Evaluation of TMED9, DNAJC1, LMAN2, COPE and KDELR1 Biomarkers in Patients with Monoclonal Gammopathy of Undetermined Significance and Multiple Myeloma

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ABSTRACT

Plasma cell dyscrasias (PCD) are a heterogeneous group of hematological diseases. The TMED9, KDELR1, DNAJC1, COPE, and LMAN2 genes identified from our transcriptome data are highly importance and specific. We determined the protein-protein interactions of these genes in STRING v11.5 higher than expected (PPI enrichment p value 1.71e-06). We evaluated the prognostic biomarker status of these genes in PCD by quantitative method (qRT-PCR) of 38 Multiple Myeloma (MM), 23 Monoclonal Gammopathy of Undetermined Significance (MGUS) and 16 control groups. We found significant gene expression levels increase among these three study groups of these genes ($p < 0.001$). As a single marker, DNAJC1 exhibited the best ability for discriminating MM from MGUS (AUC= 83%) and MM from control (AUC= 88.7%). In addition to this, the combination of five genes exhibited the highest efficacy of discriminating MM from the control (AUC= 90.90%). The combination of KDELR1, COPE, TMED9 and DNAJC1 exhibited the best ability to discriminate MM group from MGUS group (AUC= 86.80%). In conclusion, we showed that DNAJC1 alone, as well as the combination of other selected genes, can be valuable targets in the pathogenesis of myeloma. These new biomarkers have been evaluated for the first time in PCD and we think they will contribute to the discovery of potential anti-myeloma therapeutic targets in the future.

Keywords: DNAJC1, Biomarker, Multiple myeloma, MGUS, Plasma cell dyscrasias

INTRODUCTION

Plasma Cell Dyscrasias (PCD) constitute a heterogeneous group of hematological malignancies caused by the proliferation of bone marrow plasma cells.[1,2] One of the most important PCD is Monoclonal Gammopathy of Undetermined Significance (MGUS). It is defined as a premalignant precursor condition in which the plasma cell infiltration in the bone marrow is less than 10%, and there is no end-organ damage. Over the years, MGUS transforms to Multiple Myeloma (MM) with lytic bone lesions, paraproteinemia, hypercalcemia and anemia.[3,4] MM is caused by primary genetic events such as hyper diploidy (trisomy 3,5,7,9,11,15,19,21) and IGH translocations (t4;14, t6;14, t11;14) in the B cell located in the post germinal center. Other secondary genetic events such as mutations in oncogenic pathways (RAS, RAF, FGFR3, etc.), loss of tumor suppressor function (RB1, TP53) and the bone marrow microenvironment (osteoblast, T cell, Nature Killer cell) are involved in the pathogenesis of myeloma.[5]

Activation of unfolded protein response (UPR) components occurs in myeloma cells both to support paraprotein production and to resist ER stress resulting from cell differentiation.

Recent studies have been shown that endoplasmic reticulum (ER) stress is Achilles tendon in MM because it affects protein translation, proteostasis and UPR.[6]

In our previous study, we investigated transcriptome profiles by comparing genes with high and low gene expressions in newly diagnosed MM patients and healthy control groups. In this study, we found increased expression levels on TMED9, KDELR1, LMAN2, COPE, and DNAJC1 genes.[7] In silico analysis, 4 out of 5 genes (TMED9, KDELR1, LMAN2, COPE) were determined strongly related in STRING v11.5 database. The genes are located in ER and Golgi is involved in vesicular transport in terms of their biological functions.[8]

