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CHANGE CHARACTERISTICS IN SALIVA AND FECES MICROBIOTA OF A DESMINOPATHY T341P PATIENT

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Background. *A rare muscular disease, desminopathy, is caused by mutations in the DES gene. At present, information about changes in the microbiota of biological media present in such patients is very scarce.*

Purpose. *The aim of the study is to study changes in the saliva and feces microbiota of patients with desminopathy T341P in a heterozygous state.*

Materials and methods. *The retrospective investigation comprised observation of the observation of a proband with the family form of desminopathy T341P. 56 clinically significant microorganisms were numerically analyzed immediately in the obtained biological material by gas chromatography-mass spectrometry method.*

Results. *The emergence of Epstein-Barr viruses, Cytomegalovirus, Herpes spp and gram-negative rods was noted in the proband's biological media under investigation during the desminopathy progression. Excessive bacterial growth of fecal microbiota was observed along with a decrease in saliva microorganisms. There is an excess of the norm in the total number of microorganisms and an increase in their species diversity. Propionibacterium jensenii, Eubacterium spp and Eggerthella lenta predominated in the feces and Clostridium ramosum – in the saliva. An increase in fecal microbiota transient with Peptostreptococcus anaerobius 18623 dominance was observed along with the emergence and rapid 441-time growth of potentially dangerous bacterium Clostridium difficile. The total level of endotoxin in the proband's saliva and fecal microbiota increases to exceed the norm in 13.7 and 81.8 times, respectively. At the same time, a low level of plasmalogen was noted.*

Conclusion. *The investigation results can be useful for developing complex intervention tactics.*

Keywords: *desminopathy; myofibrillar myopathy; saliva microbiota; fecal microbiota; infection*

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ОСОБЕННОСТИ ИЗМЕНЕНИЯ МИКРОБИОТЫ СЛЮНЫ И ФЕКАЛИЙ У ПАЦИЕНТА С ДЕСМИНОПАТИЕЙ T341P

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Обоснование. *Десминопатии относятся к редким мышечным заболеваниям, вызываемых мутациями в гене DES. В настоящее время крайне малочисленны сведения об изменении микробиоты биологических сред у данных пациентов.*

Цель. *Изучить изменение микробиоты слюны и фекалий у пациента с десминопатией T341P в гетерозиготном состоянии.*

Материалы и методы. *В ретроспективное исследование вошло наблюдение пробанда с семейной формой десминопатии T341P. Количественный анализ 56 клинически значимых микроорганизмов осуществляли непосредственно в биологическом материале методом газовой хромато-масс-спектрометрии.*

Результаты. *С прогрессированием десминопатии в рассматриваемых биологических средах пробанда установлено появление вирусов Эпштейна-Барра, Herpes spp, цитомегаловируса и грамотрицательных палочек. Наблюдается избыточный бактериальный рост фекальной микробиоты и снижение микроорганизмов в слюне. Отмечается превышение нормы по суммарному количеству микроорганизмов и увеличение их видового разнообразия. Доминируют в кале Propionibacterium jensenii, Eubacterium spp, Eggerthella lenta, а в слюне – Clostridium ramosum. Установлено выраженное увеличение транзиторной микробиоты в кале с доминированием Peptostreptococcus anaerobius 18623, а также появление и стремительный рост в 441 раз потенциально опасной бактерии Clostridium difficile. Суммарный уровень эндотоксина в слюне и фекальной микробиоте пробанда возрастает и превышает норму соответственно в 13,7 и 81,8 раза. При этом отмечается низкий уровень плазмалогена.*

Заключение. *Результаты исследований в перспективе могут быть использованы для определения тактики комплексных вмешательств.*

Ключевые слова: *десминопатия; миофибриллярная миопатия; микробиота слюны; фекальная микробиота; инфекция*

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Introduction

Desminopathies belong to the family of muscular diseases, called myofibrillar myopathies [21], which are caused by mutations in the desmin-encoding *DES* gene [23]. Desminopathies can result in severe and often lethal degeneration of striated muscular tissue [24]. Unfortunately, to date, there is no specific treatment of this disease [20].

