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Common Genetic Variants in 11q13.3 and 9q22.33 Are Associated With Molecular Subgroups of Multiple Myeloma

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Recently, Weinhold et al.(1) reported the association of a potentially functional single nucleotide polymorphism (SNP) in the *CCND1* gene with an increased incidence of t(11;14) in multiple myeloma (MM). MM can be classified by gene expression profiling (GEP) into 7 well-defined molecular subgroups, of which 4 are strongly influenced by specific translocations involving the immunoglobulin heavy chain (IgH) locus (2). These translocations are characterized by transcriptional activation of the genes *CCND1* [t(11;14)]

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Supplementary information is available at *Leukemia's* website

(q13;q32)], *CCND3* [t(6;14)(p21;q32)], *c-MAF* [t(14;16)(q32;q23)], *MAFB* [t(14;20)(q32;q11)], and *FGFR3/MMSET* [t(4;14)(q16;q32)]. Specific cytogenetic abnormalities normally assessed by fluorescence *in situ* hybridization (FISH), including these IgH translocations, can be predicted with high accuracy by spiked gene expression levels with a technique known as *virtual karyotyping* (3;4). In the current study, we investigate whether SNPs previously associated with overall risk of MM (5-7) and t(11;14)(1) are associated with any disease-specific subset. We also performed genome-wide scans of associations between common genetic variants to molecular subgroups.

Genotypes at 777,681 SNPs in a discovery set of 972 newly diagnosed MM patients of European ancestry (EA) and 1064 cancer-free controls, plus a replication set of 252 more recent EA patients genotyped at selected SNPs, form the basis of this analysis (7). The study was conducted under the auspices of the Institutional Review Board (IRB) at UAMS. Gene expression profiling from CD138-selected plasma cells from these patients within 3 months prior to treatment (8) were available for 650 patients in the discovery set and for all 252 patients in the replication set. Patients included in specific MM subgroups served as cases and were compared to 1064 non-cancer controls. The results of this analysis showed that one SNP was significantly associated with *Cyclin D1* spiked expression (*CCND1*-hi) and one SNP was significantly associated with the proliferation (PR) subgroup (Table S1).

The SNP rs603965, located in the *CCND1* gene at 11q13.3, was significantly associated with *CCND1*-hi in the discovery set (118 patients, OR = 1.66, $p = 3.6 \times 10^{-4}$, Table 1), and this association was confirmed in the replication set (64 patients, OR = 1.90, $p = 0.001$). A significant allele dosage effect was seen on gene expression levels of *CCND1* (Figure S1). Interestingly, overall survival among the combined cohorts of *CCND1*-hi patients was poorer among non-carriers of the G allele than either heterozygous or homozygous carriers ($p = 0.008$, Figure 1).

The SNP rs73486634 at 9q22.33, located between and upstream of the forkhead box E1 (*FOXE1*) and xeroderma pigmentosum complementation group A (*XPA*) genes, was associated with the PR subgroup in the MM discovery set (78 patients, OR = 5.06, $p = 6.0 \times 10^{-9}$, Table 1). This association was confirmed in the replication set (45 patients, OR = 3.16, $p = 0.006$). A trend was observed between variant carriers of rs73486634 and poorer overall survival (Figure 1, $p = 0.115$). The PR subgroup is characterized by a significantly higher gene expression proliferation index and overexpression of genes involved in regulation of cell cycle as compared to the other molecular subgroups (2). The subgroup has poor clinical outcome and close to 70% of the cases in this subgroup have cytogenetic abnormalities (2). The *FOXE1* gene, also named thyroid transcription factor 2, is an intron less gene that encodes an evolutionary conserved transcription factor highly overexpressed in thyroid follicular cells. Changes in the gene may be involved in carcinogenesis (9). The protein encoded by the DNA excision repair gene *XPA* is crucial for global genomic nucleotide excision repair which corrects lesion in the entire genome including the non-transcribed strands of active genes and is closely coupled to DNA damage checkpoint (10). Polymorphisms in the *XPA* gene and susceptibility to several cancers have been described by a targeted SNP approach. The association we find could represent a cancer etiological

factor or a tendency to develop a proliferative behavior to be associated with disease progression.

