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SHORT COMMUNICATION

BIOINFORMATIC IDENTIFICATION OF PROTEINS WITH ALTERED PTM LEVELS IN A MOUSE LINE ESTABLISHED TO STUDY THE MECHANISMS OF THE DEVELOPMENT OF FIBROMUSCULAR DYSPLASIA

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Data from a mass spectrometry experiment of a mouse line developed to study the mechanisms of fibromuscular dysplasia and deposited by d'Escamard et al. in ProteomeXchange (PXD051750) have been analyzed. Identification of peptides with post-translational modifications (PTMs) was repeated using more stringent conditions than in the original work. The following modifications were considered during analysis of changes in the PTM levels in experimental and control groups of mice: acetylation of lysine residue and N-terminal protein peptide, ubiquitination of lysine residue, phosphorylation of serine, threonine and tyrosine residues, and deamination of asparagine and glutamine residues. The multistage analysis resulted in selection of 23 proteins with PTMs for which different levels of modification between experimental and control groups could be assumed. These included six proteins with N-terminal protein acetylation, which were particularly interesting: P80318 (T-complex protein 1 subunit gamma), P43274 (Histone H1.4), P97823 (Acyl-protein thioesterase 1), P63242 (Eukaryotic translation initiation factor 5A-1), Q3UMT1 (Protein phosphatase 1 regulatory subunit 12C), Q9D8Y0 (EF-hand domain-containing protein D2). Thus, repeated bioinformatic analysis of the data deposited in the specialized databases resulted in detection of changes in the level of N-terminal acetylation of proteins that might be functionally significant in the mechanisms underlying the development of fibromuscular dysplasia.

Key words: posttranslational modifications; fibromuscular dysplasia; bioinformatics

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INTRODUCTION

Post-translational modifications (PTMs) play an important role in the regulation of biochemical processes, including those associated with pathological conditions [1]. The study of the PTM impact on various biochemical pathways is often a much more difficult process than the study of the role of any particular protein. In many cases the change in the amount of a modified protein, is more important than the presence of a specific modification (which is easier). However, there is a large layer of data that could help in such studies. These are the raw data from mass spectrometry experiments, which are usually deposited on appropriate resources (e.g., ProteomeXchange [2]). Despite the fact that detection of modified forms of proteins can be the goal of mass spectrometric experiments [3], quite often, researches developing an algorithm for bioinformatic processing of the obtained data, are either not interested in this aspect, or they limit themselves to detecting the very fact of detection of this PTM. It is also important that to analyze changes in the ratio of amounts of modified and unmodified protein, special studies with protein quantification using tags (labels) are performed. At the same time, the so-called label-free quantitative proteomic analysis based on the comparison of the areas under the peaks (“abundance”) of the registered primary ions can also be used for pilot evaluation [4].

Important factors in the processing of deposited raw data include data quality (often depending on the instrumentation used by the authors), transparency of the experimental data design (it happens that the data are basically not described), and sufficient number of biological samples for statistical processing of the results. For example, we have previously estimated changes in the levels of a number of PTMs in experimental ischemic stroke in mice [5]; this estimate was based on available experimental data [6]. Such approach can be applied to almost any pathology. In this work, we have analyzed data from the set PXD051750 deposited in ProteomeXchange [7], presented in the article by d'Escamard et al. accepted for publication by the journal Nature Cardiovascular Research [8]. This experimental work was performed on mice created specifically to study the mechanisms of fibromuscular dysplasia (FMD) development. FMD is a rare idiopathic, segmental, non-atherosclerotic, and non-inflammatory disease of small and medium diameter arteries leading to stenosis [9] due to abnormal cell proliferation and fibrous tissue formation on the vessel walls [10]. The authors [7, 8] have identified 18 gene co-expression networks, four of which act together as a FMD supernetwork in the arterial wall. Mice that underwent selective knockout of a key driver of this network developed arterial dilation, a hallmark of FMD. Mass spectrometric

data are available [7, 8] for 10 experimental and 8 mice without gene knockout (littermate control). Which specific genes were knocked out is not mentioned in the abstract. Samples of the thoracic and abdominal sections of the aorta were taken from each animal.

It is known from the dataset abstract that analyzing mass spectrometric data, the authors considered such PTMs as oxidation of lysine, methionine, and proline, and asparagine deamination. Methionine oxidation and asparagine deamination are often considered in the analysis of mass spectrometric data without associating these phenomena with any biological functions. Hydroxyproline and hydroxylysine play an important role in the formation of collagen spatial structure [1], the disruption of which is associated with the development of FMD [11]. In this work, such modifications as acetylation, phosphorylation and ubiquitination, as well as deamination of asparagine and glutamine residues have been investigated.