The first publications about the family form of desminopathy T341P describe genealogy analysis with different clinical manifestations [15], results of histologic, cardiologic and electromyographic investigations, dynamics of immune [17] and antioxidative statuses with changes in biochemical indexes [16].

Skeletal, cardiac [11] and unstriated muscles [7] resulting, in the latter case, in digestion problems and possible changes in gastrointestinal tract [1, 2] can be involved in the desminopathological process.

The recent investigations demonstrate that intestinal microbiota helps maintaining the mass of skeletal muscles and physical function [6]. Some intestinal bacteria can increase physical performance efficiency due to their metabolism, which emphasizes the existence of microbiota-muscles axis [10]. Besides, the intestinal microbiome can also contribute to oxidative stress decrease [19].

However, microbiota are present along the whole gastrointestinal tract, the mouth cavity being the first place where a large number of microorganisms is likely to be found [5]. The complex and variable community of oral microbiota plays an important role in health, including the development of disease [25]. It is already known about the potential link between physical exercises and oral microbiome. The microbes of the mouth cavity can reflect the state of a disease in real time, including its various risks and prognosis [18].

Unfortunately, the publications characterizing the saliva and feces microbiota composition of the patients with myofibrillar myopathy are currently very scarce. In the case of rare diseases such as desminopathy, data about changes in the microbiocenosis of biological media are practically nonexistent. The relative contribution of the host genetics can be also important in the formation of organism microbiome [5].

Such investigations carried out on sick subjects will help to improve our understanding of the notion of microbiota-skeletal muscles axis, along with its underlying mechanisms (at immune, metabolic, inflammatory, neurotransmissible and hormonal levels) and pathophysiological effects [9].

The aim of the study is to study changes in the saliva and feces microbiota of patients with desminopathy T341P in a heterozygous state.

Materials and methods

The study investigated a proband with the family form of desminopathy with T341P mutation in *DES* gene (c.1021A> C) in a heterozygous state revealed in Russia (Western Siberia). The disease was first manifested at the age of 30, followed by rather slow progression over 20 years. The past medical history was studied with the examination of the available medical documents of the proband and his father with desminopathy. Father died at the age of 49 from pneumonia.

The microbiological investigation covered the proband's age from 41 up to 43. That was the most significant period in terms of progression of desminopathy, reflecting the movement ability state of the patient's organism proceeding from walking frame to wheelchair.

The new medical technology registered by Ministry of Health of the Russian Federation No. NYU-40006.2009 «Assessment of microecological status of a human by chromatography-mass spectrometry method» by G.A. Osipov [13] was applied to conduct the investigation. 56 clinically significant microorganisms - potential participants of inflammatory processes were numerically assessed in the saliva and feces on the chromatograph «Maestro» (Interlab, Russia).

Biological material was taken from the proband in the morning on an empty stomach in sterile samplers. The following microorganisms were determined in the biological material of the proband with desminopathy: cocci and bacilli - *Bacillus cereus*, *Bacillus megaterium*, *Enterococcus spp*, *Streptococcus spp*, *Streptococcus mutans* (anaerobic), *Staphylococcus aureus*, *Staphylococcus epidermidis*; anaerobes - *Bacteroides flagilis*, *Bifidobacterium spp*, *Blautia coccoides*, *Clostridium spp* (group *C. tetani*), *Clostridium difficile*, *Clostridium hystolyticum/ Streptococcus pneumonia*, *Clostridium perfringens*, *Clostridium propionicum*, *Clostridium ramosum*, *Eubacterium spp*, *Eggerthella lenta*, *Fusobacterium spp/ Haemophilus spp*, *Lactobacillus spp*, *Peptostreptococcus anaerobius 18623*, *Peptostreptococcus anaerobius 17642*, *Prevotella spp*, *Propionibacterium spp*, *Propionibacterium acnes*, *Propionibacterium freudenreichii*, *Propionibacterium jensenii*, *Ruminicoccus spp*, *Veillonella spp*; actinobacteria - *Actinomyces spp*, *Actinomyces viscosus*, *Corynebacterium spp*, *Nocardia spp*, *Nocardia asteroids*, *Mycobacterium spp*, *Pseudonocardia spp*, *Rhodococcus spp*, *Streptomyces spp*,

Streptomyces farmamarensis; enterobacteria - *Enterobacteriaceae spp (E.coli)*, *Helicobacter pylori*, *Campylobacter mucosalis*; gram-negative rods - *Alcaligenes spp/Klebsiella spp*, *Kingella spp*, *Flavobacterium spp*, *Moraxella spp/Acinetobacter spp*, *Porphyromonas spp*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*; fungi and yeast - *Aspergillus spp*, *Candida spp*, *microfungi, campesterol, sitosterol*; viruses - *Herpes spp*, *Cytomegalovirus*, *Epstein-Barr virus*.

