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MN blood group affects response of serum LDL cholesterol level to a low fat diet

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Previous studies found that MZ twin pairs who are blood group NN have greater intrapair variability in plasma lipid levels than those who are MM or MN. This led to the prediction that the response of plasma lipid levels to a low fat diet would depend on MN blood group, the greatest response being in those who are NN. The present study was based upon 254 patients who took part in the Australian Polyp Prevention Project. This was a $2 \times 2 \times 2$ randomised factorial design based upon the presence or absence of the three factors: a dietary fibre supplement, a beta-carotene supplement and reduced intake of dietary fat. The lowering of plasma, low density lipoprotein (LDL) cholesterol, in response to a low fat diet was greatest in those who were NN and least in MN heterozygotes. Overall, a reduction in LDL level was observed in the 47% of the APPP population who were on a low fat diet and who were homozygous MM or NN. The result was consistent with a balanced polymorphism at or near the GLYA locus on chromosome 4 that influences the sensitivity of plasma lipid levels to dietary fluctuations in fat intake.

Andrew J. Birley[1], Robert MacLennan[2], Mark Wahlqvist[3], Linda Gerns[4], Theresa Pangan[2] and Nicholas G. Martin[2]

[1]Queensland Institute of Medical Research, Brisbane, Australia and School of Biological Sciences, The University of Birmingham, Birmingham, UK; [3]Department of Medicine, Monash University, Melbourne; [4]Red Cross Blood Transfusion Service (Queensland), Brisbane and [2]Queensland Institute of Medical Research, Brisbane, Australia

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Dr. A. J. Birley, School of Biological Sciences, The University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK. Tel: 0044 121 414 5895. Fax: 0044 121 414 5895. email: A.J.Birley@bham.ac.uk

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The medical importance of genes which affect the incidence of a disease, level of a risk factor or other quantitative trait is established. Less attention has been paid to "variability" genes (Berg 1986, 1987) that influence the phenotypic response to environmental change or heterogeneity. Genes that encode for those functional domains of proteins associated with plasma cholesterol or triglygerides are presently permitting investigations of the contribution of allelic variations in such genes to these characters within human populations (Friedlander et al. 1995). The natural basis of such studies is the knowledge of tissue regulation and hence expression is associated with the genetic environs of structural or protein encoding regions, for example in the Apolipoprotein B region (Demmer et al. 1986). Of particular interest in public health is the efficacy of dietary interventions. Recently the results of a double crossover experiment, in which volunteers undertook two diets that differed in their levels of saturated fat intake, demonstrated the existence of two distinct groups, namely hyper- and minimal responders for low density lipoprotein (LDL) cholesterol level (Cox et al. 1995). It is generally appreciated that even apparently simple characters such as enzyme activity can be controlled from sequences associated or tightly linked to the coding region as well as by unlinked genes. In this paper we have identified a genetic basis for the results seen in the studies of Cox et al. (1995). Magnus et al. (1981) reported that variability in lipid levels within MZ twin pairs depended on their MN blood group: differences in cholesterol levels within MZ pairs who were blood group NN were significantly greater than those in pairs who were MM or MN. A later replication by Martin et al. (1983) included the lipid subfractions and indicated that the genotype-related variability effect was in LDL cholesterol but not in triglycerides or high density lipoprotein (HDL) cholesterol. It was predicted, very early in the Australian Polyp Prevention Project (APPP) trial (MacLennan et al. 1995), that patients who took up the low fat diet and were blood group NN, would be the most sensitive to variation in fat-intake and consequently show a greater reduction in serum lipid levels than other patients. This follows from the fact that any genetical association with the environmental variance of MZ twin pairs indicates a differential capacity of that genotype to respond to environmental factors

that in turn modulate the level or expression of a gene.

