1 Array design description

A brief description of the array design, feature location, information on the cDNA collection and the spotting protocols can be found on the producer website (http://www.microarray.org/sfgf/jsp/home.jsp).

Protocols for the post processing procedure can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2 Experiment description

2.1 Experimental design

2.1.1 Laboratory, authors, contact

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2.1.2 Type of experiment

Comparison of donor organs between corticosteroid (steroid group) and placebo (placebo group).

2.1.3 Experiment factor

The expression of genes were investigated and compared between the two groups steroid and placebo.

2.1.4 Hybridizations

Forty hybridizations (20 replicates in group steroid, 20 in group placebo).

2.1.5 Reference

Universal Human reference RNA (Stratagene®) was used as hybridization reference.

2.1.6 Quality control

To test for reproducibility of sample processing, RNA of three specimen was reprocessed twice.

2.2 Samples used, extract preparation and labeling

2.2.1 Bio-source properties

Organism: *Homo sapiens*
Demographic data of kidney donors can be found in the manuscript and on our website (http://www.meduniwien.ac.at/nephrogene/).

2.2.2 Biomaterial manipulations, hybridization and labeling protocol

Kidney wedge biopsies were obtained immediately before implantation of the grafts and submerged into RNAlater^{TM} to preserve RNA.

Protocols for RNA extraction, RNA labeling, hybridization and washing of microarrays can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.3 Hybridization procedures and parameters

Steroid samples	Array	Placebo samples	Array
PF-C124R	SHEO20	PF-P147L	SHEO19
PF-C134L	SHEO68	PF-P196R	SHEO210
PF-C145R	SHEO67	PF-P143R	SHEO64
PF-C157L	SHEO66	PF-P151L	SHEO141
PF-C184L	SHEO65	PF-P118R	SHEO41
PF-C124L	SHEO142	PF-P117L	SHEO40
PF-C139R	SHEO190	PF-P118L	SHEO42
PF-C386L	SHEO189	PF-P505R	SHEO191
PF-C139L	SHEO168	PF-P505L	SHEO192
PF-C389L	SHEO167	PF-P327L	SHEO166
ARF-C106R	SHEO21	ARF-P162L	SHEO18
ARF-C198R	SHEO165	ARF-P117R	SHEO22
ARF-C113L	SHEO214	ARF-P125L	SHEO39
ARF-C113R	SHEO213	ARF-P138L	SHEO38
ARF-C168R	SHEO139	ARF-P503R	SHEO193
ARF-C198L	SHEO143	ARF-P376L	SHEO164
ARF-C172R	SHEO243	ARF-P180R	SHEO212
ARF-C501R	SHEO242	ARF-P393L	SHEO211
ARF-C195R	SHEO241	ARF-P355L	SHEO140
ARF-C101R	SHEO139	ARF-P131R	SHEO240

The hybridization protocol can be found on our website (http://www.meduniwien.ac.at/nephrogene/).

2.4 Measurement data and specification of data processing

2.4.1 Raw data description
Scan hardware: GenePix Personal 4100 A
Scan software: GenePix Pro 6.0

Raw data images can be found in the data section of our website
(http://www.meduniwien.ac.at/nephrogene/).