DNAJC1 is a transmembrane heat shock protein involved in protein folding, apoptosis and immune regulation. DNAJC1 is critical for the cell surface localization of GRP78, and it has been reported that expression of GRP78 is significantly reduced when this gene is knockdown.[9,10] TMED9 is a protein expressed in the membrane of the endoplasmic re-ticulum that has a role in vesicular transport.[11] KDEL receptors,[12] and LMAN2 are proteins [13,14] that are functional between the ER and Golgi. The COPE gene encodes the coatomer protein that forms the epsilon subunit.[15] Vesicular transport and proteostasis regulate the transport of signaling pathways, receptors, and their cargo.[16] The impaired protein homeostasis of myeloma cells is the result of factors such as RNA processing, protein translation, and mutation of genes involved in the UPR or dysregulated gene transcription of these genes.[17] Although there are some drugs (Bortezomib, Carfilzomib) targeting ER stress/UPR in the treatment.[18,19] there is currently little information about ER stress and vesicular transport in MM. In particular new biomarkers on the pathogenesis of MM are very important for the discovery of potential anti-myeloma therapeutic targets and the emergence of individualized therapeutic strategies against drug response variability/resistance.[20]

Our aim in this study is to evaluate the efficacy of TMED9, KDELR1, LMAN2, COPE, and DNAJC1 genes, which are upregulated in our transcriptome data and found to be related in in silico analysis, in larger PCD patient groups. Thus, it contributes to the elucidation of the pathogenesis of MM.

PATIENTS AND METHODS

Patients and Sample Collecting

Between 2017-2020, newly diagnosed 37 MM and 23 MGUS patients without drug therapy were included in this study. Voluntary consent forms were signed in accordance with the Declaration of Helsinki, and the study was conducted in accordance with the principles of good clinical and laboratory practices. 37 newly diagnosed MM patients were diagnosed and classified according to the International Myeloma Working Group criteria. The control group consisted of a total of 16 samples, of which 9 were healthy bone marrow donors and 7 were non-MGUS. Individuals in the non-MGUS group are volunteers examined for plasma cell dyscrasia. Exclusion criteria for non-MGUS group were all other primary malignancies, microbiological-infectious diseases and rheumatological disorders. Approval for the study was obtained from the Istanbul University Clinical Research Ethics Committee (File number: 2017/1425).

The Expression of Five Candidate Genes Investigated by Quantitative Real-time PCR

MM, MGUS and control groups' bone marrow materials were separated via Ficol -Histopaque (Sigma Aldrich, USA) into mononuclear cells at a density of 1.077 g/ml. Total RNA isolation was performed with the PureLink RNA Micro kit (Invintrogen, USA). RNA quality and quantity were measured with Nano Drop ND-2000c (Thermo Fisher Scientific, USA). CDNA synthesis was made from total RNA using Transcriptor High Fidelity cDNA synthesis (Roche, Germany) kit. qRT-PCR was performed using SYBR Green with these candidate genes and TATA-Box-Binding Protein (TBP) as a housekeeping gene in a Roche Light cycler Real-Time PCR system. The expression levels of candidate genes were calculated by the $2^{-\Delta\Delta CT}$ method.

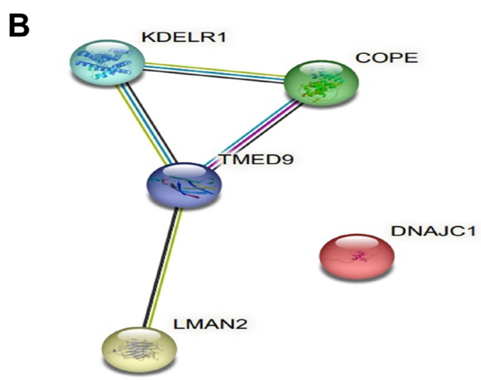
Statistical Analysis

The distribution of the data was analyzed with the Shapiro-Wilk test. The Kruskal Wallis test was used for comparisons of three independent groups that did not meet the normal distribution assumptions. Post-hoc comparisons of significant variables were

A

Biological Process (Gene Ontology) COPE, LMAN2, KDELR1, TMED9		
G0-Term	Description	False discovery rate
G0:0006888	ER to Golgi vesicle-mediated transport	0.000033
GO:0006890	Retrograde vesicle-mediated transport, Golgi to ER	2.44e-05
Cellular Component (Gene Ontology)		
G0-Term	Description	False discovery rate
GO:0000139	Golgi membrane	0.0024
GO:0005789	ER	0.00030
GO:0005798	Golgi-associated vesicle	6.10e-05
GO:0030133	Transport vesicle	0.0150
GO:0030134	COPII-coated ER to Golgi transport vesicle	0.0304