MATERIALS AND METHODS

As mentioned above, the raw data are well annotated and all procedures performed by the authors have been described in detail [7]. Peptide identification in our work was performed again using the PEAKS-X Pro program [12]. The search was performed against amino acid sequences of mouse proteins (*Mus musculus*, UniProtKB/Swiss-Prot release 2024_03, 17823 entries [13]). The mass tolerances for identification were more stringent than in [7]: 2 ppm for primary ions and 0.01 Da for fragment ions. Carbamidomethylation of cysteine was considered as a permanent modification. Acetylation of lysine and the N-terminal peptide of protein, ubiquitination of lysine, phosphorylation of serine, threonine, and tyrosine, deamination of asparagine and glutamine, and oxidation of methionine were allowed as variable ones. No more than two variable modifications per peptide and two missing cleavage sites (cleavage enzyme — trypsin) were allowed; in the final analysis only peptides containing just one examined modification were considered. The false discovery rate (FDR) for the final selection of identified peptides was 0.01%. Identification was performed for each sample independently. All options for additional filtering and chimeric spectra formation were disabled. Variants with acetylation and ubiquitination of the lysine residue were only considered provided that the modified lysine was not the last residue in the peptide. Variants with modification at the C-terminal lysine residue are likely to be misidentifications if trypsin is considered to cleave the protein with a high degree of specificity.

Based on the data from RAW files, the Progenesis LC-MS program [14] was used to align all features and normalize the value of the area under the peak for each primary ion (Normalized abundance, NA). The use of this value allowed direct comparison between data for different biological samples. For subsequent analysis, after combining the alignment results with the peptide identification results, the NA values for ions of different charge of the same peptide were summarized.

The significance of changes in the PTM levels was assessed using NA values, which were compared in mice with gene knockout (KO group, 20 values) and without knockout (WT group, 16 values). Although certain distinctions between samples of thoracic and abdominal aorta were noted for some of the peptides they were not taken into consideration and treated as individual samples thus doubling the number of analyzed biological samples. Only those peptides that were identified for at least half of the biological samples in each of the two groups were taken into the analysis. The significance of the differences in the mean values were assessed using Mann-Whitney test. In addition, the significance of NA changes between KO and WT samples was assessed (as the ratio of the mean NA values of the sample with gene knockout to the sample without knockout, NA_{KO}/NA_{WT}). For this purpose, we calculated the average NA_{KO}/NA_{WT} for peptides without modifications of the same protein. Extreme values (10% each of “bottom” and “top”) were discarded during calculation of the mean NA_{KO}/NA_{WT} for a protein, and the number of unmodified peptides with a significant difference in the mean for mice from KO and WT samples was also estimated. If the number of identified unmodified peptides for a protein was less than 5, the comparison was not considered as significant.

RESULTS AND DISCUSSION

The data obtained using the Progenesis LC-MS program resulted in selection of 89621 primary ions; for 24719 ions the 19050 peptide sequences were identified. These included peptides with medications. The peptides were registered for 2212 proteins. This study was not aimed at reliable protein identification, which was obviously performed by the authors of [8]. The final selection included proteins for which at least 6 peptides were found. Figure 1 shows the distribution in the number of biological samples for which a particular peptide was identified. The limits we set on the number of identifications in the samples corresponded to 12356 peptides. For the remaining peptides, changes in the mean values were considered insignificant. The full set of identification data is available in the Supplementary Materials.

Table 1 shows statistics on the number of identified peptides with each analyzed PTM and lists of identified proteins containing these PTMs (full data for each peptide with PTMs are given in the Supplementary

