METALLOTHIONEINS AS MARKERS FOR BIOLOGICAL ORGAN AGE IN PREIMPLANTATION KIDNEY BIOPSIES

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Background

Structure and function of the kidney deteriorate with age and age-related diseases contribute to this process, leading to the high frequency of end-stage renal disease in the elderly. The difference between chronological and biological age is a main problem in determining donor kidneys for transplantation. The ideal situation would be to match the functional capacity of a donor kidney to the patient's renal requirements. Therefore the identification of markers for biological organ age is a strong clinical need.

METHODS

Age-regulated gene expression changes in 37 zero hour kidney biopsies of non-AKI (valid informed consent available; deceased donor with no signs of AKI (serum creatinine < 0.96 for women and < 1.18 for men); complete donor characteristics available (age, sex, creatinine, cause of death, catecholamine administration yes/no); if left and right kidney samples of the same donor were available, only one was randomly selected) donors were determined using microarray technology followed by ANOVA and SAM analysis. Expression changes of selected genes were confirmed by quantitative real-time PCR. *In situ* hybridization was used to localize mRNA expression in zero hour biopsies.

RESULTS

Donors were classified into 3 age groups (<40, 40-59, >60 years). 349 genes with altered expression associated with age were identified. These genes were mostly related to Gene Ontology classes of immunity, apoptosis, cell structure and motility and stress response. 16 Transcripts were found to be significantly upregulated in group 3 (>60 years) compared to group 1 (<40). The upregulated transcripts were dominated by genes encoding for metallothionein (MT) isoforms. MTs are a family of small cysteine rich proteins, which have been implicated in diverse functions such as toxic metal detoxification, protection against oxidative stress, and in the homeostasis of both zinc and copper. *in situ* hybridization and immuno-histochemistry demonstrated localization of MT mRNA in renal proximal tubular cells. This indicates a protective role of MTs, the proximal tubule is very susceptible to a variety of factors associated with age.

PROBE	ACCNUM	SYMBL	DESCRIPTION	Gr1 mean expr	Gr2 mean expr	Gr3 mean expr	Correlation to age
A_23_P43979	M87790	IGLL5	immunoglobulin lambda-like polypeptide 5	7,13	8,80	9,18	0,39
A_23_P66241	NM_176870	MT1M	metallothionein 1M	9,14	10,21	10,69	0,38
A_23_P414343	NM_005951	MT1H	metallothionein 1H	10,80	11,98	12,50	0,45
A_23_P303242	NM_005952	MT1X	metallothionein 1X	10,82	11,96	12,48	0,45
A_24_P125096	NM_005952	MT1X	metallothionein 1X	10,27	11,38	11,86	0,42
A_23_P427703	X97261	MT1L	metallothionein 1L	10,52	11,80	12,26	0,45
A_23_P106844	NM_005953	MT2A	metallothionein 2A	11,96	13,01	13,48	0,39
A_23_P206707	NM_005950	MT1G	metallothionein 1G	9,31	10,34	10,80	0,38
A_23_P206724	NM_175617	MT1E	metallothionein 1E	10,67	11,93	12,42	0,46
A_23_P60933	NM_005950	MT1G	metallothionein 1G	11,39	12,63	13,04	0,41
A_23_P37983	NM_005947	MT1B	metallothionein 1B	10,55	11,76	12,19	0,42
A_23_P252413	NA	NA	NA	11,46	12,54	13,03	0,40
A_32_P200144	AK130614	IGHG1	IG heavy constant gamma 1	6,76	8,60	8,97	0,39
A_23_P163782	NM_001039954	NA	NA	10,33	11,45	11,91	0,41
A_24_P361896	NM_005953	MT2A	metallothionein 2A	11,65	12,81	13,33	0,43
A_23_P54840	NM_005946	MT1A	metallothionein 1A	10,83	11,85	12,30	0,42

