Variability of Individual Gene Expression Correlates with Long Term Outcomes in Heart Transplantation

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Background:
Longitudinal variation of AlloMap gene expression profiles (GEP) has been shown to be predictive of long term outcomes in heart transplantation.[1,2] We sought to determine the contribution of gene and metagene variation to the overall GEP score variation as well as the association with long term clinical outcomes.

Methods:
GEP scores were calculated from the expression levels of 11 genes in peripheral blood mononuclear cells (PBMCs). Seven of these genes can be grouped into 3 metagenes based on similarity of function (Table 1). The remaining 4 genes are not grouped into metagenes and will be considered in this analysis along with the 3 metagenes.

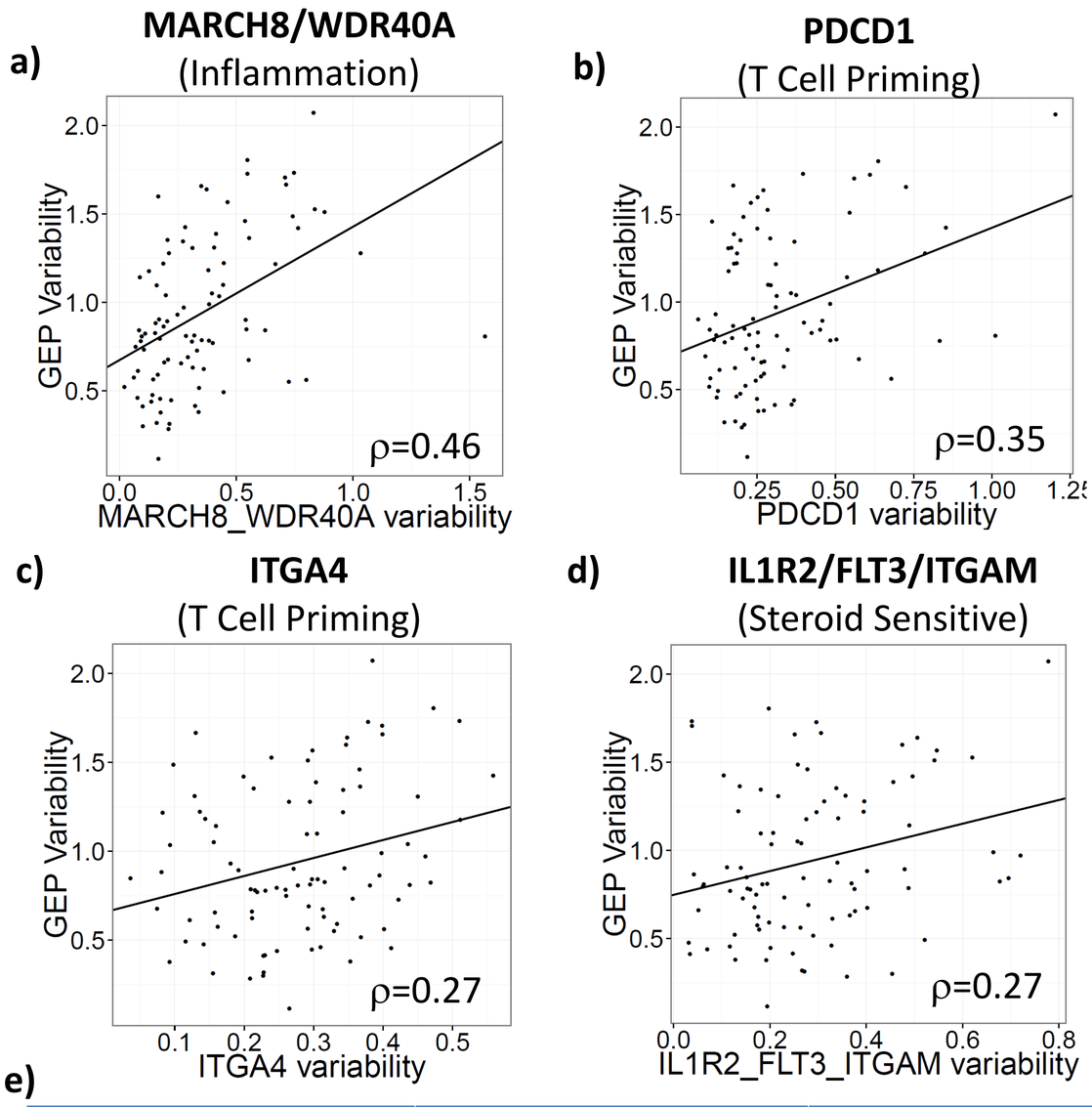
Variability of gene expression levels was calculated as the standard deviation of the expression level over 4 GEP tests starting after 315 days post transplant. Variability of GEP scores was calculated similarly. Patients were observed for a three year period following the final GEP score for events of death, graft loss, or rejection. From the CARGO II study, 36 event patients and 55 no-event patients met the inclusion criteria for this analysis.

