

SUPPLEMENTAL MATERIAL

Sample Processing and Purification

Collagen purification was performed under sterile conditions in a laboratory with no contamination with ^{14}C in order to avoid sample contamination and false increase of ^{14}C levels. For isolation of collagen from CAs, cerebral arteries and mouse tendons, tissue fragments were minced with a scalpel to increase protease accessibility. Tissue fragments were suspended in at least 1 mL of 20 mmol/L HCl. Collagens were extracted after digestion with 100 μg of pepsin/g of tissue at 4°C for 72 hours and were precipitated for 6 hours after addition of solid NaCl to a final concentration of 2.5 mol/L. After centrifugation, collagen was re-dissolved in 20 mmol/L HCl and exhaustively dialyzed against 20 mmol/L HCl. The dialysate was lyophilized using a Concentrator Plus ® (Eppendorf, Hamburg, Germany) and subjected to birth dating by ^{14}C -accelerator mass spectrometry (AMS). For selected samples, collagen content and purity were assessed by sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) and residual tissue fragments after pepsin digestion were used to quantify the amount of remaining collagens using the total collagen assay (Quickzyme biosciences, Leiden, Netherlands) according to the manufacturer's instructions.

Birth Dating of CA Collagen

All ^{14}C -AMS analyses were performed blinded to patient and CA-related data. Purified collagen samples were transferred to 6 mm O.D. quartz combustion tubes and lyophilized. Excess copper oxide was added to each dry sample and tubes were evacuated and sealed with a H_2/O_2 torch. Samples were combusted at 900°C for 3.5 hours and allowed to cool to room temperature overnight. The evolved CO_2 from each sample was cryogenically purified, trapped and reduced to graphite in the presence of iron catalyst in individual reactors.^{1,2} The $^{14}\text{C}/\text{C}$ concentration in the graphite was measured at the Centre for AMS, Lawrence Livermore National Laboratory using the 10 MV High Voltage Engineering Europa FN-class tandem electrostatic AMS spectrometer with standard measurement protocols.³ The collagen samples were measured for 30,000 ^{14}C counts per cycle for five to eight cycle repetitions to achieve measurement errors within 0.3-0.8%. Corrections for background contamination of fossil and contemporary carbon introduced during AMS sample preparation were made using standard procedures.^{3,4} The concentration of $^{14}\text{C}/\text{C}$ was expressed using the F^{14}C nomenclature ± 1 standard deviation (SD).⁵ The intercept date range corresponds to the two

SD range of atmospheric $^{14}\text{C}/\text{C}$ mapped onto the chronological record, corresponding to a chronological uncertainty of one to three years in most cases. Age of CA collagen was estimated based on intercept date ranges.

