freqüentes que nos controles [OR= 4.2 (1.1-24.8)], e portadores da deleção *GSTT1* foram de duas vezes e meia mais comuns [OR= 2.5 (1.2-4.9)];

11) O efeito da deleção *GSTT1* parece aumentar o risco de COPD em aproximadamente quatro vezes devido a presença dos genótipos *CYP1A1*1A/*2A* [(OR= 3.7 (1.1-14.6)] e *GSTP1Ile/Val* [OR= 4.0 (1.2-14.6)];

12) Estes resultados sugerem os polimorfismos *CYP1A1, CYP2E1, GSTT1* e *GSTP1* como sendo fatores predisponentes a doença pulmonar obstrutiva crônica mas não a câncer de pulmão não de pequenas células. É importante salientar que os pacientes com neoplasia pulmonar, nesta amostra, tinham idade entre 43 e 81 anos, não tendo sido possível avaliar a influência destes marcadores na manifestação precoce de tumores. A pesquisa de pacientes com câncer pediátrico poderá ajudar a esclarecer melhor o papel desses polimorfismos como fatores predisponentes na manifestação e/ou progressão de doenças ambientais.

VI. Summary and conclusions

Neo-Brazilian populations due to their varied ethnic origins, and Amerindian tribes, quite heterogeneous, with many of them living in semi-isolation conditions are especially suitable for microevolutionary studies. In Rio Grande do Sul population the incidence of environmental lung diseases (chronic obstructive pulmonary disease and non-small-cell lung cancer) is very high. These facts deserve the importance of evaluate the effects of the genes involved in xenobiotic metabolism and cycle cell control in these pathologies susceptibility. Many of the genes involved in these procedures present high interpopulation variability in allele frequencies. Therefore this work proposes to analyze the variability of *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, and *TP53* tumor suppressor genes in seven South Amerindian tribes, in three Afro-Brazilian populations and in Euro-Brazilians from RS, with the following objectives:

1. To estimate the genetic diversity and relationships of Amerindians populations, using *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1* and *TP53* polymorphisms;
2. To evaluate the distribution of CYP1A1*2C allele in Afro-Brazilians;
3. To study, in Euro-Brazilians, the genetic variability of these loci;
4. To analyze the possible effects of these markers on chronic obstructive pulmonary disease and non-small-cell lung cancer predisposition.

The following polymorphisms were investigated:

1) At *CYP1A1* locus, a point mutation at exon 7 (*CYP1A1*1A* and *CYP1A1*2C* alleles), and another at the 3' flanking region (*CYP1A1*1A* and *CYP1A1*2A* alleles);
2) At *CYP2E1* gene, two mutations in the promoter region, *Pst*UI and *Rsa*I RFLPs (*CYP2E1*1A* and *CYP2E1*5B* haplotypes);
3) The gene deletions of *GSTM1* and *GSTT1* loci, resulting in *GSTM1 (-)* and *GSTT1(-)* phenotypes;
4) A nucleotide change at *GSTP1* exon 5 (A→G: **Ala105*→ **Val105*);
5) Three *TP53* polymorphisms, a *Bst*UI and a *Msp*I RFLP at exon 4 and intron 6, respectively, and a 16bp duplication at intron 3. For all markers the *A1* allele corresponds to the absence restriction sites and of the duplication;

The research involved seven Amerindian tribes, three Afro-Brazilian populations and three samples of the Euro-Brazilian population of RS:

e) Amerindian populations: seven Brazilian groups, Xavante (n = 33), Guarani (n = 51), Wai Wai (n = 26), Zoró (n = 28), Surui (n = 21), Gavião (n = 31) and the Paraguayan Aché (n = 67);

f) Afro-Brazilians from Porto Alegre (n = 137), Rio de Janeiro (n = 64), and Salvador (n = 30);

g) Euro-Brazilian of the general population of Porto Alegre, n = 95;

h) Euro-Brazilians patients from RS with chronic obstructive pulmonary disease (n = 75) and non-small-cell lung cancer (n = 97);

The main results and conclusions were:

1) For *TP53* markers the Amerindians presented allele frequencies similar to those observed in Asia, corroborating the hypothesis of an Asian origin for these populations;

2) Among the eight possible *TP53* haplotypes, only four were verified in Amerindians. The rare haplotype *1-2-1* previously described in only two Asian samples, was verified among the Wai Wai. This combination was either lost by genetic drift or recombination in the other tribes, or it would not be present in the primitive populations which colonized South America, its presence in Wai Wai resulting from new mutation or recombination;

3) Among the Amerindians the *2-1-1* haplotype was verified in Gavião only. However it also occurs in Euro- and Afro-Brazilians, its presence in this Indians being probably due to interethnic admixture;

4) High frequencies of *CYP1A1*2A* and *CYP1A1*2C* alleles and low values of *GSTM1* and *GSTT1* deletions were observed among Amerindians. The high values of phase I alleles (*CYP1A1*2A* and *CYP1A1*2C)* may result from genetic drift or selection pressure in response to the new environmental challenges during America's colonization. These high *CYP* values could have acted against *GSTM1* and *GSTT1* deletions, reducing their frequencies;

5) Due to the high frequencies of some of these markers in Amerindian populations and to the increasing and gradual contact of these groups with the western culture it is possible that, in the future, these groups would have an increased risk of environmental diseases;