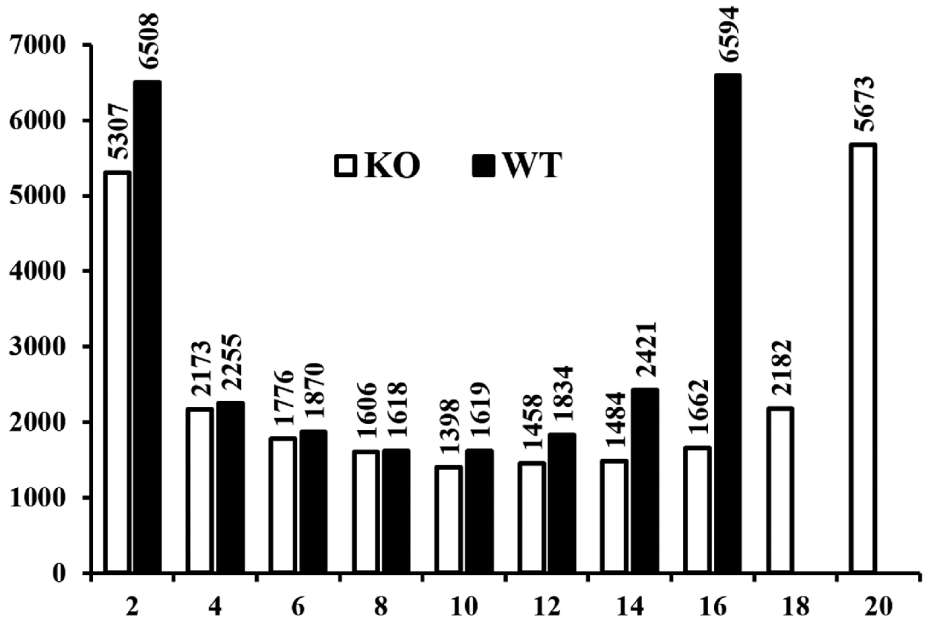

Figure 1. Distribution of peptides by the number of biological samples in the KO and WT groups of animals, for which a specific peptide was identified.

Materials). In the case of protein ubiquitination two variants of mass changes (+114.04 and +383.23) were considered due to existence of known (weak) chymotrypsin-like activity in trypsin. No peptides with a phosphorylated tyrosine residue were identified under the given selection parameters. A small number of identified peptides with PTMs can be explained by application of strict selection criteria. In this context it should be noted that in the case of the use of the identification parameters from [7] (10 ppm for primary ions, 0.02 Da for secondary fragments, and the FDR level of 1%), the number of identified peptides was 27309, while the number of peptides required to satisfy the condition of the number of samples with identifications was 17018. However, in such case the probability of error increases. In 24 of the 47 proteins for which peptides with deamination of a glutamine residue were identified, peptides with deamination of an asparagine residue were also found. This is an expected result, because the intensity of deamination processes is associated with the environmental conditions in the cells, in which the proteins are located, and the deamination rate of asparagine residues is higher [1]. Unfortunately, in the case of deamination, we cannot rule out the possibility that the process takes place during sample preparation and/or mass spectrometric determination. In this case, however, it seems unlikely that accumulation of the modified peptide would differ in mice with and without the gene knockout. In the lists of proteins containing peptides with deamination of the asparagine residue and with acetylation of the N-terminal protein residue, 67 proteins coincided. However, only for three proteins the difference for the acetylation level between the KO and WT groups can be considered as significant (P62737, P37804, P16045 — hereafter UNIPROT identifiers are used to list the proteins). These changes are determined by the changes in the content of the protein of interest.

Figure 2 shows an example that clearly illustrates the variation in the ratio of NA values for different peptides of the same protein. For protein P62737 (Fig. 2A), the shift of the mean between KO and WT groups for all peptides changed slightly and always in the same direction (NA_{KO}/NA_{WT} = 0.87±0.08), the NA_{KO}/NA_{WT} ratio for the PTM-containing peptide was almost the same (0.75). For protein P80318 (Fig. 2B), the shift in the mean NA between KO and WT peptides without modifications was insignificant and multidirectional (NA_{KO}/NA_{WT} = 1.06±0.05), and the NA_{KO}/NA_{WT} ratio for the peptide with N-terminal acetylation was 2.15. In this work, a threshold of 20% was set for changes in NA_{KO}/NA_{WT} for a PTM-containing peptide relative to the ratio NA_{KO}/NA_{WT} for non-PTM peptides of the same protein. This threshold is arbitrary; in general, the larger the difference, the more likely it is that the difference is not accidental. All peptides with PTMs that meet this criterion are listed in Table 2.

No peptides with phosphorylation or acetylation of a lysine residue have been included, and the only protein with ubiquitination (P15105, glutamine synthetase) is unlikely to be associated with FMD in any way. For six proteins, a change in the N-terminal amino acid acetylation level of can be proposed. The increase in the amount of the modified peptide was registered for: P80318 (T-complex protein 1 subunit gamma), P43274 (histone H1.4), P97823 (acyl-protein thioesterase 1), P63242 (eukaryotic translation initiation factor 5A-1). The decrease in the amount of the modified peptide was found

Table 1. Number of PTM peptides identified at different stages of selection. The second row contains the list of UNIPROT identifiers for the proteins in which peptides were identified