The total endotoxin level was found by the content of hydroxy acids (structural component of lipid A) in the biomaterial. The plasmalogen level was found by the content of hexadecanoic aldehyde in the biomaterial [14].

Results

The past medical history of the revealed case of the family form of desminopathy T341P was studied with the analysis of the available medical documents. It was found out that the proband had been fed with baby milk formula in infancy, as well as raw cow's milk without thermal treatment. From 5 years old onwards he was of ectomorphic type. At the age of 9 years, he was sick with dysentery. Between the ages of 11 and 28 years, he had digestion problems periodically manifested by liquid stool movements 1-2 times a day.

By the age of 20, the proband had become allergic to the pollen of trees and herbs (mixture of cereals, *Betula, Artemisia*, *Atriplex*), the acute period in summer being manifested by allergic rhinitis and sneezing. However, the allergic manifestations gradually decreased and by the age of 38 they had decreased considerably.

In addition, mucous tunic inflammation of the mouth was rather frequently observed with the proband, with gum bleeding periodically observed during regular teeth cleaning twice a day up until the age of 25 years. The proband's father had suffered from halitosis after the age of 40, despite cleaning his teeth every morning. Both patients were described as having good teeth.

In general, during the first three decades of his life, the proband, in common with his father who also suffered from desminopathy T341P, were physically strong and tough, rarely experiencing respiratory diseases. Both regularly engaged in sporting activities in their youth: skiing, football, attending gym.

From the age of 30, the proband, as well as his father, started stumbling, noticed progressive muscle weakness, including difficulties in climbing stairs and getting up from a sitting or lying position, rapid-onset tiredness, temporary heart rhythm disorders. The locus of the desminopathy symptoms was slowly progressing upwards to involve the muscles of the upper extremities. During most of their lives, these patients too no medications on a regular basis. From the age of 38, the proband started walking with a cane; from the age of 40, with the help of the walking frame. In 12 years after the manifestation of desminopathy, he appeared to be confined to a wheelchair as his father had been.

The results of investigation of the saliva and feces microbiota of the proband with desminopathy T341P are given in Table 1.

Table 1.

The change in saliva and feces microbiota of the proband with desminopathy T341P

Microorganism	Biological material at the age of the proband							
	saliva				feces			
	41 years	42 years	43 years	Norm	41 years	42 years	43 years	change (43-41)
1	2	3	4	5	6	7	8	9
Cocci, bacilli, x10^5 cells/gram								
Bacillus cereus	0	0	119	41	1457	2725	41326	39869
Bacillus megaterium	0	0	38	92	4168	6956	11315	7147
Enterococcus spp	0	55	5	0	48	3239	536	488
Streptococcus spp	0	0	0	45	190	1185	4993	4803
Streptococcus mutans (anaerobic)	685	527	687	114	0	3638	17411	17411
Staphylococcus aureus	487	495	343	30	841	4171	12372	11531
Staphylococcus epidermidis	0	0	0	0	0	148	646	646
Sum	1172	1077	1192	322	6704	22062	88599	81895
Anaerobes, x10^5 cells/gram								
Bacteroides flagilis	168	33	155	10	387	2145	7799	7412
Bifidobacterium spp	1209	646	683	225	1383	3422	61422	60039
Blautia coccoides	40	64	114	0	0	0	0	0
Clostridium spp (group *C. tetani*)	0	210	85	500	893	9162	21682	20789
Clostridium difficile	0	129	18	0	0	35	15447	15447
Clostridium hystolyticum/ Streptococcus pneumonia	387	280	390	50	0	112	0	0
Clostridium perfringens	61	101	56	84	780	7007	45357	44577
Clostridium propionicum	0	0	0	94	0	6488	18316	18316
Clostridium ramosum	17523	6855	3785	992	316	0	0	-316
Eubacterium spp	4055	3894	2199	565	11653	47001	147869	136216
Eggerthella lenta	267	307	167	40	6611	16761	94343	87732
Fusobacterium spp/ Haemophilus spp	0	0	135	18	0	736	3310	3310
Lactobacillus spp	455	1493	1550	659	1682	11555	39593	37911
Peptostreptococcus anaerobius 18623	265	219	693	378	10796	98118	80560	69764

