RESEARCH COMMUNICATION

Chromosomal localization of human genes for arylamine *N*-acetyltransferase

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Arylamine *N*-acetyltransferase is encoded at two loci, *AAC-1* and *AAC-2*, on human chromosome 8. The products of the two loci are able to catalyse N-acetylation of arylamine carcinogens, such as benzidine and other xenobiotics. *AAC-2* is polymorphic and individuals carrying the slow-acetylator phenotype are more susceptible to benzidine-induced bladder cancer. We have identified yeast artificial chromosome clones encoding *AAC-1* and *AAC-2* and have used the cloned DNAs as fluorescent probes for *in situ* hybridization. The hybridization patterns allow assignment of *AAC-1* and *AAC-2* to chromosome 8p21.3–23.1, a region in which deletions have been associated with bladder cancer [Knowles, Shaw and Proctor (1993) Oncogene **8**, 1357–1364].

INTRODUCTION

Arylamine *N*-acetyltransferase catalyses the acetylation of a range of xenobiotics, including hydrazine and arylamine drugs and also arylamine carcinogens (Sim et al., 1992). There are two loci encoding functional *N*-acetyltransferases (Blum et al., 1990; Ohsako and Deguchi, 1990) which have been designated *AAC-1* and *AAC-2* (Donis-Keller and Buckle, 1991). These loci have also been described as *NAT-1* (Grant, 1993), or *mNAT* (Ward et al., 1992), and *NAT-2* (Blum et al., 1991), or *pNAT* (Hickman et al., 1992). The *AAC-1* locus is an intronless gene coding for a protein of 290 amino acids. The *AAC-2* locus is a gene with a non-coding exon which is approx. 8 kb upstream of the start codon (Ebisawa and Deguchi, 1991). The coding region is 870 bases and the coding regions of *AAC-1* and *AAC-2* are 87% identical at the nucleotide level (Blum et al., 1990).

The *AAC-2* locus is multi-allelic with at least six alleles (Blum et al., 1991; Deguchi, 1992; Hickman et al., 1992; Lin et al., 1993) differing by a combination of up to three point mutations within the coding region. One of the alleles confers the fast-acetylator type, whereas the other alleles are associated with slow acetylation (Vatsis et al., 1991; Hickman et al., 1992). The *AAC-1* locus encodes what has been considered as monomorphic *N*-acetyltransferase (NAT). However, recent functional studies suggest that there is an inter-individual inherited variation which is distinct from the allele type present at the *AAC-2* locus (Cribb et al., 1991; Ward et al., 1992). Recently, different alleles at the *AAC-1* locus have been described (Vatsis and Weber, 1993). One of the alleles shows a point mutation in the coding region and there may be other alleles with deletions in the 3′ non-coding region.

Both *AAC-1* and *AAC-2* gene products are able to catalyse the acetylation of certain arylamines such as anisidine (Coroneos et al., 1991) and the carcinogen, aminofluorene. There are some substrates, e.g. *p*-aminobenzoic acid (Ohsako and Deguchi, 1990) and sulphamethoxazole (Cribb et al., 1993), which are specific for the monomorphic *N*-acetyltransferase (encoded at *AAC-1*) while other substrates, e.g. sulphamethazine, are acetylated only by the polymorphic *N*-acetyltransferase (encoded at *AAC-2*).

There is evidence to suggest that individuals of the slow-acetylator type are at increased risk of benzidine-induced bladder cancer (Cartwright et al., 1982). However, arylamine *N*-acetyltransferase, in association with N-oxidation, may be involved in activation of carcinogens (Probst et al., 1992) such as 2-aminofluorene to generate the ultimate carcinogen which has been postulated to be the *N*-acetoxyester (Flammang et al., 1987). It may be that in the absence of N-oxidation, N-acetylation of carcinogens has a protective role.

In view of the proposed association of arylamine *N*-acetyltransferase activity with cancer susceptibility, it was of particular interest to map the loci for NAT in the human genome in order to determine whether they are located in a chromosomal region, or regions which are commonly deleted or altered in bladder cancers. Here we report the confirmation of the assignment of *AAC-1* and *AAC-2* to chromosome 8 and show a more precise regional localization to 8p21.3–23.1 using yeast artificial chromosome (YAC) clones containing the genes *AAC-1* and *AAC-2*.