Materials and methods

Patients were drawn from the APPP trial (MacLennan et al. 1995). The sample, typed for MN status, comprised a control group of 158 patients who did not change their diet and a group of 157 patients who adhered to a low fat diet. These dietary regimens were allocated at random to the set of patients. A 25% reduction in dietary fat intake was made an objective for those on the low fat diet. Their diet was supplemented with a daily intake of 25 g unprocessed wheat bran (equivalent to 11 g fibre). Compliance with the diet was monitored at 3–6-month intervals (Bain et al. in preparation). Patients were monitored by diet diary, weight and total cholesterol. Supportive advice was given when appropriate. The median decrease in total fat intake accounted for more than the target of a 25% reduction in the experimental group. The average age was 56 years.

Plasma lipids were measured at 0, 6, 12 and 18 months. Measurements at 0 months were made before patients received any dietary advice. The assay of lipids was on blood drawn by venipuncture following an overnight fast of 12 h maximum duration. Samples were allowed to clot and serum was separated by centrifugation at 3000 rpm for 10 min. Serum was stored at −70°C. Total cholesterol, cholesterol and triglycerides were estimated with commercial enzymatic kits (Trace Scientific, Victoria, Australia). HDL cholesterol measurements were made after apolipoprotein B-containing lipoproteins had been precipitated with 20% polyethylene glycol 2000. LDL cholesterol was estimated by the Friedwald equation (Friedwald et al. 1972).

Results

Gene frequencies at the MN locus were homogeneous in both control and experimental groups. Similarly, genotypes were in Hardy Weinberg equilibrium. The data were analysed following a $\log_{10}$ transformation of the individual observations. Analysis of variance of genotypes, intervention groups and their interaction showed no significant differences in dietary lipid or bound and free cholesterol levels at the start of the trial. Mean values of LDL cholesterol (Table 1) indicated a fall in values with time in subjects on a low fat diet.

The multivariate approach to repeated measures analysis of variance was used to allow for the autocorrelation between measures taken in the same subjects at different times. Interactions between genotypes and the times at which LDL cholesterol was measured revealed a time-dependent change in LDL cholesterol in those patients who experienced the low fat diet. No such trend was found in the control group.

Table 1. Mean LDL cholesterol (mM/l) according to MN blood group status in the control group of patients and those undertaking the low fat diet. The times are relative to the start of the experiment (0 months). The numbers of each genotype in the control and diet groups are shown in brackets

	Control			Low fat diet		
Time (months)	MM(40)	MN(61)	NN(26)	MM(38)	MN(67)	NN(22)
0	4.5923	4.6217	4.2280	4.4026	4.2469	4.2364
6	4.6256	4.4949	4.1261	4.1316	4.1955	3.8909
12	4.4538	4.3915	4.3615	4.2056	4.1508	3.8752
18	4.4897	4.3847	4.1200	4.0389	4.2516	3.8045

Changes in scores from the starting values (Time 0) for LDL cholesterol ($\log_{10}$ mM/l) are shown for 6, 12 and 18 months (Fig. 1) for those on the low fat diet.

Discussion

The expectation of this experiment was that the dietary variation in the general population would primarily affect individuals with the NN genotype, simply because the within twin-pair variance of NN