Table 1 continued.

1	2	3	4	5	6	7	8	9
Prevotella spp	123	482	71	10	1164	4173	17346	16182
Propionibacterium spp	389	66	151	39	0	2230	0	0
Propionibacterium acnes	523	159	420	44	0	4217	43129	43129
Propionibacterium freudenreichii	1364	823	577	243	1567	4824	24563	22996
Propionibacterium jensenii	0	0	33	17	18140	41465	154043	135903
Ruminicoccus spp	0	104	143	114	0	1266	3196	3196
Veillonella spp	0	34	19	16	0	0	1254	1254
Sum	26829	15899	11444	4098	55372	260717	779229	723857
Actinobacteria, x10^5 cells/gram								
Actinomyces spp	0	9	0	21	0	0	0	0
Actinomyces viscosus	691	373	402	113	122	1638	7193	7071
Corynebacterium spp	0	63	0	35	4253	4401	18511	14258
Nocardia spp	0	86	16	94	63	556	346	283
Nocardia asteroides	127	116	132	40	28	1017	2277	2249
Pseudonocardia spp	0	63	0	12	0	0	0	0
Rhodococcus spp	476	362	334	270	86	516	1382	1296
Streptomyces spp	303	99	382	240	8897	31221	95262	86365
Sum	1597	1171	1266	825	13449	39349	124971	111522
Enterobacteria, x10^5 cells/gram								
Enterobacteriaceae spp (E.coli)	0	0	0	7	0	0	0	0
Helicobacter pylori	0	0	0	15	0	0	0	0
Campylobacter mucosalis	0	0	0	0	0	25	0	0
Sum	0	0	0	22	0	25	0	0
Gram-negative rods, x10^5 cells/gram								
Alcaligenes spp/ Klebsiella spp	0	31	0	24	0	240	970	970
Kingella spp	0	0	0	0	0	389	0	0
Moraxella spp/ Acinetobacter spp	0	0	192	40	0	144	605	605
Porphyromonas spp	0	18	31	0	0	172	644	644
Pseudomonas aeruginosa	0	0	0	0	0	47	131	131
Sum	0	49	223	64	0	992	2350	2350
Fungi, yeast, x10^5 cells/gram								
Aspergillus spp	0	493	71	980	0	3411	12137	12137

End Table 1

Candida spp	570	1088	409	520	118	0	0	-118
Microfungi, campesterol	0	49	0	115	181	1101	1827	1646
Microfungi, sitosterol	0	35	7	384	313	4259	10281	9968
Sum	570	1665	487	1999	612	8771	24245	23633
Total number of microorganisms	**30168**	**19863**	**14612**	**7330**	**76137**	**331916**	**1019394**	**943257**
Viruses, conv. units								
Herpes spp	0	6	24	0	0	448	1018	1018
Cytomegalovirus	0	17	9	0	0	191	137	137
Epstein-Barr virus	0	10	5	7	0	77	116	116
Sum (markers) of viruses	**0**	**33**	**38**	**7**	**0**	**716**	**1271**	**1271**
Plasmalogen, mcg/ml	**30,8**	**59,55**	**19,21**	**50**	**3,83**	**6,99**	**33,89**	**30,06**
Total endotoxin, nmol/ml	**4,29**	**5,52**	**6,87**	**0,50**	**1,68**	**10,18**	**40,92**	**39,24**