6) The cluster analyses involving *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1* and *TP53* polymorphisms indicated that the Aché tribe might descend from a Gê group, but had also assimilated some amount of the Guarani gene pool, maybe through intertribal admixture;

7) In the comparison among Amerindian tribes, 90% of the diversity was due to intrapopulation variability. The lower value (0,19) was observed in Guarani and the higher (0,38) in Surui;

8) The frequency of *CYP1A1*2C* (12 to 15%) was very high in Afro-Brazilians. This allele was not observed in the only two African populations so far investigated. Interethnic admixture with European descent and/or Amerindian populations and genetic drift although possibly occurring, cannot explain by themselves only these values. Our findings suggested that the *CYP1A1*2C* allele may possibly be present in Africa, although restricted to some ethnic groups, not yet investigated;

9) No effect of these markers was verified on NSCLC susceptibility in Euro-Brazilians of Porto Alegre;

10) In relation to COPD, heterozygous *CYP2E1*1A/*5B* was about 4 times more frequent than in controls [OR= 4.2 (1.1-24.8)] and the *GSTT1* null phenotype was twice more common [OR= 2.5 (1.2-4.9)];

11) The effect of *GSTT1* null phenotype on COPD susceptibility seems to be increased in around 4 times due to its interaction with *CYP1A1*1A/*2A* [(OR= 3.7 (1.1-14.6)], and *GSTP1Ile/Val* genotypes [OR= 4.0 (1.2-14.6)];

12) These results suggest that *GSTT1, GSTP, CYP2E1, CYP1A1* polymorphisms could be predictive to chronic obstructive pulmonary disease, but doesn't to non-small-cell lung cancer susceptibility, at least in this population of European ancestry. It is important to point out that the patients with lung cancer, in this sample, were 43 to 81 years old, and it was not possible to evaluate the effect of these markers on the early

development of tumors. A research of pediatric cancer patient could help to better understand the role of those polymorphisms as susceptibility factors to the development and/or the progression of environmental diseases.

VII. Bibliografia

Agarwal ML. Taylor WR. Chernov MV. Chernova OB. Stark GR. 1998. The p53 network. J Biol Chem 273: 1-4.

Ali-Osman F. Akande O. Antoun G. Mao JX. Buolamwini J. 1997. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem 272: 10004-10012.

American Thoracic Society. 1995. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 152: S77-S120.

Ames BN. Gold LS. 1997. The causes and prevention of cancer: gaining perspective. Environ Health Perspect 105 (suppl 4): 865-873.

Ames BN. Gold LS. Willett WC. 1995. The causes and prevention of cancer. Proc Natl Acad Sci USA 92: 5258-5265.

Anto JM. Vermeire P. Vestbo. J Sunyer J. 2001. Epidemiology of chronic obstructive pulmonary disease. Eur Respir J 17: 982-994.

Anzenbacher P. Anzenbacherová E. 2001. Cytochromes P450 and metabolism of xenobiotics. Cell Mol Life Sci 58: 737-747.

Arruda VR. Grignolli CE. Gonçalves MS. Soares MC. Menezes R. Saad STO. Costa FF. 1998. Prevalence of homozygosity for the deleted alleles of glutahione S-transferase mu (GSTM1) and theta (GSTT1) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet 54: 210-214.

Autrup H. 2000. Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. Mut Res 464: 65-76.

Aynacioglu AS. Cascorbi I. Mrozikiewicz PM. Roots I. 1998. High frequency of *CYP1A1* mutations in a Turkish population. Arch Toxicol 72: 215-218.

Barnes PJ. 1999. Molecular genetics of chronic obstructive pulmonary disease. Thorax 54: 245-252.

Bartsch H. Nair U. Risch A. Rojas M. Wilkman H. Alexandrov K. 2000. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. Cancer Epidemiol Biomarkers Prev 9: 3-28.

Baruzzi RG. Barros VL. Rodrigues D. Souza ALM. Pagliaro H. 2001. Helath and disease among Panará (Kreen-Akarôre) Indians in Central Brazil after twenty-five years of contact with our World, with an emphasis on tuberculosis. Cad Saúde Pública 17: 407-412.

Beckman G. Birgander R. Själander A. Saha N. Holmberg PA. Kivelä A. Beckman L. 1994. Is p53 polymorphism maintained by natural selection. Hum Hered 44: 266-270.

Birgander R. Sjalander A. Rannug A. Alexandrie AK. Sundberg MI. Seidegard J. Tornling G. Beckman G. Beckman L. 1995. P53 polymorphisms and haplotypes in lung cancer. Carcinogenesis 16: 2233-2236.

Biros E. Kalina I. Kohut A. Stubna J. Salagovic J. 2001. Germ line polymorphisms of the tumor suppressor gene p53 and lung cancer. Lung Cancer 31: 157-162.

Bouchardy C, Benhamou S, Jourenkova N, Dayer P, Hirvonen A. 2001. Metabolic genetic polymorphisms and susceptibility to lung cancer. Lung Cancer 32: 109-112.

Callegari-Jacques SM. Flowers NM. Laner NFM. Salzano FM. 2001. Demography and Genetics at the Tribal Level: The Xavante as a Test Case. Curr Anthropol 42: 154-161.

