GWAS identifies single nucleotide polymorphisms in a long non-coding RNA which are associated with severity of meningococcal disease

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EUCLIDS childhood life-threatening infectious diseases study

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Background:

Invasive meningococcal disease (MD), most commonly presenting as meningitis or septicaemia, results in significant mortality and morbidity. In the UK, 755 cases were reported between 2017-2018 with a mortality rate of 6.9% (52/755) [1]. Our previous genome wide association study (GWAS) established that genetic factors play a role in susceptibility to MD [2]. Genetic factors may also explain differences in the severity and outcome of MD.

Objective:

To identify host genetic factors which determine the severity and outcome of MD we undertook genome wide association studies comparing patients with severe or fatal disease with less severe illness.

Methods:

GWAS analyses were performed on a discovery cohort of 1236 meningococcal cases from the UK, Spain (ESP), Austria and Netherlands (CEC) (Table 1) [2] and validated in the EUCLIDS cohort (Table 2) [3], comparing patients with markers of severe disease: death, amputation, skin graft, mechanical ventilation, severity score or blood markers which are associated with severe disease such as white cell count (WCC), coagulation, base excess and platelet count.

Table 1. Discovery cohort

Cohort	Cases*	Controls	Total
UK	475	4703	5178
CEC	344	2587	2931
ESP	417	882	1332
Total	1236	8172	9441

*Cases: patients with growth of *N. meningitis* on blood/cerebrospinal fluid (CSF) culture

Table 2. Replication cohort

	N
Controls	209
Probable bacterial*	276
Meningococcus	510
Pneumococcus	222
Staphylococcus aureus	158
Group A Streptococcus	120
Escherichia coli	77
Gram negative rod	57
Gram positive cocci	35
Group B Streptococcus	22
Haemophilus influenza	21
Coagulase negative staphylococcus	9
Enterococcus	1
Influenza	1
Mixed bacteraemia	8
Other	38
TOTAL	1764

*Probable bacterial: clinical diagnosis of bacterial infection without microbiological confirmation

Results:

A locus of 8 closely linked single nucleotide polymorphisms (SNP), in a long non-coding RNA region on chromosome 22 was associated with multiple severity markers (Fig. 1).

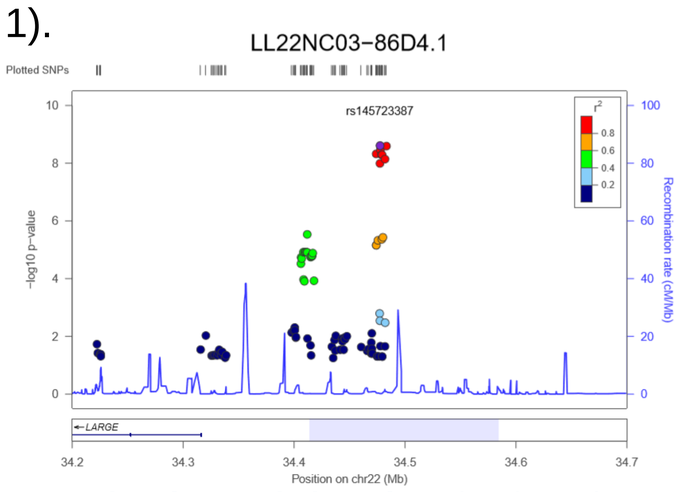

Figure 1. Regional association plot: showing a locus of 8 SNPs with strongest association with lower levels of WCC, p < 10^{-8} , purple = SNP most significantly associated, red = SNPs in high linkage disequilibrium