All of the metagenes and genes were examined for the relationship of their variability to the variability of GEP scores using linear regression. The genes, metagenes, and overall GEP scores were tested for a difference in variability between patients who had an event and those who did not have an event using t tests.

Table 1: Function of 7 Metagenes [3]

Metagene	Function
IL1R2/FLT3/ITGAM	Steroid Sensitive
MARCH8/WDR40A	Inflammation
PF4/G6b	Platelet Activation
RHOU	Cell Migration
PDCD1	T Cell Priming
ITGA4	T Cell Priming
SEMA7A	Stimulate Cytokines

Figure 1: Steroid Sensitive, Inflammation and T Cell Priming Gene Expression Variability Correlate with GEP Variability

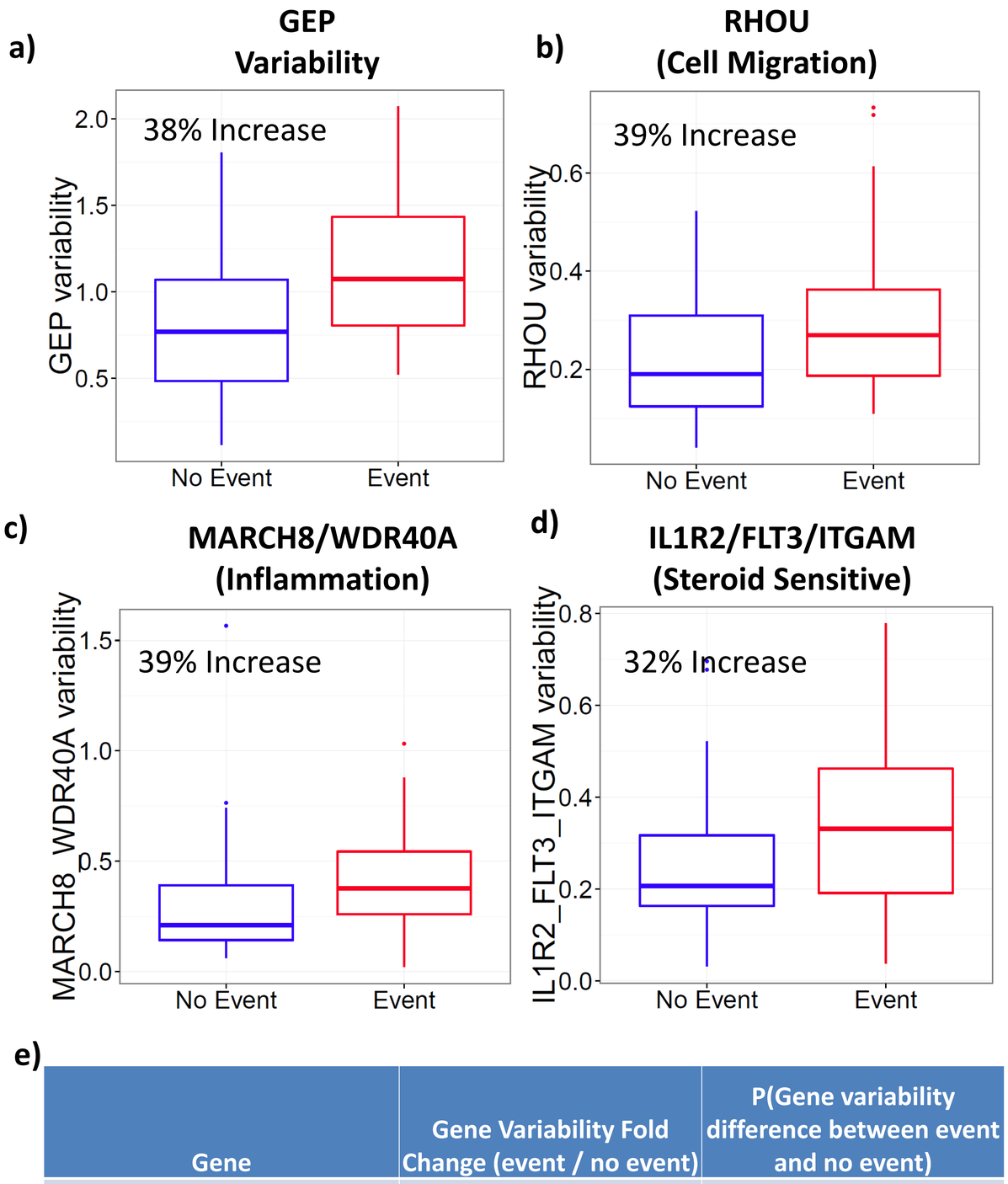


e)

Gene or Metagene	Correlation between gene variability and GEP variability	P
IL1R2/FLT3/ITGAM	0.266	**0.011**
MARCH8/WDR40A	0.456	**< 0.001**
PF4/G6B	0.135	0.202
RHOU	0.181	0.085
PDCD1	0.352	**< 0.001**
ITGA4	0.269	**< 0.001**
SEMA7A	0.113	0.285

(a – d) Shows graphically the four genes and metagenes that are most strongly correlated to the overall GEP variability (Pearson's correlation > 0.25): ITGA4, PDCD1, MARCH8/WDR40A, and IL1R2/FLT3/ITGAM. (e) Lists the correlation between the variability in expression of each metagene and gene, and the GEP variability, along with linear regression p-values for each term.

Figure 2: Cell Migration, Steroid Sensitive, and Inflammation Genes are More Variable in Patients with Adverse Long Term Outcomes



e)

Gene	Gene Variability Fold Change (event / no event)	P(Gene variability difference between event and no event)
Overall GEP score	1.38	**< 0.001**
IL1R2/FLT3/ITGAM	1.32	**0.036**