B



Number of Nodes	5
Number of Edges	4
Average Node Degree	1,6
Avegare Local Clustering Coefficient	0,667
PPI-enchriment -p-value	**1.71e-06**

made with Dunn test. Fisher-Freeman Halton test was used to evaluate the difference of categorical variables between groups. Descriptive statistics of non-normally distributed numerical variables are expressed as median (min-max). Descriptive statistics of categorical variables are given as n (%). Examination of the relationship between numerical variables was used Spearman Correlation analysis. ROC (Receiver Operating Characteristic Curve) analysis was applied to determine the distinctiveness of genes between the patients and healthy groups and to calculate the cut-off value, and the cut-off values were determined according to the Youden J index. AUC (Area Under Curve) values were calculated to evaluate the performance of the genes. The sensitivity and specificity for the gen combinations were estimated by identifying the cut-off point of the predicted probability that yielded the highest sum of sensitivity and specificity. Predicted probability was calculated with binary logistic regression. Significance was evaluated at $p < 0.05$ levels.

RESULTS

Analysis of Demographic and Clinical Parameters of MM and MGUS Patients

A total of 76 participants were enrolled in this study. It consisted of 37 (48.7%) MM patients, 23 (30.3%) MGUS patients and 16 (21.1%) control group. The gender distribution were 47 men and 29 women. The mean age was 61.03±14.25 (min: 30-max: 84). Gender and age variables showed a homogeneous distribution according to the groups ($p > 0.05$).

In Silico Analyzes for TMED9, LMAN2, KDELR1, COPE, DNAJC1 Genes

The biological properties of TMED9, LMAN2, KDELR1, COPE genes were found to be associated with Golgi and ER vesicular transport in the PANTHER v17.0 program (http://www.pantherdb.org) [20]. The protein-protein interaction of these genes was found to be higher than expected (PPI enrichment p value 1.71e-06) in STRING v11.5 (https://string-db.org/).[7] This means that the proteins have more interactions among themselves than would be expected for a random set of proteins of similar size in the genome. This enrichment shown in Figure 1 indicates that the proteins as a group are at least partially biologically related.

Expression of Five Candidate Genes in the MM and MGUS Group

Expression of five genes was determined by qRT-PCR in the bone marrow in all groups. KDELR1, COPE, TMED9, DNAJC1 and LMAN2 genes had

Table 1. Evaluation of expression of 5 genes between groups

	MM1 (n= 37)	MGUS2 (n= 23)	Groups Control3 (n= 16)	p-value	Post-hoc p-value
KDELR1	12.99(0.08-61.39)	4.92(0.03-21.55)	5.57(0-10.55)	<0.001**	1-2:0.001 1-3:0.001 2-3:1
COPE	8.57(0.05-24.25)	3.60(0.02-16)	4.80(0-15.13)	0.007**	1-2:0.013 1-3:0.085 2-3:1
TMED9	6.86(0-43.71)	3.09(0.02-8.81)	4.01(0-8.57)	<0.001**	1-2:0.001 1-3:0.035 2-3:1
DNAJC1	10.70(0.43-72.50)	2.96(0.08-12.04)	1.95(0-5.89)	<0.001**	1-2:<0.001 1-3:<0.001 2-3:1
LMAN2	3.97(0.15-13.73)	2.31(0.14-5.50)	1.98(0.01-4.99)	<0.001**	1-2:0.003 1-3:0.009 2-3:1

** It was the p-value of the Kruskal Wallis test. Post-hoc comparisons were analyzed with Dunn's test. Numerical data are given as median (min-max).

