A pilot study correlating IDH-1/2 gene status with 2-Hydroxyglutarate concentration in plasma and urine from patients with glioma

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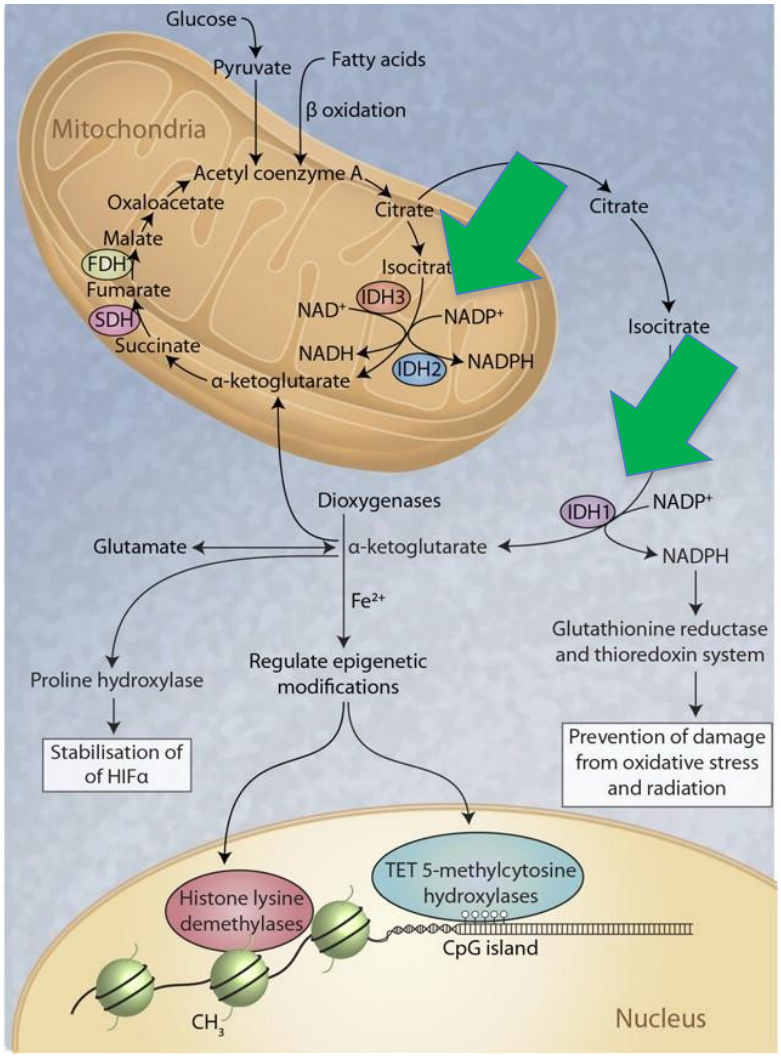
Disclosure

- **No conflict of interest to declare**

Background



Gupta et al, JCP 2011

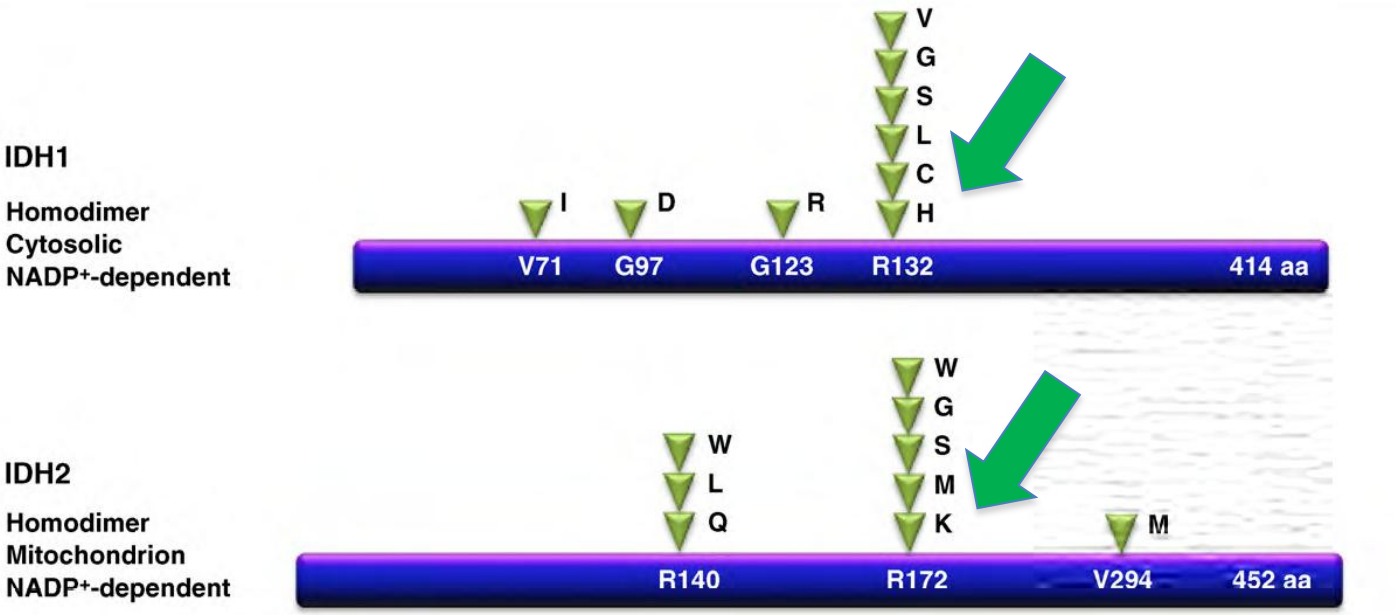
- Isocitrate dehydrogenase (IDH) 1 and 2 are enzymes involved in the citric acid cycle and catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate

- Mutations of IDH1 and IDH2 genes are found in 70-80% of grade II and III astrocytomas, oligodendrogliomas, oligoastrocytomas and secondary glioblastomas[1]

- Mutations of IDH1 and IDH2 genes are considered to be indipendent prognostic factors in these patients[2]

