nature genetics April

Righting an inherited wrong

A gene-therapy trial meets with success, and genetic mapping becomes an increasingly sophisticated but nonetheless accessible process.

THE April issue of *Nature Genetics* contains a landmark paper — the report by James Wilson and colleagues[1] at the University of Michigan Medical Center of gene therapy to treat a lethal condition, familial hypercholesterolaemia. Although this work comes more than ten years after the first (albeit unauthorized) human gene therapy procedures — carried out by Martin Cline in Italy and Israel — it is the first description of an extended follow-up period (18 months). The team concerned has documented, for the first time, the successful long-term correction of a genetic defect and the associated clinical improvement.

Familial hypercholesterolaemia is an inherited deficiency of the low-density lipoprotein (LDL) receptors which leads to dramatically increased levels of serum cholesterol and subsequent coronary artery disease. The female patient described in the article suffered a myocardial infarction at age 16, required a coronary artery bypass at 26, and did not respond to the cholesterol-lowering drug lovastatin. With a total serum cholesterol concentration of 545 mg dl^{-1} (some five times greater than normal) and a ratio of LDL to high-density lipoprotein (HDL) cholesterol of around 11, she would have been at grave risk of further cardiac failure; moreover there were signs that one of her earlier bypass grafts had begun to deteriorate. But after gene therapy her LDL levels dropped by some 17 per cent, and the LDL:HDL ratio fell to just below 8 and is now responding to lovastatin.

There are a number of points that make Wilson and colleagues' account all the more intriguing. First, there is the sheer scale of the procedure. About one-fifth (250 g) of the patient's liver was removed and dissociated to yield 3.2×10^9 hepatocytes, which were plated out onto 800 cell-culture dishes. After a 12–18-hour exposure to recombinant retrovirus containing the LDL receptor gene, 150 ml of cell suspension was infused, through a catheter inserted in the mesenteric vein during the earlier surgery, back into the patient. As David Weatherall comments in an accompanying News and Views article[2], the procedure is "rather hair-raising".

The second point of interest is the overall inefficiency of the transfection process. Of the three billion liver cells removed for culture, only two billion survived, and of these only 20 per cent showed any uptake of virus expressing the LDL receptor. A biopsy after four months suggested that as few as 1 in 1,000 to 1 in 10,000 liver cells expressed the transgene.

In all, these results give cause both for concern and for optimism. The worry lies in the overall inefficiency of this cumbersome *ex vivo* protocol, despite the opportunities it provides to manipulate and quantify the experimental procedure. This probably means that *in vivo* techniques — which would in some respects be preferable — are a long way off, given that they can be subject to far less control. The good news is that, for all the drawbacks of the process described in the paper, it worked — the patient's condition patently improved. Presumably even slight refinement of this first trial will yield even better results.

In a more immediate and less dramatic vein, two other reports in the same issue[3,4] describe new tools to help generate meaningful genetic maps from the abundance of primary linkage data now being produced.

The first is an expert system computer program, MULTIMAP[3]. Based on a map-building algorithm, this program (available by sending email to multimap@genome1.hgen.pitt.edu) aims to avoid some of the drudgery and associated errors that result from the many hours it takes to assemble a linkage map. There are also qualitative benefits. At present, when different groups choose to combine their 'local' maps it is done by comparing the various maps rather than the raw data; reworking the raw data would usually entail a computational and manual burden beyond the means of most groups. But MULTIMAP combines the heuristic aspects of previous map-building programs and a novel algorithm that sequentially builds a map from the primary data. The authors demonstrate the application of their program by producing, from scratch, a total linkage map of the human genome using genotypes of a total of 1,663 markers (data produced by Weissenbach *et al.*[5], the NIH/CEPH Collaborative Mapping Group[6] and six other smaller groups). The map, presented as a fold-out poster, offers nearly complete coverage of the autosomes with a sex-averaged map resolution of 6.2 centimorgans, using the 639 markers that could be allocated a unique map location with odds of greater than 1,000:1.

In the same vein, Kenneth Buetow and colleagues[4] present an integrated genome-wide map, this time employing genotypes mostly generated by the National Center for Human Genome Research[7] and Genethon[5], complemented by many smaller data sets, and again using the CEPH reference panel. A program resembling MULTIMAP, but less fully automated, uses a new algorithm to assemble and integrate genotypes for more than 2,800 markers. The outcome is two sets of maps: high-confidence 'skeletal' maps, with an average density of 6.7 centimorgans, incorporating 544 simple tandem-repeat polymorphic markers; and annotated 'framework' maps representing the addition of a further 579 loci. Both maps and map-building software are available from linkage-server@chlc.org.

In the accompanying News and Views article[8], Mark Lathrop discusses some of the steps involved in map construction and comments on the similarities between the two programs. Both build on the extensively tested CRI-MAP program[9] and make extensive use of the option of checking the map for errors during its construction, thereby avoiding the repercussions of small initial errors that can lead to incorrect marker and locus orders.

Lathrop also points out that the groups have, most importantly, made sure that the software and data are available to others. This will allow individual groups to annotate and improve loci of local interest, and incorporate new markers as they become available, and enable the production of higher resolution maps which might prompt new investigations into mechanisms of interference, variable recombination rates and other parameters of the map. Such questions can be tackled with confidence only by using high-resolution, high-confidence maps.

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