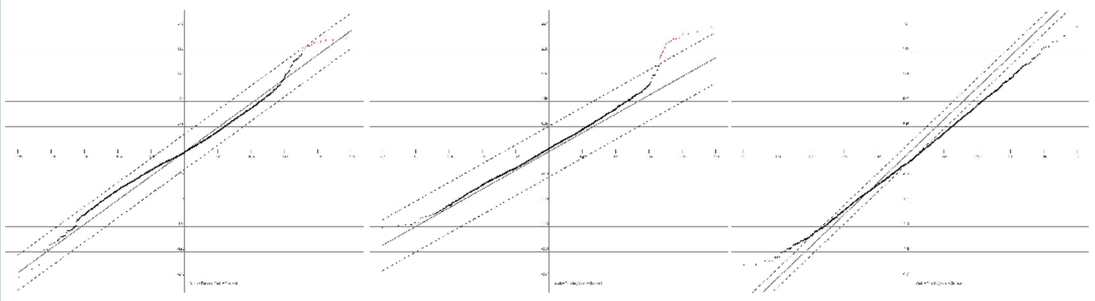

Fig. 1 To optimize the number of features and thus the number of statistical test, a filter to focus only on those features having a coefficient of variation being one standard deviation above the mean coefficient of variation of all 41091 features across all 37 arrays was applied. After this filter 4164 features remained in the dataset. SAM analysis yielded the plots above, identifying 16 features as statistically upregulated in group 3 as compared to group 1.

