



#### Genomic amplification of Dp140 first exon

Dystrophin exon 55 was co-amplified in each reaction tube. The agarose gel was stained with ethidium bromide. Dystrophin gene deletions are reported on the upper end of the figure. M=marker size. C=control patient. Lane 1 to lane 6 patients' IQ was 110, 72, 68, 68, 74, 61, respectively.

retardation. In BMD the most common deletions are clustered around exons 45-53. At present, it is unknown whether these mutations also extend to involve Dp140 promoter and first exon located within intron 44. If so, the result would be the complete absence of Dp140 in the presence of an altered—but still partially functional—full-length dystrophin.

The study was done on 24 BMD patients. Selection was determined by the presence of deletions of variable length either with a 3'-end breakpoint in exon 44 or with a 5'-end breakpoint in exon 45. Full IQ (FIQ) was evaluated by means of Wechsler Intelligence Scale. Patients with an FIQ less than or equal to 75 were considered mentally retarded. Six of the 24 (25%) individuals showed mental retardation. All patients were screened for deletions in Dp140 first exon and promoter region by PCR amplification (figure).

Mean FIQ level in the whole BMD group was 88.2. All patients with FIQ>75 presented no deletions in the Dp140 regulatory sequences. Five out of the six mentally-retarded patients presented a deletion including Dp140 regulatory region. The correlation between the absence of Dp140 regulatory regions and the presence of mental retardation was statistically significant ( $\chi^2$  test,  $p<0.001$ ).

Three patients had 3'-end deletion breakpoint in exon 44. Interestingly, these deletions extended to the Dp140 regulatory elements only in the two mentally-retarded patients, whereas the other patient presented spared cognitive functions and Dp140 isoform.

With regard to deletions located downstream to exon 45, it should be noted that 17 patients presented deletions not including the Dp140 translation start site (exon 51). Their cognitive profile correlates with the presence or absence of Dp140 regulatory sequences, with the only exception of a patient coming from a very low socioeconomic background.

Finally, in four patients with deletions encompassing exon 51, mental retardation was not observed. Yet, sequence analysis showed that different in-frame start codons are present in exons 52 through 56. In the absence of the first start codon in exon 51 and upon shortening of the 5'-untranslated region, the downstream start codons might serve as translation initiation sites.<sup>5</sup>

In conclusion, our study provides evidence that the absence of Dp140 might determine the occurrence of cognitive impairment in BMD patients. Other authors have already highlighted the importance of Dp140 for mental retardation in DMD patients.<sup>4</sup> Yet, the absence of full-length

dystrophin transcripts in DMD makes it more difficult to understand the functional role for each single isoform. The close correlation between Dp140 and mental retardation in BMD indirectly throws light on the role of Dp140 in DMD as well.

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## Genetic variation of CYP2A6, smoking, and risk of cancer

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Smoking is the major cause of lung cancer, but genetic variation in metabolism of substances in tobacco smoke may influence susceptibility to cancer. Pianezza, Sellers, and Tyndale reported that genetically deficient CYP2A6, an enzyme involved in metabolism of nicotine reduces the tendency to smoke and queried whether lung-cancer risk might be reduced by this mechanism.<sup>1</sup>

We examined whether the association between the presence of a single CYP2A6 reduced activity allele and lower prevalence of smoking held among 460 people enrolled in a case-control study of lung cancer in Los Angeles County, California.<sup>2</sup> We collected detailed lifetime smoking histories on all participants. CYP2A6 alleles were identified by the method of Fernandez-Salguero et al.<sup>3</sup> The CYP2A6\*2 variant allele has a T to A substitution that creates an XcmI restriction site, leads to a leucine to histidine change at codon 160 in exon 3 and is associated with reduced activity. The CYP2A6\*3 allelic variant appears to have been created by gene conversion between the wild-type (CYP2A6\*1) allele and the neighbouring CYP2A7 and is identified by a unique DdeI restriction site in exon 3. It is thought that CYP2A6\*3 confers reduced activity because of sequence similarity to CYP2A7 which codes for an inactive enzyme.

Compared with subjects without a reduced activity allele, those with two reduced activity alleles were slightly over-represented among never smokers (table) ( $p=0.057$ ). However, in contrast to the finding of Pianezza, Sellers, and Tyndale,<sup>1</sup> participants with a single reduced activity allele were not less likely to smoke (table). Further, we found no evidence that the presence of reduced activity alleles decreased the usual number of cigarettes consumed per day by smokers (table).

CYP2A6 is also involved in metabolic activation of carcinogenic nitrosamines in tobacco smoke including NNK, NNAL, NDEA, and NDMA.<sup>4,5</sup> Diminished CYP2A6 activity might decrease production of genotoxic metabolites of these nitrosamines and potentially reduce the risk of lung cancer by this mechanism. We tested the hypothesis that carriers to reduced activity CYP2A6 alleles are at decreased risk of lung cancer among 182 cases of recently diagnosed lung cancer and the 460 population controls.<sup>2</sup> Participants

	Number of CYP2A6 alleles with decreased activity			
	None	One	Two	Total
<b>Smoking</b>				
Never*	75.8%	19.9%	4.3%	161
Ever	77.9%	20.7%	1.3%	299
<b>Ever Smokers</b>				
Cigarettes per day†	23.5	27.1	27.5	
<b>Lung Cancer</b>				
Cases	142	38	2	182
Controls	355	94	11	460
RR (95% CI)‡	1.0	1.0 (0.6-1.6)	0.5 (0.1-2.1)	

\*p=0.057 for difference between proportion of controls with two versus no inactive alleles comparing never and ever smokers. p=0.113 for difference between proportion of controls with one versus no inactive alleles comparing never and ever smokers. Ever smokers are defined by having smoked at least 100 cigarettes. †Mean of cigarettes per day usually smoked, p=0.35 for difference by CYP2A6 genotype. ‡Relative risk of lung cancer and 95% CI are calculated relative to participants with no inactivating alleles.

