CONDITIONING THERAPY-INDUCED ORAL MUCOSITIS- ASSOCIATED SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IDENTIFIED BY EXOME SEQUENCING ANALYSIS: A PILOT STUDY

J.L. Mougeot¹, B. Hasséus², K. Garming-Legert³, N. Rozario⁴, A. Hovan⁵, I. von Bültzingslöwen⁶, M. Brennan¹, F. Mougeot¹

¹Carolinas HealthCare System, Oral Medicine, Charlotte, USA; ²Institute of Odontology- University of Gothenburg, Oral Medicine and Pathology, Gothenburg, Sweden; ³Karolinska Institute, Dental Medicine, Stockholm, Sweden; ⁴Carolinas HealthCare System, IAS CORE Analytics, Charlotte, USA; ⁵University of British Columbia, Radiation Oncology, Vancouver, Canada; ⁶University of Gothenburg, Department of Oral Microbiology and Immunology, Gothenburg, Sweden

Abstract

INTRODUCTION: Oral mucositis (OM) is a common dose-limiting side effect of conditioning therapy used in cancer or immunodeficiency treatment of hematopoietic stem cell transplant (HSCT) patients. There is a need to identify SNPs predictive of risk and severity of OM.

OBJECTIVE: Using exome-sequencing, our objective was to identify candidate SNPs associated with OM in HSCT patients.

METHODS: Saliva DNA was extracted from HSCT patients (n=63) with WHO-OM scores 2-4 (Group-2-4; n=24), or 0-1 (Group-0-1; n=39). Illumina HiSeq2000-paired-end platform was used for exome-sequencing. SNP calling was determined with GATK pipeline. Using a simplified logit model, a list of 309 SNPs/209 genes was obtained (uncorrected p-value<0.01). Assuming a “toxic gain in function”, homozygous variant alleles overrepresented by more than 20% in Group-2-4 (possibly representing true positives independent from diagnosis) were determined. Proportions differences were evaluated by two-tailed 2-sample z-test.

RESULTS: Ten genes with SNP(s) were identified, including LAMC1 and ABCA4. LAMC1, essential for basal cell adhesion, had 8 SNPs that encompass 28kbp. All SNPs combined were either heterozygous or homozygous for reference or variant genotype. Ten patients, eight in Group-2-4 and one with WHO-OM score 1, had variant homozygous genotype in LAMC1. Also, an overrepresented intronic homozygous SNP variant (100% penetrance) was found in ABCA4, a gene adjacent to ARHGAP29 gene. In a previous cleft lip/palate association study, a different intronic SNP in ABCA4 was found within a long distance regulatory element of ARHGAP29. ARHGAP29 is critical for craniofacial development and involved in oral epithelial cell adhesion.

CONCLUSIONS: Exome-sequencing has potential to uncover SNPs relevant to OM.

Results

HSCT patients’ saliva DNA samples (N=63, ~50% M/F, age range ~25-65)

NGS and GATK pipeline

Quality check

Cleaning/Trimming

Quality Control

Alignment to Human Genome hg19

Pre-processing - GATK

Variant Calling - GATK

All 63 saliva DNA samples data were valid

Association analysis logit (p<0.01):
OM scores 0-1 (myelosuppressive) [N=39]
vs. 2-4 (myeloablative) [N=24]
⇒ VCF file with list of 309 SNPs/ 209 genes

For the 209 LRM-determined genes, two-tailed two-sample z-test was used to identify genes with predominance of the homozygous variant genotype in 2-4 OM score group

ABCA4	AA	AG	GG
OM 0-1 [N=39]	58.97%	41.03%	0%
OM 2-4 [N=24]	29.17%	37.5%	33.33%
Predominance OM 2-4	29.80%		33.33%
Penetrance OM 2-4	100%		
z-value	3.9		
P-value	0.0001		

⇒ 10 candidate genes identified

ABCA4:

Intronic regulation of ARHGAP29 essential for appropriate oral epithelium adhesion

ACTL7B:

Unknown function related to OM

COPB2:

Unknown function related to OM

FAAH2:

Unknown function related to OM

FBXO10:

Unknown function related to OM

LAMC1:

Oral mucosa basement membrane adhesion

MIR548I2:

Unknown function related to OM

MRPS22:

Unknown function related to OM

ORM1:

Immunosuppression/ anti-inflammatory

SF3B6:

Unknown function related to OM

Methods

-HSCT patients with hematological disorders (myelo-suppressive or -ablative conditioning)

-Saliva DNA isolation ⇒ Illumina PE₁₀₀ Exome-sequencing ⇒ GATK SNP calling = ~73,000 informative SNPs ⇒ Simplified conditional logistic regression model (LRM):

$\text{logit} [P(M = 1 | c = 1)] = \alpha + \beta g$

-Variables in model are: M: Mucositis grade (M = 0, 1), c: chemotherapy group (c = 0, 1); g: genotype or SNV at a specific locus, *i.e.*, dose of the variant allele (g = 0, 1, 2)

⇒ Question asked: is beta significantly different from 0 (H0: beta = 0)?

Conclusion

- ❑ SNPs in ABCA4 (regulatory of ARHGAP29) and LAMC1 are relevant to OM
- ❑ Need for validation with independent method
- ❑ Extend study with larger sample size

References

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