a statistically significant difference between MM, MGUS and control groups (p< 0.05) (Table 1). In paired comparisons, the KDELR1 gene was found to be significantly higher in the MM group compared to the MGUS and control groups (respectively; p= 0.001, p= 0.001). The KDELR1 gene did not have a statistically significant difference between the MGUS and control groups (p= 1). The COPE gene was found to be statistically significant and higher in the MM group than in the MGUS group (p= 0.013). No significant difference was observed be-tween MGUS and control, and between MM and control, and similar results were obtained (p> 0.05). TMED9, DNAJC1 and LMAN 2 genes were found to be significantly higher in the MM group compared to the MGUS and control groups (p< 0.05). It was concluded that there was no statistically significant difference between MGUS and control group (p> 0.05). When the correlation of genes with each other in all patients was examined, it was concluded that five genes had a significant and positive relationship with each other. In addition, the KDELR1 gene appeared to be significantly, positively and highly correlated with COPE, TMED9, DNAJC1 and LMAN2. As a result of the increase in KDELR1 gene, COPE, TMED9, DNAJC1 and LMAN2 genes will also increase significantly (respectively; r= 0.750, p< 0.001; r= 0.709, p< 0.001; r= 0.735, p< 0.001; r=0.800, p< 0.001). Statistical analyzes showing the relationship of five genes with laboratory parame-ters in the MM group was given in Table 2. According to Table 2, while the KDELR1 gene had a statistically significant, negative and low-level relationship with eGFR in the MM group (r= -0.351, p= 0.036) it had a significant positive and low-level relationship with the percentage of plasma cells (r= 0.450, p= 0.005).

In the MM group, DNAJC1 gene and RBC, HGB, HCT, eGFR variables had a statistically significant, low and negative relationship (p< 0.05). While B2M, UREA, CR showed a significantly low and positive relationship, the percentage of plasma cells had a significant positive and medium relationship. Both TMED9 gene and LMAN2 genes had significant positive and low-grade associations with plasma cell percentage (TMED9 r= 0.421, p= 0.009) (LMAN2 r= 0.496, p= 0.002). In the MGUS group, there was no significant relationship be-

Table 2. Investigation of the relationship between genes and laboratory parameters in the MM group

MM Group		KDELR1	DNAJC1	COPE	TMED9	LMAN2
RBC (10/6 ul)	r	-0.295	-0.560	-0.18	-0.302	-0.286
	p value	0.077	<0.001	0.286	0.069	0.086
B2M (mg/L)	r	0.012	0.506	-0.11	0.220	0.138
	p value	0.953	0.010	0.602	0.291	0.51
HGB (g/dl)	r	-0.300	-0.482	-0.182	-0.312	-0.275
	p value	0.071	0.003	0.282	0.060	0.100
HCT (%)	r	-0.213	-0.430	-0.113	-0.256	-0.223
	p value	0.205	0.008	0.505	0.125	0.184
UREA (mg/dl)	r	0.161	0.366	-0.100	0.118	0.070
	p value	0.342	0.026	0.555	0.486	0.679
CR (mg/dl)	r	0.298	0.399	0.129	0.056	0.129
	p value	0.073	0.014	0.448	0.741	0.448
CK (U/L)	r	-0.117	-0.047	-0.276	0.280	0.045
	p value	0.596	0.83	0.203	0.196	0.839
ALB (g/dl)	r	0.065	-0.079	0.027	-0.099	0.206
	p value	0.705	0.647	0.876	0.564	0.228
CA (mg/dl)	r	-0.235	0.163	-0.283	0.145	-0.112
	p value	0.162	0.334	0.090	0.391	0.509
eGFR (mL/min/1.73 m^2)	r	-0.351	-0.402	-0.228	-0.062	-0.170
	p value	0.036	0.015	0.182	0.719	0.322
CRP (mg/L)	r	-0.226	-0.085	-0.099	-0.083	-0.159
	p value	0.179	0.617	0.561	0.624	0.349
LDH (U/L)	r	-0.063	0.108	-0.186	0.155	-0.077
	p value	0.713	0.532	0.276	0.366	0.655
PLASMA CELLS (%)	r	0.450	0.651	0.129	0.421	0.496
	p value	0.005	<0.001	0.448	0.009	0.002

p-value: *It is the p-value of the Spearman correlation coefficient.* ***r***: *correlation coefficient*

tween the KDELR1, COPE, TMED9 and LMAN2 genes and the laboratory parameters of five genes ($p > 0.05$). As a result of the statistical analysis performed according to Durie Salmon staging, there was no significant difference between gene expression values of KDELR1, COPE, DNAJC1, TMED9 and LMAN2 ($p > 0.05$). The results of the ROC analysis performed to determine the discrimination of genes between MM-MGUS, MM-control and MGUS-control groups were shown in Table 3.

