Validation of *CYP4F11* as a marker of accommodation in biopsies from recipients of an ABO-incompatible renal transplant

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Transplant accommodation occurs when the transplanted organ acquires a resistance to immune-mediated damage and was first identified in ABO incompatible transplant patients. These individuals have circulating anti-AB antibodies, A and/or B antigens expressed on endothelium and usually C4d deposition on the endothelium, yet with adequate immunosuppressive treatment, this seldom leads to antibody-mediated rejection. Previous work using RNA sequencing compared ABO-incompatible (ABOi) and ABO-compatible (ABOc) surveillance biopsies with normal histology and identified a number of differentially expressed genes¹ (Table 1). Following on from sequencing, we now present the validation of *CYP4F11* as a marker of accommodation using NanoString nCounter technology in an independent set of biopsies.

NanoString nCounter

Genes with the most significant p-value or fold change from sequencing analysis¹ were selected for replication by inclusion in an nCounter XT CodeSet (NanoString Technologies, Seattle, WA). *CD1e* included different exons because evidence suggested that there may have been differential expression of different transcript variants. RNA was extracted from formalin fixed paraffin embedded biopsy samples up to 8 years old. Expression in ABOc (n=20) and ABOi (n=9) was measured and normalised to reference (housekeeping) genes. Quality control and normalisation of raw gene expression counts were performed with nSolver™ Analysis Software Version 3.0. Default parameters for quality control flagging were used for imaging (field of view registration <75%), binding density (<0.05 or >2.25), positive control linearity (R2 value <0.95), and positive control limit of detection (0.5fM positive control ≤2 standard deviations above the mean of the negative controls). Background subtraction was performed for each sample by subtracting the mean of the negative controls from all data points. Differences in expression between the two test groups were measured using the non-parametric Mann-Whitney U test. Results are shown in Table 1 and Figure 1.

Table 1: Comparison of gene expression in ABOi and ABOc transplant recipients

Gene	RNA-seq		Nanostring replication (Mann-Whitney test)
	Fold Change	P	
<i>ANKRD1</i>	0.26	1.21x10 ⁻⁷	0.129
<i>ANKRD45</i>	0.82	5.72x10 ⁻³	0.105
<i>C11orf21</i>	0.41	2.71x10 ⁻⁴	0.069
<i>C4orf22</i>	3.83	3.89x10 ⁻²	0.220
<i>CD1e</i>	0.32	2.16x10 ⁻⁶	Exon 1 0.390 Exon 4 0.444 Exon 6 0.274
<i>CRYBA4</i>	22.06	8.19x10 ⁻⁴	0.556
<i>CSF2RB</i>	0.55	3.37x10 ⁻⁵	0.594
<i>CYP4F11</i>	0.15	5.72x10 ⁻³	0.0005
<i>FPR2</i>	4.07	2.67x10 ⁻⁶	0.999
<i>GSTT2B</i>	6.06	7.74x10 ⁻³	0.764
<i>KLHL1</i>	0.4	8.55x10 ⁻⁴	0.645
<i>MMRN1</i>	0.35	6.41x10 ⁻³	0.945
<i>MYO1G</i>	0.33	7.80x10 ⁻²	0.248
<i>RASSF9</i>	0.82	7.80x10 ⁻²	0.908
<i>SLC25A18</i>	1.56	4.57x10 ⁻⁴	0.971
<i>XCR1</i>	0.17	3.41x10 ⁻²	0.317

Figure 1: Expression of *CYP4F11* in ABOi and ABOc transplant recipients

CYP4F11

Most of the substrates of cytochrome p450 4F isoforms are eicosanoids, which play important roles in the inflammatory response. Cytochrome 4F enzymes metabolize arachidonic acid to 20-hydroxyeicosatetraenoic acid (20-HETE). *CYP4F11* is known to have high expression in the kidney and 20-HETE inhibitors have demonstrated improved renal microvascular function in hypertension. The down-regulation of *CYP4F11* in ABOi transplants may be likewise protecting the microvasculature, suggesting possibilities for anti-rejection drugs which inhibit 20-HETE in order to promote a state of accommodation.

Further Discussion

There was no replication of the sequencing findings for 15 of the 16 genes tested for replication. A significant limitation to these analyses is the relatively low frequency of stable ABOi biopsy samples that are available for study. Larger sample numbers would improve the power of detection. It is also possible that specific transcript variants are contributing to the accommodation phenomenon, which are not individually detected in the Nanostring system but are identified during sequencing. Further work is required to determine whether any other genes identified during sequencing are genuine markers of accommodation.

¹Use of RNA Sequencing for Discovery of Markers of Accommodation in Surveillance Biopsies from Recipients of an ABO-Incompatible Renal Transplant
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