[1]Dang et al, Nature 2009
[2]Yan et al, NEJM 2009

Background



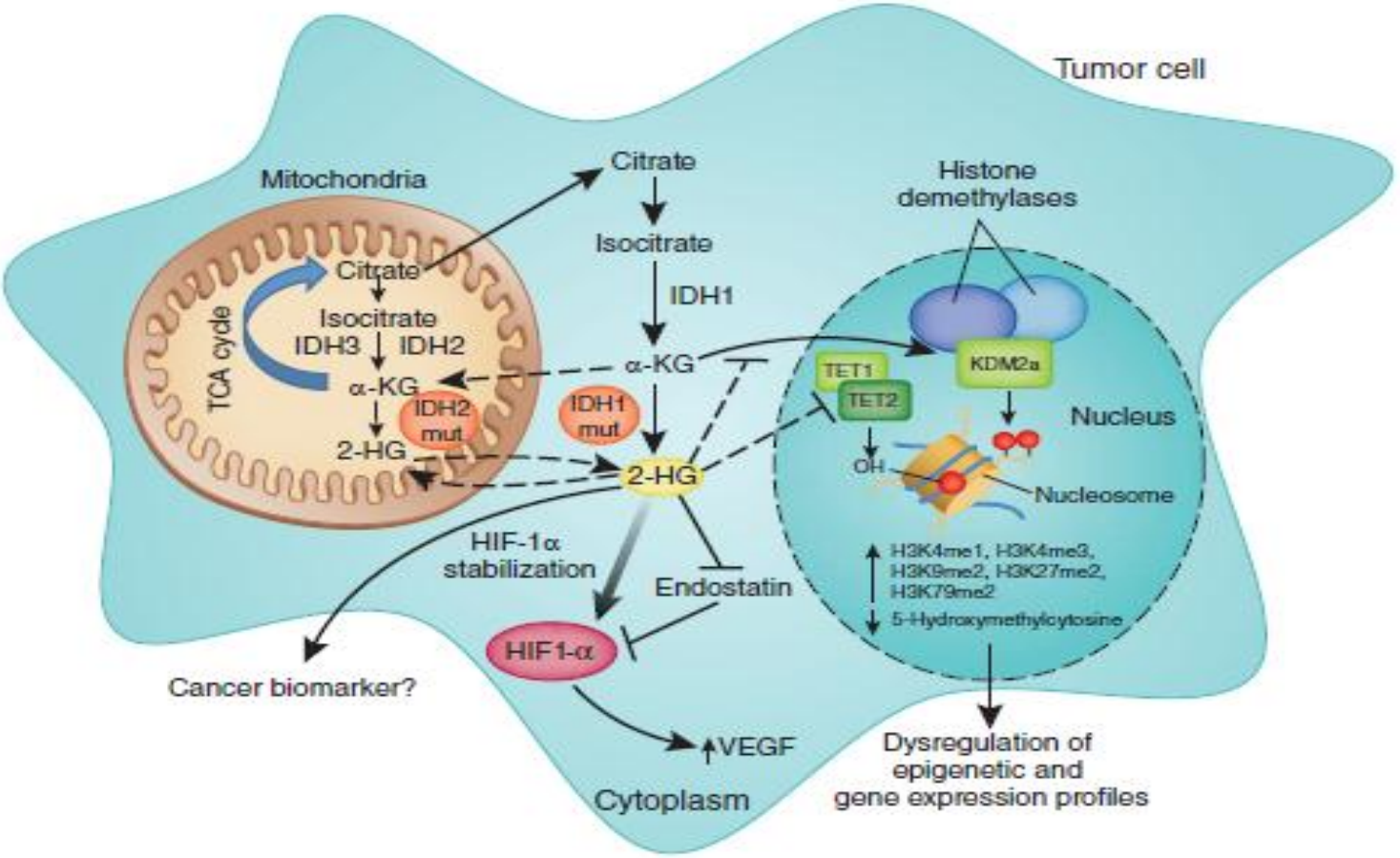
Dang et al, Trend in Mol Med 2010

- Mutations are typically found in **codon 132 in exon 4 of IDH1** and **172 in exon 4 of IDH2**. Of the mutations in IDH1, 95% are of a single variant R132H, resulting in substitution of arginine by histidine[1]

- Mutations of IDH1 and IDH2 are mutually exclusive in glioma patients[1]

[1]Ichimura, Brain Tum Pathol 2012

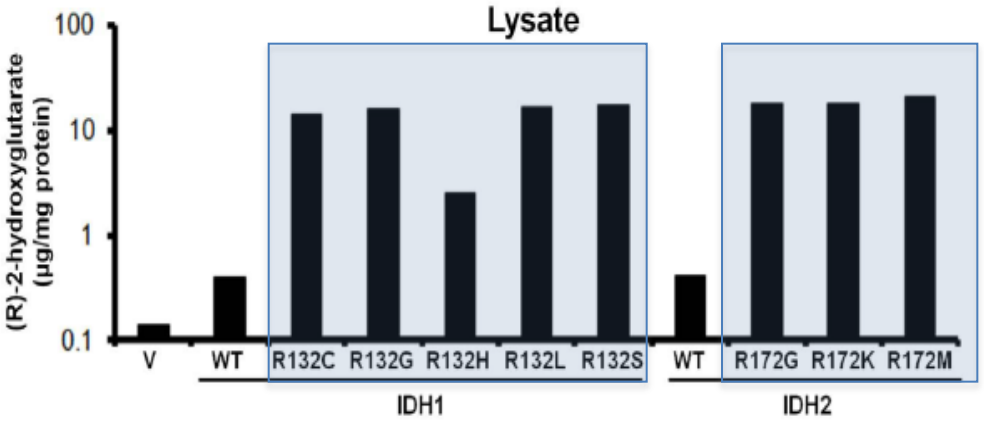
Background

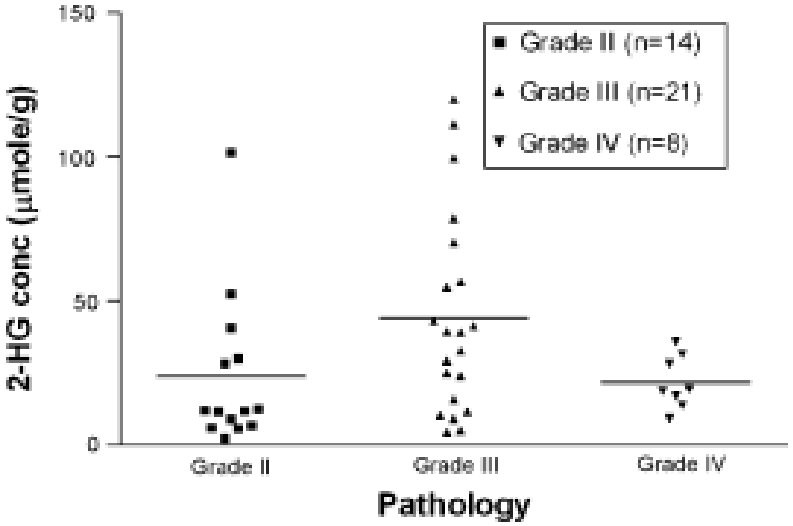


- Mutant IDH1 and IDH2 enzymes show a neomorphic enzymatic **capacity to convert α-KG into D-2HG**, which can be considered an oncometabolite[1]

