Commentaries

See article on page 787

Genetics of inflammatory bowel disease: a puzzle with contradictions?

Genetic factors have a well established role in the aetiology of inflammatory bowel disease (IBD). The mode of inheritance suggests a polygenic disease with penetrance of the genetic factors being strongly influenced by the lifestyle of an industrialised society. The development of linkage analysis has allowed the generation of important molecular clues to the location of putative disease genes.¹

The inheritance of genomic DNA can be traced by identification of highly polymorphic regions ("microsatellites"). Ideally, polymorphic regions should be used which are present in a high number of variations ("alleles") in the population (that is, are highly informative).

If a phenotype (for example, disease) is inherited, the use of linkage analysis assumes that it will be possible to trace the piece of DNA which contains the putative disease gene. In affected relative pairs of individuals (sharing the same phenotype), certain areas of the DNA which contain disease genes should therefore be co-inherited more frequently in comparison with random distribution. In a systematic analysis ("genome scan"), a densely spaced set of microsatellites is used throughout the entire genome to identify the origin of DNA and follow them through the inheritance process. Statistical measures can be used to qualify the degree of over proportional sharing (of polymorphic alleles). This "LOD" score (which roughly corresponds to a logarithmised p value) is usually displayed as a function of the genomic map (for example, fig 1 in Lesage and colleagues²). Linkage analysis typically leads to the description of a genomic area (often as large as one third of a chromosome), which most likely contains a disease gene.

It is obviously easier to detect disease genes in a monogenic disorder in which inheritance follows Mendelian rules. In many cases analysis of a few large families has led to the localisation of disease genes. In polygenic disorders, multiple disease genes are present and therefore linkage analysis has to be conducted using a population based approach. The method has to account for the fact that multiple disease genes may be present in varying combinations.

Siblings should share 50% of their DNA with each of their parents and also with each another. These DNA pieces should be randomly distributed, and sharing (in a sufficiently large sample) should not exceed 50% for any of the regions traced with microsatellites. Using siblings who share a disease will allow assessment of an over proportional amount of sharing which then can be used to describe a region in which disease genes are suspected.

In IBD a series of linkage studies have been conducted. The study by Hugot and colleagues³ described a region of chromosome 16 ("IBD1") which has been confirmed in a large number of subsequent studies.⁴⁻¹² Satsangi and colleagues¹³ found a region on chromosome 12 ("IBD2") which was also confirmed in other studies.^{8,9,14,15} We have described IBD3 on chromosome 6^{9,16} which was confirmed by Rioux and colleagues,¹⁷ and Brant and coworkers have suggested IBD4 (personal communication). In these stud-

ies linkage was considered as verified based on the criteria of Kruglyak and Lander¹⁸ if the LOD score exceeded 3.6 in a single study and exceeded 2.0 in subsequent populations.

Using stochastic instruments for a population based analysis will always carry a significant amount of statistical uncertainty. Little is known of the theoretical specificity and power of linkage analysis. Therefore, we constructed a computer model of a six locus disease (three strong and three weak disease genes) and simulated transmission through random mating.¹⁹⁻²¹ Using a virtual population, repeat linkage studies can be simulated and different strategies for finding disease genes can be explored. From these studies it has become apparent that results from repeated linkage studies in the same population will result in LOD scores varying in a certain range.²¹ The power of linkage to detect disease genes is fairly high and increases with the number of sib pairs used (fig 1).²¹ However, using linkage for fine mapping of disease genes requires a high number of sib pairs, beyond technical and epidemiological feasibility.

In the study presented in this issue of *Gut*, Lesage and coworkers have conducted a linkage experiment limited to chromosome 12 (see page 787).² They used families from all over Europe. In their study they could not replicate linkage on chromosome 12 which had been described previously by Satsangi and colleagues.¹³ Taking into account the results from mathematical modelling described above, the differences are not as surprising as they first appear: variance is expected in repetitive linkage studies which is greater in studies with small family numbers and in disease genes with a low impact on the susceptibility to disease development, respectively. Lesage and coworkers used 95 families, which is in the lower range of power in comparison with larger studies.² All other studies which found linkage to chromosome 12 reported LOD scores much smaller than those described

Figure 1 Variations in linkage results in repeated studies are dependent on the genetic impact of a locus and sample size. A population was simulated with a six locus disease, with three contributing loci of high frequency but low impact (scenario F) and three contributing loci with high impact but low frequency (scenario R). LOD score as a function of the number of analysed sib pairs is shown, as calculated by the SIPP AIR program in 60 replicate studies, which were conducted in sib pairs randomly drawn from the population. The analysis of locus 1 under both disease model scenarios (F=frequent, locus specific heritability $h^2=0.37$; R=rare, $h^2=0.25$) is shown. Boxes denote median (interquartile range), and whiskers indicate minimum and maximum values (reprinted from Hampe and colleagues²¹).

by Satsangi *et al* in the initial analysis.¹³ As concluded by Lesage and colleagues² further studies are needed to determine if chromosome 12 contains a major disease gene or if the putative gene in this region has only a small influence on the risk of developing IBD.