MATERIALS AND METHODS

PCR and screening of the YAC library

Screening of the YAC library (Anand et al., 1990) was carried out on pools, as supplied by the Human Genome Mapping Project (HGMP) Resource Centre, using PCR with primers specific for *AAC-1* and *AAC-2*. Specificity of amplification was confirmed by restriction enzyme digestion, with *Hin*cII, or its isoschizomer *Hin*dII, and *Hin*dIII digesting *AAC-2* and *AAC-1* respectively (Kelly and Sim, 1991). The conditions for amplification were as described previously for Nat-Hu7 and Nat-Hu8 (Kelly and Sim, 1991), Nat-Hu14 and Nat-Hu16 (Hickman and Sim, 1991) and Mono A and Mono C (Coroneos and Sim, 1993). The conditions for amplification using Nat-Hu20 as sense primer with either Nat-Hu21 or Nat-Hu22 as anti-sense primer (see Table 1) were

Abbreviations used: HGMP, human genome mapping project; TE, 10 mM Tris/HCl/1 mM EDTA (pH 7.5); SSC, 0.15 M NaCl/0.015 M sodium citrate (pH 7.4); SSPE, 0.15 M NaCl/10 mM sodium phosphate/0.17 mM EDTA (pH 7.0); f.i.s.h., fluorescence *in situ* hybridization; YAC, yeast artificial chromosome; NAT, *N*-acetyltransferase.

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Table 1 Oligonucleotide primers for amplification of the open reading frame of *AAC-1* and *AAC-2*

The numbers correspond to the base position in the cDNA. These primers have engineered restriction sites (lower case) for *Eco*RV (Nat-Hu20) and *Eco*RI (Nat-Hu21 and Nat-Hu22) and underlining indicates where the sequence has been altered from the *AAC* template sequence. Bases in bold type at the 3′ end are specific for amplification of either *AAC-1* (Nat-Hu22) or *AAC-2* (Nat-Hu21).

Primer	Sequence
Nat-Hu20 (*AAC-1, AAC-2*)	5′$^{-10}$TTAGGGgatatcGGACATTGAA^{12} 3′ sense
Nat-Hu21 (*AAC-2*)	5′^{881}TCCgaattcTAAATAGTAA**GGG**^{860} 3′ antisense
Nat-Hu22 (*AAC-1*)	5′^{883}ACTCCgaattcTAAATAGTAA**AAA**^{860} 3′ antisense

as follows: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation (94 °C, 0.5 min); annealing (54 °C, 1 min); extension (72 °C, 1.5 min); and a final extension time of 10 min. For each PCR reaction 2 μl of DNA template was used in a final 25 μl reaction volume. *Taq* polymerase was purchased from Boehringer (Mannheim, Germany) and the buffer, which was diluted from a 10-fold concentrated stock supplied by Boehringer, contained 1.5 mM $MgCl_2$. For the tertiary screen, the initial denaturation time at 94 °C was increased to 5 min and the annealing temperature was decreased to 52 °C.

The primary screen was carried out on 40 pools of the YAC library (Anand et al., 1990). The secondary screen was carried out on nine aliquots of the single positive pool from the first screen. The tertiary screen was carried out on the single positive sample from the secondary screen using pooled rows and columns from microtitre plates containing, in each well, an individual YAC clone in a yeast cell suspension (Anand et al., 1990). Restriction enzyme digestion of the products of the PCR was carried out as described previously (Hickman et al., 1992). All restriction enzymes were purchased from Boehringer.