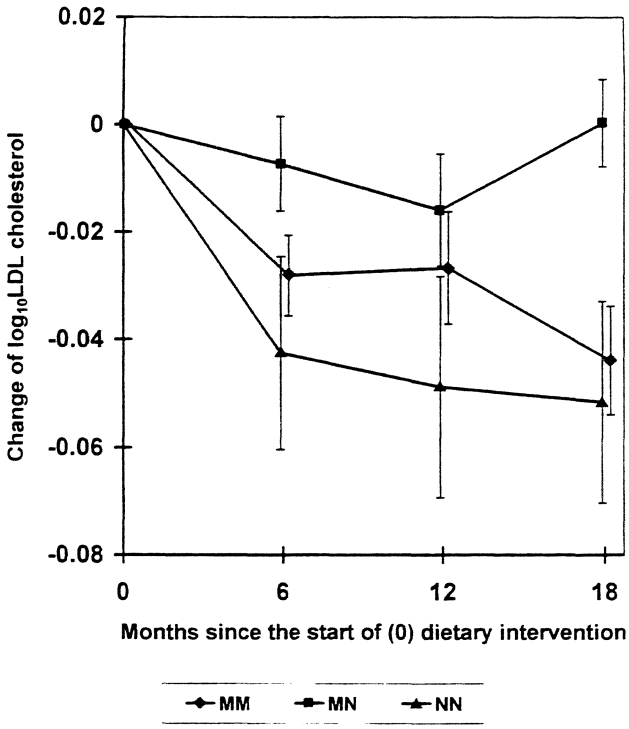

Fig. 1. Changes of LDL ($\log_{10}$ mm l^{-1}) cholesterol at 6, 12 and 18 months, together with standard errors of the change scores, for MM, MN and NN genotypes.

genotypes was seen to be greatest in earlier studies (Magnus et al. 1981, Martin et al. 1983). Individuals who have the NN genotype and experience variation in dietary lipids in their normally varied diets will respond to variations in level more readily or sensitively than the other two genotypes. This is because the within MZ twin pair variance must be due to environmental factors, and thus any systematic trend in intrapair variance with a measured genotype will indicate an environmental sensitivity or "variability gene" (Berg 1986, 1987). The prediction that the greatest reduction in LDL cholesterol would be seen in the NN blood group appears to be borne out, though the particular comparison of NN genotype vs. remaining (MM and MN) genotypes and the interaction of NN genotype with time was not significant. It is apparent from Fig. 1 that the more important contrast may be between the heterozygous MN genotype and the two homozygotes, MM and NN, with time. This contrast measures how the degree of additivity of gene effects at the locus changes with time. In the analysis of variance, this contrast was significant. The same contrast was significant at 6 ($P=0.0031$), and 18 months ($P=0.0002$) and almost so at 12 months ($P=0.0936$) in an analysis that tested for the interaction of each sampling time with the measurements at the start of the experiment (Time 0). It is also noteworthy that the variances of the change scores at each time point were greater for NN than for either of MN or MM genotypes and in agreement with our earlier prediction. This also implies that the "variability gene effect" continues to amplify sources of environmental variability other than those introduced by the dietary intervention. The experiment does not identify all sources of the environmental variability effect, nor can it exactly prescribe the dietary habits of those in the trial. LDL cholesterol levels in individuals with an MN genotype in the dietary intervention group mirrored those in the overall results of the control group, which were themselves genotype independent. There was effectively little or no response of heterozygous MN individuals to the low fat diet. Since this class accounted for 53% of those in the dietary intervention group, about half of the population at large would not show a reduction in LDL cholesterol if they were to be given that dietary advice. Conversely the statistically similar responses of the MM and NN genotypes account for nearly all of the lowering of LDL cholesterol level following dietary intervention. There was no evidence for an interaction between sex and the differential response of LDL cholesterol seen for the MN genotypes on the low fat diet. Similar results were not found for HDL cholesterol nor for triglycerides. Whilst HDL cholesterol level was both *per se* and as a time-dependent marker of diet, independent of genotype at the MN locus, it was about 2.5-fold higher in females than males ($P=0.0035$). Triglyceride levels were about 1.75 times higher in males than in females, and were both genotype independent and lowest in the group that experienced the low fat diet. Triglyceride levels did show a dependence on sex such that they fell slightly more rapidly in females ($P=0.0075$) on the low fat diet, although the exact nature of the trend was unclear. Age correlations were weak (Pearson's correlation coefficient, $r=0.15$; $P=0.05$ in the sample at time 0). There was no evidence to suggest that the responses were age dependent.