Campos SS. 1961. Estudos sobre o câncer nos índios do Brasil. Rev Bras Cancerol 2: 33-50.

Carbone DP. 1997. The biology of lung cancer. Semin Oncol 24: 388-401.

Cardoso AM. Mattos IE. Koifman RJ. 2001. Prevalence of risk factors for cardiovascular disease in the Guarani-Mbyá population of the State of Rio de Janeiro. Cad Saúde Pública 17: 345-354.

Cascorbi I. Brockmöller J. Roots I. 1996. A C4887A polymorphism in exon 7 of human *CYP1A1*: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res 56: 4965-4969.

Chen C. Liu Q. Relling MV. 1996. Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American Whites and Blacks. Pharmacogenetics 6: 187-191.

Coimbra Jr. CEA. Chor D. Santos RV. Salzano FM. 2001. Blood Pressure Levels in Xavánte adults from the Pimentel Barbosa Indian Reservation, Mato Grosso, Brazil. Ethn Dis 11: 232-240.

Coimbra Jr. CEA. Santos RV. 2000. Saúde, minorias e desigualdade: Algumas teias de inter-relações, com ênfase nos povos indígenas. Ciência & Saúde Coletiva 5: 125-132

Coimbra Jr. CEA. Wanke B. Santos RV. do Valle AC. Costa RL. Zancope-Oliveira RM. 1994. Paracoccidioidin and histoplasmin sensitivity in Tupi-Monde Amerindian populations from Brazilian Amazonia. Ann Trop Med Parasitol 88: 197-207.

Corchero J. Pimprale S. Kimura S. Gonzalez FJ. 2001. Organization of the *CYP1A* cluster on human chromosome 15: implications for the gene regulation. Pharmacogenetics 11: 1-6.

Crawford MH. 1998. The Origins of Native Americans: evidence from anthropological genetics. United Kingdom: Cambridge University Press.

Dahl M. Nordestgaard BG. Lange P. Vestbo J. Tybjaerg-Hansen A. 2001. Molecular diagnosis of intermediate and severe alpha(1)-antitrypsin deficiency: MZ individuals with chronic obstructive pulmonary disease may have lower lung function than MM individuals. Clin Chem 47: 56-62.

Duzhak T. Mitrofanov D. Ostashevskii V. Gutkina N. Chasovnikova O. Posukh O. Osipova L. Lyakhovich VV. 2000. Genetic polymorphisms of CYP2D6, CYP1A1, GSTM1 and p53 genes in a unique Siberian population of tundra. Pharmacogenetics 10: 1-7.

Eaton DL. Bammler TK. 1999. Concise review of the glutathione S-transferases and their significance to toxicology. Toxicol Science 49: 156-164.

Esteller M. Garcia A. Martinez-Palones JM. Xercavins J. Reventos J. 1997. Susceptibility to endometrial cancer: influence of allelism at p53, glutathione S-

transferase (GSTM1 and GSTT1) and cytochrome P-450 (CYP1A1) loci. Br J Cancer 75: 1385-1388.

Fan R. Wu MT. Miller D. Wain JC. Kelsey KT. Wiencke JK. Christiani DC. 2000. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 9: 1037-1042.

Feldmann D. Bard M. Chauve C. Couderc R. 2000. A new case of alpha-1-antitrypsin frameshift mutation (1123insT) causing severe deficiency and emphysema. Hum Mut 16: 447.

Ford JG. Li Y. O'Sullivan MM. Demopoulos R. Garte S. Taioli E. Brandt-Rauf PW. 2000. Glutathione S-transferase M1 polymorphism and lung cancer risk in African-Americans. Carcinogenesis 21: 1971-1975.

Garte SJ. Trachman F. Crofts Toniolo P. Buxbaum J. Bayo S. Taiolo E. 1996. Distribution of composite *CYP1A1* genotypes in Africans, African-Americans and Caucasians. Hum Hered 46:121-127.

Gattas GJ. Soares-Vieria JA. 2000. Cytochrome P450-2E1 and glutathione S-transferase mu polymorphisms among Caucasians and mulattoes from Brazil. Occup Med 50: 508-511.

Gauderman WJ. Morrison JL. Carpenter CL. Thomas DC. 1997. Analysis of gene-smoking interaction in lung cancer. Genet Epidemiol 14: 199-214.

Gonzalez FJ. Nebert DW. 1990. Evolution of the P450 gene superfamily: animal-plant 'warfare', molecular drive and human genetic differences in drug oxidation. Trends Genet 6: 182-186.

González MV. Alvarez V. Pello MF. Menendez MJ. Suarez C. Coto E. 1998. Genetic polymorphism of N-acetyltransferase-2, glutathione S-transferase-M1, and cytochromes P450IIE1 and P450IID6 in the susceptibility to head and neck cancer. J Clin Pathol 51: 294-298.

Griese EU. Ilett KF. Kitteringham NR. Eichelbaum M. Powell H. Spargo RM. LeSouef PN. Musk AW. Minchin RF. 2001. Allele and genotype frequencies of polymorphic cytochromes P4502D6, 2C19 and 2E1 in Aborigines from Western Australia. Pharmacogenetics 11: 69-76.