For screening hamster/human or mouse/human hybrid cell lines with a defined human chromosome content, genomic DNA (50 ng) was used as a template for PCR with Nat-Hu20 and Nat-Hu21 and with Nat-Hu20 and Nat-Hu22 or Mono A and Mono C as primers. Hybrids were obtained from the MRC Human Biochemical Genetics Unit or through the HGMP Resource Centre, Harrow, U.K. The sources of the hybrids were as follows: MCP6BRA, 7628a (Shephard et al., 1991); Clone21E, 1aA9602+VE, 289, HORLI, 2860H7 (Zhong et al., 1992), D.T.1.2.4 (Swallow et al., 1977), C4a/G (Edwards et al., 1986), JICI4 (Kao et al., 1976) and all other hybrids were from the Human Genetic Mutant Cell Respiratory (Cornell Institute for Medical Research, Camden, NJ, U.S.A.).

Yeast culture and preparation of YAC DNA

Colonies from two of the positive YAC clones (designated 10BG11 and 10DF9) were streaked on to plates containing SD medium [which contains, per litre, 6.7 g of yeast nitrogen base, 55 mg of adenine sulphate, 55 mg of tyrosine, 20 g of glucose and 14 g of casamino acids (Difco, East Molesey, U.K.)] from slopes provided by the HGMP Resource Centre. The cultures were grown for 48 h. Single colonies, grown overnight from SD medium (5 ml), were used to inoculate 50 ml cultures which were harvested (1000 ***g***, 20 min, 4 °C) after 24 h. All yeast cultures were maintained at 30 °C and liquid cultures were shaken.

DNA was prepared from yeast cells from 50 ml cultures and was extracted three times with 1 ml of phenol/chloroform/isoamyl alcohol (25:24:1, by vol.), once with chloroform/isoamyl alcohol (24:1, v/v) and precipitated with ammonium acetate and ethanol (Sambrook et al., 1989). The precipitate was resuspended in 100 μl of 10 mM Tris/HCl/1 mM EDTA, pH 7.5 (TE).

Southern-blot analysis

DNA (2 μg) prepared from yeast clones G11 or F9 was digested for 15 h with 5 units of either *Kpn*I or *Eco*RI, as previously described (Kelly and Sim, 1991). The DNA fragments were separated by electrophoresis on an 0.8% agarose gel and were blotted on to Hybond N^+ (Amersham) by capillary transfer for 16 h in 0.4 M NaOH (Brown, 1991). The membrane was rinsed (2 min) in 0.3 M NaCl/0.03 M sodium citrate, pH 7.4 (2 × SSC).

The probe was a fragment of cDNA covering bases 20–871 of polymorphic *NAT* generated by amplification of genomic DNA from a homozygous fast acetylator in a PCR reaction mixture using Nat-Hu7 and Nat-Hu8 as primers (Kelly and Sim, 1991). The probe was labelled with [α-^{32}P]dCTP (50 μCi) using nick translation (Amersham) to a final specific radioactivity of 5×10^8 c.p.m. per μg. Prehybridization of the membrane was at 65 °C for 4 h in 20 ml of 10% (w/v) dextran sulphate/6 × SSC/5 × Denhardt's reagent/1% (w/v) SDS/1 mM EDTA, pH 8.0, containing 500 μg/ml sonicated herring sperm DNA (Sigma, Poole, Dorset, U.K.). The radiolabelled probe (400 μl, containing 2.5×10^7 c.p.m.) was added and hybridization was for 16 h at 65 °C. Membranes were washed at 65 °C for 1 min and then for 10 min in 0.9 M NaCl/60 mM sodium phosphate/1 mM EDTA, pH 7.0 (6 × SSPE), twice for 10 min in 4 × SSPE, once for 10 min in 2 × SSPE and then once in 2 × SSPE for 5 min at 20 °C. Autoradiographs were exposed at −70 °C for 5 h.

Fluorescence *in situ* hybridization (f.i.s.h.)

