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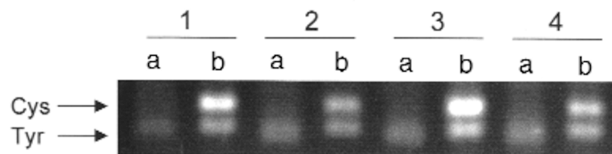

Fig. 2 *Rsa*I digest of amplified genomic DNA from four 5474A/5569A compound heterozygotes. PCR reactions are shown using the intron 3 sense primer and the intron 4 antisense primer that included the 5569G/A polymorphism site[4] ('a'), and the intron 3 sense primer and the alternative intron 4 antisense primer that avoided the 5569G/A polymorphism site ('b'). PCR product 'a' is 387 bp and 'b' is 393 bp. Normal DNA *Rsa*1 cleavage of product 'a' produces 247-bp and 140-bp fragments, and cleavage of product 'b', 247-bp and 146-bp fragments. The presence of the 5474A (C282Y) mutation results in cleavage of the 140-bp fragment ('a') into 111-bp and 29-bp fragments and cleavage of the 146-bp fragment ('b') into 117 bp and 29 bp. *Rsa*1 digestion of reaction 'a' produced a single fragment of 111 bp (Tyr) that incorrectly indicated a 5474A/5474A homozygote. In contrast, *Rsa*1 digestion of reaction 'b' produced fragments of 146 bp (Cys) and 117 bp (Tyr), correctly identifying 5474A heterozygotes. DNA from two 5569A homozygotes initially did not amplify with the original Feder *et al.* primers, but were later identified by repeat PCR using the new primer assay. We performed other experiments using the Feder *et al.* primers at annealing temperatures of 62 °C, 60 °C, 58 °C and 56 °C with DNA from five 5474A/5569A compound heterozygotes. We correctly identified only one of these as heterozygous for 5474A by the *Rsa*1 assay at an annealing temperature of 60 °C or less. This suggests that further modifications of assay conditions may reduce the possibility of false-positive genetic testing in the presence of the 5569A polymorphism.

hood estimate of linkage disequilibrium D (ref. 5) was 0.71 ($P<0.005$), confirming the presence of moderate to strong linkage disequilibrium between the 2 variant sites. It is unlikely that the 5569A polymorphism has functional significance, because it is found within intron 4 and does not disrupt a splice-site consensus sequence. Moreover, all 5474A/5569A compound heterozygotes had a transferrin saturation in the normal range (mean 31%, range 20–40%). In our population study, the prevalence of haemochromatosis was reduced from 1 in 168 to 1 in 327 by the use of the new primers. These results have major public health implications regarding the use of population screening for haemochromatosis[6,7]. Individuals previously considered to be non-expressing 5474A homozygotes on the basis of a PCR-based restriction endonuclease digestion assay using the original Feder *et al.* primers require confirmatory testing.

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FDA comments on phase I clinical trials without vector biodistribution data

In the June issue of *Nature Genetics*, Haig Kazazian discussed calculations presented at a Recombinant DNA Advisory Committee (RAC) meeting held on 12 March 1999 on inadvertent germline alteration[1]. He mentioned a proposed safety limit on genetic insertions, which was attributed to the Food and Drug Adminstration (FDA). We would like to clarify the FDA's policy. Rather than a specific limit, policy decisions about risks of genetic insertion are based on disease severity, age, life expectancy and reproductive status, as well as vector biology and animal safety data.

The FDA agrees with Kazazian that the natural rate of insertional mutations should be considered when developing regulations, and his presentation at the meeting was a key factor enabling a decision on policy to be reached. There are also other relevant factors to consider. Public reaction to a harmful germline mutation caused, even inadvertently, by gene therapy might differ from reaction to insertional mutations in nature. In addition, expression of therapeutic genes in inappropriate tissues or stages of development may have harmful consequences.

Germline alterations are a theoretical risk of somatic cell gene therapy that extends beyond consenting patients to unborn generations. Germline gene therapy[2] does not presently have societal endorsement[3,4]. Previously, when cells were transduced by gene therapy vectors outside the body and used to treat serious diseases, inadvertent germline effects were not considered a significant risk. With direct administration of gene therapy vectors to patients, however, potential distribution to unintended tissues raises concern about effects on sperm or ova genomes. Concern is also heightened by extension of gene therapy to patients with milder conditions and greater reproductive potential.

The FDA determined that public discussion of inadvertent germline alteration was necessary to reach a consensus on policy. At the RAC meeting held in March, the FDA reviewed vector biodistribution data from pre-clinical studies, including unexpectedly persistent PCR signals in gonads http://www.nih.gov/od/orda/3-99sum.htm). RAC and public representatives concluded that a low risk of germline alteration was acceptable for some phase I trials. While PCR signals in gonads do not demonstrate that vector is in germ cells, further studies were recommended in such cases. Based on this public discussion, the FDA now allows certain therapies to enter initial clinical trials despite incomplete biodistribution data or detection of gonadal presence of the vector. Investigators are required, however, to address vector biodistribution and potential germline transmission during product development before licensure.

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