#	PTM	Total	Con. 1	Con. 2	$p<0.05$	Average is higher than expected[1]	Average is lower than expected[1]	Identified in proteins[2]
1	Lys acetylation	12	12	11	0	NA	NA	11
	P11087, P45952, P62737, P84228, P84244, Q61425, Q6ZWY9, Q8BMS1, Q8BTM8, Q8BWT1, Q9D2G2							
2	N-terminal acetylation	207	206	143	22	4	2	190
	O35344, O35639, O35737, O54724, O55222, O70318, O70400, O70435, O88543, O88952, P04117, P06151, P07091, P07356, P08249, P0DP27, P10107, P10922, P11404, P11983, P13542, P14148, P14206, P14733, P14873, P15105, P15864, P16045, P17225, P18760, P19096, P19253, P20065, P21107, P21981, P23927, P24270, P26231, P26350, P29341, P31725, P31786, P37804, P40124, P40142, P41105, P41216, P42669, P43274, P43275, P43276, P43277, P45591, P47754, P47757, P48678, P48758, P48787, P48962, P51125, P51880, P51881, P54775, P55264, P56212, P56391, P56812, P57776, P59999, P60710, P61982, P62075, P62077, P62082, P62141, P62196, P62259, P62281, P62627, P62737, P62849, P62874, P62878, P62880, P62960, P63085, P63242, P68040, P68134, P68510, P70362, P80313, P80316, P80318, P84089, P97379, P97461, P97823, P99028, Q01853, Q05816, Q08091, Q3UMT1, Q4KML4, Q5SX40, Q60605, Q60864, Q60876, Q60932, Q60936, Q60972, Q61171, Q61206, Q61792, Q61937, Q62093, Q62189, Q62318, Q62422, Q62426, Q62523, Q63844, Q68FD5, Q6IRU2, Q6ZWY8, Q7TMF3, Q7TPR4, Q8BFS6, Q8BFW7, Q8BG05, Q8BG32, Q8BH64, Q8BZF8, Q8C1B7, Q8CGN5, Q8CI51, Q8CI94, Q8K124, Q8K1M6, Q8R3B1, Q8VEK3, Q91VJ2, Q91VM5, Q91YR1, Q921U8, Q93092, Q99JI1, Q99KC8, Q99KP3, Q99PT1, Q9CPQ1, Q9CPQ3, Q9CPZ8, Q9CQJ8, Q9CQR2, Q9CR00, Q9CR68, Q9CRB6, Q9CXW4, Q9CYT6, Q9D0E1, Q9D1M0, Q9D7J4, Q9D8U8, Q9D8Y0, Q9DAK9, Q9DCL8, Q9DCL9, Q9EQP2, Q9ET01, Q9JIF9, Q9JII6, Q9JJ28, Q9JKF1, Q9JKP5, Q9JL62, Q9JLR9, Q9JMG1, Q9QXS6, Q9QYG0, Q9QZB9, Q9R0P5, Q9WTI7, Q9WU78, Q9WUA3, Q9WV32, Q9WVA4, Q9WVQ5, Q9Z0P5, Q9Z1Q5							
3	Ser phosphorylation	44	44	38	3	0	0	32
	A2ASS6, D3YXK2, E9Q0S6, O08638, O70251, P11087, P11499, P14602, P14873, P29699, P35486, P42208, P48678, P51859, P62737, P70460, Q03173, Q5SWU9, Q60668, Q61235, Q62417, Q64727, Q7TPW1, Q7TT50, Q80XU3, Q8BH64, Q8BTM8, Q8BVA4, Q8BZF8, Q8CGN5, Q91V92, Q9CQS8							
4	Thr phosphorylation	4	4	3	0	NA	NA	4
	E9Q0S6, O55028, Q01149, Q99PT1							
5	Ubiquitination	11	11	4	1	0	1	10
	P15105, P15864, P62737, P62984, Q60597, Q61879, Q64523, Q8BFW7, Q8BTM8, Q8R1M2							
6	Asn deamination	699	694	667	55	7	8	374
	A2ARA8, A2ASQ1, A2ASS6, E9PV24, E9Q0S6, O08553, O08583, O08638, O08709, O08749, O09164, O09165, O09167, O35129, O35206, O35226, O35658, O35685, O35737, O35855, O54724, O55029, O55131, O70325, O70373, O70400, O88207, O88342, O88569, O88851, O88952, O89086, P00920, P01027, P01872, P01942, P02088, P02468, P02469, P02535, P02762, P04117, P05064, P05202, P05213, P05977, P06151, P06745, P06801, P07724, P07901, P08074, P08113, P08249, P09411, P09541, P09542, P09671, P0DP27, P10107, P10126, P10493, P10649, P11087, P11276, P11404, P11983, P12787, P13020, P13707, P14131, P14148, P14152, P14211, P14873, P15105, P15655, P15864, P16015, P16045, P16546, P16858, P17182, P17183, P17225, P17742, P17751, P18608, P19096, P19157, P19253, P20029, P20108, P20152, P21550, P21956, P21981, P22599, P23492, P23927, P24270, P24369, P24549, P25444, P26039, P26041, P26350, P26443, P27659, P28481, P28650, P28653, P28654, P29758, P30416, P31001, P31938, P35486, P35700, P37804, P38647, P39447, P41216, P43025, P43275, P43406, P45952, P47754, P47911, P47934, P47963, P48036, P48678, P48962, P49290, P49312, P49722, P50136, P50516, P50544, P51174, P51410, P51885, P52196, P52480, P52825, P56391, P56480, P57776, P57780, P58252, P58771, P58774, P58871, P60710, P61022, P61079, P61161, P61255, P62082, P62242, P62270, P62737, P62774, P62814, P62960, P62962, P63017, P63037, P63038, P63101, P63158, P63242, P63260, P63330, P68033, P68040, P68134, P68254, P80316, P80318, P82198, P84104, P97314, P97315, P97807, P97873, P97927, P99024, P99027, P99029, Q00896, Q00915, Q01149, Q01339, Q01730, Q01853, Q02566, Q02788, Q03173, Q03265, Q04857, Q05793, Q05816, Q05920, Q05BC3, Q07417, Q08091, Q3TEA8, Q3TJD7, Q3UHD6, Q3UPL0, Q3UTY6, Q5SWU9, Q5SX40, Q60597, Q60605, Q60759, Q60854, Q60864, Q60899, Q60930, Q60932, Q61166, Q61235, Q61316, Q61598, Q61646, Q61735, Q61765, Q61937, Q62009, Q62059, Q62165, Q62188, Q62219, Q62261, Q62264, Q62417, Q62523, Q63932, Q640N1, Q64521, Q64523, Q64727, Q68FD5, Q6P069, Q6PDN3, Q6ZQ38, Q6ZWY9, Q7M6Y3, Q7TMF3, Q7TPR4, Q7TPW1, Q80X19, Q8BFW7, Q8BG05, Q8BH95, Q8BMS1, Q8BPB5, Q8BTM8, Q8BWF0, Q8BWT1, Q8BZF8, Q8C1B7, Q8C6K9, Q8CAY6, Q8CC88, Q8CDN6, Q8CHT0, Q8CJ53, Q8JZR0, Q8K0E8, Q8K1M6, Q8K2B3, Q8K310, Q8K3J1, Q8K4G1, Q8QZT1, Q8R081, Q8R0Y6, Q8R164, Q8R3B1, Q8VCM7, Q8VDQ1, Q8VE95, Q8VEK3, Q8VHQ9, Q8VHX6, Q91V92, Q91VD9, Q91VT4, Q91YE8, Q91Z83, Q91ZJ5, Q921G7, Q921I1, Q921U8, Q93092, Q99JR1, Q99JR5, Q99JY0, Q99K51, Q99KD5, Q99KI0, Q99KR7, Q99LB2, Q99LC5, Q99LX0, Q99M87, Q99MQ4, Q99PL5, Q9CPU0, Q9CPW4, Q9CPX8, Q9CQ19, Q9CQ62, Q9CQA3, Q9CXU9, Q9CXZ1, Q9CZ13, Q9CZ44, Q9CZU6, Q9CZX8, Q9CZY3, Q9D051, Q9D0F9, Q9D0K2, Q9D0M3, Q9D1H9, Q9D1J3, Q9D1M0, Q9D2G2, Q9D7N9, Q9D8S4, Q9D9V3, Q9DAW9, Q9DB20, Q9DB77, Q9DBG9, Q9DBR7, Q9DC70, Q9DCB1, Q9DCD0, Q9DCJ5, Q9DCN2, Q9EQ20, Q9ERG2, Q9JHI5, Q9JI91, Q9JII6, Q9JJV2, Q9JK53, Q9JKB3, Q9JLJ2, Q9QUP5, Q9QXT0, Q9QYB1, Q9QZQ8, Q9R0P5, Q9R0Y5, Q9R1P3, Q9WTI7, Q9WUM4, Q9WV32, Q9WVA4, Q9WVH9, Q9WVK4, Q9Z0X1, Q9Z1E4, Q9Z1P6, Q9Z2I8, Q9Z2V4, Q9Z2Z6							
7	Gln deamination	53	53	41	3	0	1	47
	A2ASS6, O09164, O35685, O55029, P01837, P06745, P08228, P14211, P16858, P19096, P20152, P35278, P47738, P48678, P60122, P62315, P68433, Q01730, Q08091, Q2TPA8, Q3TEA8, Q60875, Q61292, Q62523, Q64727, Q68FD5, Q8BFW7, Q8BMF4, Q8BTM8, Q8C6K9, Q8CGN5, Q8K2B3, Q8VCN6, Q8VDD5, Q8VDQ1, Q8VHQ9, Q921U8, Q923D2, Q99L88, Q9CQC7, Q9D6J6, Q9EP89, Q9EPC1, Q9QVP4, Q9QXS1, Q9QZE5, Q9WV32							

