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Identification of disruptive germline and somatic variants in intronic splicing sequences and their confirmation by RNA expression in 2,489 cancer patients

CONTRIBUTING AUTHORS

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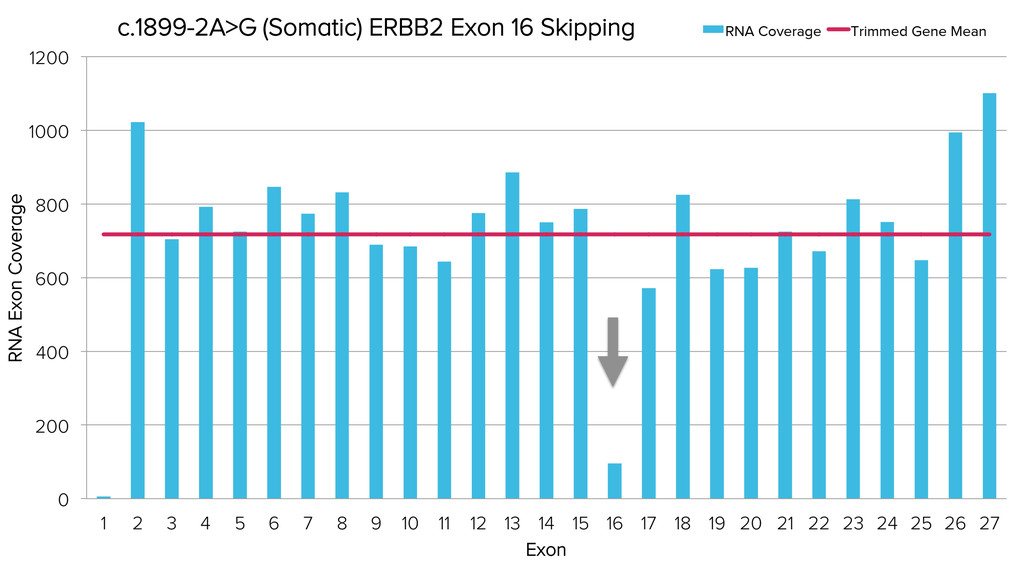
BACKGROUND

- RNA splicing is an integral step in mRNA processing in which introns are removed from the pre-mRNA and exons are subsequently ligated together
- Mutations at the acceptor and donor splice sites, branch point, and polypyrimidine tract can disrupt splicing and lead to deleterious events including exon skipping
- Genomic variants that disrupt pre-mRNA splicing have been shown to play a role in some cancers and rare diseases

METHODS

- We obtained RNA exon coverage for 328 cancer genes in 2,484 clinical samples processed by next-gen DNA and RNA sequencing and calculated background statistics
- Variants at acceptor and donor splice sites were identified by running all clinical samples through our research pipeline
- For each splice site variant, a z-score was calculated and used to identify exons with significantly lower coverage than expected
- Introns in the 328 cancer genes were scanned for candidate branch points using known motif characteristics and patterns:

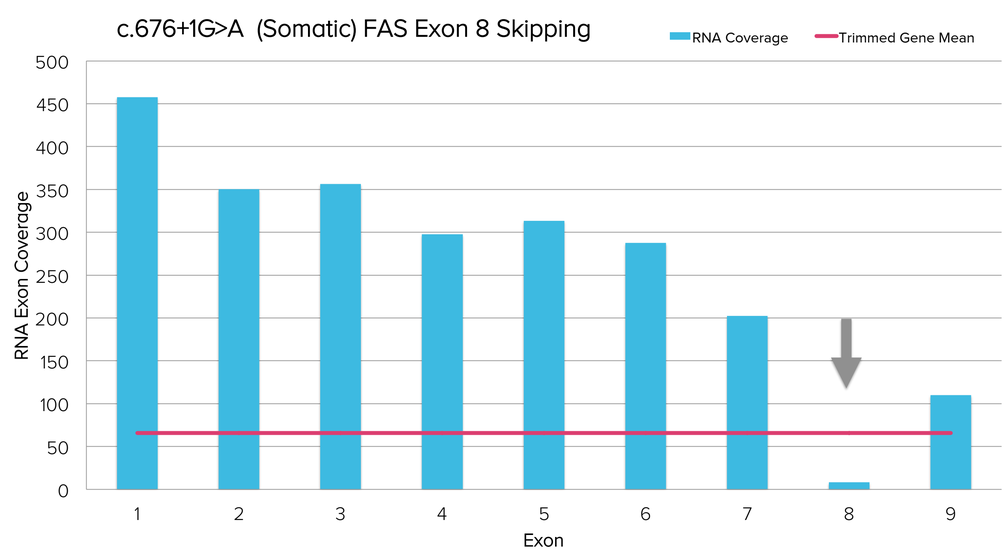
RESULTS



Exon	Coverage	Normalized Coverage	Z-Score
11	643.7	1.04	0.29
12	775.7	1.15	1.37
13	886.0	1.09	0.29
14	750.5	0.85	-0.73
15	786.5	0.87	-1.66
16	**95.3**	**0.19**	**-4.71**
17	571.2	0.76	-2.15
18	824.8	1.03	-0.36
19	623.0	1.19	0.61
20	626.6	1.16	1.27
21	724.5	1.19	1.45