Gsur A. Haidinger G. Hollaus P. Herbacek I. Madersbacher S. Trieb K. Pridun N. Mohn-Staudner A. Vetter N. Vutuc C. Micksche M. 2001. Genetic polymorphisms of CYP1A1 and GSTM1 and lung cancer risk. Anticancer Res 21: 2237-2242.

Guengerich FP. Shimada T. 1998. Activation of procarcinogens by human cytochrome P450 enzymes. Mutat Res 400: 201-13.

Gugelmin SA. Santos RV. 2001. Human ecology and nutritional anthropometry of adult Xavánte Indians in Mato Grosso, Brazil. Cad Saúde Pública 17: 313-322.

Hamada GS. Sugimura H. Suzuki I. Nagura K. Kiyokawa E. Iwase T. Tanaka M. Takahashi T. Watanabe S. Kino I. Tsugane S. 1995. The heme-binding region polymorphism of cytochrome P450IA1 (CypIA1), rather than the RsaI polymorphism of IIE1 (CypIIE1), is associated with lung cancer in Rio de Janeiro. Cancer Epidemiol Biomarkers Prev 4: 63-67.

Hanrahan JP. Sherman CB. Bresnitzf EA. Emmons KM. Manion DM. 1996. Cigarette smoking and health. Am J Respir Crit Care Med 153: 861-865.

Harries LW. Stubbins MJ. Forman D. Howard GC. Wolf CR. 1997. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. Carcinogenesis 18: 641-644.

Harris MJ. Coggan M. Langton L. Wilson SR. Board PG. 1998. Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. Pharmacogenetics 8: 27-31.

Harrison DJ. Cantlay AM. Rae F. Lamb D. Smith CA. 1997. Frequency of glutathione S-transferase M1 deletion in smokers with emphysema and lung cancer. Hum Exp Toxicol 16: 356-360.

Hasler JA. Estabrookb R. Murrayc M. Pikulevad I. Watermand M. Capdevilae J. Hollae V. Helvige C. Falckb JR. Farrellf G. KaminskygLS. Spivackg SD. Boitierh E. Beauneh P. 1999. Human cytochromes P450. Mol Aspects Med 20: 1-137.

Hayashi SI. Watanabe J. Kawajiri K. Kawajiri K. 1991. Genetic linkage of lung cancer-associated *Msp*I polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. J Biochem 110: 407-411.

Hecht SS. 1999. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 91: 1194-1210.

Hill K. Hurtado AM. 1996. Ache life history. The ecology and demography of a foraging people. New York: Aldine de Gruyter.

Houlston RS. 1999. Glutathione S-transferase M1 Status and Lung Cancer Risk: A Meta-Analysis. Cancer Epidemiol Biomarkers Prev 8: 675-682.

Hu X. Xia H. Srivastava SK. Herzog C. Awasthi YC. Ji X. Zimniak P. Singh SV. 1997. Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. Biochem Biophys Res Commun 238: 397-402.

Ilett KF. McCormick N. Carpenter DS. Spargo RM. Le Souef PN. Musk AW. Minchin RF. 2000. Genetic polymorphisms in glutathione S-transferase M1 and T1 in an Australian Aborigine population. Pharmacogenetics 10: 477-80.

INCA: Instituto Nacional de Câncer. http://www.inca.org.br/.

Indulski JA. Lutz W. 2000. Metabolic genotype in relation to individual susceptibility to environmental carcinogens. Int Arch Occup Environ Health 73: 71-85.

Ingelman-Sundberg M. 2001. Genetic susceptibility to adverse effects of drugs and environmental toxicants: the role of the CYP family of enzymes. Mut Res 482: 11-19.

Inoue K. Asao T. Shimada T. 2000. Ethnic-related differences in the frequency distribution of genetic polymorphisms in the *CYP1A1* and *CYP1B1* genes in Japanese and Caucasian populations. Xenobiotica 30: 285-295.

Ishii T. Matsuse T. Teramoto S. Matsui H. Miyao M. Hosoi T. Takahashi H. Fukuchi Y. Ouchi Y. 1999. Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease. Thorax 54: 693-696.

Jaiswal AK. Nebert DW. 1986. Two RFLPs associated with the human P(1)450 gene linked to the MPI locus on chromosome 15 (HGM8D15S8). Nucleic Acids Res 14: 4376.

Kato S. Shields PG. Caporoso NE. Hoover RN. Trump BF. Sugimura H; Weston A. Harris CC. 1992. Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res 52: 6712-6715.

Kawajiri K. Nakachi K. Imai K. Watanabe J. Hayashi S. 1993. The CYP1A1 gene and cancer susceptibility. Crit Rev Oncol Hematol 14: 77-87.

Ketter B. 1988. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mut. Res 202: 343-361.

Khaliq S. Hameed A. Khaliq T. Aybur Q. Qamar R. Mohyuddin A. Mazhar K. Mehdi Q. 2000. p53 mutations, polymorphisms, and haplotypes in Pakistani ethnic groups and breast cancer patients. Genet Test 4: 23-29.

Kim KS. Ryu SW. Kim YJ. Kim E. 1999. Polymorphism analysis of the CYP1A1 locus in Koreans: presence of the solitary m2 allele. Mol Cells 28: 78-83.