MM Group Versus MGUS Group

In Table 3, it was shown that the five genes whose discrimination characteristics were analyzed according to the MM and MGUS groups had a statistically significant difference ($p < 0.001$). The highest AUC value of DNAJC1 gene was found to be 0.830, and the AUC values of the other four genes were between 0.718 and 0.788. In Table 2, each gene had a cut-off value. If it was above this value, it could indicate that the patient had a MM disease. Binary logistic regression was used to evaluate different combinations of five genes. Among the different discrimination combinations, the combination of KDELR1, COPE, TMED9, DNAJC1 and COPE, TMED9, DNAJC1 could significantly discriminate MM patients from MGUS patients with the highest AUC value of 0.868 and 73% sensitivity and 91.30% specificity (Table 4). In addition, the AUC values of the other evaluated combinations ranged from 0.792 to 0.866, with the best discrimination.

Table 3. Diagnostic potential of KDELR1, COPE, TMED9, DNAJC1 and LMAN2 biomarkers between patients and control group

	Cut-Off Value	AUC	95%CI	p-value	Sensitivity	Specificity	PPV	NPV
MM versus MGUS								
KDELR1	>6.23	0.788	0.664-0.883	<0.001	69.57	81.08	69.60	81.10
COPE	>7.41	0.718	0.587-0.827	<0.001	91.30	56.76	56.80	91.30
TMED9	>5.57	0.774	0.648-0.872	<0.001	91.30	56.76	56.80	91.30
DNAJC1	>6.77	0.830	0.710-0.914	<0.001	91.30	64.86	61.80	92.30
LMAN2	>3.18	0.759	0.631-0.860	<0.001	86.96	59.46	57.10	88
MM versus Control								
KDELR1	>10.55	0.815	0.685-0.908	<0.001	56.76	100	100	50
COPE	>6.54	0.694	0.553-0.813	0.013	59.46	75	84.60	44.40
TMED9	>8.57	0.715	0.574-0.830	0.003	37.84	100	100	41
DNAJC1	>5.89	0.887	0.770-0.957	<0.001	67.57	100	100	57.10
LMAN2	>2.08	0.747	0.609-0.857	<0.001	83.78	56.25	81.60	60
MGUS versus Control								
KDELR1	>0.09	0.533	0.366-0.694	0.740	95.65	25	64.70	80
COPE	≤4.28	0.535	0.369-0.696	0.727	65.22	62.50	71.40	55.60
TMED9	≤3.43	0.543	0.377-0.704	0.674	65.22	62.50	71.40	55.60
DNAJC1	>5.89	0.630	0.461-0.779	0.151	26.09	100	100	48.50
LMAN2	>0.207	0.522	0.356-0.684	0.827	91.30	31.25	65.60	71.40

AUC: *Area Under Curve, The cutoff point was determined according to the Youden j index.*

MM Group Versus Control Group

It was shown in Table 4 that the five genes whose distinctive features were determined according to the MM and Control groups had a statistically significant difference ($p< 0.001$). The DNAJC1 gene had the highest AUC value of 0.887, and the other four genes was exhibited AUC values between 0.715 and 0.815. Among the different combinations, the combination of KDELR1, TMED9, DNAJC1, LMAN2 and the combination of all five genes KDELR1, COPE, TMED9, DNAJC1, LMAN2 significantly discriminated MM patients from healthy control patients with the highest AUC value of 0.909, sensitivity of 86.50% and specificity of 81.20% (Table 5) and was shown in the Figure 2. AUC values for all combinations between MM and control ranged from 0.824 to 0.902, with the best discrimination.

