

# Genetic variation in ADME genes is associated with thalidomide related peripheral neuropathy in multiple myeloma patients

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## Introduction

Peripheral neuropathy is a major adverse effect seen in multiple myeloma (MM) patients treated with thalidomide, with rates varying between trials from 15% to 70%. Peripheral neuropathy can often lead to ongoing impairment of quality of life and can lead to discontinuation of treatment. Identifying patients at risk of neuropathy and understanding its pathogenesis would be a significant step forward in management of thalidomide based therapy.

## Materials and Methods

We took a nested case control approach, using peripheral blood DNA from 388 Caucasian MM patients, all who had received induction thalidomide (200 mg) as part of the Myeloma IX trial. Samples from 80 patients that developed sensory-motor peripheral neuropathy in response to thalidomide therapy were available for this analysis, these were age and sex matched 1:4 case to controls. We assayed 3403 SNPs in coding and predicted regulatory regions selected in 1266 Ensembl genes shown to be involved in the pathogenesis, treatment response, and/or side effects associated with myeloma and its therapy. SNPs were present on a custom-built Affymetrix® targeted genotyping chip (designed by “Bank on a Cure” (BOAC)), which utilizes molecular inversion probe technologies (Figure 1 and 2). Data analysis including Fischer exact tests (Table 1 and Figure 3) and Risk Score (Figure 5) was carried out in PLINK<sup>1</sup>.

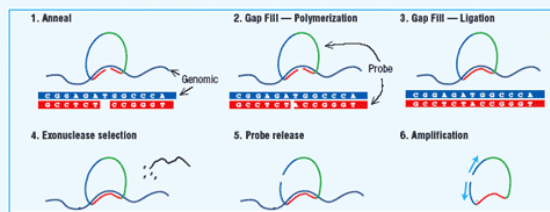


Figure 1: Experimental stages of molecular inversion probe protocol

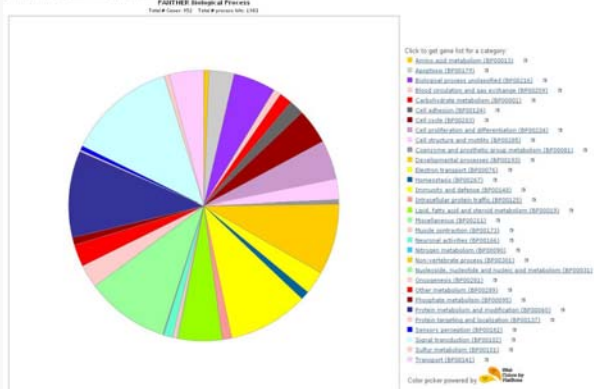


Figure 2: Pathway coverage of the BOAC chip using PANTHER<sup>2</sup>

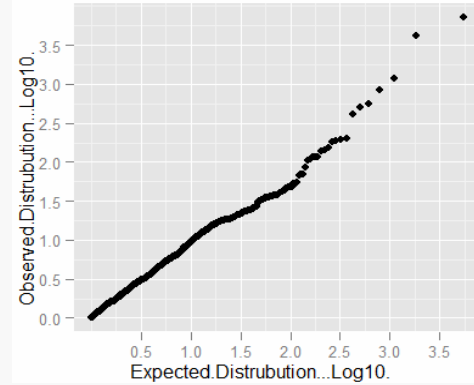


Figure 3: Quantile-quantile plot results of 3403 SNP association tests from the thalidomide related neuropathy cases versus controls.