A third possibility is that we are looking at a population specific difference. Although it appears unlikely that the population in the UK is substantially different from that in France or Belgium, only a repeat study in the UK could clarify this issue. Within our own linkage study⁹ much of the power for the weak linkage seen on chromosome 12 resulted from the sib pairs recruited from the UK which would support this theory (unpublished). A consortial analysis, which drew about 50 sib pairs from each sample collected world wide,²² confirmed linkage to chromosome 12 although at a much lower significance level than that reported initially.

The work of Lesage and colleagues² further indicates that linkage to chromosome 12 is not as powerful as initially thought. Identification of the first disease genes for IBD is still eagerly awaited. Although most likely in the distant future, only the definition of the causative mutations will allow a precise construction of a disease model and more exact predictions of the number and influence of additional genetic alterations.

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See article on page 820

Gastrin, growth, and colon neoplasia

The ability of the gastric hormone gastrin to stimulate gut epithelial cell proliferation has been appreciated since the late 1960s.¹ However, aside from the special case of gastric carcinoid tumours arising from enterochromaffin-like cells, the contribution of gastrin to gastrointestinal neoplasia has been uncertain. Several developments now suggest a role for gastrin in both gastric and colorectal cancer. In the case of gastric cancer, recent evidence indicates a synergy between gastrin and *Helicobacter* infection in accelerating progression to atrophy and cancer.² Different issues are involved in colorectal cancer. The important emerging concepts here are that (a) the gastrin gene is expressed in colorectal cancer cells, but (b) the main products of gene expression in these cells are not ligands for the gastrin-cholecystokinin receptor B (CCKB), although (c) they do act as colon growth factors. The paper by Smith and Watson³ now shows that gastrin mRNA, detected by reverse transcription-polymerase chain reaction, is expressed in early stage polyps, that the main forms of gastrin detected by immunohistochemistry are biosynthetic precursor peptides, and that there is also parallel expression of the gastrin-CCKB receptor (see page 820).

It used to be thought that the only biologically active peptides generated from the gastrin gene were COOH terminally amidated peptides such as G34 and G17, and that

carboxy terminal amidation was essential for the biological activity of members of the gastrin family. The amidated gastrins are generated from a precursor, progastrin, via biosynthetic intermediates, the Gly-gastrins (fig 1). Neither progastrin nor Gly-gastrins have a carboxy terminal amide group and therefore they do not have high affinity for the gastrin-CCKB receptor, which is the receptor mediating acid secretory responses to gastrin. It seems, however, that these peptides exhibit other biological properties, notably stimulation of colon cell growth. This effect has been found in both transgenic mice over expressing progastrin and Gly-gastrin, and in colon cancer cell lines.^{4–6} The precise receptors mediating the trophic actions of progastrin and Gly-gastrin remain uncertain. It is not clear if there is one (the putative gastrin-CCKC receptor) or several. It is conceivable that a modified version of the gastrin-CCKB receptor, perhaps generated by alternative mRNA splicing, mediates the effects of progastrin and Gly-gastrin; at present this idea is largely speculative. It is unlikely, however, that the progastrin and Gly-gastrin found by Smith and Watson in early stage polyps act at authentic gastrin-CCKB receptors.

The pattern of relatively high abundance of progastrin and Gly-gastrin compared with amidated gastrin that occurs in colonic neoplasms differs markedly from that in normal pyloric antral G cells where the amidated gastrins predominate. There are several plausible explanations for this. There may, for example, be differences in the expression of enzymes required for each conversion step in

Figure 1 Schematic representation of the pathway of gastrin biosynthesis and the biological properties of the products. Open arrows show the conversion steps by which preprogastrin is converted to amidated gastrins. This sequence of events is completed in G cells but in colorectal cancer cells the main products are progastrin and Gly-gastrin. Progastrin and Gly-gastrins stimulate colon proliferation. Amidated gastrins play a minor role in control of colon proliferation but stimulate acid secretion and gastric proliferation via the gastrin-CCKB receptor (at which progastrin and Gly-gastrin have low affinity).

gastrin biosynthesis (fig 1). In normal G cells, the enzymes responsible for production of carboxy terminal amidated gastrins mostly act in vesicles of the so called regulated pathway of exocytosis⁷; but these vesicles are scarce or absent in polyp and adenoma cells. As a consequence, these cells are poorly equipped both to store hormonal peptides and to complete the full sequence of events giving rise to carboxy amidated gastrins. Instead, peptides mostly corresponding to progastrin and Gly-gastrin are likely to pass directly from the Golgi complex to the cell surface by what is known as the constitutive route of secretion.

