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.

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 0.06 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.

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 CTNNB1 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.

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