SNP	CHR	BP	GENE	FUNCTIONAL TYPE	Minor Allele	Freq affected	Freq controls	Major Allele	Odds Ratio	L95	U95	Unadjusted P	Empirical P	Number of Permutations
rs3740066	10	101594197	ABCC2	coding-synon	T	0.488	0.326	C	1.972	1.386	2.806	1.38E-04	9.71E-05	329670
rs17646069	13	41701263	DGKH	coding-nonsynon	C	0.074	0.018	T	4.269	1.848	9.865	2.43E-04	1.05E-03	28536
rs735943	1	238356192	EXO1	coding-nonsynon	A	0.594	0.450	G	1.788	1.254	2.547	1.21E-03	1.44E-03	20769
rs2266782	1	167808624	FMO3	coding-nonsynon	A	0.323	0.461	G	0.557	0.385	0.807	1.81E-03	2.55E-03	11748
rs717620	10	101532568	ABCC2	untranslated,TagSNP-ABCC2	T	0.290	0.180	C	1.866	1.252	2.782	1.98E-03	1.71E-03	17568
rs1029702	14	74010365	NPC2	3' UTR	T	0.093	0.194	C	0.424	0.240	0.749	2.44E-03	1.62E-03	18487
rs560096	11	68435538	IGHMBP2	coding-nonsynon	T	0.099	0.192	C	0.460	0.264	0.802	5.17E-03	4.15E-03	7224
rs4646421	15	72803245	CYP1A1	intron	A	0.148	0.077	G	2.087	1.231	3.537	5.42E-03	7.53E-03	3984
rs858521	17	7470872	SAT2	intron	C	0.278	0.396	G	0.586	0.400	0.857	5.60E-03	5.37E-03	5586
rs4149963	1	238361423	EXO1	coding-nonsynon	T	0.025	0.087	C	0.265	0.094	0.744	0.007	0.013	2301
rs4252596	17	35109360	ERBB2	intron	A	0.066	0.148	C	0.405	0.205	0.799	7.33E-03	8.95E-03	3352
rs1736565	1	167844148	FMO6	locus	C	0.537	0.421	T	1.593	1.124	2.258	8.63E-03	1.08E-02	2781
rs1800566	16	68302646	NQO1	coding-nonsynon,TagSNP-NQO1	A	0.259	0.168	G	1.729	1.144	2.611	8.75E-03	8.03E-03	3736
rs854569	7	94594706	PON1	intron,TagSNP-PON1	T	0.154	0.252	G	0.543	0.341	0.864	9.15E-03	1.06E-02	2826
rs1801132	6	152357636	ESR1	coding-synon	G	0.136	0.229	C	0.529	0.325	0.862	0.010	0.017	1772
rs2938395	3	12404468	PPARG	intron	G	0.303	0.411	A	0.621	0.427	0.901	0.012	0.016	1920
rs491591	4	70141987	UGT2B7C	coding-synon	G	0.062	0.131	A	0.435	0.220	0.861	0.014	0.017	1796
rs11725880	4	2162240	POLN	coding-nonsynon	A	0.044	0.013	G	3.351	1.197	9.386	0.015	0.012	2457
rs1034809	7	94696303	PON2	intron,TagSNP-PON2	A	0.333	0.242	G	1.570	1.077	2.289	0.018	0.028	1066
rs7731453	5	162843653	HMMR	coding-nonsynon	A	0.173	0.104	C	1.803	1.097	2.964	0.019	0.026	1150
rs3735481	7	44610562	PPIA	intron	A	0.259	0.177	C	1.629	1.081	2.455	0.019	0.020	1510
rs3759259	12	10673382	STYK1	coding-nonsynon	T	0.456	0.356	C	1.515	1.064	2.158	0.021	0.028	1076
rs7164902	15	32338374	SLC12A6	coding-synon	A	0.213	0.305	G	0.614	0.405	0.931	0.021	0.021	1412
rs2287780	5	7942304	MTRR	coding-nonsynon	T	0.073	0.032	C	2.418	1.116	5.237	0.021	0.021	1420
rs506504	11	125030405	CHEK1	coding-nonsynon	A	0.056	0.022	G	2.673	1.121	6.371	0.021	0.023	1304
rs1052637	2	118291445	DDX18	coding-nonsynon	G	0.438	0.341	C	1.510	1.061	2.151	0.022	0.033	916
rs9282564	7	86874091	ABCB1	coding-nonsynon	C	0.167	0.102	T	1.754	1.074	2.866	0.023	0.020	1492
rs2071724	22	41901546	TTL12	intron	A	0.312	0.413	C	0.645	0.440	0.944	0.024	0.025	1210
rs1052536	17	30355688	LIG3	untranslated	T	0.544	0.444	C	1.495	1.052	2.125	0.024	0.020	1472

Table 1: Univariate analysis by Fischer exact tests – associated SNPs with p-value<0.025 with MAF>5%. Correction for multiple testing using label swapping permutation using the program PLINK.

## Conclusions

**Patients at risk of developing a thalidomide related neuropathy can be stratified by inherited genetic variation**

Possible mechanisms of thalidomide related neuropathy include: reduced nerve blood supply through anti-angiogenic properties of thalidomide; direct toxic effects of thalidomide on posterior root ganglia and dysregulation of neurotrophin activity through effects of thalidomide on NF-kB. Our results indicate the importance of thalidomide dose and cumulative exposure, highlighting the metabolism of thalidomide as playing a pivotal role in dictating neuropathy events and open the way for predictive testing and dose adjustment. The results also implicate a direct toxicity mechanism for thalidomide related peripheral neuropathy, as we see a number of associations with SNPs in genes with known importance in peripheral neuron function. We have also shown that a risk score can be built on inherited variation using the significant thalidomide related association to stratify cases and controls (Figure 5). Importantly this Risk score analysis included weak associations and rare alleles (data not shown), we are currently validating this risk panel prospectively in separate datasets.