Kodavanti UP. Costa DL. Bromberg PA. 1998. Rodent Models of cardiopulmonary disease: their potential applicability in studies of air pollutant susceptibility. Environ Health Perspect 106 (suppl): 111-130.

Koifman S. Ferraz I. Viana TS. Silveira CL. Carneiro MT. Koifman RJ. Sarcinelli PN. Mattos Rde C. Lima JS. Silva JJ. Moreira JC. Ferreira Mde F. Fernandes C. Bulcao AC. 1998. Cancer cluster among young Indian adults living near power transmission lines in Bom Jesus do Tocantins, Para, Brazil. Cad Saúde Pública 14 (Suppl 3): 161-172.

Kubbutat MHG. Vousden KH. 1998. Keeping an old friend under control: regulation of p53 stability. Mol Med Today 4: 250-256.

Khuder SA. 2001. Effect of cigarette smoking on major histological types of lung cancer: a meta-analysis. Lung Cancer 31: 139-148.

Kvitko K. Nunes JCD. Weimer TA. Salzano FM. Hutz MH. 2000. Cytochrome P4501A1 polymorphisms in South American Indians. Hum Biol 72: 1039-1043.

Kyohara C. Hirohata T. Inutsuka S. 1996. The relationship between aryl hydrocarbon hydroxilase and polymorphisms of the CYP1A1 gene. Jpn J Cancer Res 87: 18-24.

Landi MT. Bertazzi PA. Shields PG. Clark G. Lucier GW. Garte SJ. Cosma G. Caporaso NE. 1994. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics 4: 242-246.

Landi S. 2000. Mammalian class theta GST and differential susceptibility to carcinogens: a review. Mutat Res 463: 247-283.

Lang M. Pelkonen O. 1999. Chapter 3: metabolism of xenobiotics and chemical carcinogens. IARC Sci Publ 148: 13-22.

Lewis DFV. Watson E. Lake BG. 1998. Evolution of the cytochrome P450 superfamily: sequence and alignments and pharmacogenetics. Mut Res 410: 245-270.

Lin HJ. Han C. Bernstein DA. Hsiao W. Lin BK. Hardy S. 1994. Ethnic distribution of the glutathione transferase Um 1-1 (GSTM1) null genotype in 1473 individuals and application to bladder cancer susceptibility. Carcinogenesis 15: 1077-1081.

Liu S Park JY Schantz SP Stern JC Lazarus P. 2001. Elucidation of CYP2E1 5' regulatory RsaI/Pstl allelic variants and their role in risk for oral cancer. Oral Oncol 37: 437-45.

London SJ. Daly AK. Cooper J. Carpenter CL. Navidi WC. Ding L. Idle JR. 1996. Lung cancer risk in relation to the CYP2E1 RsaI genetic polymorphism among African-Americans and Caucasians in Los Angeles County. Pharmacogenetics 6: 151-158.

Lucas D. Ferrara R. Gonzales E. Albores A. Manno M. Berthou F. 2001. Cytochrome CYP2E1 phenotyping and genotyping in the evaluation of health risks from exposure to polluted environments. Toxicol Lett 124: 71-81.

Lucas D. Menez C. Girre C. Berthou F. Bodenez P. Joannet I. Hispard E. Bardou LG. Menez JF. 1995. Cytochrome P450 2E1 genotype and chlorzoxazone metabolism in healthy and alcoholic Caucasian subjects. Pharmacogenetics 5: 298-304.

MacNee W. Rahman I. 2001. Is oxidative stress central to the pathogenesis of chronic obstructive pulmonary disease? Trends Mol Med 7: 55-62.

Masimirembwa CM. Dandara C. Sommers DK. Snyman JR. Hasler JA. 1998. Genetic polymorphism of cytochrome P4501A1, microsomal epoxide hydrolase, and glutathione S-transferase M1 and T1 in Zimbabweans and Venda of Southern Africa. Pharmacogenetics 8: 83-85.

Matthias C. Bockmuhl U. Jahnke V. Harries LW. Wolf CR. Jones PW. Alldersea J. Worrall SF. Hand P. Fryer AA. Strange RC. 1998. The glutathione S-transferase GSTP1 polymorphism: effects on susceptibility to oral/pharyngeal and laryngeal carcinomas. Pharmacogenetics 8: 1-6.

Mcbride OW. Merry D. Givol D. 1986. The gene for human p53 cellular tumor is located on chromosome 17 short arm (17p13). Proc Natl Acad Sci USA 83: 130-134.

McLellan RA. Oscarson M. Alexandrie AK. Seidegard J. Evans DA. Rannug A. Ingelman-Sundberg M. 1997. Characterization of a human glutathione S-transferase mu cluster containing a duplicated GSTM1 gene that causes ultrarapid enzyme activity. Mol Pharmacol 52: 958-965.

Menezes AMB. 2001. Epidemiologia da DPOC. In: Corrêa da Silva LC, organizador. Condutas em Pneumologia. Rio de Janeiro: REVINTER. p: 326-327.

Miller MC 3rd. Mohrenweiser HW. Bell DA. 2001. Genetic variability in susceptibility and response to toxicants. Toxicol Lett 120: 269-280.

