



Figure 2 The Giemsa stain in (A) as well as the spectrum orange painting probe which detects the euchromatic region of chromosome 9 by FISH (B) showing the G positive bands' resistance to undercondensation and the heterochromatic decondensation after the induction of 5-azacytidine (see text).

location of the breakpoints, and the number of breaks are crucial variables that play a significant role in the formation. The chromosome 9 may possess one or two distinct alpha signals⁸ in conjunction with either one or two beta signals.⁹ The number of breaks that occur are usually two or three and the location of breakpoints, which have been described by Macera's group, can either occur at the p or q arms, at pericentromeric regions within alpha and beta satellite DNA, and also within the qh region.³

It is possible that the extra bands in the three cases which have been highlighted earlier were transcriptionally inactive. The inactivation scenario suggested in these reports is probably the result of different factors depending upon the position effect or the chromatin packaging order. More extra chromosomal material has been found to be transcriptionally active and can be detrimental, resulting in abnormal fetal development.¹⁰ Nevertheless, this is not the case in the three aforementioned studies since the offspring were phenotypically normal. Obviously, the molecular approach should be taken to elucidate the heterogeneity of such variants showing the various inactivation mechanisms of euchromatic bands integrated within heterochromatin whose structure and function remain to be unravelled. The reproductive fitness of people with euchromatic bands embedded within selfish DNA (heterochromatin) will reflect the evolutionary dynamics of repetitive DNA sequences whose parasitic nature will continue to stir interest among biologists and clinicians alike in resolving the controversy concerning pathological significance. Molecular tools have just begun to play an important role in the discrimination of heteromorphisms from

chromosomal abnormalities, thus avoiding unnecessary fetal wastage. Reverberations will continue as new technology flourishes and the discovery of rare variants will no longer be as rare as we think. The clinical significance of these variants has been obscure since the function of heterochromatin remains unknown.^{11,12} This meaningless filler, when it surrounds functional DNA, may further dictate the iconoclastic nature of the so called junk DNA where one day we may discover treasure in "trash".

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Mild cystic fibrosis phenotype in patients with the 3272-26A>G mutation

The molecular defect in the cystic fibrosis (CF) gene appears to contribute to the heterogeneity of the CF phenotype, as certain mutations have severe and others mild clinical manifestations. During the investigation of 186 Greek CF patients to determine the type and frequency of CF mutations in Greece, three patients were characterised as compound heterozygotes for the mutation 3272-26A>G. In comparison to other patients, these three had a milder clinical phenotype as indicated by advanced age of diagnosis,

Clinical and laboratory findings in three CF patients with the 3272-26A>G mutation

	Case 1	Case 2	Case 3
	3272-26A>G/ Δ F508	3272-26A>G/ Δ F508	3272-26A>G/unknown
Current age	11 y	6 y	20 y
Age at diagnosis	4 y	9 y 6 mth	14 y
Sex	M	M	F
Sweat chloride (mEq/l)	110	105	95
Meconium ileus	—	—	—
Pancreatic function	PS	PS	PS
Other GI symptoms*	(-)	(-)	(-)
Shwachman score†	85	94	89
Chronic cough	Mild	Mild	Mild
Obstructive lung disease	Mild	None	None
CXR score	19	22	20
Respiratory failure	(-)	(-)	(-)
Nasal polyps	(+)	(-)	(+)
Pneumothorax	(-)	(-)	(-)
Haemoptysis	(-)	(-)	(-)
<i>Pseudomonas</i> colonisation	(-)	(-)	(-)
Dehydration	(+)	(+)	(+)
Lung function parameters‡			
FVC	113	—	98
FEV ₁	124	—	92

* Intestinal obstruction, gallbladder disease, liver cirrhosis.

† Shwachman-Kulczycki score of clinical status.⁷

‡ % of predicted.

absence of pancreatic insufficiency, absence of or mild obstructive lung disease, and good general condition, although one patient is still young (6 years old). We conclude that the 3272-26A>G mutation is not a severe CF allele and appears to be associated with a mild type of CF.