#### Effect of number of CYP2A6 inactive alleles smoking and lung cancer risk

carrying only one reduced activity allele (genotypes CYP2A6\*1/CYP2A6\*2 and CYP2A6\*1/CYP2A6\*3) were not at decreased risk of lung cancer compared with participants with no reduced activity alleles (genotype CYP2A6\*1/CYP2A6\*1) (RR=1.0). Among the few participants carrying two reduced activity alleles a 50% decreased risk of lung cancer was observed, a result virtually unchanged upon restriction of the analysis to smokers (OR=0.4). However, given the small number of participants with two reduced activity alleles this association is quite compatible with chance.

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## Appearance of IMP-1 metallo-β-lactamase in Europe

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The *bla*<sub>IMP</sub> gene was the first transferable metallo-β-lactamase determinant identified in clinical isolates of various *Enterobacteriaceae* (*Serratia marcescens*, *Klebsiella pneumoniae*, *Citrobacter freundii*), *Pseudomonas aeruginosa*, and other non-fastidious gram-negative non-fermenters.<sup>1-3</sup> Owing to the broad substrate profile of its product (the IMP-1 enzyme), which includes expanded-spectrum cephalosporins and carbapenems,<sup>1</sup> spreading of *bla*<sub>IMP</sub> among similar pathogens is

a matter of major concern for the future of antimicrobial chemotherapy. Thus far the *bla*<sub>IMP</sub> determinant has been detected in only clinical isolates from Japan<sup>2,3</sup> and South Korea,<sup>4</sup> while metallo-carbapenemase-producing *P. aeruginosa* strains occasionally isolated in Europe have been found to harbour determinants other than *bla*<sub>IMP</sub>.<sup>5</sup>

In April, 1997, a multidrug resistant *Acinetobacter baumannii* strain (AC-54/97), which also showed an unusually high-level resistance to imipenem (minimum inhibitory concentration of 256 µg/mL), was isolated from the bronchial aspirate of an Italian patient admitted to our intensive care unit. The patient reported no history of recent travel abroad. The AC-54/97 strain was susceptible to only polymyxin B, and was resistant to fluoroquinolones, amikacin, tobramycin, gentamicin, fosfomycin, trimethoprim/sulfamethoxazole, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanate, aztreonam, ceftazidime, cefepime, imipenem, and meropenem. A crude extract prepared from this strain showed imipenem-hydrolysing activity susceptible to inhibition by EDTA. Isoelectric focusing analysis of the crude extract, followed by β-lactamase detection via a bioassay, revealed an imipenem-hydrolysing enzyme with an isoelectric pH of 8.5. A *bla*<sub>IMP</sub>-specific probe containing the 0.5-kb *Hind*III fragment internal to the gene<sup>1</sup> recognised AC-54/97 in a colony-blot assay carried out under stringent hybridisation conditions. In a Southern blot analysis of the genomic DNA of AC-54/97, the same probe hybridised to the band of undigested chromosomal DNA and to single restriction fragments of not variable sizes after digestion with several enzymes that do not cut into the *bla*<sub>IMP</sub> gene,<sup>1</sup> including *Bgl*II, *Cla*I, *Eco*RI, *Pst*I, *Kpn*I, *Sal*I, *Xba*I, *Xho*I. An *A. baumannii* strain with an identical resistance phenotype and similarly recognised by the *bla*<sub>IMP</sub>-specific probe was again isolated from this patient in June, 1997. The macrorestriction pattern of the *Sma*I-digested chromosomal DNA, analysed by pulsed-field gel electrophoresis, was identical for both strains supporting their clonal relatedness. The patient subsequently died from pneumonia and septic shock.

To our knowledge, this is the first report of a clinical isolate producing an enzyme that is closely related to the IMP-1 metallo-β-lactamase in Europe. Since the determinant was not sequenced, it remains to be established whether it is identical to or subtly different from *bla*<sub>IMP</sub> found in Japanese isolates. In this case, the host of the *bla*<sub>IMP</sub> allele was different from those encountered in the Far East where this resistance determinant has never been reported in *Acinetobacter* spp.<sup>2,4</sup> A similar finding strongly suggests an autochthonous origin of this *bla*<sub>IMP</sub>-positive *A. baumannii* strain, which could have independently acquired the β-lactamase determinant from a yet unknown environmental source. Molecular characterisation of the *bla*<sub>IMP</sub> locus carried by AC-54/97 is currently in progress to investigate this hypothesis, which implies that the environmental reservoir of *bla*<sub>IMP</sub> alleles could be more widespread than that initially suggested by the restricted geographical distribution of IMP-1-producing clinical isolates.

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