DISCUSSION

In MM, the production of high levels of paraprotein exposes the cell to continuous ER stress. Increased protein load affects the survival of myeloma cells by causing changes in many genes involved in protein synthesis, protein folding, and degradation, including intracellular protein transport of MM patients.[17] There is an urgent need for new biomarkers that can mediate new therapeutic approaches in both the diagnosis and treatment of MM.[20] This study for that reason is important in that TMED9, KDELR1, LMAN2, COPE, and DNAJC1 genes, which are associated with vesicular transport and ER stress, are new targets in myeloma pathogenesis.[21] We evaluated the genes and their combinations that were not previously associated with myeloma pathogenesis. As a result, we reported that KDELR1, COPE, TMED9, DNAJC1, and LMAN2 combination had the best ability to discriminate MM from the control group. Also, the combination

Table 4. Performance of genome biomarker combinations to discriminate MGUS patients from MM patients

	Sensitivity (%)	Specificity (%)	AUC	%95CI	p-value
MM versus MGUS					
KDELR1+COPE+TMED9	73	82.6	0.823	0.702-0.909	<0.001
KDELR1+COPE+DNAJC1	73	91.3	0.852	0.737-0.930	<0.001
KDELR1+COPE+LMAN2	59.46	91.30	0.792	0.668-0.886	<0.001
COPE+TMED9+DNAJC1	73	91.30	0.868	0.756-0.942	<0.001
COPE+TMED9+LMAN2	70.30	82.60	0.807	0.685-0.898	<0.001
TMED9+DNAJC1+LMAN2	86.50	78.30	0.864	0.751-0.939	<0.001
KDELR1+ TMED9+ DNAJC1	83.80	78.30	0.866	0.753-0.940	<0.001
KDELR1+ TMED9+LMAN2	81.10	73.90	0.824	0.704-0.910	<0.001
KDELR1+COPE+TMED9+DNAJC1	73	91.30	0.868	0.755-0.941	<0.001
KDELR1+COPE+TMED9+LMAN2	67.57	82.61	0.824	0.704-0.910	<0.001
COPE+TMED9+DNAJC1+LMAN2	73	91.30	0.865	0.752-0.939	<0.001
KDELR1+ TMED9+ DNAJC1+LMAN2	67.60	91.30	0.860	0.764-0.936	<0.001
KDELR1+ COPE+ DNAJC1+LMAN2	73	91.30	0.853	0.738-0.931	<0.001
KDELR1+ COPE+ TMED9+DNAJC1+LMAN2	73	91.30	0.865	0.752-0.939	<0.001

AUC: Area Under Curve

of KDELR1, COPE, TMED9, and DNAJC1 exhibited the highest efficacy for discriminating MGUS from the control group.

DNAJC1 showed the best ability to discriminate MM from the control group and MGUS when the discrimination abilities of the genes were examined one by one. We presented experimental evidence that these 4 genes may be associated with DNAJC1, which has not been previously reported in in silico analyses. In our analysis, the fact that we found these genes were upregulated and correlated with each other. It confirms that there is a strong relationship between them, just like in silico analyses. It has been shown that lung cancer cells with high level of GRP78/DNAJC1 activation, especially cancer stem cells, can be effective in drug resistance. It has been found that high expression of DNAJC1 can also lead to cancer aggressiveness.12 DNAJC1 has been reported in the single-cell leukemia transcriptome.[22] The DNAJC1 gene is upregulated in the TCGA Pan-Cancer expression dataset[23] and thyroid samples[24] but moderately expressed in primary cutaneous melanoma.[25] Similar to other studies, we found that the DNAJC1 gene was upregulated in MM compared to the control and MGUS. However, the fact that DNAJC1 was a biomarker with the best ability to discriminate both alone and in combinations in our results showed that this gene may have an important role in myeloma pathogenesis. However, there are very few studies on the DNAJC1 gene, and its role in cancer is not yet known.