Miller MS. McCarver DG. Bell DA. Eaton DL. Goldstein JA. 1997. Genetic polymorphisms in human drug metabolic enzymes. Fundam Appl Toxicol 40: 1-14.

Moreira JS. Porto N. Camargo JJ. Geyer GR. 2001. Carcinoma Brônquico. In: Côrrea da Silva LC, organizador. Condutas em Pneumologia. Rio de Janeiro: REVINTER. p: 553-570.

Morita S. Yano M. Shiozaki H. Tsujinaka T. Ebisui C. Morimoto T. Kishibuti M. Fujita J. Ogawa A. Taniguchi M. Inoue M. Tamura S. Yamazaki K. Kikkawa N. Mizunoya S. Monden M. 1997. CYP1A1, CYP2E1 and GSTM1 polymorphisms are not associated with susceptibility to squamous-cell carcinoma of the esophagus. Int J Cancer 71: 192-195.

Mukanganyama S. Masimirembwa CM. Naik YS. Hasler JA. 1997. Phenotyping of the glutathione S-transferase M1 polymorphism in Zimbabweans and the effects of chloroquine on blood glutathione S-transferases M1 and A. Clin Chim Acta 265:145-155.

Müllauer L. Gruber P. Sebinger D. Buch J. Wohlfart S. Chott A. 2001. Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutat Res 488: 211-231.

Munõz S. Vollrath V. Vallejos MP. Miquel JF. Covarrubias C. Raddatz A. Chianale J. 1998. Genetic polymorphisms of CYP2D6, CYP1A1 and CYP2E1 in the South-Amerindian population of Chile. Pharmacogenetics 8: 343-351.

Murata M. Tagawa M. Kimura H. Kakisawa K. Shirasawa H. Fujisawa T. 1998. Correlation of the mutation of p53 gene and the polymorphism at codon 72 in smoking-related non-small cell lung cancer patients. Int J Oncol 12: 577-581.

Murata M. Tagawa M. Kimura M. Kimura H. Watanabe S. Saisho H. 1996. Analysis of a germ line polymorphism of the p53 gene in lung cancer patients; discrete results with smoking history. Carcinogenesis 17: 261-264.

Murata M. Watanabe M. Yamanaka M. Kubota Y. Ito H. Nagao M. Katoh T. Kamataki T. Kawamura J. Yatani R. Shiraishi T. 2001. Genetic polymorphisms in cytochrome P450 (CYP) 1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett 165: 171-177.

Murray JF. Nadel JA. 1994. Textbook of respiratory medicine. 2nd ed. Philadelphia: Saunders Company. pp.1332-1535.

Nebert DW, Roe AL. 2001. Ethnic and genetic differences in metabolism genes and risk of toxicity and cancer. Sci Total Environ 274: 93-102.

Nebert DW. 1991. Role of genetics and drug metabolism in human cancer risk. Mut Res 247: 267-281.

Nebert WN. 1997. Polymorphisms in drug-metabolizing enzymes: what is their clinical relevance and why do they exist? Am J Hum Genet 60: 265-271.

Neel JV. Weiss KM. 1975. The genetic structure of a tribal population. The Yanomama Indians. Am J Phys Anthropol 42: 25-52.

Nelson HH. Wiencke JK. Christiani DC. Cheng TJ. Zuo ZF. Schwartz BS. Lee BK. Spitz MR. Wang M. Xu X. et al. 1995. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. Carcinogenesis 16: 1243-1245.

Omura T. 1999. Forty years of cytochrome P450. Biochem Biophys Res Commun 266: 690-698.

Oyama T. Mitsudomi T. Kawamoto T. Ogami A. Osaki T. Kodama Y. Yasumoto K. 1995. Detection of CYP1A1 gene polymorphism using designed RFLP and distributions of CYP1A1 genotypes in Japanese. Int Arch Occup Environ Health 67: 253-256.

Park JY. Muscat JE. Ren Q. Schantz SP. Harwick RD. Stern JC. Pike V. Richie Jr JP. Lazarus P. 1997. CYP1A1 and GSTM1 polymorphisms and oral cancer risk. Cancer Epidemiol Biomarkers Prev 6: 791-797.

Pearson WR. Vorachek. Xu SJ. Berger R. Hart I. Vannais D. Patterson D. 1993. Identification of class-um glutathione transferase genes *GSTM1-GSTM5* on human chromosome 1p13. Am J Hum Genet 53: 220-233.

Peluso M. Airoldi L. Armelle M. Martone T. Coda R. Malaveille C. Giacomelli G. Terrone C. Casetta G. Vineis P. 1998. White blood cell DNA adducts, smoking, and NAT2 and GSTM1 genotypes in bladder cancer: a case-control study. Cancer Epidemiol. Biomarkers Prev 7: 341-346.

Pemble S. Schroeder KR. Spencer SR. Meyer DJ. Hallier E. Bolt HM. Ketterer B. Taylor JB. 1994. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J 300: 271-276.

Perera FP. 1996. Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. J Natl Cancer Inst 88: 496-509.

Persson I. Johansson I. Bergling H. Dahl ML. Seidegard J. Rylander R. Rannug A. Högberg J. Ingelman-Sundberg M. 1993. Genetic polymorphism of cytochrome P450IIE1 in a Swedish population: relationship to incidence of lung cancer. FEBS 319: 207-211.

