

laboratory aspects are simplified. Accepted international guidelines and counselling protocols should be adhered to when using direct repeat size determination in doing pre-symptomatic or prenatal testing.<sup>18</sup>

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## Rare variants of chromosome 9 with extra G positive band within the qh region are not alike

The intriguing findings in a recent investigation by Fernandez *et al*<sup>1</sup> prompted us to re-evaluate our previous case<sup>2</sup> that had a similar extra G positive band within the 9qh region by additional molecular techniques. To our knowledge, these two studies, in addition to a third by Macera *et al*,<sup>3</sup> are the only ones that have used various molecular techniques which showed satellite DNA locations in this particular so-called rare variant of chromosome 9. Many other reports which have focused on this seeming paradox, stated that the variant chromosome 9 appeared to be similar by conventional banding techniques. Nevertheless, a molecular approach using the FISH technique suggested that each of these three chromosome 9 variants apparently have different structural arrangements. These three plus approximately 11 other people who have had this variant are phenotypically normal.<sup>3</sup> The structural organisation of the qh region in these rare variants have been shown by alpha, beta, and satellite III DNA probes by the FISH technique. In the study of Fernandez *et al*,<sup>1</sup> the chromosome 9 displayed one alpha and one beta signal. Verma *et al*<sup>2</sup> determined that a chromosome 9 had two alpha and two beta signals and their additional case<sup>3</sup> showed that a chromosome 9 had one alpha and two beta signals which shed some light on the possible mechanism and origin of a variety of these so-called rare heteromorphisms.<sup>3</sup> Three types of mechanism of the origin of such heteromorphic variants have been described<sup>3</sup> which can account for the alpha, beta, and satellite III DNA rearrangements within chromosome 9. Briefly, they are as follows. *Type I*: a break in each chromosome homologue is required, with one break on a chromosome at q21.2 and the other break in the homologue at or near the alpha/beta junction. *Type II*: initially, a pericentric inverted chromosome is essential with one break each at the p12/p13 interface and within the alphoid region; the satellite III/p13 border is the breakage site on the homologue. *Type III*: a break in the satellite III region of one chromosome and one break at band q21.2 on the homologue is necessary. Type I could account for the chromosome 9 in the report of Macera *et al*,<sup>3</sup> type II for Verma's group,<sup>2</sup> and type III closely resembles the variant chromosome 9 of Fernandez *et al*.<sup>1</sup>

The extra band observed by Fernandez *et al*<sup>1</sup> was resistant to *AluI* digestion and when introduced to 5-azacytidine treatment the band homogeneously undercondensed. However, in the investigation of Verma *et al*<sup>2</sup> the extra band was digested by *AluI*. All human centromeres contain alpha satellite DNA but not all centromeres are resistant to *AluI* treatment. This may be because of an evolutionary divergence at the DNA base pair level,<sup>4</sup> but the reason why the G positive band in the case of Verma *et al*<sup>2</sup> was not resistant to *AluI* digestion is apparently because of its euchromatic aetiology. The chromosome 9 from our previous case<sup>2</sup> was subjected to 5-azacytidine incorporation and then examined by the FISH technique using a D9Z1 probe which detects classical satellite III DNA of chromosome 9 and a spectrum orange painting probe which detects the euchromatin of

chromosome 9 (figs 1 and 2). Again, as in the *AluI* treatment, the result was the opposite of that of Fernandez *et al* and showed that the extra band was not affected and remained condensed. The induction of 5-azacytidine ( $3.5 \times 10^{-7}$  mol/l) during the last seven hours of incubation apparently hypomethylates the late replicating classical satellite constitutive heterochromatin regions of chromosomes 1, 9, 15, 16, and Y, and causes undercondensation owing to its incorporation during this period.<sup>5,6</sup> It is believed the undercondensation of the heterochromatin that is caused by the induction of 5-azacytidine is possible owing to the relatively high variation in the number of repeated units within the classical satellite DNA region; generally this is not the case in the alpha and beta satellite DNA of chromosome 9.<sup>4</sup> However, it has been shown that there are differences in the condensation inhibition behaviour of centromeric heterochromatin of chromosome 9 when treated with 5-azacytidine.<sup>7</sup> As stated earlier<sup>2</sup> the extra band in the variant chromosome 9 was "euchromatic in nature" and originated from chromosome 9, as indicated by the whole chromosome 9 painting probe (figs 1 and 2). Also, the fact that the extra band did not undercondense, as the heterochromatin did, suggests that this band is not late replicating and hence euchromatic in behaviour.

In the case of Fernandez *et al*,<sup>1</sup> the extra band in the qh region of the variant chromosome 9 undercondensed owing to 5-azacytidine treatment. This may suggest that the band is late replicating and is not euchromatic in behaviour. In this case, the decondensing effects of the 5-azacytidine treatment may have spread into euchromatic bands integrated into large blocks of classical satellite DNA. In contrast, the G band material may be insensitive to decondensation if it is immediately juxtaposed with alpha or beta satellite DNA. The results from the *AluI* treatment also indicate the same properties. It is important to note the significant events which resulted in this rare chromosome 9 variant. The particular type of chromosome 9 that is involved in the rearrangement, the

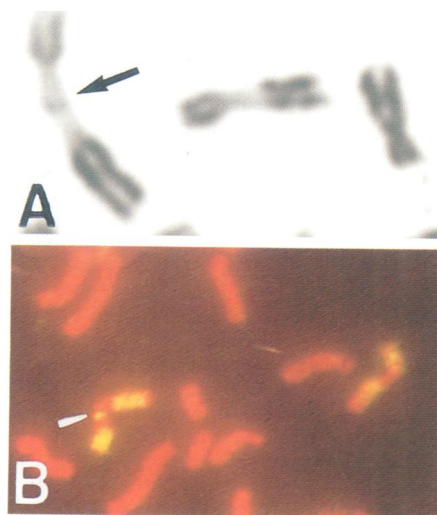


Figure 1 A D9Z1 probe by FISH detected classical satellite III DNA of chromosome 9 showing that the G positive band remains condensed in control (A) as well as after the induction of 5-azacytidine (B), while the heterochromatin decondensed after treatment (B).



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 signals<sup>8</sup> in conjunction with either one or two beta signals.<sup>9</sup> 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.<sup>3</sup>

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.<sup>10</sup> 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.<sup>11,12</sup> 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,