Chromosome preparations were obtained from normal male peripheral blood and f.i.s.h. of YAC clones F9 and G11 was performed essentially as described (Buckle and Rack, 1993). Probe DNA was labelled with biotin by nick translation and hybridized at 300 ng of DNA per slide, with 7.5 μg of Cot1 DNA as competitor. After probe detection with layers of fluorescein-conjugated avidin (Vector Laboratories, Peterborough, U.K.) and biotinylated anti-avidin antibodies (Vector), the slides were mounted with 0.5 μg of diamidinophenylindole and 0.5 μg of propidium iodide per ml of anti-fade medium (Vector). In this way the fluorescein isothiocyanate signal from the YAC can be seen against propidium iodide-stained red chromosomes and the same chromosomes can be viewed banded with diamidinophenylindole under u.v. illumination. A confocal laser microscope (Bio-Rad MRC600) was used for the analysis. The banded chromosomes were photographed on a standard Olympus Vannox fluorescence microscope. The identity of chromosome 8 was confirmed by staining with chromosome 8-specific alpha satellite DNA purchased in the biotinylated form (Oncor, Alpha Labs, Eastleigh, Devon, U.K.).

RESULTS AND DISCUSSION

Specificity of primers for *AAC-1* and for *AAC-2*

The primer pairs Mono A and Mono C and Nat-Hu7 and Nat-Hu8 have already been shown to be specific for *AAC-1* (Coroneos and Sim, 1993) and *AAC-2* (Kelly and Sim, 1991) respectively. Nat-Hu14 and Nat-Hu16 have also been shown to be specific for *AAC-2* (Hickman and Sim, 1991). Nat-Hu20 can be used with either primer Nat-Hu21 or with Nat-Hu22 as the anti-sense primer. The specificity of the primer pairs 20 and 21 for *AAC-2*

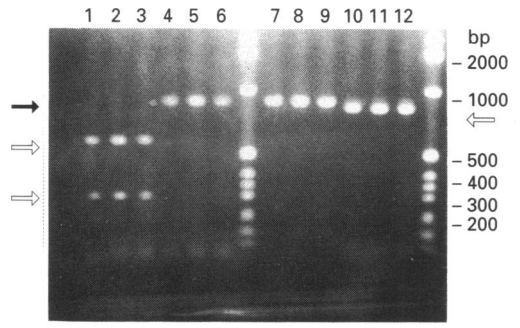

Figure 1 Specificity of amplification of genomic DNA using sense primer Nat-Hu20 with either Nat-Hu21 or Nat-Hu22 as anti-sense primer

Genomic DNA prepared from peripheral white blood cells from three different individuals was amplified with either Nat-Hu20 and Nat-Hu21 (tracks 1–6) or with Nat-Hu20 and Nat-Hu22 (tracks 7–12). The amplification product was digested with either *Hin*cII (tracks 1–3 and 7–9) or with *Hin*dIII (tracks 4–6 and 10–12). The closed arrow denotes the position of the undigested product and the open arrows denote the positions of informative bands. A 1 kb DNA ladder as a molecular-mass marker is shown after tracks 6 and 12. Samples from the same individual are shown in tracks 1, 4, 7 and 10; 2, 5, 8 and 11; or 3, 6, 9 and 12.

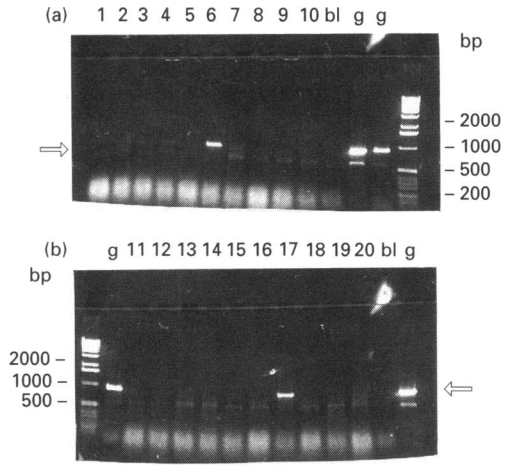

Figure 2 Tertiary screen of HGMP YAC library for *ACC-1* by PCR

Rows (tracks 1–12) and columns (tracks 13–20) screened with Nat-Hu20 and Nat-Hu22 as primers are shown. Human genomic DNA used as positive control is illustrated by 'g'. A negative control with no added DNA is shown as 'bl'. The position of molecular-mass markers are indicated adjacent to the marker track. The open arrow denotes the position of the *AAC-1* band.

is demonstrated in Figure 1 (tracks 1–6), by using a comparison of the susceptibility of the products to digestion with *Hin*cII and *Hin*dIII (see Sim and Hickman, 1991, for a summary). The amplification product with human genomic DNA as template is 900 bp and the expected size is 893 bp. The 900 bp product is resistant to digestion with *Hin*dIII but is digested with *Hin*cII to give fragments of 300 bp and 600 bp. This pattern of digestion is diagnostic of polymorphic *NAT* or *AAC-2* and the expected sizes of the fragments are 304 bp and 587 bp.

