Identification of genetic risk factors for drug-induced liver injury

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Background and Aims

Drug-induced liver injury (DILI) is a leading cause of withdrawal of newly developed drugs. DILI may involve inappropriate immune responses or direct toxicity from drug metabolites (1). This project was concerned with DILI due to two separate drugs, flucloxacillin and azathioprine.

Flucloxacillin is prescribed for the treatment of *Staphylococcus aureus* infections. A genome wide association study (GWAS) identified the presence of HLA-B*57:01 as a genetic risk factor for development of DILI in patients taking flucloxacillin (2). In a new GWAS (Daly and Nicoletti, unpublished) another SNP, rs141194478, was identified as a potential factor which could lead to flucloxacillin DILI. This SNP is located between the genes RECK and GLIPR2. However the association with rs141194478 had been detected via imputation (a statistical prediction of genotype via haplotypes) and only by directly genotyping the samples could this be confirmed as an accurate result.

Azathioprine is commonly prescribed in the treatment of autoimmune diseases e.g. arthritic conditions and inflammatory bowel diseases. rs3218760, a SNP in the gene POLD1, which codes for DNA polymerase and has been associated recently with azathioprine-induced DILI in a GWAS, again by imputation (Daly and Nicoletti, unpublished).

The objectives of the current study were to further investigate the association of both rs141194478 and rs3218760 with DILI by genotyping directly for these SNPs in both the samples already used in the GWAS and some recently recruited additional DILI cases.

Methods

DILI cases were recruited to the study via the iDILIC consortium; flucloxacillin cases came from the UK and Sweden and azathioprine DILI from a total of 8 centres, mainly in Europe. Population control DNA samples from North East England (n=124) (3) and Crohn's disease patient DNA samples (n=79) (supplied by Dr Sally Coulthard) were also analyzed.

Cases were genotyped for rs2395029 by PCR-RFLP analysis to determine HLA B*57:01 genotype. An example of this analysis is shown in figure 1.

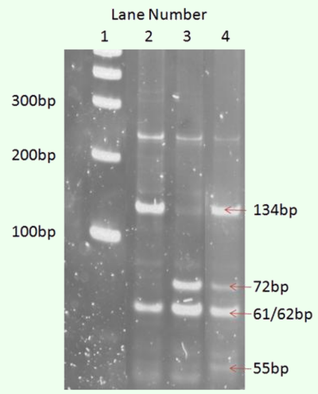
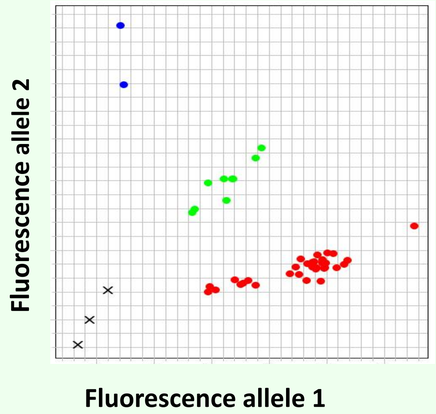


Fig.1 Typical PCR-RFLP result with digestion products separated on a polyacrylamide gel
Lane 1 shows a molecular ladder, at 100bp intervals, Lane 2 shows a homozygous wild type (TT) genotype, Lane 3 shows a homozygous mutant (GG) genotype, Lane 4 shows a heterozygote (TG) genotype.

Two SNPs (rs141194478 and rs3218760) were genotyped using allelic discrimination (Taqman) assays. Figure 2 is an example of a result for a set of control data.

Fig. 2 Allelic discrimination (Taqman) plot
Red circles show Wt/Wt genotype, Green circles show a Heterozygous (Wt/Mt) genotype, Blue circles show Mt/Mt genotype, X denotes blank/undetermined sample



Two tailed Fisher's exact tests were used to analyse possible differences in genotype frequency between cases and controls.

Results

141 patients and 282 population controls had already been genotyped for the SNP rs2395029 (4). An additional 61 patients were now genotyped and this data combined (see Table 1).

Table 1. Genotyping data for rs2395029

	TT (Wt/Wt)	TG (Heterozygous)	GG (Mt/Mt)
Controls (n=282)	252 (89.4)	30 (10.6)	0 (0.0)
Cases (n=202)	41 (20.3)	156 (77.2)	5 (2.5)

P-Value 1.063×10^{-56}, Odds ratio 32.99 (95% CI 19.79-54.98)

We genotyped the same 202 patients for rs3218760. Control data for this SNP was obtained from the 1000 Genomes project (http://www.1000genomes.org). The data is summarised in Table 2.

Table 2. Genotyping data for rs141194478

	TT (Wt/Wt)	TC (Heterozygous)	CC (Mt/Mt)
Controls (n=379)	365 (96.3)	14 (3.7)	0 (0.0)
Cases (n=202)	180 (89.1)	22 (10.9)	0 (0.0)

P-Value = 0.001, Odds ratio 3.19 (95% CI 1.59-6.38)

Of the 22 patients who were heterozygous for rs14119447, 18 (81.8%) were also heterozygous or homozygous mutant for rs2395029 (i.e. predicted to be B*57:01-positive).

We genotyped 27 azathioprine DILI cases and 124 population controls for rs3218760. The data obtained is summarised in Table 3. Interestingly all the patients which tested heterozygous or homozygous mutant for the SNP rs3218760 also had Crohn's disease. We therefore also genotyped 79 patients with Crohn's disease (but without DILI) for rs3218760 in order to see if there was any correlation between this SNP and Crohn's disease but we still observed a significant difference in genotype frequency between the cases and these controls (p=0.027).

Table 3. Genotyping data for rs3218760

	AA (Wt/Wt)	AG (Heterozygous)	GG (Mt/Mt)
Controls (n=124)	103 (83.1)	19 (15.3)	2 (1.6)
Cases (n=27)	15 (55.55)	10 (37.03)	2 (7.40)

P-Value = 0.0037, Odds ratio 3.924 (95% CI 1.61-9.58)

Discussion

There is a statistically significant difference in percentage of patients who were heterozygous for the SNP rs141194478 in those who have flucloxacillin DILI (10.89%) vs controls (3.69%), confirming the new GWAS findings. Therefore it appears that rs141194478 increases a patient's risk of DILI though the effect is modest compared with that from HLA-B*57:01 as the odds ratio is approx. 10 fold lower. Of the 22 patients who tested heterozygous for the SNP rs141194478, 18 (81.8%) of these patients also tested heterozygous or homozygous mutant for rs2395029 so the new SNP was not a stronger risk factor for DILI in B*57:01-negative cases. Both genes adjacent to rs141194478 could potentially have an effect on DILI as they have been associated with fibrosis and autophagy. It is clear there are other factors which can cause flucloxacillin DILI which were not detected in either the previous or current GWAS.

An association with azathioprine DILI was also confirmed for rs3218760 and we confirmed that this association did not appear to relate to this SNP affecting risk of Crohn's disease. POLD1 is an interesting candidate as a risk factor for azathioprine DILI since azathioprine is a cytotoxic drug which will cause DNA damage and there is already data showing that the POLD1 gene product may be important in repair of DNA damage. The functional effect of the SNP which is in the upstream non-translated region still needs further study. It is also part of a haplotype so may not be itself functionally significant. Confirmation of the association in larger numbers of cases is also still needed.

References

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