Con. (Condition) 1 – peptide was identified for at least half of the samples in both KO and WT groups; Con. (Condition) 2 – at least 5 unmodified peptides were identified for the protein with the modified peptide; [1] – differences in ratio are greater than 20%, NA – not applicable; [2] – more than one peptide with PTM was identified for part of the proteins, for the same position with PTM there could be more than one peptide due to "missing cleavage".

Table 2. List of proteins with PTM-containing peptides for which the NA_{KO}/NA_{WT} value of the modified peptide differs from the average NA_{KO}/NA_{WT} for unmodified peptides by more than 20%

#	Protein (UNIPROT ID)	Peptide sequence	PTM	Significance of difference in the mean between groups KO and WT (*p*)	Direction of change from the expected	% changes of NA_{KO}/NA_{WT}	NA_{KO}/NA_{WT} (PTM)	Mean of NA_{KO}/NA_{WT} of peptides without PTM	Standard deviation	Number of peptides without PTM[1]	Minimum of NA_{KO}/NA_{WT} of peptides without PTM	Maximum of NA_{KO}/NA_{WT} of peptides without PTM
1	P80318	MMGHRPVLVLSQNTK	[1] M(+42.01)	0.007	Higher	95.30%	2.150	1.062	0.048	8	1.006	1.137
2	P43274	SETAPAAPAAPAPAEKTPVK	[1] S(+42.01)	0.029	Higher	47.50%	1.767	1.092	0.129	4	0.946	1.248
3	Q3UMT1	SGEDGPAAGPGAAAAAAAAR	[1] S(+42.01)	0.005	Lower	30.30%	0.458	0.690	0.023	5	0.667	0.721
4	Q9D8Y0	ATDELASK	[1] A(+42.01)	0.004	Lower	29.90%	0.524	0.813	0.047	5	0.768	0.876
5	P97823	SAPMPAVVPAAR	[1] S(+42.01)	0.018	Higher	23.70%	1.365	1.078	0.051	3	1.019	1.109
6	P63242	ADDLDFETGDAGASATFPMQCSALRK	[1] A(+42.01)	0.021	Higher	22.50%	1.389	1.001	0.092	8	0.896	1.163
7	P15105	TCLLNETGDEPFQYK	[15] K(+114.04)	0.006	Lower	23.00%	0.681	1.055	0.200	8	0.924	1.466
8	Q9Z0X1	VSAQDLPNIENGGVAVLTGK	[11] N(+0.98)	0.004	Higher	536.00%	8.075	1.237	0.119	11	1.074	1.446
9	A2ASQ1	ALETEGLLLYNGNAR	[11] N(+0.98)	0.001	Higher	96.10%	3.089	1.386	0.175	11	1.123	1.757
10	P99029	SMVIDNGIVK	[6] N(+0.98)	0.037	Higher	95.60%	1.960	0.975	0.044	7	0.889	1.028
11	Q01730	LDVNGLFSLAHITQLVLSHNK	[4] N(+0.98)	0.022	Higher	79.40%	2.316	1.155	0.222	8	0.731	1.399
12	P0DP27	VFDKDGNGYISAA	[7] N(+0.98)	0.046	Lower	78.00%	0.076	1.044	0.099	8	0.891	1.167
13	P68254	AVTEQGAELSNEER	[11] N(+0.98)	0.034	Higher	53.10%	1.831	1.149	0.050	4	1.114	1.221
14	Q7TPR4	MAGTNPYTTITPQEINGK	[16] N(+0.98)	0.005	Lower	52.70%	0.284	0.843	0.074	53	0.728	1.037
15	P47934	VSDDVYRNHVAGQMLHGGGSK	[8] N(+0.98)	0.023	Higher	51.80%	1.733	1.044	0.069	14	0.948	1.192
16	P17742	NAGPNTNGSQFFICTAK	[7] N(+0.98)	0.013	Lower	48.70%	0.355	0.820	0.049	9	0.754	0.875
17	Q9JK53	LDSNKIETIPNGYFK	[11] N(+0.98)	0.006	Higher	44.30%	1.761	1.049	0.090	24	0.878	1.296
18	O88207	NQDTIFEGIGGPR	[1] N(+0.98)	0.034	Lower	31.40%	0.548	1.035	0.181	4	0.873	1.288
19	Q640N1	GGCQAQDYTSGMGIVNGAK	[16] N(+0.98)	0.015	Lower	30.50%	0.571	0.983	0.054	16	0.871	1.048
20	Q8CAY6	TAIGSFNGALSTVPVHEMGTTVIK	[7] N(+0.98)	0.009	Lower	30.00%	0.607	0.909	0.029	5	0.880	0.956
21	Q05793	TGSGLYLGQCELCECNGHSDLCHPETGACSR	[16] N(+0.98)	0.002	Lower	25.30%	0.468	0.867	0.071	115	0.687	0.993
22	Q8K1M6	KNVPSAGGGIGDGGQEPTTGNWR	[2] N(+0.98)	0.043	Lower	24.00%	0.657	0.909	0.045	3	0.875	0.960
23	P14211	GQTLVVQFTVK	[2] Q(+0.98)	0.004	Lower	24.90%	0.694	1.009	0.039	16	0.946	1.055