[1]Prensner et al, Nature Medicine, 2011

Background

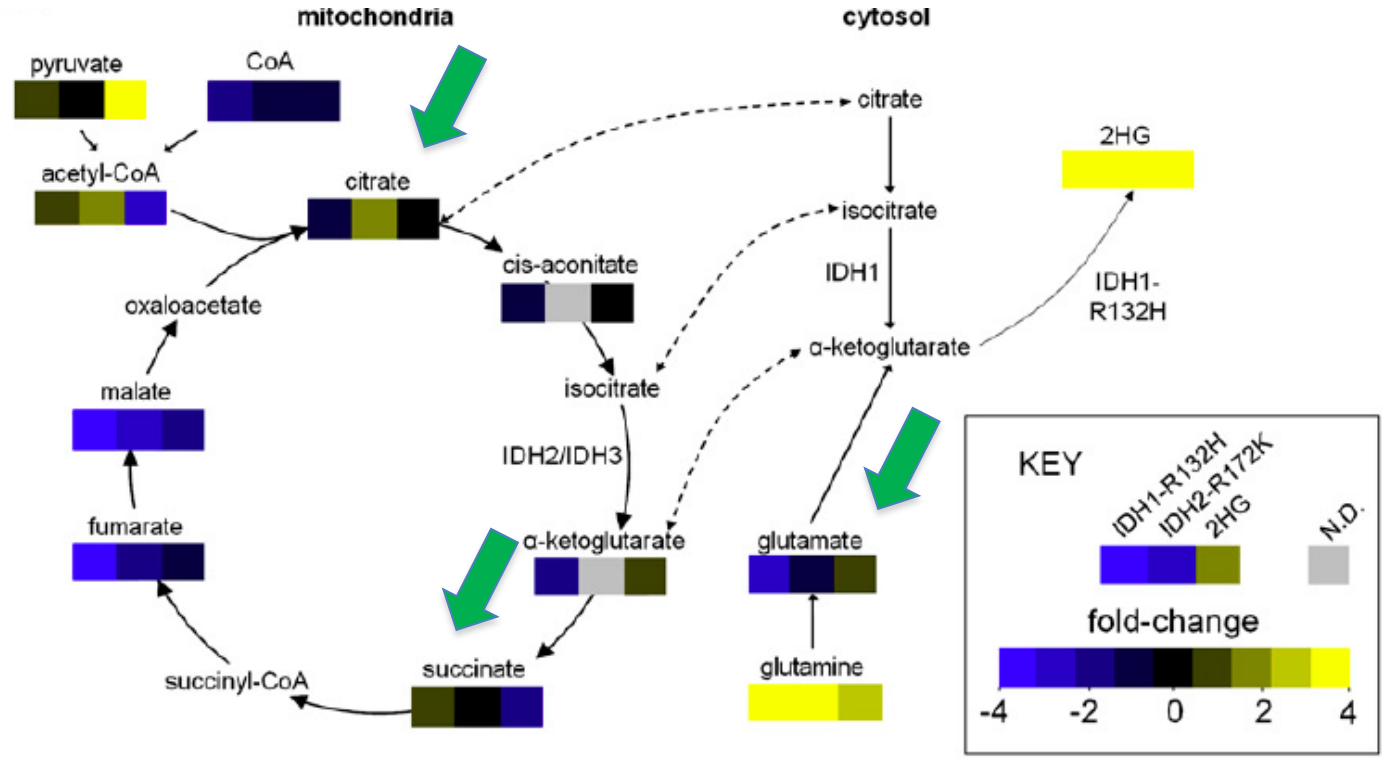




- Patients with IDH mutations have **highly elevated amounts of intracellular D-2HG** (up to 100-fold higher than wild-type IDH)[1]
- **Tumor grade** does not correlate with intracellular 2HG concentration in IDH mutant gliomas [2]

[1]Jin et al, Plos One 2011
[2]Dang Pope et al, J Neurooncol 2011

Background



- In addition to intracellular 2HG accumulation, levels of **other citric acid cycle intermediates** such as glutamate, succinate and citrate can be altered in mutant IDH1/2-expressing cells[1]

[1]Reitman et al, PNAS 2011

Aims of the Study

- Investigate the effect of mutant and wild-type IDH1/2 genes on **plasma and urinary 2HG** concentration in patients with glioma

- Investigate the correlation between **tumor volume/grade and 2HG concentration** in plasma and urine

- Investigate whether mutant IDH1/2 may influence the plasma and urinary concentrations of **other metabolites** involved in the citric acid cycle

Methods

Major Inclusion Criteria:

- A prior biopsy/ surgery of the brain tumor and histological confirmation of glioma
- Neoplastic tissue available for analysis of IDH1/2 genes by PCR and sequence analysis
- A recent brain MRI (within 2 weeks) showing the neoplastic lesion
- Written consent

Major Exclusion Criteria:

- Absence of neoplastic lesions on brain MRI
- Any chemotherapy performed within 28 days prior
- Other neoplastic and metabolic diseases
- Renal and/or liver failure