Persson I. Johansson I. Ingelman-Sundberg M. 1997. In vitro kinetics of two human CYP1A1 variant enzymes suggested to be associated with interindividual differences in cancer susceptibility. Biochem Biophys Res Commun 231: 227-230.

Persson I. Johansson I. Lou YC. Yue QY. Duan LS. Bertilsson L. Ingelman-Sundberg M. 1999. Genetic polymorphism of xenobiotic metabolizing enzymes among Chinese lung cancer patients. Int J Cancer 81: 325-329.

Pierce LM. Sivaraman L. Chang W. Lum A. Donlon T. Seifried A. Wilkens LR. Lau AF. Le Marchand L. 2000. Relationships of TP53 codon 72 and HRAS1

polymorphisms with lung cancer risk in an ethnically diverse population. Cancer Epidemiol Biomarkers Prev 9: 1199-1204.

Puga A. Nebert DW. Mckinnon RA. Menon AG. 1997. Genetic polymorphisms in human drug-metabolizing enzymes: potential uses of reverse genetics to identify genes of toxicological relevance. Crit Rev Toxicol 27: 199-222.

Quiñones L. Berthou F. Varela N. Simon B. Gil L. Lucas D. 1999. Ethnic susceptibility to lung cancer: differences in *CYP2E1*, *CYP1A1* and *GSTM1* genetic polymorphisms between French Caucasian and Chilean populations. Cancer Lett 141: 167-171.

Quinones L. Lucas D. Godoy J. Caceres D. Berthou F. Varela N. Lee K. Acevedo C. Martinez L. Aguilera AM. Gil L. 2001. CYP1A1, CYP2E1 and GSTM1 genetic polymorphisms. The effect of single and combined genotypes on lung cancer susceptibility in Chilean people. Cancer Lett 174: 35-44.

Rannug A. Alexandrie AA. Persson I. Ingelman-Sundberg M. 1995. Genetic polymorphism of cytochromes P450 1A1. 2D6 and 2E1: regulation and toxicological significance. JOEM 37: 25-36.

Rebbeck TR. 1997. Molecular epidemiology of the human glutathione S-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 6: 733-743.

Ribas DLB. Sganzerla A. Zorzarro JR. Phillipi ST. 2001. Child health and nutrition in a Teréna indigenous community, Mato Grosso do Sul, Brazil. Cad Saúde Pública 17: 323-331.

Rossi AM. Guarnieri C. Rovesti S. Gobba F. Ghittori S. Vivoli G. Barale R. 1999. Genetic polymorphisms influence variability in benzene metabolism in humans. Pharmacogenetics 9: 445-451.

Sakao S. Tatsumi K. Igari H. Shino Y. Shirasawa H. Kuriyama T. 2001. Association of tumor necrosis factor α gene promoter polymorphism with the presence of chronic obstructive pulmonary disease. Am J Crit Care Med 163: 420-422.

Salzano FM. Callegari-Jacques SM. 1988. South American Indians. A case Study in Evolution. Oxford: Clarendon Press.

Santos RV. Flowers NM. Coimbra Jr. CEA. Gugelmin SA. 1996. Human ecology and health in the context of change: the Xavante Indians of Mato Grosso, Brazil. In: Follér M-L. Hansson LO. (eds). Human ecology and health adaptation to a changing world. Göteborg University, Göteborg, p: 94-117.

Schwarz D. Kisselev P. Schunck WH. Chernogolov A. Boidol W. Cascorbi I. Roots I. 2000. Allelic variants of human cytochrome P450 1A1 (CYP1A1): effect of T461N and I462V substitutions on steroid hydroxylase specificity. Pharmacogenetics 10: 519-530.

Sellers TA. Weaver TW. Phillips B. Altmann M. Rich SS. 1998. Environmental factors can confound identification of a major gene effect: results from a segregation analysis of a simulated population of lung cancer families. Genet Epidemiol 15: 251-262.

Silverman EK. Speizer FE. 1996. Risk factors for the development of chronic obstructive pulmonary disease. Med Clin North Am 80: 501-522.

Själander A. Birgander R. Kivelä A. Beckman G. 1995. P53 polymorphisms and haplotypes in different ethnic groups. Hum Hered 45: 144-149.

Själander A. Birgander R. Saha N. Beckman L. Beckman G. 1996. p53 polymorphisms and haplotype show distinct difference between major ethnic groups. Hum Hered 46: 41-48.

Smith CA. Harrison DJ. 1997. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. Lancet 350: 630-633.

Smith CM. Bora PS. Bora NS. Jones C. Gerhard DS. 1995. Genetic and radiation-reduced somatic cell hybrid sublocalization of the human GSTP1 gene. Cytogenet Cell Genet 71: 235-239.

Song N. Tan W. Xing D. Lin D. 2001. CYP1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China. 22: 11-16.

Sprenger R. Schlagenhaufer R. Kerb R. Bruhn C. Brockmoller J. Roots I. Brinkmann U. 2000. Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation Pharmacogenetics 10: 557-565.

Stephens EA. Taylor JA. Kaplan N. Yang CH. Hsieh LL. Lucier GW. Bell DA. 1994. Ethnic variation in the CYP2E1 gene: polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. Pharmacogenetics 4: 185-192.