What regulates gastrin gene expression in colon polyps and adenomas? Studies by Nakata *et al* have shown that oncogenic Ras stimulates the mitogen activated protein kinase pathway and so increases gastrin gene expression.⁸ Recently, Koh *et al* demonstrated that the β -catenin/T cell factor 4 pathway also stimulates gastrin gene expression.⁹ Acquisition of mutations that activate the latter pathway is likely to be an early event in the progression to colorectal cancer and could account for the increased gastrin gene expression found by Smith and Watson. Whether or not

similar mechanisms account for upregulation of the gastrin-CCKB receptor is not known.

Recently, Singh *et al* reported that mice with elevated plasma progastrin exhibit increased aberrant crypt foci, adenomas, and adenocarcinomas after treatment with azoxymethane.^{10 11} Aberrant crypt foci are considered to be a marker for progression to colon cancer. Singh *et al* suggest progastrin is a cocarcinogen, that is, on its own it is not carcinogenic but it increases the pool of transformed cells and so exacerbates the oncogenic progression. Interestingly, mice with elevated plasma concentrations of amidated gastrin did not exhibit increased aberrant crypt foci, adenomas, or carcinomas in response to azoxymethane. The picture emerges then that mutations acquired early in the progression to colorectal cancer lead to increased local production of progastrin and Gly-gastrin that then act as auto- or paracrine growth factors expanding the number of transformed cells. This analysis leaves open the role of the gastrin-CCKB receptor, and further work will certainly be needed on this point. Whether or not these findings can be developed into novel therapeutic strategies will depend on characterisation of the receptor mechanisms mediating the effects of progastrin or Gly-gastrin on colon epithelial proliferation.

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See article on page 825

Alcohol and retinoid metabolism

The complex interactions between the metabolism of retinoids and ethanol have been reported for a long time. Clinically, chronic ethanol consumption leads to vitamin A deficiency but also to enhanced toxicity of vitamin A and beta-carotene when supplemented. Changes in retinol metabolism due to alcohol may have a pathophysiological impact in both alcoholic liver disease and alcohol associated cancer as retinoic acid, the most active form of vitamin A, is an important regulator of normal epithelial cell growth, function, and differentiation. Under normal conditions, ingested retinol is metabolised to retinaldehyde

via cytosolic alcohol dehydrogenase (ADH), microsomal retinol dehydrogenase (three types), and several types of cytosolic retinol dehydrogenases, and retinaldehyde is further oxidised to retinoic acid via aldehyde dehydrogenase (ALDH). Retinoic acid binds to retinoic acid receptors (RAR), initiating intracellular signal transduction leading to a cascade of events and finally to a decrease in cell regeneration. The main molecular action of retinoic acid involves either transactivation through direct binding to retinoic acid response elements (RARE) in target gene promoters, thereby transcriptionally activating a series of genes with distinct antiproliferative activity, or transrepression of activator protein (AP-1) and regulation of apoptosis. It is not surprising that this complex interaction between ethanol and metabolism of retinoids occurs as

both substrates share common pathways, namely (a) ADH, (b) ALDH, and (c) cytochrome P4502E1 (CYP2E1).

It has been shown that chronic ethanol consumption decreases hepatic retinol and retinoic acid concentrations due to various mechanisms including, increased mobilisation of retinyl esters to extrahepatic tissues and enhanced hepatic metabolism of retinol and retinoic acid to polar metabolites, predominantly via induced CYP2E1.^{1,2} These metabolites include 18-OH-retinoic acid, 4-OXO-retinoic acid, and some unidentified metabolites, possibly with fibrogenic and toxic properties. Decreased hepatic retinoic acid concentrations are associated with functional down-regulation of RAR, enhanced expression of AP-1 gene (c-jun and c-fos), and increased hepatic cell regeneration, all of which return to normal following retinoic acid supplementation.^{2,3} In contrast, retinol concentrations in extrahepatic tissues such as the oesophageal and colonic mucosa were found to be increased rather than decreased following chronic ethanol consumption.⁴ This was also confirmed in alcoholics with oropharyngeal cancer where normal retinol concentrations were found in normal oral mucosa adjacent to cancerous tissue.⁵ It was believed that one mechanism for this observation was increased mobilisation of retinyl esters from the liver to the oral mucosa.