Methods

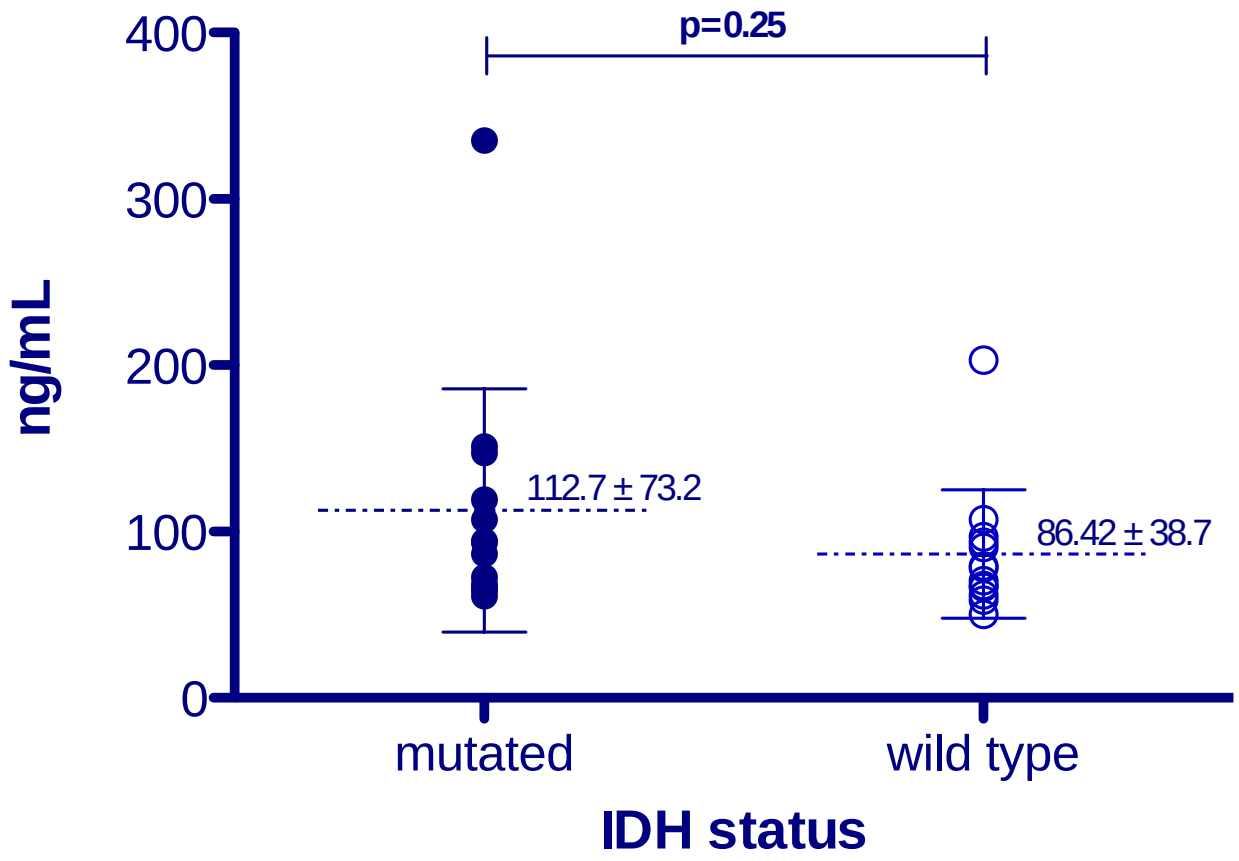
- **Plasma and overnight-urine samples** were taken from all the patients
- **Exon 4 of IDH1/2 genes** were analyzed by Sanger sequencing
- **Metabolite concentrations** in urine and plasma were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS)
- **Mann-Whitney test** was used to test for differences in metabolite concentrations between mutant and wild-type IDH1/2 patients
- **Tumor volume** was estimated based on Flair imaging for LGGs and on contrast-enhanced tumor area for HGGs using the formula for an ellipsoid

Results

Patients with mutant IDH1/2	
Sex	5 Females, 8 Males
Average Age	51.5 ys
Histology	11 HGGs *(4 AO, 3 AOA, 1 AA, 3 GBM)* 2 LGGs (grade 2 A, grade 2 OA)
Mutations	IDH1_R132H (all patients)
Average size of the tumor	64 cm^3

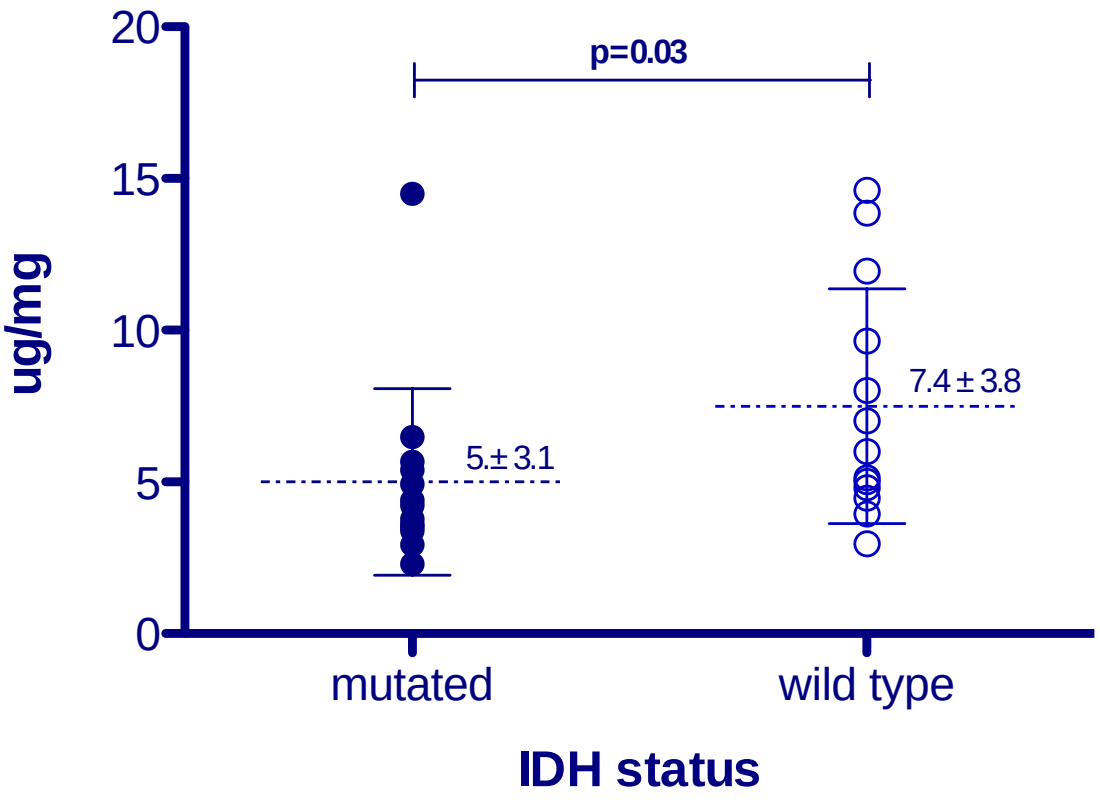
Patients with wild type IDH1/2	
Sex	5 Females, 8 Males
Average Age	60 ys
Histology	13 HGGs *(1 AO, 1 AA, 11 GBM)*
Mutations	none
Average size of the tumor	61 cm^3

2HG concentration in plasma



No statistically significant difference of 2HG concentration between patients with mutant and wild-type IDH was detected *(p=0.25, Mann-Whitney test)*