[1] – Taking into account the elimination of 10% of the extreme values of NA_{KO}/NA_{WT} in the calculation of the average.

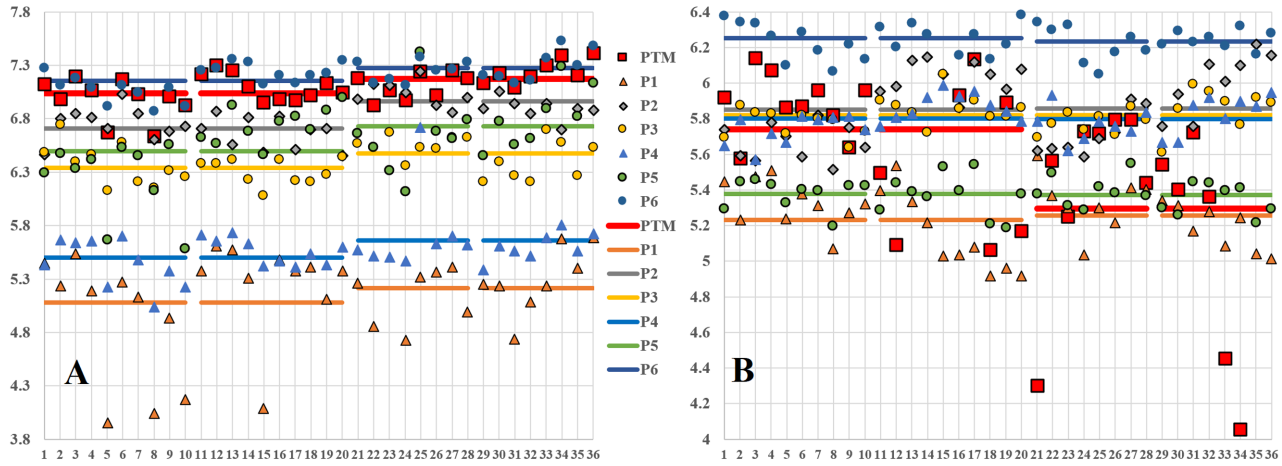

Figure 2. An example demonstrating the variants of the NA ratio for different peptides of the same protein. The abscissa axis shows biological sample numbers, 1 to 20, KO group, 21 to 36, WT group, 1 to 10 and 21 to 28 are samples of the abdominal part of aorta, 11 to 20 and 29 to 36 are samples of the thoracic part of aorta, and the ordinate axis shows log(NA). Horizontal lines designate mean NA values for the peptide across KO and WT groups. **A.** Protein P62737, PTM is E(+42.01)EEDSTALVCDNGSGLCK peptide, P1-P6 are unmodified peptides. **B.** Protein P80318, PTM is M(+42.01)MGHRPVLVLVLSQNTK peptide, P1-P6 are unmodified peptides.