Cystic fibrosis is the most common autosomal recessive disorder in white populations and is characterised by wide heterogeneity at the clinical level, which seems to correlate with the extreme heterogeneity at the molecular level.

In the last few years several studies have been published which attempt to correlate the clinical phenotype with the genotype. Although there is evidence from these studies that some mutations are clearly correlated with mild clinical manifestations, results have been mixed owing to the fact that most of the mutations have only been found in a very small number of cases. However, in an extensive study published recently by the CF Genotype-Phenotype Consortium (1993) it was concluded that the only prognostic value of genotype information is for the prediction of pancreatic function.¹⁻³

The 3272-26A>G mutation constitutes a splicing defect in intron 17a; the nucleotide substitution A>G at position 3272-26 creates an alternative cryptic acceptor splice site that competes with the normal acceptor splice site during RNA processing and probably reduces splicing from the correct site. Thus at least a proportion of the mature mRNA may contain improperly spliced intron sequences. An example of the preferential use of a mutated 3' acceptor splice site, which compares with the 3272-26A>G mutation, is the G>A substitution in base pair 110 of intron 1 of the β globin gene.⁴ The globin mRNA formed from this gene is elongated and a β^+ thalassaemia phenotype results because the correct acceptor site is still used 10% of the time.

Mutation 3272-26A>G was first described in a compound heterozygote for the severe W806X mutation. This patient had a mild clinical phenotype with normal pancreatic function and mild pulmonary disease.⁵ In this report we present the clinical phenotype of three more CF patients, who have the 3272-26A>G mutation; two are compound heterozygotes with the common Δ F508 mutation and the third patient carries an as yet un-

identified CF mutation on his other chromosome.

The three patients were identified in the course of the CFTR genotype analysis of 186 CF patients attending the CF unit of St Sophia's Children's Hospital. The method of molecular analysis was based on denaturing gradient gel electrophoresis (DGGE) screening² of CFTR exons 4, 10, 11, 17b, 19, 20, and 21 and direct sequencing of any DNA samples showing a shift in DGGE mobility.⁶ Through this analysis we were able to identify 21 mutations covering about 75% of the CF alleles in Greece (submitted for publication). The patients included in this report all showed a distinct DGGE pattern for the PCR fragment of exon 17b and the 3272-26A>G mutation was identified by direct sequencing following asymmetrical PCR.

The clinical assessment of the three patients was based on the age of diagnosis, respiratory status, pancreatic function and other GI symptoms, and also the Shwachman-Kulczycki clinical score⁷ (patient activity, physical examination, growth, and nutrition). The clinical and basic laboratory data of the three patients are summarised in the table. They are at present 6, 11, and 20 years old and were diagnosed at ages 4, 9, and 14 years respectively. They are all pancreatic sufficient without other gastrointestinal symptoms, have an average S-K clinical score, and quite good general clinical status. They all have mild respiratory disease with normal CRX score, no lung colonisation with bacterial pathogens, very mild cough, and no obstructive lung disease, respiratory failure, pneumothorax, or haemoptysis. The two older patients (cases 1 and 3) have developed nasal polyps. Of interest is that all three patients have a history of dehydration, a cause for hospitalisation at least once during the summer months.

These severe episodes of salt loss and dehydration are more consistent with the manifestation of a severe CF allele.⁸ They also have quite high sweat chloride concentrations, which again is not in agreement with other reports of patients with mild alleles such as compound heterozygotes for R117H/ Δ F508,⁹ although a patient carrying Δ F508 with the mild allele 3850-3T>G (also a splice mutation) was diagnosed by greatly raised chloride concentrations at the age of 14 years.⁸ These observations indicate that levels

of chloride concentration in sweat tests may not correlate well with the clinical presentation of CF alleles.

The oldest patient in this study (case 3) was diagnosed in her teens and has the mildest phenotypic manifestations of CF, indicating that along with the 3272-26A>G allele she carries another mild CF allele.

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