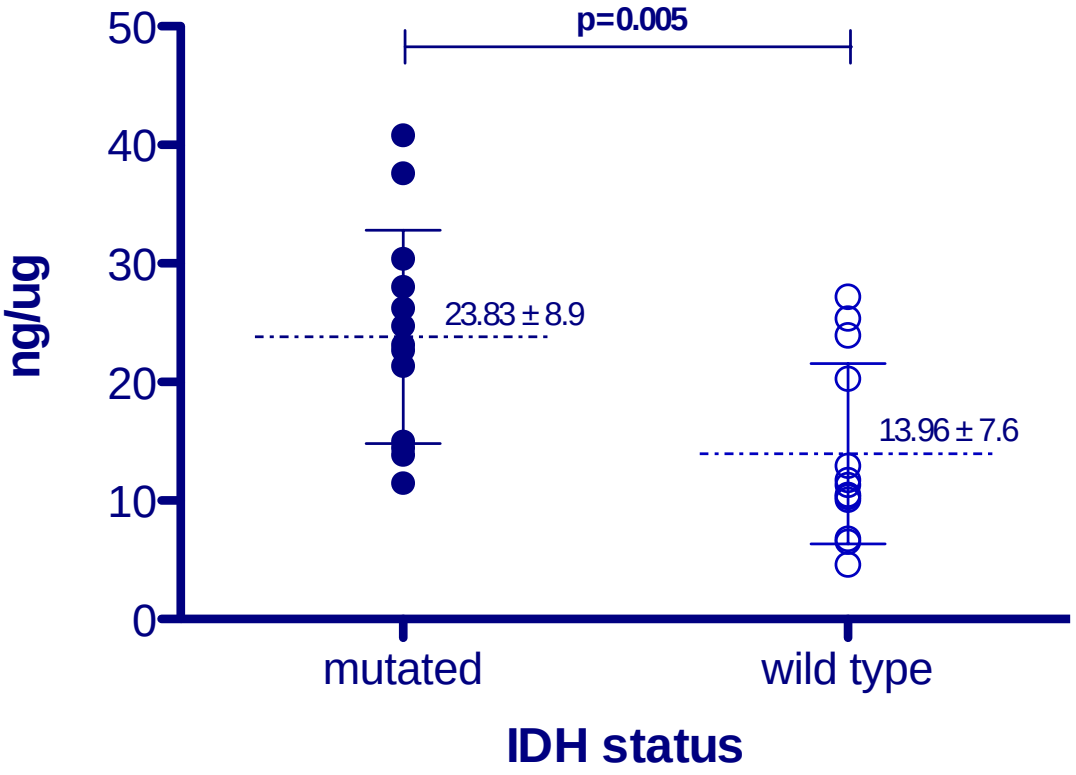
2HG concentration in urine*



*normalized by creatinine concentration

A statistically significant difference of urinary 2HG concentration between patients with mutant and wild-type IDH was detected *(p=0.03, Mann-Whitney test)*

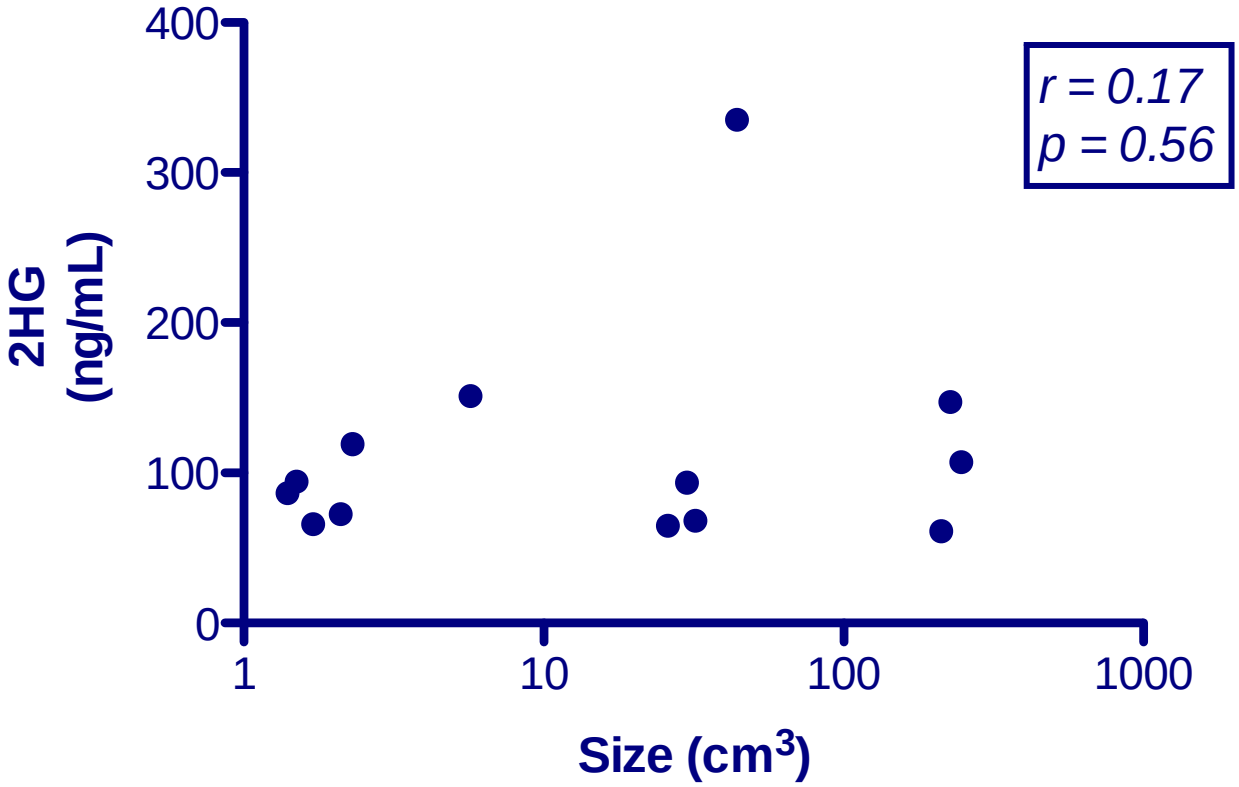
Ratio 2HG concentrations in plasma/urine*