The variety of the investigated microorganisms in the saliva of the proband with desminopathy at the age of 41 years was determined to be 21 species, with the absence of viruses; at the age of 43, 35 species were identified, including *Epstein-Barr viruses*, *Herpes spp* and *Cytomegalovirus*. Within the last two years under the analysis, the following microorganisms were found in the saliva for the first time: anaerobes – *Clostridium difficile* (exceeding the norm up to 129 times), *Clostridium spp* (group *C. tetani*), *Fusobacterium spp/ Haemophilus spp* (exceeding the norm up to 7,5 times), *Propionbacterium jensenii*, *Ruminicoccus spp*, *Veillonella spp*; cocci, bacilli - *Bacillus cereus* (exceeding the norm up to 2.9 times), *Bacillus megaterium*, *Enterococcus spp*; gram-negative rods - *Moraxella spp/Acinetobacter spp* (exceeding the norm by up to 4.8 times), *Porphyromonas spp* (exceeding the norm up to 31 times); actinobacteria - *Nocardia spp*; fungi, yeast - *Aspergillus spp*, *sitosterol*.

While the total number of microorganisms detected in the patient's saliva decreased by 2.1 times during the last three years of the patient's life, it still exceeded the norm by 2 times. The total number of microorganisms of saliva microbiota of the proband with desminopathy decreased mainly due to the 4.6-time decrease in anaerobe *Clostridium ramosum*, which was predominating within 3 years of the investigations under consideration.

During the analyzed period, the relative number of anaerobes decreased by 10.6% in the composition of saliva microorganisms, but actinobacteria increased by 3.4%, cocci and bacilli increased by 4.3%, fungi and yeasts increased by 1.4%; gram-negative rods – by 1.5% (Fig. 1).

During the last analyzed year, the following microorganisms disappeared in the saliva: *Actinomyces spp*, *Corynebacterium spp*, *Pseudonocardia spp*, *Alcaligenes spp*, *Klebsiella spp*, *campesterol*. And, in general, the following microorganisms were absent in the saliva during the considered time period: *Streptococcus spp*, *Staphylococcus epidermidis*, *Clostridium propionicum*, *Kingella spp*, *Pseudomonas aeruginosa*, *Enterobacteriaceae spp (E.coli)*, *Helicobacter pylori* and *Campylobacter mucosalis*.

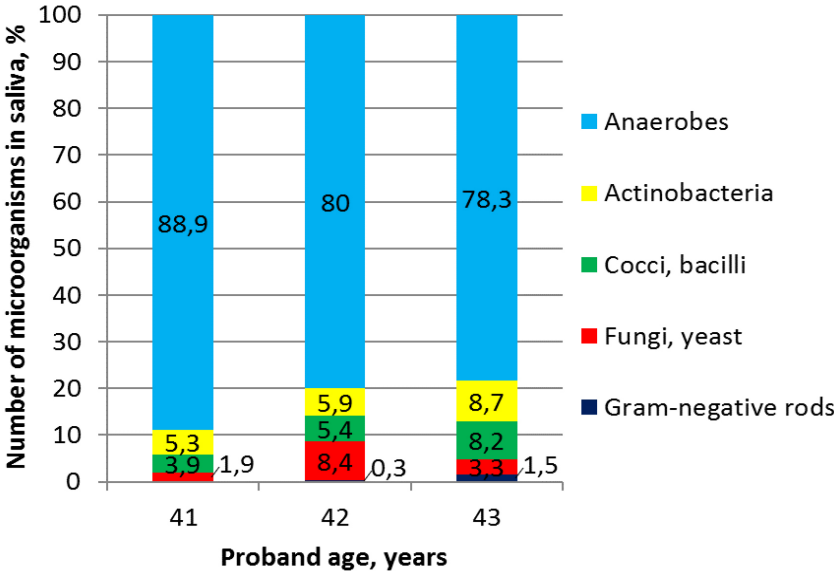

Fig. 1. The change in the relative composition of saliva microorganisms of the proband with desminopathy T341P

The total level of endotoxin in the saliva microbiota of the proband with desminopathy in the considered age interval increased by 1.6 times to reach a level exceeding the norm by 13.7 times. The plasmalogen in saliva decreased by 1.6 times to fall 2.6 times lower than the reference level.