Array	Laser power		PMT Gain		Lines Averaged	Background Subtraction	Scan region
	635 nm	532 nm	635 nm	532 nm			
SHEO139	3.42	3.48	485	495	1	LocalFeature	62,537,2013,7049
SHEO21	3.45	3.54	600	520	1	LocalFeature	155,458,2060,6979
SHEO214	3.50	3.44	530	530	1	LocalFeature	101,454,1977,7057
SHEO213	3.42	3.43	485	495	1	LocalFeature	101,477,1977,7002
SHEO139	3.41	3.52	555	490	1	LocalFeature	122,474,2021,7069
SHEO243	3.71	3.54	580	550	1	LocalFeature	88,454,1966,7017
SHEO241	3.52	3.55	550	580	1	LocalFeature	86,469,1994,7057
SHEO143	3.43	3.49	670	430	1	LocalFeature	122,474,2003,7101
SHEO165	3.55	3.56	580	530	1	LocalFeature	130,474,2011,7036
SHEO242	3.46	3.51	470	460	1	LocalFeature	86,477,1994,7049
SHEO22	3.41	3.50	610	515	1	LocalFeature	188,409,2070,7011
SHEO39	3.33	3.48	610	550	1	LocalFeature	204,618,2053,6987
SHEO240	3.48	3.52	515	520	1	LocalFeature	86,469,1994,7064
SHEO38	3.37	3.49	570	555	1	LocalFeature	216,488,2058,6970
SHEO18	3.42	3.52	610	505	1	LocalFeature	172,458,2077,7003
SHEO212	3.45	3.49	510	595	1	LocalFeature	101,477,1977,7057
SHEO140	3.39	3.51	540	480	1	LocalFeature	122,482,2021,7069
SHEO164	3.47	3.54	600	460	1	LocalFeature	130,474,2011,7085
SHEO211	3.36	3.46	585	560	1	LocalFeature	101,548,1973,7049
SHEO19	3.52	3.41	600	560	1	LocalFeature	124,578,1973,7033
SHEO142	3.45	3.53	580	510	1	LocalFeature	122,458,2021,7069
SHEO20	3.50	3.57	600	450	1	LocalFeature	179,458,2070,7020
SHEO68	3.31	3.45	565	520	1	LocalFeature	139,475,2070,6946
SHEO168	3.46	3.52	565	500	1	LocalFeature	130,474,2011,7061
SHEO190	3.39	3.52	580	450	1	LocalFeature	122,474,2008,7061
SHEO67	3.29	3.46	580	520	1	LocalFeature	163,335,2043,6847
SHEO66	3.29	3.42	560	525	1	LocalFeature	154,507,2046,6987
SHEO65	3.27	3.46	580	525	1	LocalFeature	170,507,2062,7003
SHEO189	3.42	3.52	555	460	1	LocalFeature	127,474,2008,7085
SHEO167	3.54	3.52	600	480	1	LocalFeature	122,474,2003,7069
SHEO40	3.36	3.47	590	575	1	LocalFeature	190,483,2064,6913
SHEO42	3.40	3.48	585	550	1	LocalFeature	188,499,2062,6987
SHEO41	3.32	3.48	600	550	1	LocalFeature	172,581,2053,6929
SHEO64	3.37	3.47	590	565	1	LocalFeature	164,458,2045,6987
SHEO193	3.41	3.52	610	535	1	LocalFeature	172,458,2077,7069
SHEO141	3.40	3.49	540	490	1	LocalFeature	122,474,2021,7069

Array	Laser power		PMT Gain		Lines Averaged	Background Subtraction	Scan region
	635 nm	532 nm	635 nm	532 nm			
SHEO210	3.36	3.44	585	565	1	LocalFeature	101,462,1973,7010
SHEO166	3.50	3.58	590	460	1	LocalFeature	130,474,2011,7110
SHEO192	3.49	3.51	585	540	1	LocalFeature	124,462,1973,7010
SHEO191	3.49	3.44	585	570	1	LocalFeature	136,477,1984,7033

2.4.2 Image analysis and quantitation

Image griding and calculation of spot intensity was performed with GenePix Pro 6.0 software.

2.4.3 Normalized and summarized data

Normalization:
Normalization was done through the default computed normalization by SMD (see http://genome-www5.stanford.edu/help/results_normalization.shtml). For data retrieval the log2 (R/G normalized ratio [mean]) was used.

Computation of missing values:
Missing values were obtained through computation of k-nearest-neighbor (k=10) with the EMV module (http://cran.r-project.org/src/contrib/Archive/EMV) of the R software package (http://cran.r-project.org).

Cluster analysis:
Before cluster analysis different standard deviation filters were applied (program Cluster ©1998-9 (http://rana.lbl.gov/EisenSoftware.htm). Software used for cluster analysis was TIGR Multi Experiment Viewer 3.0.3 (http://www.tm4.org/mev.html).

Linkage rule: Complete linkage
Distance measure: 1 – Cosine Correlation

Significance analysis:
We used the significance analysis of microarrays (SAM) to determine significant differentially expressed genes (DEGs) between steroid and placebo treatment. The number of permutations was set to one hundred and genes with a fold change over 2 and a delta value over 1.2 were assigned as DEGs resulting in a false discovery rate (median) of 0.47%. Software used for SAM analysis was TIGR Multi Experiment Viewer 3.0.3 (http://www.tm4.org/mev.html).