High throughput genome study to identify predictors of aggressiveness in patients with sporadic desmoid tumor who undergo a wait and see approach

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Background

Wait and see approach for desmoid-type fibromatosis (DF) patients has become part of the routine treatment strategy. Two parallel European prospective studies have been conducted to validate this approach. However, predictive factors to select the risk of progressive disease in the individual patient are still lacking.

Aim

The goal of the current study (founded by DTRF) was to identify genomic signatures associated to specific behaviors in patients enrolled within the Italian prospective observational study.

Methods

DF fresh frozen samples from enrolled patients who have been biopsied at our Institution were collected for translational studies. Whole exome sequencing was performed on DNA extracted from 12 fresh frozen biopsies using Nextseq500 (Illumina, CA) sequencer. Deep sequencing of *CTNNB1*, *APC* and *LAMTOR2* was performed on additional 11 FFPE cases of WT DF using Truseq custom amplicon low input kit (Illumina) for library preparation and sequenced on MiSeq instrument (Illumina). Mutations were validated through PCR and Sanger sequencing.

Conclusions

DF is characterized by a low load of mutational events, which do not seem to be associated to the clinical course of the disease. A minority of DF is wild type for either *CTNNB1, APC* or any other gene involved in the WNT pathway. Approximately half of them harbor low frequencies clonal *CTNNB1* mutations. Additionally, long intragenic deletions were observed in exon 3 of *CTNNB1* gene. These type of deletions are difficult to be discovered also using NGS. This is a novel finding and molecular meaning needs further investigation.

Exome sequencing analysis of fresh frozen biopsy					
#ID	*CTNNB1* status	# of somatic mutations	Mutational Burden	Other somatic events	Shifting to active treatment
#1	T41A	6	0,168	NRP2 (p.R572Q)	No
#2	T41A	6	0,168	LPA (p.Y1728H)	No
#3	T41A	5	0,143	-	No
#4	T41A	7	0,204	-	No
#5	S45F	9	0,255	-	Yes
#6	WT	10	0,286	APC (p.D1696N)	No
#7	T41A	12	0,337	RELN (p.S2062K)	No
#8	S45F	12	0,348	-	Yes
#9	WT	13	0,363	LAMTOR2 (p.V92M)	No
#10	T41A	14	0,392	EGR2 (p.A358S)	No
#11	T41A	15	0,427	SETDB1 (p.R495X); PDX1 (p.V220G)	No
#12	T41A	30	0,887	-	No

Table 1. Twelve fresh frozen biopsies were analyzed through exome sequencing. Using Sanger sequencing 10 mutated DF (8 T41A and 2 S45F) and 2 WT were identified. In WT cases, two genes were found to be mutated: *APC* in one case (p.D1696N and p.D1670H) and *LAMTOR2* (p.V92M) in the other. Globally, DF exhibited low somatic sequence mutation rate (mean 0.36 mutations per megabase), and in the *CTNNB1*-mutated group no other recurrent mutational event was identified. Overall, in this group, only 2/12 patients were shifted from an observational approach to a specific treatment for progressive disease.

Custom amplicon analysis for FFPE WT samples						
#ID	TIPO	Medium coverage	Medium coverage of *CTNNB1* exon3	*CTNNB1 mutation (altered allele frequency %)*	APC mutation	CTNNB1 Deletion
#1	FFPE	1752	2272	A121G - T41A; 17%		
#2	FFPE	2483	3174	A107C - H36P; 17%		
#3	FFPE	330	490	A121G - T41A; 15%		
#4	FFPE	3071	2907	A121G - T41A; 13%		c.14-79_128del
#5	FFPE	1292	1921	T133C - S45P; 14%		
#6	FFPE	2925	3815	WT	C2377T - Q793X 30%; c.4607delA -p.E1536fs 29%	
#7	FFPE	2031	3143	WT	c.4526delT - p.L1508fs (84%)	
#8	FFPE	3774	4884	WT		
#9	FFPE	1945	2524	WT		
#10	FFPE	960	792	WT		c.68_241+21del
#11	FFPE	755	1060	WT		

Table 2. In order to enlarge the study on WT DF subtype and identified new potential mutations, high deep sequencing of *CTNNB1*, *APC* and *LAMTOR2* was conducted on a retrospective series of 11 additional WT DF. No other mutation of *LAMTOR2* was detected. *APC* mutation was detected in 2 cases, while low-frequencies *CTNNB1* mutations were found in 5 samples (50%) (mean of 16% reads). However, 4 cases (42%) remained WT for *CTNNB1* or *APC* mutations. Through in-depth analysis of NGS data, we discovered also the presence of 2 intragenic deletions of *CTNNB1* exon 3 that were validated through PCR. They occurred in 2 samples: one carrying a T41A low frequency mutation and one wild type.

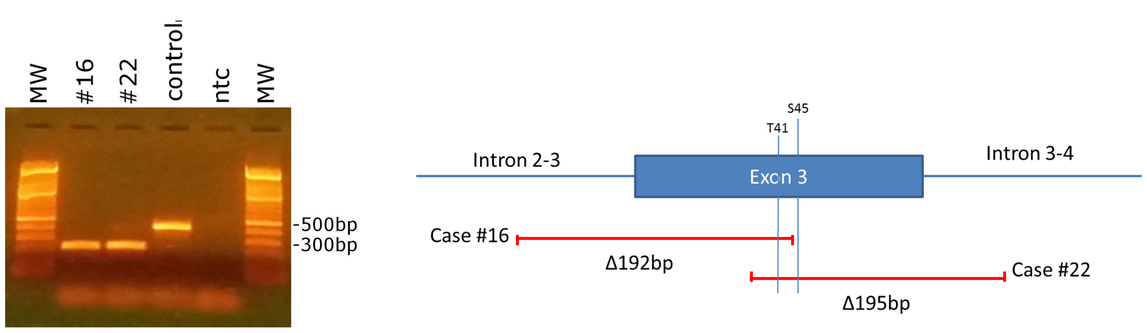

Fig 1. We discovered large intragenic deletions (approximately 200bp) of *CTNNB*1 exon 3: one in the 5' terminal and one in the 3' terminal. This type of deletions is difficult to be found also using NGS analysis when the position is not known *a priori.*

This work was supported by DTRF. This sponsor had no involvement in the collection, analysis and interpretation of data or in the writing of the report.

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