In addition to testing SNPs known to be associated with overall risk of MM, we also performed genome-wide scans of GEP-defined subgroups using the genotype data previously described (7). First, an MM-case-only analysis was performed on each GEP-defined subgroup, with MM patients not belonging to the subgroup serving as controls (Table S2). By using MM patients as controls, we ensured that genetic associations were specific to a subgroup and were not simply a recapitulation of associations to overall MM risk. In Figure S2, we also show the results of a confirmatory analysis using cancer-free individuals, instead of non-subgroup MM patients, as controls.

These Genomic scans identified one region containing multiple SNPs significantly associated with risk of the *cMAF*-hi subgroup. The most significant SNP, rs4666188, was associated with risk of belonging to the *cMAF*-hi subgroup when compared to cancer-free controls (OR 4.1; $p = 1.2E-7$) (Table S2). The SNP is located in an intron in *ALK* (anaplastic lymphoma receptor tyrosine kinase) at 2q23.2 and is in linkage disequilibrium (LD) with the SNP rs35093491, which results in an amino acid change at residue 476. An allele dose effect of rs4666188 in the *ALK* gene was found for gene expression levels of *cMAF* and *ALK* (Figure S3). Risk allele carriers had higher gene expression levels of *cMAF* in contrast to the gene expression level of *ALK* where the levels were highest among homozygous wild-type G-allele carriers. The transmembrane tyrosine kinase encoded by the *ALK* gene is involved in development of the brain and nervous system. Rearrangements at locus 2p21.2 are found in many cancers but no translocations in the *ALK* break point at 2p23 were found by metaphase cytogenetic analysis among patient in the *cMAF* subgroup. Elevated expression levels of *cMAF* are found in MM with t(14;16) and t(4;14) and inhibition of MEK has been shown to deplete *MAF* expression levels both in patients with *MMSET* overexpression and in those with t(14;16) (11).

The SNP rs16897930 (at 6q27) was associated to risk of the *MMSET*-hi subtype (OR 2.5; $p = 1.2E-05$). The SNP rs16897930 is located in an intron of *PDE10A* (phosphodiesterase 10A) and encodes a protein that is important for signal transduction by regulating the intracellular concentration of cyclic nucleotides. The phosphodiesterase encoded by *PDE10A* gene has recently been shown to be a target of curcumin, a drug that has shown apoptotic effect in myeloma cell lines including those with t(4;14) and t(14;16)(12;13).

Unfortunately, the allele frequency of the significant SNPs, rs4666188 and rs16897930, was too low for validation of the results in the small number of patients in the replication set.

In conclusion, we confirm the association of rs603965 to t(11,14) and our results indicate that this SNP may have prognostic importance. We also identify a novel association between the SNP rs73486634 and the PR molecular subgroup as an inherited contributor to the development of proliferative disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Kaplan-Meier plot of overall survival for A: CCND1-hi patients by rs603965 genotype and B: PR subgroup patients by rs73486634 (*FOXE1/XPA*) genotype

Table 1
Two SNPs associated with molecular subgroups of multiple myeloma

	Risk Allele	MM cases					Controls								
		N	Genotypes			RAF	N	Genotypes			RAF	OR	P	P(adj)	
			GG	GA	AA			GG	GA	AA					
rs73486634 (9q22.33)		PR molecular subgroup													
G	Discovery	78	2	12	64	0.10	1064	0	47	1017	0.02	5.06	6.0E-09	2.0E-06	
	Replication	45	0	6	39	0.07						3.16	6.0E-03		
rs603965 (11q13.3)		CCND1-hi, t(11;14)													
G	Discovery	118	51	55	12	0.67	1064	309	542	213	0.55	1.66	3.6E-04	1.7E-04	
	Replication	64	29	31	4	0.70						1.90	7.6E-04		

OR, odds ratio; RAF, risk allele frequency.
P, Cochran-Armitage trend test.
P(adj), logistic regression assuming additive inheritance and including 4 genome-wide multidimensional scaling parameters as covariates.