## Results

**ABC genes play a role in thalidomide neurotoxicity**

The most significant associated SNP is *ABCC2*:Ile1324Ile, rs3740066. The *ABCC2* variant (-24)C>T, rs717620 is also associated with risk. rs717620 has been shown to decreased *ABCC2* function *in vitro*, and is associated with other several toxic induced complications. The “silent” polymorphism: rs3740066, may modulate substrate specificity via codon usage influencing the translation rate, a process shown to occur in another ABC gene *ABCB1* (P-gp).<sup>3</sup> Analysis of 12 SNPs in *ABCC2* on the BOAC chip reveal a four SNP haplotype (Figure 4), with three SNPs additively contributing to thalidomide related neuropathy risk. Evidence that thalidomide can modulate the function of both *ABCC2* and *ABCB1* hints at the mechanism by these genes modulate thalidomide related neuropathy risk.<sup>4</sup> Weaker associations were seen with other ABC genes: *ABCB1*, *ABCB11*, and *ABCC1*.

## A.

\*\*\* Proxy haplotype association report for rs2273697 \*\*\*

SNP	MAF	Call rate	KB	R-SQUARED	Odds Ratio	CHISQ	P
rs717620	0.203	0.992	-21.2	0.0692	1.88	9.88	0.00167
rs2273697	0.214	0.995	0	*	0.797	1.05	0.306
rs3740066	0.358	0.997	40.4	0.115	2	16	6.37E-05

	Frequency	Odds Ratio	CHISQ	P
TGT	0.199	1.87	9.59	0.00195
CGT	0.148	1.51	3.4	0.0653
CAC	0.204	0.776	1.32	0.251
CGC	0.435	0.587	9.02	0.00267

HAP	Frequency	R-SQUARED	Odds Ratio	CHISQ	P
C C	0.639	0.117	0.491	17	3.82E-05
. T	0.358	0.115	2	16	6.37E-05

## B.



Figure 4: Haplotype analysis of thalidomide neuropathy associated *ABCC2* polymorphisms: A. Proxy association around rs2273697 B. *ABCC2* locus SNPs related on the BOAC chip viewed in Haploview 4.0.

## Results

**Additional evidence of ADME genes and neuronal damage modulating risk**

Associations were also seen in a number of ADME genes (drug absorption, distribution, metabolism, and excretion): *CYP1A1*, *CYP20A1*, *CYP2C9*, *CYP3A7*, *CYP4F2*, *FMO2*, *FMO3*, *FMO6*, *SLC12A6*, *SLC22A3* and *SLC7A7*. Significant associations were also seen in genes important in neurological system processes and central nervous system development: *ERBB2*, *NQO1*, *MYO3A*, *PPARD*, *DBH*, *NGFR*, *GSTP1*, *TCF8* and *ICF1R*. Associations between SNPs in *DGKH*, *EXO* and *NPC2* with risk were seen, these genes have all been previously reported to play a role in peripheral neurotoxicity.

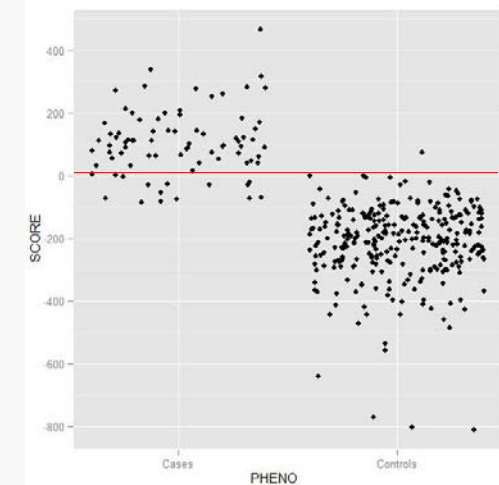


Figure 5: Risk score (SCORE) of developing thalidomide related neuropathy in European myeloma patients against observed phenotype (cases or controls). SCORE for an individual patient = SUM of the Log10 (Odds ratio of significant associated SNP with thalidomide neuropathy) x number of significant associated alleles per patient x 10<sup>4</sup>.

## References

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