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL Table I

| Sample No. | Sex | Patient age | Aneurysm Location | Diameter (mm) | AR | Aneurysm Irregularity | Risk factor(s) | SAH | Aneurysm excision | F ¹⁴ C (±1 SD) | Intercept year range | Estimated collagen age (years) |
|------------|-----|-------------|-------------------|---------------|-----|-----------------------|----------------|-----|-------------------|---------------------------|----------------------|--------------------------------|
| 1 | F | 56 | Pcom | 10 | 1.8 | yes | No | Yes | 2009 | 1.068 ± 0.0051 | 2004-2007 | 4 |
| 2 | M | 77 | PICA | 12 | 4.0 | no | Yes | Yes | 2009 | 1.057 ± 0.0039 | 2005-2009 | 2 |
| 3 | M | 77 | PICA | 12 | 4.0 | no | Yes | Yes | 2009 | 1.052 ± 0.0038 | 2006-2010 | 1 |
| 4 | F | 53 | MCA | 5 | 1.4 | yes | No | Yes | 2010 | 1.035 ± 0.0036 | 2010- 2015 | <1 |
| 5 | F | 74 | MCA | 13 | 1.3 | no | Yes | No | 2010 | 1.053 ± 0.0039 | 2007-2010 | 1 |
| 6 | M | 57 | PICA | 8 | 1.8 | no | Yes | Yes | 2010 | 1.043 ± 0.0038 | 2008-2013 | 1 |
| 7 | F | 47 | Pcom | 12 | 1.3 | no | No | Yes | 2010 | 1.062 ± 0.0090 | 2003-2010 | 4 |
| 8 | F | 47 | Pcom | 12 | 1.3 | no | No | Yes | 2010 | 1.109 ± 0.0108 | 1994-2002 | 12 |
| 9 | F | 61 | MCA | 7 | 0.6 | no | Yes | Yes | 2010 | 1.067 ± 0.0104 | 2002-2010 | 4 |
| 10 | M | 48 | MCA | 40 | 7.0 | no | Yes | No | 2010 | 1.050 ± 0.0068 | 2005-2012 | 2 |
| 11 | F | 74 | PCA | 15 | 0.9 | no | No | No | 2010 | 1.036 ± 0.0039 | 2010-2015 | <1 |
| 12 | F | 49 | MCA | 5 | 1.4 | yes | Yes | Yes | 2010 | 1.064 ± 0.0123 | 2002-2011 | 5 |
| 13 | F | 53 | MCA | 8 | 2.2 | yes | Yes | Yes | 2011 | 1.037 ± 0.0039 | 2010-2014 | <1 |
| 14 | M | 51 | MCA | 6 | 1.0 | yes | Yes | Yes | 2011 | 1.057 ± 0.0085 | 2004-2011 | 3 |
| 15 | F | 43 | MCA | 4 | 1.5 | no | Yes | Yes | 2011 | 1.037 ± 0.0052 | 2009-2016 | <1 |
| 16 | M | 52 | Acom | 5 | 1.2 | yes | Yes | Yes | 2011 | 1.051 ± 0.0047 | 2006-2011 | 3 |
| 17 | M | 54 | Pcom | 12 | 1.8 | yes | Yes | Yes | 2011 | 1.039 ± 0.0044 | 2009-2014 | <1 |
| 18 | F | 62 | PICA | 6 | 2.3 | no | Yes | Yes | 2011 | 1.043 ± 0.0037 | 2008-2013 | 1 |
| 19 | F | 48 | Pcom | 6 | 1.7 | no | No | No | 2011 | 1.053 ± 0.0046 | 2006-2010 | 3 |
| 20 | F | 62 | MCA | 10 | 3.2 | no | Yes | No | 2011 | 1.038 ± 0.0039 | 2010-2015 | <1 |
| 21 | F | 41 | Acom | 9 | 2.3 | yes | Yes | Yes | 2011 | 1.036 ± 0.0049 | 2010-2014 | <1 |
| 22 | F | 51 | ACA | 7 | 2.8 | no | Yes | Yes | 2011 | 1.033 ± 0.0044 | 2011-2015 | <1 |
| 23 | F | 52 | MCA | 9 | 2.0 | no | Yes | No | 2012 | 1.043 ± 0.0045 | 2008-2014 | 1 |
| 24 | M | 45 | MCA | 6 | 1.3 | yes | Yes | Yes | 2012 | 1.053 ± 0.0144 | 2003-2017 | 2 |
| 25 | M | 51 | MCA | 6 | 1.7 | yes | Yes | Yes | 2012 | 1.044 ± 0.0067 | 2007-2014 | 2 |
| 26 | F | 47 | MCA | 5 | 1.9 | yes | Yes | Yes | 2012 | 1.048 ± 0.0059 | 2006-2012 | 3 |
| 27 | F | 82 | MCA | 16 | 3.0 | no | Yes | Yes | 2012 | 1.026 ± 0.0082 | 2010-2020 | <1 |
| 28 | F | 47 | MCA | 6 | 2.1 | no | Yes | Yes | 2012 | 1.041 ± 0.0049 | 2008-2014 | 1 |