KDELR1 26 and TMED9 are involved in vesicular transport.[10] Yuna et al. have been defined increased gene expression of KDELR1 as a prognostic biomarker in glioma patients and reported its relationship with immune filtration and microenvironment.[27] Similarly, the microenvironment is very important in MM.[28] KDELR1, which was upregulated in the MM group compared to the other groups, was also found to be significant with the eGFR parameter, which is closely related to the kidneys in the MM group. In the literature, the loss of KDEL receptors has been shown that it is effective in preventing cancer by inhibiting cell proliferation. In addition, decreased expression of KDELR1 has been reported in cases such as kidney transplant rejection.[26] Future investigation of the relationship of up-regulated KDELR1 in MM with the microenvironment, which we reported for the first time in our study, may contribute to the treatment response.

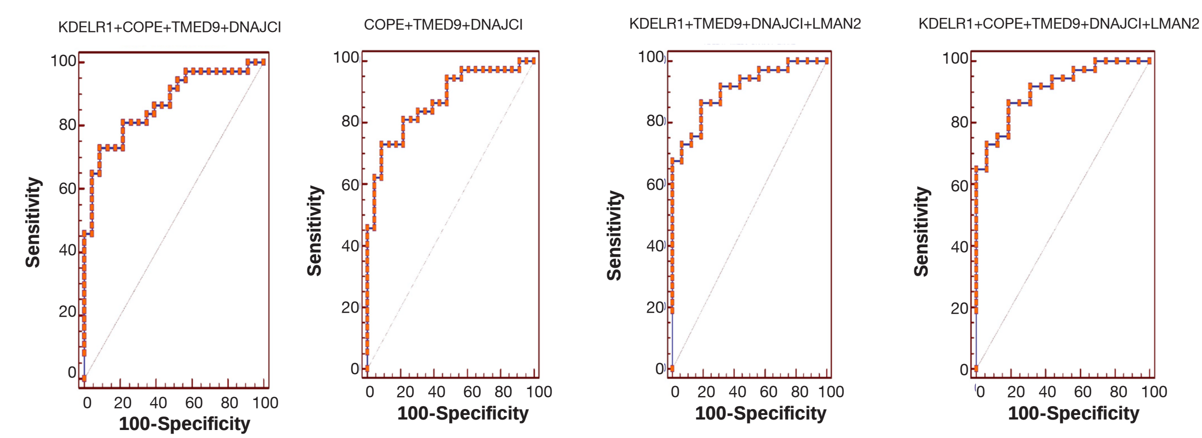
Table 5. Performance of genome biomarker combinations to discriminate MM patients from controls					
	Sensitivity %	**Specificity %**	**AUC**	**%95CI**	**p-value**
MM versus Control					
KDELR1+COPE+TMED9	64.90	93.70	0.836	0.709-0.924	<0.001
KDELR1+COPE+DNAJC1	64.90	100	0.902	0.789-0.967	<0.001
KDELR1+COPE+LMAN2	56.80	100	0.826	0.697-0.916	<0.001
COPE+TMED9+DNAJC1	70.30	100	0.892	0.776-0.960	<0.001
COPE+TMED9+LMAN2	59.50	87.50	0.777	0.642-0.880	<0.001
TMED9+DNAJC1+LMAN2	70.30	100	0.892	0.776-0.960	<0.001
KDELR1+ TMED9+ DNAJC1	70.30	100	0.900	0.787-0.966	<0.001
KDELR1+ TMED9+LMAN2	59.50	100	0.824	0.695-0.915	<0.001
KDELR1+COPE+TMED9+DNAJC1	67.60	100	0.900	0.787-0.966	<0.001
KDELR1+COPE+TMED9+LMAN2	59.50	100	0.838	0.711-0.925	<0.001
COPE+TMED9+DNAJC1+LMAN2	70.30	100	0.892	0.776-0.960	<0.001
KDELR1+ TMED9+ DNAJC1+LMAN2	86.50	81.20	0.909	0.797-0.970	<0.001
KDELR1+ COPE+ DNAJC1+LMAN2	86.50	81.20	0.902	0.789-0.967	<0.001
KDELR1+ COPE+ TMED9+DNAJC1+LMAN2	86.50	81.20	0.909	0.797-0.970	<0.001

