COMMON GENETIC VARIATION IN NATURAL KILLER CELL RECEPTOR PROTEIN G2D DOES NOT MODIFY SUSCEPTIBILITY TO SPORADIC CHOLANGIOCARCINOMA

Wadsworth CA1, Dixon PH2, Zabron AA1, Wong JH1, Chapman MH1, McKay SC4, Sharif A1, Spalding DR4, Pereira SP3, Wasan HS3, Thomas HC1, Taylor-Robinson SD1, Whittaker J1, Williamson C2 and Khan SA3
1Department of Hepatology & Gastroenterology, Faculty of Medicine 2Institute of Reproductive and Developmental Biology, Imperial College London, 3Institute of Hepatology, University College London, 4Department of Surgery, 5Department of Oncology, Imperial College London, 6Statistical Genetics Unit, Royal School of Hygiene & Tropical Medicine, London, UK

Background
○ Cholangiocarcinoma (CC) is increasing in incidence globally and its pathogenesis remains poorly understood
○ Primary sclerosing cholangitis (PSC) is the commonest known risk factor for CC in Western populations. However, ~70% of CC cases are sporadic and are not PSC related
○ Natural Killer (NK) cells play a critical role in innate immunity, including tumour surveillance
○ NK cell receptor protein G2D (NKG2D) is involved in activation of NK cells (Figure 1). Reduced NKG2D expression has been associated with increased risk of malignancy in mouse models and human studies1–3
○ Genetic polymorphisms in NKG2D have been associated with increased risk of CC in patients with PSC
○ A recent study compared 49 subjects with PSC and CC to 365 subjects with PSC and no CC. Two single nucleotide polymorphisms (SNPs) in NKG2D were associated with an increased risk of CC: rs11053781 (OR 2.08, cor. p=0.011) and rs2617167 (OR 2.32, cor. p=0.0020)4

Method
○ To investigate whether common genetic variation in NKG2D is associated with altered susceptibility to non-PSC related, sporadic CC

Method continued
○ Haploview v4.2 was used to select SNPs capturing the majority of common genetic variation around the NKG2D gene (MAF >0.05, pairwise comparisons)
○ This identified 7 SNPs to be genotyped, including rs11053781 and rs2617167 (Table 1)
○ Genotyping was undertaken with a competitive allele-specific PCR based robotic genotyping system (KasPar, KBioscience, Herts, UK)
○ Confirmation of Hardy-Weinberg equilibrium and Cochran-Armitage trend testing were performed using PLINK v1.07 in R V2.10.1
○ Haplotype frequencies were compared using Haplo Stats v1.4.4

Results
○ Case and control groups were well matched (Table 2)
○ All 7 SNPs were in Hardy-Weinberg Equilibrium
○ None of the individual 7 SNPs in NKG2D were associated with altered susceptibility to CC (Table 3)
○ Haplotype analysis of the genotyped SNPs in NKG2D identified no difference in haplotype frequencies between cases and controls

References

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Table 1 – SNPs selected for genotyping by gene, RS number and location on chromosomes

Table 2 – Demographic data, case and control groups

Table 3 – Summary results for SNPs genotyped in NKG2D, including allelic trend testing results

Figure 1 – NK cells lacking expression of the activating receptor NKG2D fail to recognise transformed epithelial cells expressing Rae-1 proteins. Transformation of normal epithelium and other cell types often leads to the expression of ligands for the activation receptor NKG2D. In mice, such ligands belong to a family of several Rae1 – as well as H60 and MULT1 proteins. This figure illustrates the destruction of tumour transformed epithelial cells expressing Rae-1 proteins. Transformation of normal epithelium and other cell types often leads to the expression of ligands for the activating receptor NKG2D. In mice, such ligands belong to a family of several Rae1, including allelic trend testing

Conclusion
○ First study of NKG2D in sporadic cholangiocarcinoma
○ The reported association of rs11053781 and rs2617167 with PSC related CC was not replicated
○ No association detected in any of the SNPs genotyped, or with haplotype analysis
○ Common genetic variation in NKG2D does not modify susceptibility to non-PSC related, sporadic CC
○ This may represent an important difference between the pathogenesis of sporadic CC and that of PSC related CC

Method continued
○ A power calculation was performed, assuming the same risk demonstrated in the PSC related CC study, desired p=0.05, desired power = 80% and case:control ratio of 1:2. This generated required sample sizes of 112 cases, 223 controls
○ DNA was obtained from 164 Caucasian patients with confirmed sporadic CC
○ A control group was formed of 254 healthy Caucasian individuals with normal LFTs

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