Out of 56 species of microorganisms 26 species with the absence of viruses were found in the fecal microbiota of the proband with desminopathy in the age of 41 years, and in the age of 43-37 species already with the presence of *Epstein-Barr viruses*, *Herpes spp* and *Cytomegalovirus*. During the last analyzed year the emergence of the following 13 microorganisms was observed in the feces: gram-negative rods - *Pseudomonas aeruginosa*, *Moraxella spp*, *Acineto-*

bacter spp, *Porphyromonas spp*, *Alcaligenes spp*, *Klebsiella spp*; fungi - *Aspergillus spp*; cocci - *Staphylococcus epidermidis*, *Streptococcus mutans* (anaerobic); anaerobes - *Fusobacterium spp*, *Haemophilus spp*, *Ruminicoccus spp*, *Clostridium difficile*, *Clostridium propionicum*, *Propionibacterium acnes*, *Veillonella spp*.

During the last three years the total amount of microorganisms of the proband's fecal microbiota increased in 13.4 times. First of all, this is conditioned by the increase in anaerobes by 76.6%, and in actinobacteria by 11.8%. The following predominated in the feces of the patient during the last year of the analysis: *Propionbacterium* – 15.1%; *Eubacterium spp* – 14.5%; *Eggerthella lenta* – 9.3%; *Peptostreptococcus anaerobius (18623)* – 7.9%; *Bifidobacterium spp* – 6.0%; *Clostridium perfringens* – 4.4%; *Propionibacterium acnes* – 4.2%; actinobacteria *Streptomyces spp* – 9.3%; in total constituting 70.7% of the sum of microorganisms. At the same time, the separate growtsh of the first three and the remainder of the abovementioned bacteria exceeded the total sum of fecal microbiota microorganisms when the proband was 41 years old.

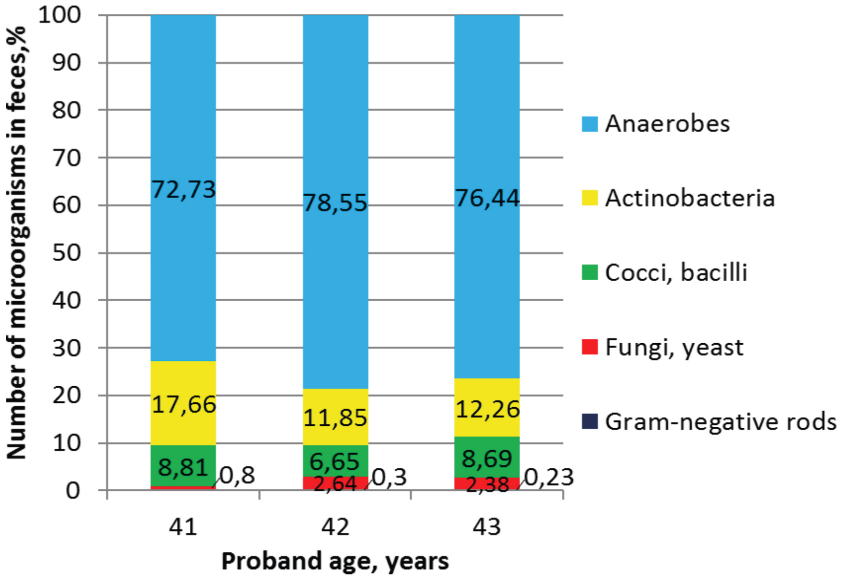

Fig. 2. The change in the relative composition of feces microorganisms of the proband with desminopathy T341P

During the analyzed period, the relative amount of anaerobes increased by 3.71% in the composition of feces microorganisms, fungi and yeast increased by

1.58%; gram-negative rods – by 0.23%; but actinobacteria decreased by 5.4%; cocci and bacilli – by 0.12% (Fig. 2).

Out of transient microorganisms, *Peptostreptococcus anaerobius 18623* having a relative content from 7.9 up to 29.56% predominated in the fecal microbiota during the considered proband's age period (Table 2).

Table 2.