AUC: Area Under Curve

TMED9 has an oncogenic role and promoted cell proliferation and migration in Hepatocellular Carcinoma (HCC).[10] It has been known that TMED9 interacts directly with COPE, while knockdown of TMED9 in the breast cell line has been reported to suppress proliferation, drug resistance, and migration abilities.[29] Similarly, there was a significant correlation between COPE and TMED9 in our results. However, there has been limited information on COPE. The oncogenic role of TMED9 has been demonstrated in head and neck cancer[30] HCC[11] breast cancer[29] and Epithelial Ovarian Cancer[31] but is not yet known in hematological cancers. We reported for the first time that TMED9 was upregulated in MM.

Similar to paraprotein in MM, its common feature in neurodegenerative diseases is the accumulation of misfolded proteins. It has been reported that LMAN2 is up regulated in Alzheimer patients.[32] We found LMAN2 gene upregulated in the MM group compared to the other groups. Grams et al reported that LMAN2 was associated with eGFR in a cohort of patients with progressive kidney injury, and high LMAN2 levels were significant in the progression of chronic kidney disease.[33] On the contrary, in our results, there was no relationship between eGFR and LMAN2 in the MM group, but a relationship was found between KDELR1 and DNAJC1. Nevertheless, the reporting of LMAN2 as a biomarker in patients with chronic kidney disease has been shown that this gene may be important in myeloma pathogenesis, considering the factor that affects kidney functions, which is one of CRAB (hypercalcemia, renal failure, anemia, or lytic bone lesions), in the diagnosis of MM.[34]

Apart from COPE, we also proved that the expression of four other genes related to vesicular transport and ER stress response, which we screened in larger patient groups was consistent with our transcriptome data.[7] However, there were some limitations in this study. The limitation was that the number of Smoldering Myeloma patients within the PCD group could not be obtained in the study. We will measure the expression of five genes by a providing sample size to further explore the clinical significance of these genes in our future studies.



a) The combination of KDELR1, COPE, TMED9 and DNAJCI used to dicriminete MM patients from MGUS patients.
AUC value: 86.80%
Sensitivity: 73%
Specificity: 91.30%

b) The combination COPE, TMED9 and DNAJCI used to dicriminete MM patients from MGUS patients.
AUC value: 86.80%
Sensitivity: 73%
Specificity: 91.30%

c) The combination KDELR1, TMED9 and DNAJCI and LMAN2 used to dicriminete MM patients from controls.
AUC value: 90.90%
Sensitivity: 86.50%
Specificity: 81.20%

d) The combination KDELR1, COPE, TMED9 and DNAJCI and LMAN2 used to dicriminete MM patients from controls.
AUC value: 90.90%
Sensitivity: 86.50%
Specificity: 81.20%

Figure 2. Receiver operating characteristic (ROC) analysis of genes biomarker combina-tions with AUC values, specificity, and sensitivity. **A** and **B**: Shows the different combi-nations between MM and MGUS with the highest AUC value. **C** and **D**: Shows the different combinations with the highest AUC value between MM and the control group.

Conclusion

Our study, groups included MGUS, MM from plasma cell dyscrasias, and also the control group. We found a significant gene expression increase among these three groups of KDELR1, DNAJC1, LMAN2, TMED9, and COPE genes. As a single marker, DNAJC1 exhibited the best diagnostic value for discriminating MM from MGUS and MM from the control group. However, the combination of KDELR1, COPE, TMED9, DNAJC1, and LMAN2 had the highest efficiency in discriminating MM patients from the control group, while the combination of KDELR1, COPE, TMED9, and DNAJC1 showed the best ability to discriminate the MM group from MGUS group.

As a result, we concluded that these genes, which were evaluated for the first time in PCD, are valuable targets in the pathogenesis of myeloma, especially DNAJC1 alone and other genes in combination. We report that the role of these genes in the ER stress/vesicular transport mechanism should be investigated in the future.

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