*normalized by creatinine concentration

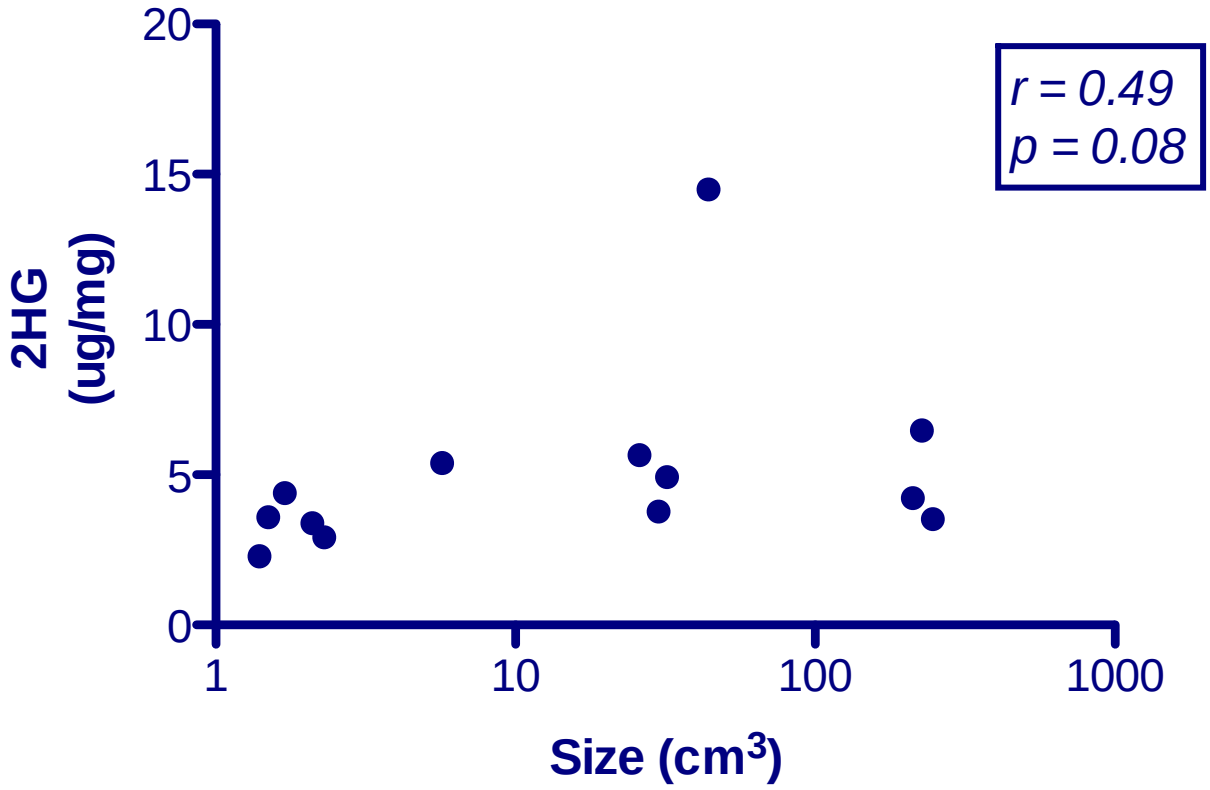
A statistically significant difference in the ratio of 2HG concentrations in plasma and urine between patients with mutant and wild-type IDH was detected *(p=0.005, Mann-Whitney)*

Correlation between plasma 2HG and tumor size in IDH mutated



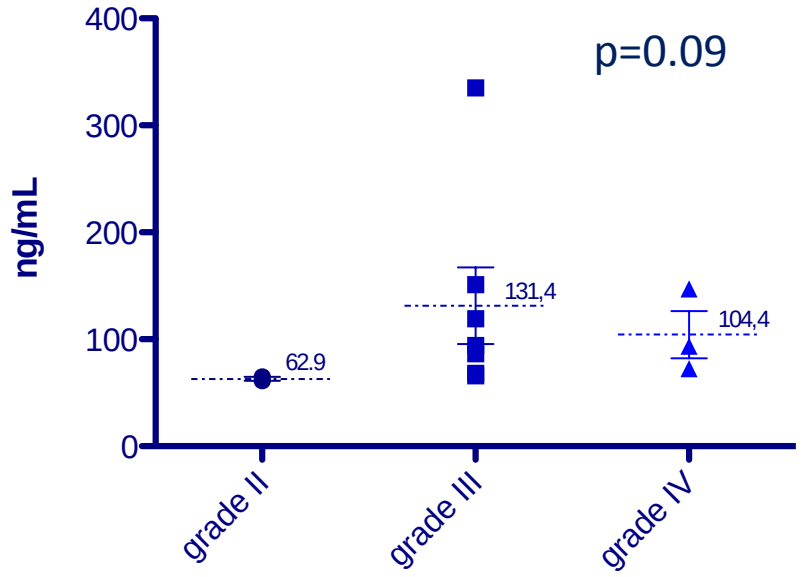
No statistically significant correlation of plasma 2HG levels and tumor volume was detected *(p=0.56, Spearman r)*

Correlation between urinary 2HG and tumor size in IDH mutated

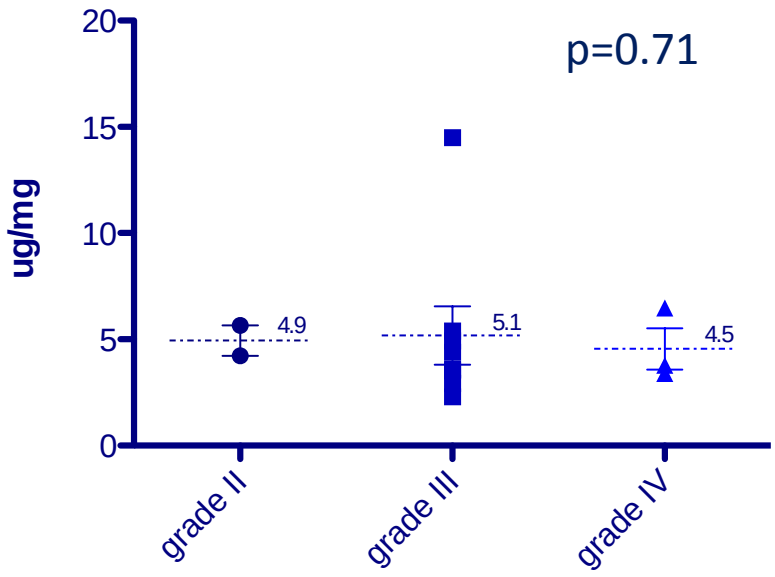


Although there is a trend for an association (p=0.08, Spearman r), no statistically significant correlation of urinary 2HG levels and tumor volume was detected