The product-primer pair Nat-Hu20 and Nat-Hu22 amplifies a similarly sized product (Figure 1, track 7), which is resistant to digestion with *Hin*cII but is digested with *Hin*dIII to give a product of 840 bp. This pattern of digestion is diagnostic of monomorphic *NAT* or *AAC-1* and products of 842 and 51 bp would be expected; however, the 51 bp band would be lost on agarose gel electrophoresis.

Screening of YAC library by PCR

The primary screen was carried out on 40 individual YAC pools supplied by the HGMP as described by Anand et al. (1990). The primer pairs used were Mono A and Mono C for *AAC-1* and Nat-Hu20 and Nat-Hu21 for *AAC-2*. Each of these pairs of primers amplified a band of the correct size from tube 10. The secondary screen was carried out on nine tubes (10A–10I) which correspond to microtitre plate positions of the original library. The secondary screen carried out with the primer pair Nat-Hu20 and Nat-Hu21 or Nat-Hu14 and Nat-Hu16 (*AAC-2*) gave a positive result with pool 10B as template, while the primer pair Nat-Hu20 and Nat-Hu22 or Mono A and Mono C (*AAC-1*) gave a positive result with pool 10D as template. In the tertiary screen, 20 combinations of rows or columns, (a–h and 1–12) were used as templates and three positives were obtained with the primer pairs Nat-Hu20 and Nat-Hu21 or Nat-Hu14 and Nat-Hu16, identifying two YAC clones containing *AAC-2*, namely 10BG6 and 10BG11. These clones were not amplified by Nat-Hu20 and Nat-Hu22 or by Mono A and Mono C.

Using the primer pair Nat-Hu20 and Nat-Hu22, on the 10D pool, two positive results were obtained, identifying one YAC clone containing *AAC-1*, namely 10DF9 (Figure 2). No amplification of 10DF9 was obtained with the primer pair Nat-Hu20 and Nat-Hu21 or with Nat-Hu14 and Nat-Hu16.

DNA from clones positive for *AAC-2* (G6 and G11) was amplified with Nat-Hu20 and Nat-Hu21 or Nat-Hu14 and Nat-Hu16, and the genotype (Hickman et al., 1992) of *AAC-2* in these clones has been determined as 'F1' as they are digested by *Kpn*I, *Taq*I, *Bam*HI and *Dde*I to give 'F1'-specific fragments.

With the F9 clone, amplification was obtained with Nat-Hu20 and Nat-Hu22 as primers and this was susceptible to digestion with *Hin*dIII but was resistant to *Hin*cII digestion. *AAC-1* is therefore present in the F9 clone.

Southern-blot analysis

In order to have independent evidence that the YAC clones identified from the tertiary screen contained *NAT*, Southern blotting of DNA from these clones with a probe specific for *NAT* was carried out. After digestion of DNA from the YAC clone G11 with *Eco*RI, a band corresponding to 1.9 kb was obtained. After digestion of DNA from the F9 clone with *Eco*RI, a minor band corresponding to 5 kb and a major band corresponding to 1.3 kb were detected (Figure 3).