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|----|---|----|------|----|-----|-----|-----|-----|------|--------------------|-----------|----|
| 29 | M | 64 | Acom | 12 | 2.5 | yes | Yes | Yes | 2012 | 1.051 ± 0.0104 | 2004-2014 | 3 |
| 30 | M | 47 | Pcom | 7 | 1.8 | no | No | Yes | 2012 | 1.061 ± 0.0077 | 2004-2010 | 6 |
| 31 | M | 45 | MCA | 10 | 2.0 | yes | No | Yes | 2012 | 1.040 ± 0.0045 | 2009-2014 | 1 |
| 32 | F | 45 | PCA | 10 | 3.0 | no | Yes | Yes | 2012 | 1.039 ± 0.0055 | 2009-2014 | 1 |
| 33 | M | 29 | MCA | 7 | 1.8 | yes | No | Yes | 2012 | 1.068 ± 0.0059 | 2003-2007 | 7 |
| 34 | F | 62 | MCA | 6 | 1.2 | yes | Yes | Yes | 2012 | 1.030 ± 0.0052 | 2011-2017 | <1 |
| 35 | F | 59 | MCA | 7 | 2.4 | no | Yes | No | 2012 | 1.049 ± 0.0103 | 2005-2015 | 2 |
| 36 | F | 48 | MCA | 8 | 1.4 | no | Yes | No | 2012 | 1.052 ± 0.0046 | 2006-2010 | 4 |
| 37 | M | 66 | PICA | 3 | 2.0 | no | Yes | Yes | 2012 | 1.029 ± 0.0040 | 2012-2017 | <1 |
| 38 | M | 66 | PICA | 5 | 3.3 | no | Yes | Yes | 2012 | 1.039 ± 0.0040 | 2009-2014 | 1 |
| 39 | F | 45 | ACA | 4 | 0.7 | no | No | Yes | 2012 | 1.060 ± 0.0060 | 2004-2009 | 5 |
| 40 | M | 53 | Acom | 6 | 1.1 | yes | Yes | Yes | 2012 | 1.030 ± 0.0050 | 2011-2018 | <1 |
| 41 | F | 50 | Pcom | 6 | 1.8 | yes | Yes | Yes | 2012 | 1.031 ± 0.0143 | 2006-2020 | <1 |
| 42 | F | 47 | MCA | 8 | 2.1 | yes | No | No | 2012 | 1.045 ± 0.0060 | 2007-2014 | 2 |
| 43 | M | 53 | ICA | 6 | 1.2 | no | Yes | Yes | 2013 | 1.022 ± 0.0053 | 2013-2020 | <1 |
| 44 | F | 72 | Pcom | 8 | 2.0 | yes | no | No | 2013 | 1.039 ± 0.0034 | 2009-2014 | 2 |
| 45 | F | 78 | Pcom | 8 | 2.7 | no | Yes | yes | 2013 | 1.039 ± 0.0039 | 2009-2014 | 2 |
| 46 | F | 44 | Acom | 6 | 1.6 | no | Yes | yes | 2013 | 1.039 ± 0.0037 | 2009-2014 | 2 |

Epidemiological data, radiological and chronological aneurysm measurements for every patient and CA. The incidence of potential risk factors for aneurysm formation and rupture, such as hypertension, cigarette smoking, cocaine use or their combination were concluded and dichotomized (yes vs. no). The chronological range of each CA depends upon the measurement precision and slope of the bomb curve at the intercept. Abbreviations: ACA indicates Anterior Cerebral Artery; AR, aspect ratio (aneurysm dome/neck diameter); ACom, Anterior Communicating Artery; F, female; M, male; MCA, Middle Cerebral Artery; PCom, Posterior Communicating Artery; PICA, Posterior Inferior Cerebral Artery; PCA, Posterior Cerebral Artery.