Plasma 2HG concentration and tumor grade

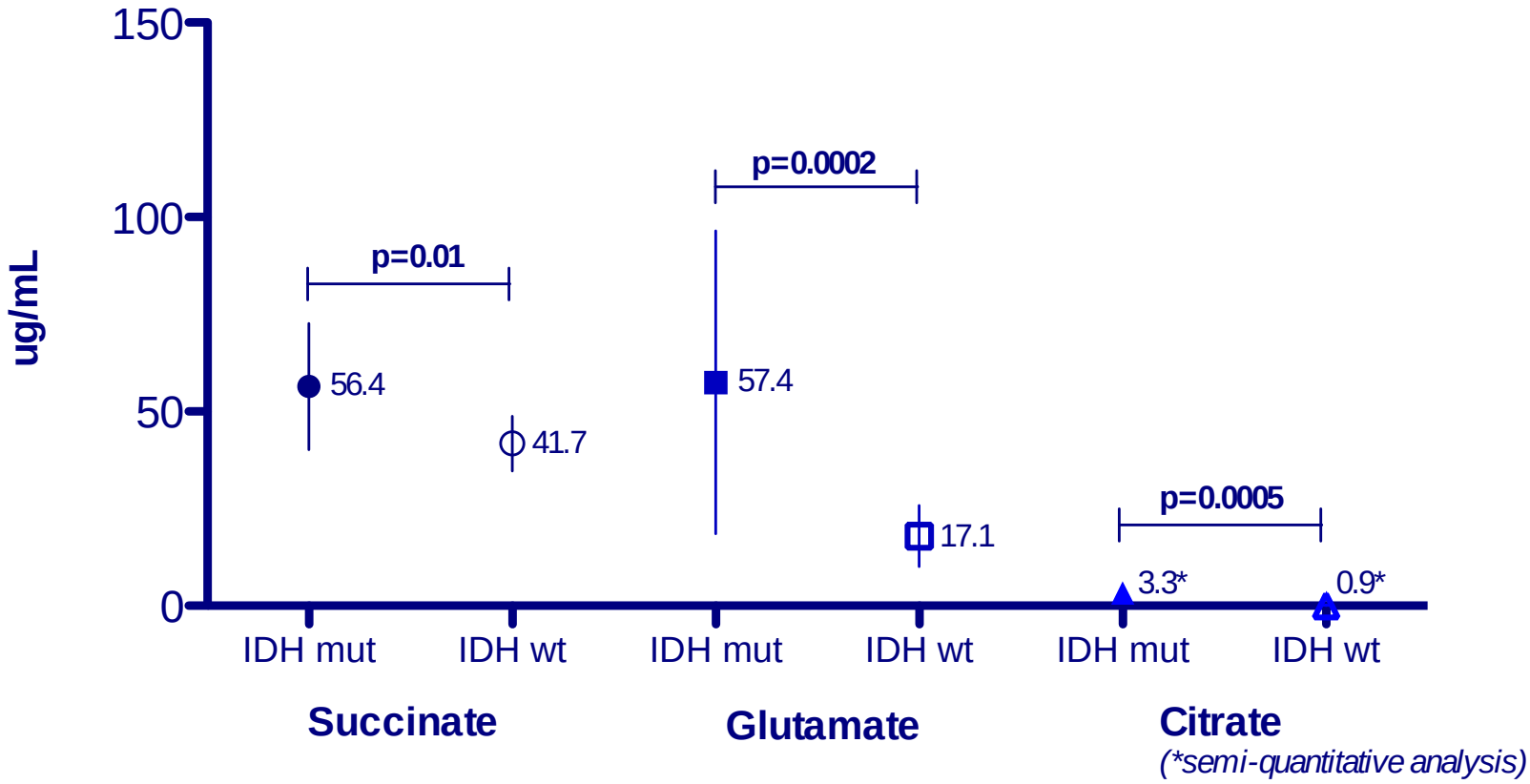


Urinary 2HG concentration and tumor grade



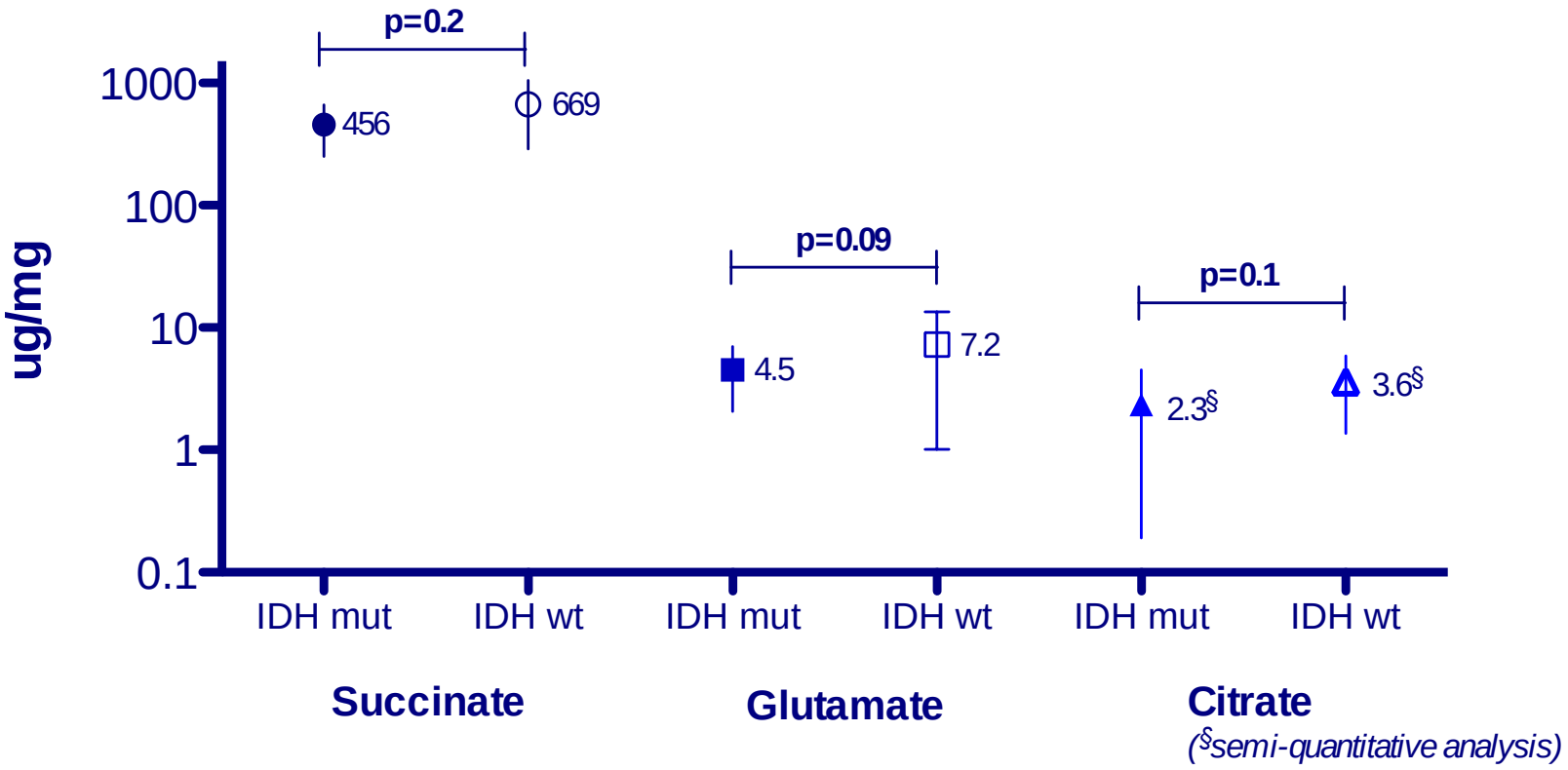
No statistically significant differences of plasma and urinary 2HG concentrations among gliomas with different tumor grades