The change in the content of transient microorganisms in fecal microbiota of the proband with desminopathy T341P

Transient microorganisms	% from the total number of microorganisms of fecal microbiota in the proband's age		
	41 years	42 years	43 years
Bacillus cereus	1,91	0,82	4,05
Enterococcus spp	0,06	0,98	0,05
Bacteroides flagilis	0,51	0,65	0,77
Clostridium difficile	0	0,01	1,52
Clostridium hystolyticum/ Streptococcus pneumonia	0	0,03	0
Peptostreptococcus anaerobius 18623	14,18	29,56	7,90
Campylobacter mucosalis	0	0,01	0
Kingella spp	0	0,12	0
Porphyromonas spp	0	0,05	0,06
Pseudomonas aeruginosa	0	0,01	0,01
Sum	16,66	32,24	14,36

The sum of transient microorganisms in the feces was 14.36-32.24% from the total number of microorganisms of fecal microbiota at the known norm below 1%. The twofold growth and decrease in the abovementioned index were observed.

At the same time, 2 microorganisms disappeared in the feces during the period under consideration: *Candida spp* and *Clostridium ramosum*. Thus, the following microorganisms were not revealed in the proband's feces during the analyzed years: anaerobes - *Blautia coccoides*, *Peptostreptococcus anerobius 17642*; gram-negative rods - *Flavobacterium spp*, *Stenotrophomonas maltophilia*; actinobacteria - *Actinomyces spp*, *Mycobacterium spp*, *Pseudonocardia spp*, *Streptomyces farmamarensis*; enterobacteria - *Enterobacteriaceae spp (E.coli)*, *Helicobacter pylori*.

The total level of endotoxin in fecal microbiota of the proband with desminopathy within the considered age interval increased in 24.4 times to exceeds the norm in 81.8 times. While plasmalogen increased by 8.8 times in the feces, it was still below the reference level by 1.5 times.

Discussion

The conducted retrospective investigations demonstrated that from the childhood the proband had had problems with digestion and mouth mucous tunic inflammation. Thus, the two-forked interaction between the host's microbiota and immune system starts from birth and developed throughout the host's life [6]. In this context, the emergence of allergies by the end of the second decade of his life was not coincidental at all.

As it is known, intestinal microbiota also directly affects outside of the gastrointestinal tract, especially the organs interdependent on the glycaemia level, including brain, liver, fatty tissue and skeletal muscles [9].

It was previously established that decreased metabolic, phagocytic and oxidative activity of monocytes and granulocytes occurs in terms of immunosuppression with the progression of the revealed case of desminopathy T341P [17]. Phagocytes are particularly responsible for utilization of killed cells [3]. As it is known, regeneration of muscles is connected with immunity [8], while dysbacteriosis or negative changes in the microbe composition of intestines can disturb the regulation of immune reactions, causing inflammation and oxidative stress [26]. Thus, taking the abovementioned into account, as well as the forced decrease in the motion and physical activity of the patients, the microbiocenosis of the organism biological media can change with progression of the considered disease.

During the middle period of the course of desminopathy, at a time when the proband still moved with the help of a walking frame, the investigated viruses and gram-negative rods, as well as anaerobes: *Clostridium difficile*, *Clostridium propionicum*, *Fusobacterium spp*, *Haemophilus spp*, *Ruminicoccus spp*, *Veillonella spp*, were absent in the saliva and feces microbiota. After the middle period, when the proband appeared to already be confined to a wheelchair, the occurrence of viruses and gram-negative rods, as well as the abovementioned anaerobes, was found in the considered biological media.

The total amount of microorganisms of the proband's saliva microbiota during the analyzed age period demonstrated a two-fold decrease, while fecal microbiota, conversely exhibited a more than 13-fold increase. Thus, excessive bacterial growth of fecal microbiota was observed. At the same time, a total amount of microorganisms exceeding of the norm and increase in their specimen variety in the examined biological materials of the proband was noted. *Propionibacterium jensenii*, *Eubacterium spp*, *Eggerthella lenta* predominated in the fecal microbiota, and *Clostridium ramosum* - in the saliva microbiota.

The following 7 microorganisms were absent in the proband's saliva and feces microbiota: gram-negative rods - *Flavobacterium spp*, *Stenotrophomonas*

maltophilia; actinobacteria - *Mycobacterium spp*, *Streptomyces farmamarensis*; anaerobes - *Peptostreptococcus anaerobius 17642*; enterobacteria - *Enterobacteriaceae spp (E.coli)*, *Helicobacter pylori*.