In this issue of *Gut*, Parlesak and colleagues⁶ demonstrate another explanation for the lack of loss of retinol in gastrointestinal mucosa of alcoholics (see page 825). These authors showed inhibition of retinol oxidation by ethanol concentrations frequently observed after social drinking in an in vitro study using cytosol from rat liver and intestine. This effect was due to inhibition of ADH. As this inhibition was found not only with low ethanol concentrations (8.6 mM) but also increasingly with higher ethanol concentrations (17 mM, 34 mM), a low K_m ADH (rat ADH3 corresponding to class I ADH) and an ADH with higher K_m (ADH2 corresponding to class III ADH or ADH1 corresponding to class IV ADH, not present in the rat liver and colon, but in the rat rectum⁷) seem to be involved. This inhibition of retinol metabolism by ethanol seems especially relevant in the colon. This may explain the accumulation of retinol in this tissue which may lead to a reduction in retinoic acid levels; however, this needs to be proved. In contrast with the colon, both retinol and retinoic acid concentrations were found to be significantly decreased in rat liver following chronic ethanol ingestion.² Thus this finding cannot be explained by inhibition of retinol oxidation by ethanol. Indeed, at least in the liver, a variety of other pathways for oxidation of retinol are involved, as mentioned above.

In humans it has been shown that retinol is a physiological substrate for ADH3 (class I ADH) in the gastrointestinal mucosa. ADH3 has a low K_m for ethanol (1–2 mM) and is the only class I ADH gene that contains an RARE in the promoter region. It has been suggested that retinoic acid activation of ADH constitutes a positive feedback loop regulating retinoic acid synthesis.⁸ Ethanol was found to be a competitive inhibitor of retinol for class I ADH, but also for classes II and IV ADH.^{9,10} Indeed, it has been shown that class IV ADH (only present in the mucosa of the upper gastrointestinal tract) has a low K_m for all-trans-retinol of 15–60 μ M and has the highest catalytic efficiency of 3800–4500 mmol/min.^{10,11} In vitro studies using class IV ADH enzyme preparations have shown strong inhibition of metabolism of all-trans-retinol and 9-cis-retinol by ethanol with a K_i of 6–10 mM.¹⁰ Oxidation of retinol to retinaldehyde is probably the rate limiting step in the generation of retinoic acid. However, recently it was shown in the rat oesophagus that in addition to ethanol, acetaldehyde also inhibits generation of retinoic acid, possibly by inhibition at the retinaldehyde level.¹²

The data of Parlesak *et al* extended these in vitro experiments with isolated ADH by using rat cytosol from liver and colonic mucosa. An important next step would be to measure retinoic acid in colonic mucosa following chronic ethanol consumption and, as there are differences in ADH patterns between rats and humans, to also measure retinoic acid in the mucosa of alcoholics.

It is interesting that class IV in contrast with class I ADH is not expressed in human colorectal mucosa. However, it was found recently that in a number of biopsies from colorectal polyps of alcoholics, class IV ADH was expressed.¹³ One explanation for such de novo expression of class IV ADH could be retinoic acid deficiency in a critical premalignant condition to guarantee increased generation of retinoic acid.

Chronic alcohol consumption is associated with an increased risk of both hepatic and colorectal cancer. Whereas for the liver, cirrhosis is possibly the most important precondition for cancer development, mechanisms for colorectal carcinogenesis are complex and less clear. One important morphological feature of chronic ethanol consumption in rats and humans is colorectal cellular hyperproliferation associated with extension of the proliferative compartment of the crypt towards the lumen, a condition associated with increased cancer risk.¹⁴ This early event in colorectal carcinogenesis was thought to be related to acetaldehyde induced cell injury as acetaldehyde (produced during mucosal and bacterial ethanol metabolism) and crypt cell production rate showed a significant positive correlation.¹⁴ However, this important alteration in cell cycle behaviour, which was also observed in the upper alimentary tract, could also be due to retinoic acid deficiency, and acetaldehyde may contribute by preventing its generation, as discussed above.

In summary, the paper by Parlesak *et al* shows the importance of ethanol in the inhibition of retinol metabolism in the liver and colon. However, it raises more questions than it answers. One major question arising from these data is whether decreased retinoic acid concentrations can be found in extrahepatic tissues, especially in the colorectal mucosa, following chronic ethanol ingestion of rodents, and even more important in alcoholic. If so, a new mechanism for alcohol associated carcinogenesis has to be considered.

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Web extra

A new website has been developed to help gastroenterologists calculate CDAI (Crohn's Disease Activity Index) and PCDAI (Paediatric Crohn's Disease Activity Index).

The user registers his/her patient under a name/code and registers the sex, age, and weight of the patient, and is then asked to fill in raw data. The website will then calculate CDAI (and for patients below 19 years, PCDAI). The site enables the user to store indices over time, and the disease activity of patients will also be presented as a diagram. The data are stored on the Örebro Medical Centre Hospital for one year. You can visit the website at:

http://www.orebroll.se/rso/barn/crohn/Home_Eng.asp

Users must first register; this is done by entering a user name and password, which the user chooses (user names and passwords are not distributed by Dr Ludvigsson).

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