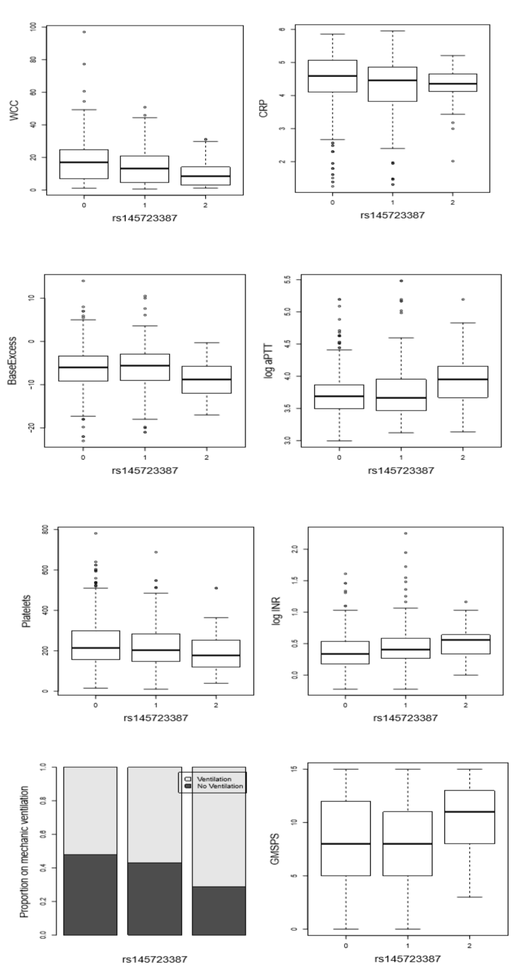

Figure 2. Discovery cohort:
Box-plots showing levels of WCC, platelets, INR, CRP, base excess and APTT, proportion of mechanical ventilation and GMSPS in different genotypes:
0 = no risk allele, 1 = 1 risk allele, 2 = 2 risk alleles.
P values as stated in the text, otherwise not significant.

The risk alleles were associated with lower levels of WCC ($p<5x10^{-9}$), platelets (p=0.003), base excess (p=0.025), CRP (p=0.009) and higher levels of INR (p=0.005) and APTT (p=0.041) (Fig 2). The direction of these blood markers are all indicative of a more severe disease process. The risk alleles were also associated with poorer outcomes including: higher Glasgow meningococcal septicaemia prognostic scores (GMSPS) (p=0.008), increased mechanical ventilation (p=0.002) and increased death rates (p=0.05) (Fig. 2).