These results confirm that there are sequences in these YAC clones that correspond to *NAT*. The probe will hybridize with *AAC-1* and *AAC-2*, due to the similarity between them over this region (Ohsako and Deguchi, 1990). The sizes of the bands observed on Southern blotting agree with studies which have been carried out by Deguchi and colleagues (Ohsako and Deguchi, 1990; Deguchi et al., 1990) in which genomic DNA from different individuals was cleaved with *Eco*RI and visualized with a *NAT* probe specific for *AAC-1* and for *AAC-2*. They showed that the 1.9 kb band, which is present in G11, is due to *AAC-2* and the band at 1.3 kb which is present in F9 is due to *AAC-1*. The identification of *AAC-2* in G11 and *AAC-1* in F9 are also in agreement with the amplification of DNA from the YAC clones using specific primers. The minor band at 5 kb observed in F9 corresponds to a band which was observed by Deguchi and colleagues (Ohsako and Deguchi, 1990) when they used a

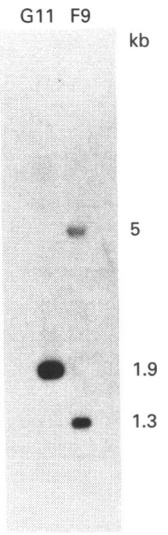

Figure 3 Southern blot of DNA from YAC clones G11 and F9

The clones were digested with *Eco*RI, blotted and probed as described in the Materials and methods section. The sizes of the fragments are indicated by comparison with a 1 kb ladder.

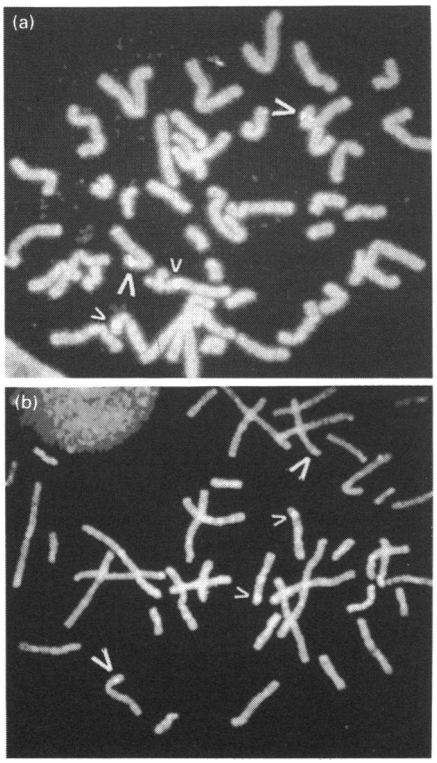

Figure 4 F.i.s.h. of (a) YAC F9 for *AAC-1* and (b) YAC G11 for *AAC-2*

(**a**) F9 hybridizes to chromosome 8 short arm in the region p21.3–p23.1 (large arrowheads) and chromosome 10 short arm (short arrowheads). (**b**) G11 hybridizes to chromosome 8 short arm in the region p21.3–p23.1 (large arrowheads) and to the long arm of chromosome 11 (short arrowheads).

complete cDNA for *NAT* as the probe, but it was not observed with either *AAC-1*- or *AAC-2*-specific probes. It may be that the 5 kb band is due to an unrelated cross-hybridizing sequence, as

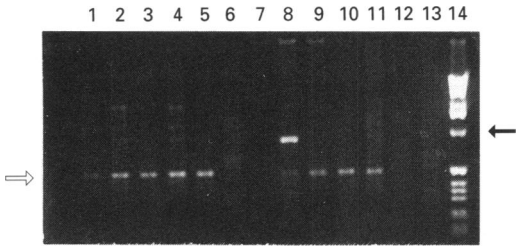

Figure 5 PCR analysis of genomic DNA from hybrids with a known human chromosomal content

The lanes contain the product of PCR amplification using primers Nat-Hu20 and Nat-Hu21 with 1 μg of DNA from each of the following hybrids: lane 1, GM07299; lane 2, GM10826B; lane 3, GM10253; lane 4, HHW4164; lane 5, GM10114; lane 6, MCP6BRA; lane 7, CLONE21E; lane 8, C4a/G; lane 9, GM10611; lane 10, 7628a; lane 11, JICI4; lane 12, 1aA9602+VE; lane 13, HORLI. The chromosomal content of the hybrids is shown in Table 2. The filled arrow denotes the expected size of the product (920 bp) and the open arrow denotes a 500 bp product which is due to hamster genomic DNA. Lane 14 shows molecular-mass markers. The marker band closest to the filled arrow is 1 kb.