The clear fall in LDL cholesterol levels shown by the groups of individuals who were either homozygous MM or NN provided a deviation from our original prediction and led us to re-examine the twin data from the 1983 study by Martin et al. Following Magnus et al. (1981), we had only examined the MM + MN vs. NN contrast in MZ intrapair variances. In fact, the intrapair variances in $\log_{10}$LDL are 0.0021 for 27 MM pairs, 0.0016 for 33 MN pairs, and 0.0032 for 21 NN pairs. These variances are in the same order as the response to reduced fat intake and, when expressed on the natural scale, those of the two homozygous genotypes are homogeneous and the variance of the heterozygote is significantly smaller than that of the homozygotes ($P=0.0025$). The differences between prediction and observation therefore appear to resolve themselves in accordance with expectation derived from the within twin-pair variances for LDL cholesterol. It is also possible that the apparent discrepancy between the present results for the MM genotype in our data, compared to those predicted by Magnus et al. (1981), reflects a population heterogeneity in the genetic background. This is suggested from the within twin-pair variances of total cholesterol levels in MMS_ and MMss genotypes (Berg 1983).

Conclusion

We conclude that MN gene status does indeed have an important effect on or rather association with the variability of response to a low fat diet and is a relevant factor in the likely success of prescribing such a diet. The finding that a blood group locus has manifested itself as a quantitative trait locus for an apparently unrelated molecular phenotype might seem surprising. However, it is striking that these results used as their basis the results of Magnus et al. (1981) and Martin et al. (1983), both of which were supportive of the notion that the MN locus could reflect the "variability gene" (Berg 1986, 1987) status of this locus for LDL cholesterol level.

The effects and predictions of the response by a variability gene have been verified for the MN locus: the lowering of plasma LDL cholesterol in response to a low fat diet was greatest in those who were NN and least in MN heterozygotes. Further "variability genes" concerned with levels of dietary lipids (HDL bound cholesterol and apolipoprotein A-I) have been implied from associations of RFLP's in the cholesteryl ester transfer protein gene and MZ co-twin variance (Berg et al. 1989). Polymorphisms, associated with but flanking rather than within, the apolipoprotein B gene, have also been reported to be related to a lowering of total cholesterol level and LDL cholesterol following dietary challenge (Friedlander et al. 1995). Whilst the overall response in LDL cholesterol may be polygenic, these observations indicate a major contributing locus that is unlinked to the apolipoprotein gene. That the response is due to the MN blood group locus itself is unclear. However, the present finding raises the question of the most appropriate methodological screen for variability genes. It may be that in addition to further studies of loci that encode protein domains and which are known to influence lipid levels, more general genome scans will be useful in the search for "variability genes". The most likely explanation for these results is a polymorphic DNA domain that influences lipid levels and is in linkage disequilibrium with the MN locus.

Given the epidemiological evidence that relates modifications of mood, particularly increased depression and aggression (Muldoon et al. 1990, Weder 1991, Fowkes 1992, Hibbeln et al. 1995), as well as hypertension (Maes et al. 1994), with a lowered intake of cholesterol, these findings have a wider behavioural genetic significance. They also raise some evolutionary questions that help us to understand the evolutionary genetic basis of a result that could have considerable clinical application or significance. It will be recalled that the heterozygous MN genotype showed exceptional stability for LDL cholesterol level in the diet group and similarly, as judged from its associated environmental variance, in the general population. The heterozygote vs. homozygote contrast in lipid response was also statistically significant. This finding is consistent with a balanced polymorphism at or near the GLYA locus on chromosome 4 that influences the sensitivity of plasma lipid levels to dietary intervention; this is the classical manifestation of heterosis and if these characteristics are related to Darwinian fitness, of heterozygote advantage. It is also noteworthy that a likely discrete basis in terms of hyper- and hypo-responders has been recognised amongst individuals on cholesterol-enhanced diets (Beynon & Katan 1986, Cox et al. 1995). This is perhaps the most striking, broad biological feature of the data and is indicative of selection in past or even in recent and present times upon a gene or gene complex. However, the immediate clinical significance is more apparent. Provided these results are applicable to other gene-pools or human evolutionary lineages, the MN locus is a simple predictor of the response of LDL level to a low fat diet. It will also be a genetical avenue to the molecular biology and physiology of the effects of a low fat diet. Effective advice based upon the genetic risks to individual patients rather than to the population *per se* may soon be realistic for diseases related to dietary lipid intake.

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