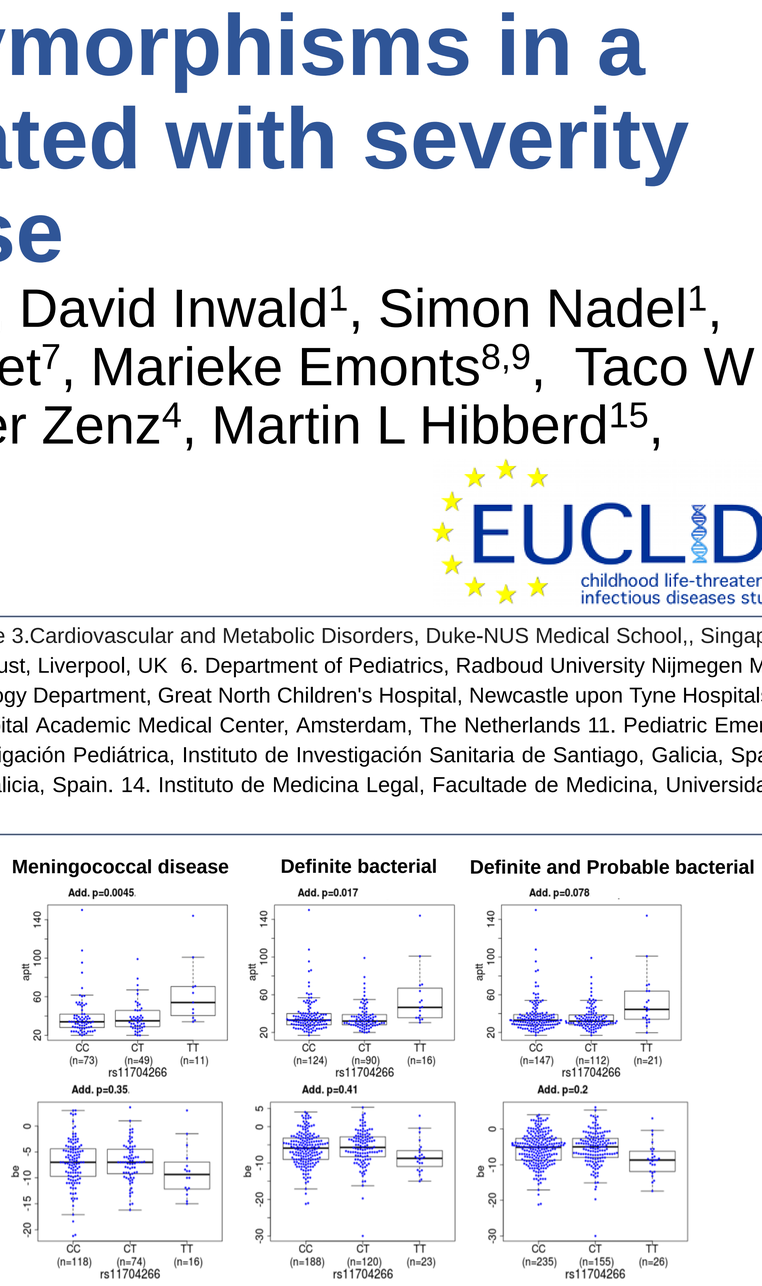

Figure 3. Replication cohort: Box-plots of APTT and base excess levels
CC = homozygous (0 risk allele),**CT** = heterozygotes (1 risk allele), **TT** = (2 risk alleles)
Definite bacterial: all patients with bacteria isolated from a sterile site
Add. = additive model

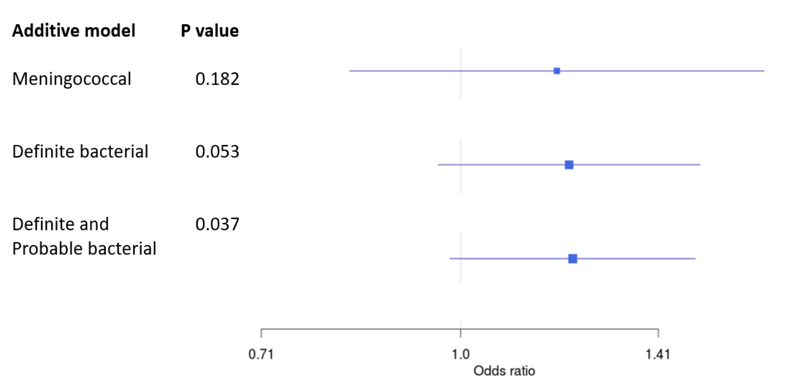

Figure 4. Replication cohort: Odds of mechanical ventilation in an additive model
P value for one-sided test of risk

In the replication cohort, the risk alleles were associated with higher levels of APTT in definite bacterial patients (p=0.017), with a more significant effect seen in the meningococcal subgroup (p=0.005) (Fig. 3). A trend of lower levels of base excess in patients with two risk alleles was seen across all phenotypes (Fig. 3). A significantly increased risk of mechanical ventilation was observed in definite and probable bacterial patients (p = 0.037) (Fig. 4).

Conclusions:

A long non-coding RNA locus on chromosome 22 may confer increased susceptibility to severe meningococcal disease. This risk may extend to other bacterial infections. Exploring the biological role of this SNP will allow better understanding of the disease, patient risk stratification and tailored management.

References:

1. Public Health England (2018) Health Protection Report Volume 12 Number 38
2. Davila et al (2010) *Nature Genetics*, 42(9), 772-776.
3. Martinón-Torres, F. (2018). *Lancet Child & Adolescent Health*, 2(6), pp.404-414.