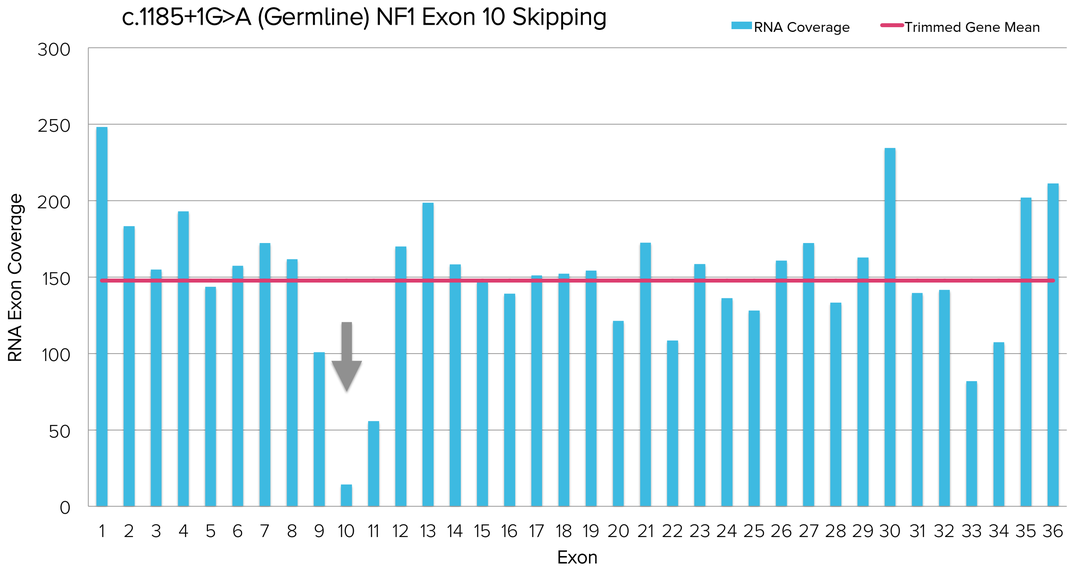
RNA exon coverage in the ERBB2 gene for a patient with breast cancer. A loss of coverage of exon 16 is seen with a Z-score of -4.71 due to an acceptor site mutation. Exon 16 skipping has been detected in breast cancers and is associated with trastuzumab resistance.

Patient Group	# of Patients	% of Total Patients
With Germline SS Mutations in cancer genes	2,484	100
With Low Exon Coverage	47	1.89
With Exon Skipping	1	0.04
With Somatic SS Mutations in cancer genes	419	16.86
With Low Exon Coverage	25	1.00
With Exon Skipping	2	0.08



RNA exon coverage in the FAS gene in a patient with lung cancer. A loss of coverage of exon 8 is seen with a Z-score of -2.13 due to a donor site mutation.

Exon	Coverage	Normalized Coverage	Z-Score
1	457.6	1.73	1.36
2	350.3	1.32	0.79
3	356.5	1.35	-0.02
4	297.6	1.12	-0.42
5	313.4	1.18	-0.21
6	287.6	1.09	0.39
7	202.4	0.76	-0.21
8	**8.2**	**0.03**	**-2.13**
9	110.2	0.42	-0.78



Exon	Coverage	Normalized Coverage	Z-Score
5	143.6	0.97	1.42
6	157.4	1.07	1.43
7	172.2	1.17	0.84
8	161.7	1.10	-0.53
9	100.9	0.68	-2.22
10	**14.2**	**0.10**	**-5.09**
11	55.7	0.38	-2.36
12	170.0	1.15	-0.28
13	198.7	1.35	0.87
14	158.3	1.07	-0.27
15	147.5	1.00	1.47

RNA exon coverage in the NF1 gene for a patient with soft tissue sarcoma. A loss of coverage of exon 10 is seen with a Z-score of -5.09 due to a donor site mutation. Variant is associated with neurofibromatosis I and may contribute to the development of soft tissue sarcomas.

CONCLUSIONS

- One germline and two somatic splice site mutations with putative deleterious effects were identified in three patients
- The impact of splice site mutations in two of these patients were relevant to the patient's cancer pathology, displaying the important role this analysis plays in the whole genome study of cancer patients
- Estimating branch points and polypyrimidine tracts in intronic regions enabled us to focus our intronic sequence analysis to specific regions

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