Table 2 Identification of chromosomal assignment of *AAC-1* and *AAC-2* by PCR

Genomic DNA (50 ng) from each of the hybrids was used as template for PCR with either *AAC-1*-specific primer pairs (Nat-Hu20, 22 or Mono A, Mono C) and with the *AAC-2*-specific primer pair (Nat-Hu20, 21). + denotes a product, as illustrated in Figure 5.

Hybrid	Hu chromosome	*AAC-1*	*AAC-2*
GM07299	1, X	–	–
GM10826B	2	–	–
GM10253	3	–	–
HHW4164	4	–	–
GM10114	5	–	–
MCP6BRA	6p21–qter, Xq	–	–
CLONE21E	7	–	–
C4a/G	8	+	+
GM10611	9	–	–
D.T.1.2.4.	3, 6, 10, 11, 12, 15, 17, 18, 20, 21, X	–	–
7628a	10, Y	–	–
JICI4	11	–	–
1aA9602+VE	12, X, 21	–	–
GM10479	14, 16	–	–
HORLI	15, 11q, X	–	–
2860H7	16	–	–
PGME25nu	22, X	–	–

Deguchi and colleagues suggest. A 5 kb *Eco*RI fragment is also compatible with the partial restriction map of a cDNA phage clone containing a *NAT* pseudogene identified by Blum and colleagues (Blum et al., 1990). The pseudogene would hybridize with the probe as it is 79% identical with *AAC-1* and 80% identical with *AAC-2* (Blum et al., 1990).

F.i.s.h.

Although lambda and cosmid clones can be used for f.i.s.h., YAC clones have also been used successfully (Buckle and Rack, 1993). Both YAC clones F9 and G11 hybridized using f.i.s.h. to the mid short arm of chromosome 8. Comparison with DAPI-banded photographs of the same metaphase spreads suggest that both YAC signals are localized to 8p21.3–23.1 (Figure 4, large

arrowheads). In addition, F9, which is thought from PCR analysis to contain *AAC-1*, hybridizes to the short arm of chromosome 10, while G11, containing *AAC-2*, hybridizes to the long arm of chromosome 11 (Figure 4, short arrowheads).

Using primers specific for *AAC-1* and for *AAC-2* and, as template for PCR, genomic DNA from a series of somatic cell hybrids with a defined human chromosomal content, it has been shown clearly that only the hybrid cell line C4a/G (Edwards et al., 1986), which contains chromosome 8, was amplified. No *AAC-1* or *AAC-2* was identified in hybrids containing human chromosome 10, namely 7628a (Shephard et al., 1991) and D.T.1.2.4. (Swallow et al., 1977). No amplification was obtained from DNA from two hybrids which contain human chromosome 11 (JICI4) (Kao et al., 1976) or the long arm of chromosome 11 (HORLI) (Zhong et al., 1992). These results are illustrated for *AAC-2* in Figure 5 and are summarized for *AAC-1* and *AAC-2* in Table 2.

The results of the screening of the somatic cell hybrids confirm the chromosomal localization of *AAC-1* and *AAC-2* to chromosome 8 and indicate that the hybridization of the YAC clones to the short arm of chromosome 10 and the long arm of chromosome 11 are likely to represent either chimerism within the YAC clones or cross-hybridization of sequences within the YACs to secondary sites in the genome. We conclude that both *AAC-1* and *AAC-2* are localized to the region 8p21.3–23.1.

It has been demonstrated that there is a correlation between slow acetylation catalysed by *AAC-2* and susceptibility to bladder cancer (Cartwright et al., 1982) and recently it has been demonstrated that in bladder cancer there is a correlation with a deletion in chromosome 8 covering a region including 8p21.3 (Knowles et al., 1993). The localization of *AAC-1* and *AAC-2* close to or within this region indicates that these genes have functional importance in determining susceptibility to bladder cancer. The use of highly polymorphic *AAC-2* as a marker for defining more precisely the extent of chromosome 8 deletions in tumours will be of importance in determining the role of arylamine *N*-acetyltransferase as a bladder-cancer-susceptibility factor.

