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

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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 studies[1, 2]
- 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)[3]

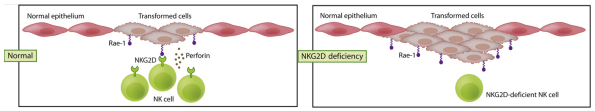

Figure 1 – NK cells lacking expression of the activating receptor NKG2D fail to recognise transformed epithelial cells expressing Rae-1 proteins. Transformation of normal epithelium and other cell types often leads to the expression of ligands for the activation receptor NKG2D. In mice, such ligands belong to a family of several Rae1 as well as H60 and MULT1 proteins. This figure illustrates the destruction of tumor cells expressing NKG2D ligands by NK cells. In the absence of the NKG2D receptor, these cells cannot be rejected by NK cells or other NKG2D-expressing immune cells. The result is aggressive tumour development[1,4] *Adapted from Ljunggren et al, Immunity, 2008*

Aim

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

Method

- 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

Method continued

- Haploview v4.2 was used to select SNPs capturing the majority of common genetic variation around the *NKG2D* gene (MAF >0.05, pair-wise 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

Ref	RS number	Chromosome	BP location
1	rs7397310	12	10412260
2	rs10772271	12	10415387
3	rs1049172	12	10417007
4	rs11053781	12	10428536
5	rs12819494	12	10442808
6	rs2617165	12	10445197
7	rs2617167	12	10450498

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

	n	Female (%)	Male (%)	Median age (range)
Controls	254	119 (46.8%)	135 (53.1%)	66 (55-80)
Cases	164	71 (43.4%)	93 (56.7)%	68 (30-92)

Table 2 – Demographic data, case and control groups

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

Ref	SNP	A1	Freq CC	Freq control	A2	OR	L95	U95	Allelic χ2	p
1	rs7397310	T	0.1925	0.19	C	1.017	0.712	1.451	0.00822	0.9278
2	rs10772271	G	0.4317	0.3947	A	1.165	0.876	1.549	1.1	0.2942
3	rs1049172	G	0.2625	0.286	A	0.8886	0.648	1.218	0.5383	0.4632
4	rs11053781	T	0.4938	0.4592	C	1.149	0.867	1.523	0.9335	0.334
5	rs12819494	T	0.1415	0.127	C	1.133	0.752	1.708	0.3577	0.5498
6	rs2617165	A	0.1398	0.1537	G	0.8946	0.6	1.334	0.2986	0.5848
7	rs2617167	A	0.2112	0.259	G	0.7661	0.549	1.07	4.454	0.1173

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

Results continued

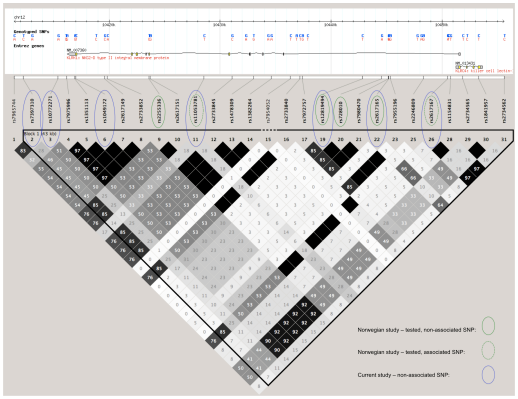

Figure 2 – LD Plot, *NKG2D*, HaploView V 4.1, HapMap data V3 build R2, CEU, Chr12, 10410KB-10460KB. SNPs genotyped in current study, and prior PSC related CC study are circled

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

References

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Acknowledgements

This work has been supported by generous donations from: The British Liver Trust, Imperial College Healthcare Charity, The Alan Moremont Memorial Fund and Mr and Mrs Barry Winter

AMMF RAISING FUNDS FOR RESEARCH INTO CANCER

Imperial College Healthcare Charity Supporting St Mary's and Hammersmith Hospitals

BRITISH LIVER TRUST