in the case of: Q3UMT1 (protein phosphatase 1 regulatory subunit 12C), Q9D8Y0 (EF-hand domain-containing protein D2). No data on direct association of these proteins with FMD were found in the literature. Nevertheless the functions of these proteins that may be relevant to this pathology include: chaperone-mediated protein folding (P80318, the following protein roles are taken from the corresponding entry in UNIPROT), regulation of macroautophagy and protein transport from the Golgi system to the plasma membrane (P97823), regulation of apoptosis (P63242, Q9D8Y0), and regulation of actin cytoskeleton assembly (Q3UMT1). We also selected 16 proteins with significant changes in the level of deamination (Table 2). There are indications in the literature that deamination of asparagine residues of extracellular matrix proteins, particularly fibronectin and tenascin C, has been associated with FMD and may result in increased monocyte adhesion [15]. For tenascin, only one unmodified peptide was identified in this study. For fibronectin (P11276), 88 peptide variants were identified, 11 of which contained a deaminated asparagine residue. None of them showed a significant difference in the mean NA between the KO and WT groups.

Changes in the deamination level are determined by the conditions at the site of protein localization in the cell [1]. This is an indirect parameter, dependent on the condition of a particular animal. The variation of NA values within each group is very large. For example, for protein Q9Z0X1 (Apoptosis-inducing factor 1) NA varies within 2 orders of magnitude in the KO group and up to 2.5 orders of magnitude in the WT group. For unmodified peptides the range of NA variation is not more than 1.5 orders of magnitude. In addition, deaminated peptides are more often not identified in any particular sample. Nevertheless, these data may also be useful. For example, occurrence of a deaminated form of the VSAQDLPNIENGGVAVLTGK peptide of the Q9Z0X1 protein in the KO group is 5 times higher than in the WT group. Taking into consideration that that one of the main biological functions of this protein consists in the regulation of apoptosis, this may be important. Other proteins in this group also have functions that may be related to FMD. These include argin (A2ASQ1, argin), which regulates microtubule cytoskeleton organization; calmodulin 2 (P0DP27, calmodulin 2), part of the calcium signal transduction pathway, which controls a large number of enzymes, ion channels, aquaporins, and other proteins; structural proteins (Q7TPR4, Alpha-actinin-1) and collagen (O88207, Collagen alpha-1), which are also crucial for the arterial wall.

In conclusion, it is important to note that the results obtained in this study cannot be considered as direct evidence of the involvement of these proteins in the process of FMD development. Especially since the study [8] used mice with knockout of genes only presumably important for FMD development. Nevertheless, repeated bioinformatic analysis of data deposited in the specialized databases resulted in identification of changes in the level of N-terminal acetylation of proteins that could be functionally important in the mechanism of fibromuscular dysplasia development.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving humans or the use of animals as objects.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

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БИОИНФОРМАТИЧЕСКАЯ ИДЕНТИФИКАЦИЯ БЕЛКОВ С МЕНЯЮЩИМСЯ УРОВНЕМ ПТМ У ЛИНИИ МЫШЕЙ, СОЗДАННОЙ ДЛЯ ИЗУЧЕНИЯ МЕХАНИЗМОВ РАЗВИТИЯ ФИБРОМУСКУЛЯРНОЙ ДИСПЛАЗИИ

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Проанализированы данные масс-спектрометрического эксперимента линии мышей, созданной для изучения механизмов развития фибромускулярной дисплазии, которые были депонированы d'Escamard и соавт. в ProteomeXchange (PXD051750). Идентификация пептидов с посттрансляционными модификациями (ПТМ) была проведена заново с использованием более жёстких условий, чем в оригинальной работе. При анализе изменения уровня ПТМ у экспериментальной и контрольной групп мышей рассматривали: ацетилирование остатка лизина и N-концевого пептида белка, убиквитинирование остатка лизина, фосфорилирование остатков серина, треонина и тирозина, дезаминирование остатков аспарагина и глутамина. В результате многоступенчатого отбора были отобраны 23 белка с ПТМ, для которых можно предположить разный уровень модификации между экспериментальной и контрольной группами. Из них наибольший интерес представляют 6 белков с N-концевым ацетилированием белка: P80318 (T-complex protein 1 subunit gamma), P43274 (Histone H1.4), P97823 (Acyl-protein thioesterase 1), P63242 (Eukaryotic translation initiation factor 5A-1), Q3UMT1 (Protein phosphatase 1 regulatory subunit 12C), Q9D8Y0 (EF-hand domain-containing protein D2). Таким образом, повторный биоинформатический анализ данных, депонированных в специализированных базах данных, позволил выявить изменения в уровне N-концевого ацетилирования белков, которые могут быть функционально значимыми в механизме развития фибромускулярной дисплазии.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: посттрансляционные модификации; фибромускулярная дисплазия; биоинформатика

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