Concentrations of other metabolites in plasma



Statistically significant differences of succinate, glutamate and citrate concentrations between patients with mutant and wild-type IDH were detected *(Mann-Whitney test)*

Concentrations of other metabolites in urine*



*normalized by creatinine concentration

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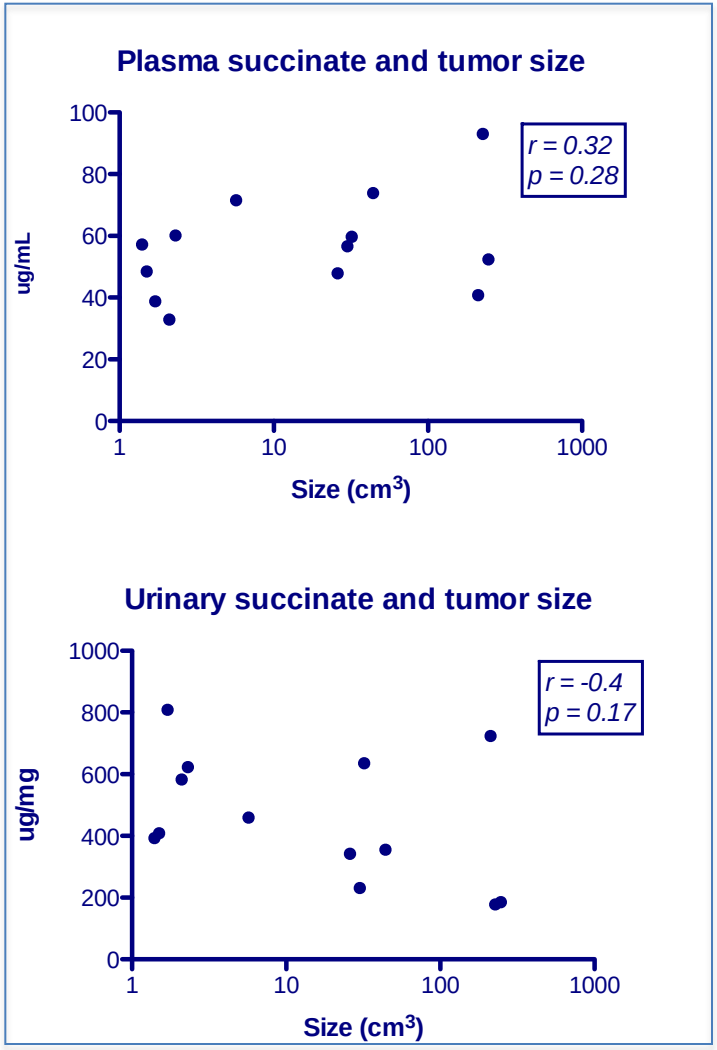

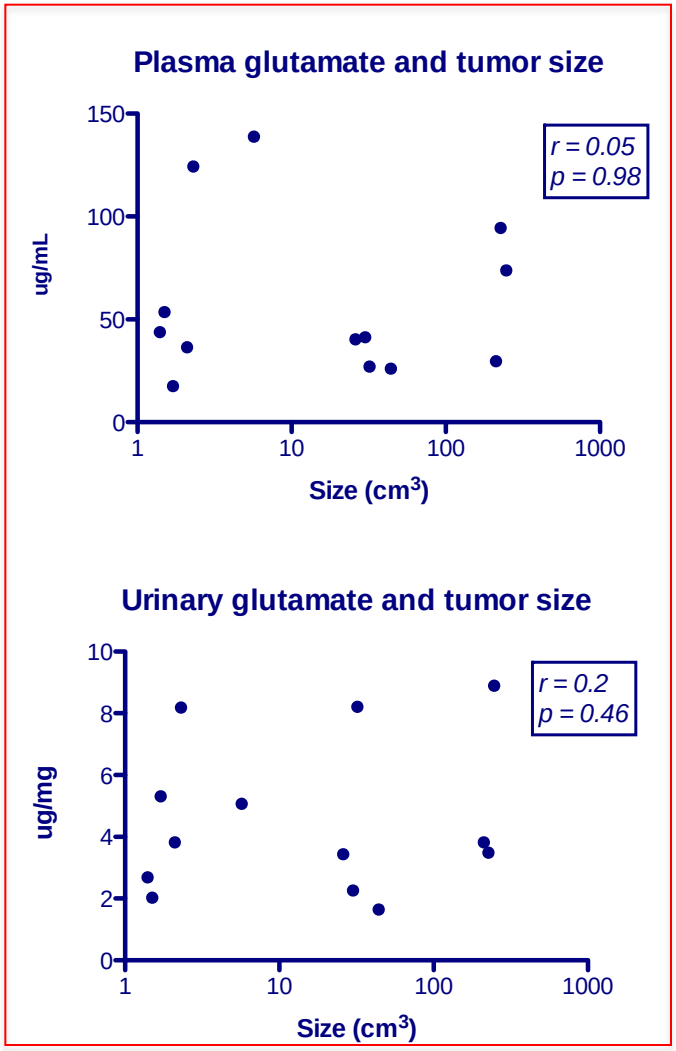

No significant correlations of plasma and urinary levels of the metabolites and tumor volume were detected *(Spearman r)*

Summary

- **Urinary 2HG concentration** in patients with mutant IDH was statistically lower than patients with wild-type IDH and there was a trend for a correlation with tumor size

- No statistical difference in **plasma 2HG concentration** was observed between mutant and wild-type IDH and no association with tumor volume was found

- In patients with mutant IDH **succinate, glutamate and citrate** had statistically higher plasma concentrations, but no associations with tumor volume were detected

- **Tumor grade** did not correlate with plasma and urinary 2HG concentrations in mutant IDH gliomas

Conclusions

This is the first study analyzing the concentrations of the metabolites involved in the citric acid cycle in both plasma and urine from glioma patients with mutant IDH gene.

However, a larger study is ongoing to draw final conclusions, in particular to analyze whether:

- These specific metabolic alterations might serve as **surrogate markers** for IDH mutations
- **Urinary 2HG concentration** might be used to monitor tumor growth and response to the treatment

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