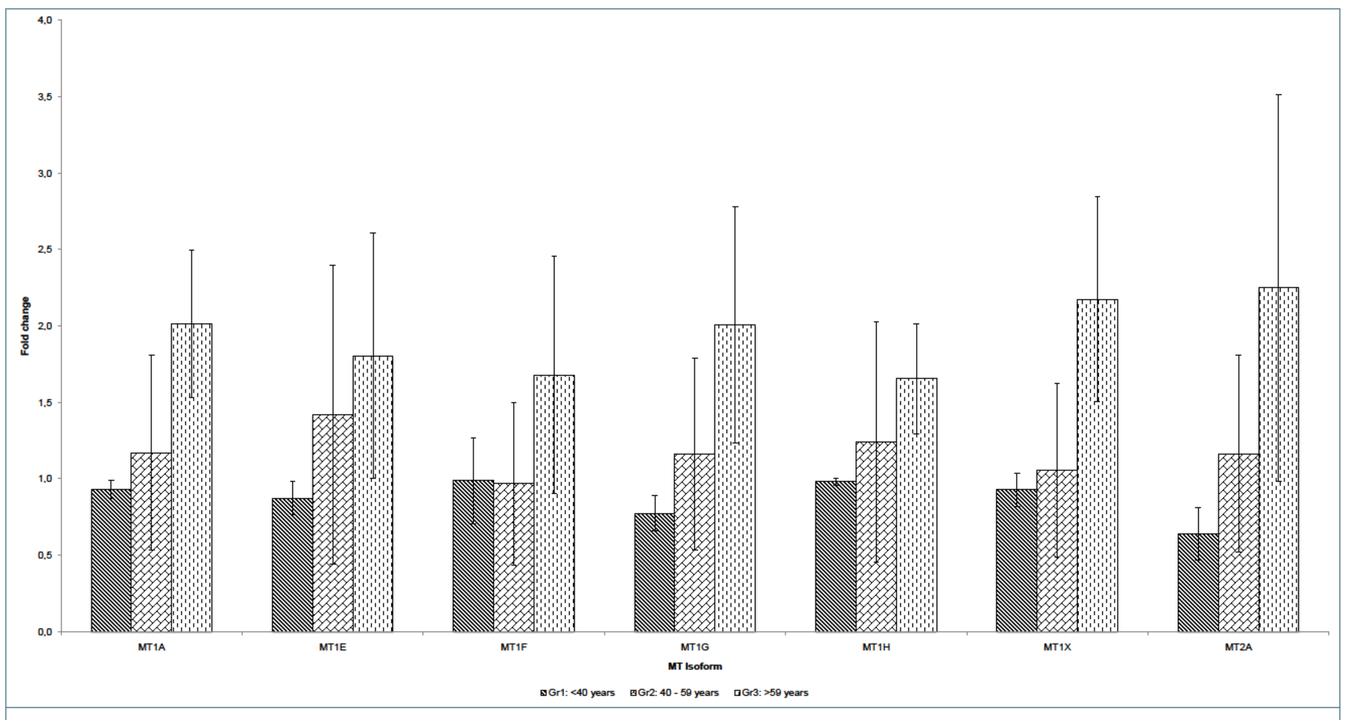

Fig. 2 mRNA levels of Metallothionein isoforms compared in three age groups measured by quantitative real time PCR.

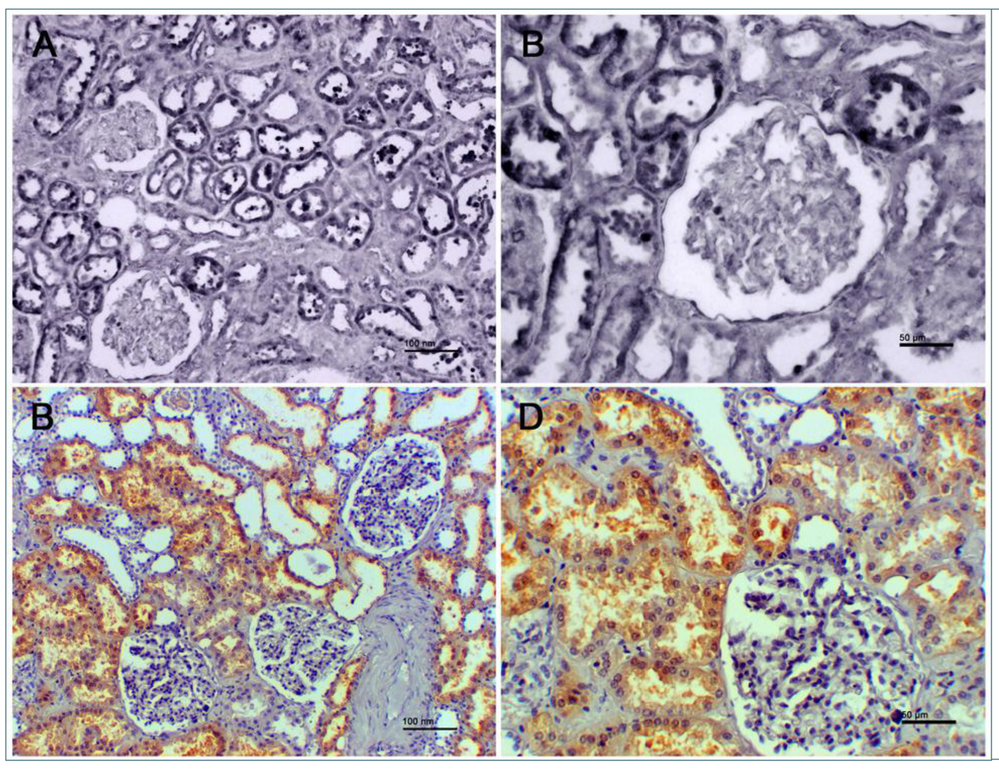

Fig. 3 *in situ* hybridization (A, B) and immuno-histochemistry (C, D) for MT. On the mRNA level (dark blue/black labels in A,B) as well as on protein level (brown colored in C,D) MTs seem to be located in (epithelial cells of) proximal tubuli.

CONCLUSIONS

Metallothionein expression might serve as a marker for renal biological age. Additionally, a functional role of MTs can be postulated as Metallothioneins contribute to detoxification of heavy metals and homeostasis of essential metals and protect from ROS mediated oxidative stress and prevent apoptosis.

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