The increased level of the proband's transient fecal microbiota with the prevalence of *Peptostreptococcus anaerobius 18623* was observed during the period under investigation. As it is known, transient microorganisms can influence the resident microbiota with the help of different mechanisms [4].

The presence of bacterium *Clostridium difficile* in the saliva and feces microbiota of the proband with desminopathy progression is especially concerning, since it was absent before in the middle of the disease course. Moreover, its rapid growth in the feces in 441 times was observed only during the last year. As it is known [22], the infection of large intestine *Clostridium difficile* is potentially dangerous for life, especially in patients having intestinal microbiota dysbiosis. Based on the foregoing, the fact that the proband's grandfather died from the rectal adenocarcinoma in the age of 72 years draws attention [15].

The total level of endotoxin in the proband's saliva and fecal microbiota increases, moreover, more intensively in the latter, exceeding the norm in more than 13 and 81, respectively. As it is known, dysbacteriosis is often accompanied by the increased number of gram-negative bacteria [19], which possess endotoxic properties and activate proinflammatory cytokines, such as IL-6 [12]. Besides, dysbacteriosis can decrease physiological adaptation, increasing the formation of active oxygen forms leading to the destruction of macromolecules by free radicals, which contributes to atrophy of skeletal muscles [19]. As was observed earlier, the increased level of IL-6 [17] and growth in oxidative stress indexes were observed with the proband while he was approaching the middle stage of the desminopathy course [16].

It is significant that an observed increase in the proband's muscle mass and physical force during the month in the age of 40 years after he stopped cleaning the teeth with toothpaste. He continued cleaning the teeth twice a day with a tooth brush with water. During that period, the proband was not taking any medications. However, 30 days later the state of his muscles had returned to their earlier emaciated state. However, the abandonment of toothpaste use clearly resulted in the changed microbiota of the mouth cavity, which temporarily, but for a significant period, improved the state of the patient's skeletal muscles. It can be considered that the majority of bacteria colonizing the mouth cavity are necessary to maintain a general state of health [5].

It is notable that, at the age of 33 years, the proband was diagnosed with lambliosis, for the treatment of which the following complex therapy was prescribed: *Cynarae scomuli* foliae extract, Mebeverine, gastro-resistant capsules

with *Bifidobacterium longum* and *Enterococcus faecium*, intestinal sorbent from lactulose with hydrolyzed lignin, antibiotic Albendazole. Due to two relapses, the lambliosis was treated twice more at the age of 34 years following the same scheme only using antibiotics Ornidazole and Nifuratel. It is notable that the indicated complex therapy of lambliosis each time resulted in temporary increase in the proband's muscle mass and physical force in the period from 1 to 3 months. At the same time, the same way as in the case of toothpaste-use cessation, we observed increased stamina, decreased tiredness, rapid revival with the decreased sleep duration, decreased muscle stiffness, absence of arrhythmia, improved appetite and intestinal motor skills with the emergence of borborygmus when hungry, increased voice production, improvement of skin fitness.

As was discovered, quite various approaches to the microbiota of the mouth cavity or intestine of the patient with desminopathy demonstrated temporary effectiveness. Thus, a complex approach to the change in oral and intestinal microbiota can become a powerful tool to prevent and treat muscle diseases, especially those manifesting late in life.

Conclusion

The conducted retrospective investigation was used to determine changes in saliva and feces microbiota of the desminopathy T341P patient in a heterozygous state. The emergence of the investigated viruses and gram-negative rods in the considered biological media was established to accompany progression of the disease, while excessive bacterial growth of fecal microbiota occurred along with an observed increase in transient microorganisms, including endotoxins, and a reduced plasmalogen level. In perspective, the investigation results can be used by neurologists, gastroenterologists, infectiologists, dietitians, immunologists, geneticists to find the tactics of complex interventions.

The study was conducted in accordance with the principles of the provisions of the Declaration of Helsinki of the World Medical Association.

Informed consent. Informed consent was obtained from all subjects participating in the study.

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