MARCH8/WDR40A	1.39	**0.032**
PF4/G6B	1.22	0.152
RHOU	1.39	**0.008**
PDCD1	1.34	0.051
ITGA4	1.09	0.318
SEMA7A	1.23	0.051

(a) Shows an increase of nearly 40% in overall GEP variability in patients who had an event. (b – d) Shows three genes with variability which had the most statistically significant increase in patients with long term outcomes. The most significant with an increase of nearly 40% is RHOU (p= 0.008). Two other terms also significantly increased, MARCH8/WDR40A (p=0.032), and IL1R2/FLT3/ITGAM (p=0.036), each by greater than 30%. (e) Shows in tabular form the increase in variabiiity of each gene or metagene, and the statistical significance of the t test.

Conclusions:
Most of the individual genes and metagenes are correlated with the GEP variability. RHOU, a cell morphology gene, is the most correlated with long term outcomes despite being not significantly correlated to GEP variability. Two other metagenes were marginally associated with outcomes, representing an inflammation pathway and the steroid responsiveness pathway. These data provide insight into the primary gene contributors to overall longitudinal variation of AlloMap and correspondingly to long term outcomes.

References

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2. Crespo-Leiro et al (2015) . Performance of gene-expression profiling test score variability to predict future clinical events in heart transplant recipients. BMC Cardiovacsular Disorders 15:120
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Disclosures
Off label use of the AlloMap ® test will be discussed.
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