CRPP radiz - Rare Disease Initiative Zurich

Absence of Somatic Mutations in Linear Localized Scleroderma

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INTRODUCTION

LLS is a rare connective tissue disorder characterised by chronic inflammation and dysregulation of collagen metabolism. This results in initial hardening and later atrophy of the region. LLS starts in early childhood although rare cases of congenital onset have been described. LLS is distinguished from morphea by sharply delimited and linear plaques (Figure 1) that are unilaterally oriented along embryological cellular migration routes. This suggests that LLS could be caused by *de novo* somatic mutation(s) resulting in a mosaic state as has been described previously in other skin conditions.

METHODS

19 patients clinical trial number NCT02222038. "Affected" tissue DNA from lesional FFPE skin or 1-2mm of snapfrozen skin tissue. "Unaffected" tissues are blood and saliva.

Samples are prepared for whole exome sequencing (WES) as per SureSelect XT and exon capture v6 kits (Agilent Technologies) and sequencing was performed on an Illumina HiSeq4000. Resulting data analyzed as per Figure 2.

ANALYSIS OF HLA

Scleroderma (SSc) is associated with HLA allotypes. We compared allotypes of our patients (*VBSeq* and *Optityper*) to 50 Swiss controls and SSc data.

Results

- 1) No SSc allotype was in common between patients.
- 2) No significant HLA signal was found when compared to the Swiss population.

Exome library preparation of extracted and sheared DNA

Massively parallel sequencing on Illumina HiSeq4000

Raw output aligned to reference genome (b37) using *BWA*

Removal of PCR artefacts using *Picard*

Realign reads and indels with *GATK-3.6*

Somatic analysis

Germline analysis

Varsan2.3.9, Mutect2 and Strelka1.2.14 to call somatic variants

Search for common somatic mutation or gene affected in patient pairs

Annotation (CADD/ VEP/ Annovar; MAF in TG and ES projects)

Visual control of alignment / called mutations using IGV

Figure 2. Visualization of WES bioinformatic analysis

DEEP SEQUENCING

Hypothesis: Heterogeneity in the sample could lead to causative somatic mutation being undetectable by somatic mutation callers.

- 1) Test the sensitivity of somatic mutation callers using BAMsurgeon to create mutations at desired allelic fraction at various read depths. These files were fed through all somatic callers. (Table 1)
- 2) Perform deep sequencing to a read depth >200.

Results:

- 1) Somatic mutation callers vary in sensitivity based on default setting parameters and read depth.
- 2) Increased scrutiny into low allelic fraction mutations did not yield any damaging or

Genotype caller

Allelic fraction

	0.05	0.1	0.15	0.2	0.3	0.4	0.5	...	1
Varscan (High)	-	-	-	-	-	+	+	...	+
Varscan (Mid)	-	-	+	+	+	+	+	...	+
Varscan (Low)	-	-	+	+	+	+	+	...	+
Strelka (DP 20)	-	-	-	+	+	+	+	...	+
Strelka (DP 100)	-	+	+	+	+	+	+	...	+
Strelka (DP 100, Q5)	+	+	+	+	+	+	+	...	+
MuTest (DP 20)	+	+	+	+	+	+	+	...	+
MuTest (DP 100)	+	+	+	+	+	+	+	...	+

Table 1. Allelic fraction at which somatic variant callers will call a mutation at various stringency settings. DP = read depth

rare somatic snp of interest.

3) Several snp mutations found in maximum 2/4 patients. Visualization of mutations using IGV did not produce promising candidates for future experimental focus.

SOMATIC ANALYSIS

Affected skin and corresponding blood samples were sequenced and analyzed pairwise per patient and then called variants were analyzed between patients.

Results:

- 1) Analysis of high confidence somatic mutations called by 3 somatic variant callers found that no somatic snp variant was present in more than 5 patients.
- 2) No indel has been found present in all sample pairs. This indicates no single somatic mutation is responsible for LLS.
- 3) Gene restricted analysis found no gene that is affected by any rare and damaging mutation or indel common amongst all patients.
- 4) 1 gene has been found to be affected with a rare mutation in 10 patients. 5 genes have been affected with a rare mutation in 6 patients. These mutations within the genes were all examined and found to be artefacts (IGV).

CONCLUSION

- ❖ No single somatic snp or indel is driving LLS as previously suspected.
- ❖ No gene of significance has been found to be burdened with a damaging mutation in all patients.
- ❖ No specific HLA allotype associated with LLS
- ❖ CGH of affected skin has not found any large duplication or deletion in patients.

❖ An associated study based on mRNA analysis of 3 patients showed no increase in expression in any gene in common.

Future of the project:

- ❖ Expansion of the study to investigate possible causative germline mutations. Comparison of clinical cohort to data of >3,000 healthy European controls.

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Figure 1. Linear localized scleroderma on a) right leg b) trunk and c) right arm all showing atrophy and fibrosis