Strange RC. Lear JT. Fryer AA. 1998. Glutathione S-transferase polymorphisms: influence on susceptibility to cancer. Chem Biol Interact 111-112: 351-364.

Sundberg K. Johansson AS. Stenberg G. Widersten M. Seidel A. Mannervik B. Jernstrom B. 1998. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis 19: 433-436.

Tan W. Song N. Wang GQ. Liu Q. Tang HJ. Kadlubar FF. Lin DX. 2000. Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. Cancer Epidemiol Biomarkers Prev 9: 551-556.

Thomas M. Kalita A. Labrecque S. Pim D. Banks L. Matlashewski G. 1999. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Moll Cell Biol 19: 1092-1100.

To-Figueras J. Gene M. Gomez-Catalan J. Pique E. Borrego N. Corbella J. 2001. Lung cancer susceptibility in relation to combined polymorphisms of microsomal epoxide hydrolase and glutathione S-transferase P1. Cancer Lett 173: 155-162.

Torres MR. Dellazzeri L. Gaspar PA. Kvitko K. 2001. Análise do polimorfismo do gene *GSTP1, GSTM1* e *GSTT1* em caucasóides, negróides e pacientes que desenvolveram câncer de mama. Anais: V Congresso da Sociedade Brasileira de Mutagênese, Carcinogênes e Teratogênese Ambiental, Gramado. p: 85.

Umeno M. Mcbride OW. Yang CS. Gelboin HV. Gonzales FJ. 1988. Human ethanol-inducible P450IIE1: complete gene sequence, promoter characterization, chromosome mapping, and cDNA-directed expression. Biochem 27: 9006-9013.

Venitt S. 1994. Mechanisms of carcinogenesis and individual susceptibility to cancer. Clin Chem 407: 1421-1425.

Viegi G. Scognamiglio A. Baldacci S. Pistelli F. Carrozzi L. 2001. Epidemiology of chronic obstructive pulmonary disease (COPD). Respiration 68: 4-19.

Vieira Filho JPB. 1994. Malignant tumors among Gavião Indians. Proximity of electromagnetic fields. Rev Ass Med Bras 40: 137-139.

Walter R. Gottlieb DJ. O'Connor GT. 2000. Environmental and genetic risk factors and gene-environment interactions in the pathogenesis of chronic obstructive lung disease. Environ Health Perspect 108: 733-742.

Wang YC. Chen CY. Chen SK. Chang YY. Lin P. 1999. p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. Clin Cancer Res 5: 129-134.

Watanable J. Hayashi S. Kawajiri K. 1994. Different regulation and expression of the human CYP2E1 gene due to the RsaI polymorphism in the 5'-flanking region. J Biochem 116: 321-326.

Watanable J. Yang JP. Eguchi H. Hayashi SI. Imai K. Kawaliri K. 1995. An *Rsa*I polymorphism in the *CYP2E1* gene does not affect lung cancer risk in a Japanese population. Jpn J Cancer Res 86: 245-248.

Watson MA. Stewart RK. Smith GB. Massey TE. Bell DA 1998. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 19: 275-280.

Weston A. Ling-Cawley HM. Caporaso NE. Bowman ED. Hoover RN. Trump BF. Harris CC. 1994. Determination of the allelic frequencies of an L-myc and a p53 polymorphism in human lung cancer Carcinogenesis 15: 583-587.

Weston A. Pan CF. Ksieski HB. Wallenstein S. Berkowitz GS. Tartter PI. Bleiweiss IJ. Brower ST. Senie RT. Wolff MS. 1997. p53 haplotype determination in breast cancer. Cancer Epidemiol Biomarkers Prev 6: 105-112.

Wilce MCJ. Parker MW. 1994. Structure and function of glutathione S-transferases. Biochem Biophys Acta 1205: 1-18.

Wild CP. Yin F. Turner PC. Chemin I. Chapot B. Mendy M. Whittle H. Kirk GD. Hall AJ. 2000. Environmental and genetic determinants of aflatoxin-albumin adducts in the Gambia. Int J Cancer 86: 1-7.

Wilkinson J. Clapper ML. 1997. Detoxification enzymes and chemoprevention. Proc Soc Exp Biol Med 216: 192-200.

Xu S. Wang Y. Roe B. Pearson WR. 1998. Characterization of the human class Mu glutathione S-transferase gene cluster and the GSTM1 deletion. J Biol Chem 273: 3517-3527.

Yamada N. Yamaya M. Okinaga S. Nakayama K. Sekizawa K. Shibahara S. Sasaki H. 2000. Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. Am J Hum Genet 66: 187-195.

Yim JJ. Park GY. Lee CT. Kim YW. Han SK. Shim YS. Yoo CG. 2000. Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. Thorax 55: 121-125.

Zhang ZY. Fasco MJ. Huang L. Guengerich FP. kaminsky LS. 1996. Characterization of purified human recombinant cytochrome P4501A1-Ile^{462} and Val^{462}: assessment of a role for the rare allele in carcinogenesis. Cancer Res 56: 3926-3933.

Zimniak P. Nanduri B. Pikula S. Bandorowicz-Pikula J. Singhal SS. Srivastava SK. Awasthi S. Awasthi YC. 1994. Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymatic properties. Eur J Biochem 224: 893-899.