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REFERENCES

Anand, R., Riley, J. H., Butler, R., Smith, J. C. and Markham, A. F. (1990) Nucleic Acids Res. **18**, 1951–1956
Blum, M., Grant, D. M., McBride, W., Heim, M. and Meyer, U. A. (1990) DNA Cell Biol. **9**, 193–203
Blum, M., Demierre, A., Grant, D. M., Heim, M. and Meyer, U. A. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5237–5241
Brown, T. A. (1991) Molecular Biology: Labfax, p. 284, Bios Scientific, Oxford, U.K.
Buckle, V. J. and Rack, K. A. (1993) in Human Genetic Disease Analysis: A Practical Approach (Davies, K. E., ed.), 2nd edn., pp. 59–82, Oxford University Press, Oxford
Cartwright, R. A., Glasham, R. W. and Rogers, H. J. (1982) Lancet **ii**, 842–846
Coroneos, E. and Sim, E. (1993) Biochem. J. **294**, 481–486
Coroneos, E., Gordon, J., Kelly, S. L., Wang, P. D. and Sim, E. (1991) Biochim. Biophys. Acta **1073**, 593–599
Cribb, A. E., Grant, D. M., Miller, M. A. and Spielberg, S. P. (1991) J. Pharm. Exp. Therap. **259**, 1241–1246
Cribb, A. E., Nakamura, H., Grant, D. M., Miller, M. A. and Spielberg, S. P. (1993) Biochem. Pharmacol. **45**, 1277–1282
Deguchi, T. (1992) J. Biol. Chem. **267**, 18140–18147
Deguchi, T., Mashimo, M. and Suzuki, S. (1990) J. Biol. Chem. **265**, 12757–12760
Donis-Keller, H. and Buckle, V. (1991) Cytogenet. Cell Genet. **58**, 382–402
Ebisawa, T. and Deguchi, T. (1991) Biochem. Biophys. Res. Commun. **177**, 1252–1257
Edwards, Y. H., Barlow, J. H., Konialis, C. P., Povey, S. and Butterworth, P. H. W. (1986) Ann. Hum. Genet. **50**, 123–129
Flammang, T. J., Yamazoe, Y., Guengerich, F. P. and Kadlubar, F. F. (1987) Carcinogenesis **8**, 1967–1970
Grant, D. M. (1993) Pharmacogenetics **3**, 45–50
Hickman, D. and Sim, E. (1991) Biochem. Pharmacol. **42**, 1007–1014
Hickman, D., Risch, A., Camilleri, J. and Sim, E. (1992) Pharmacogenetics **2**, 217–226
Kao, F. T., Jones, C. and Puck, T. T. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 193–197
Kelly, S. L. and Sim, E. (1991) Human Exp. Toxicol. **10**, 33–38
Knowles, M. A., Shaw, M. E. and Proctor, A. J. (1993) Oncogene **8**, 1357–1364
Lin, H. J., Han, C.-Y., Lin, B. K. and Hardy, S. (1993) Am. J. Human Genet. **52**, 827–834
Ohsako, S. and Deguchi, T. (1990) J. Biol. Chem. **265**, 4630–4634
Probst, M. R., Blum, M., Fasshauer, I., D'Orazio, D., Meyer, U. A. and Wild, D. (1992) Carcinogenesis **13**, 1713–1717
Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, pp. 9.22, Appendix E, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
Shephard, E. A., Povey, S., Spurr, N. K. and Phillips, I. R. (1991) Genomics **11**, 302–308
Sim, E., Hickman, D., Coroneos, E. and Kelly, S. L. (1992) Biochem. Soc. Trans. **20**, 304–309
Swallow, D. M., Solomon, E. and Pajunen, L. (1977) Cytogenet. Cell Genet. **18**, 136–148
Vatsis, K. P. and Weber, W. W. (1993) Arch. Biochem. Biophys. **301**, 71–76
Vatsis, K. P., Martell, K. J. and Weber, W. W. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 6333–6337
Ward, A., Hickman, D., Gordon, J. W. and Sim, E. (1992) Biochem. Pharmacol. **44**, 1099–1104
Zhong, S., Wolf, C. R. and Spurr, N. K. (1992) Human Genet. **90**, 435–439