Supplementary Information

Ancient DNA reveals monozygotic newborn twins from the Upper Paleolithic

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Supplementary Fig. 1: Morphology of maxillary right second deciduous incisors (i2). Ind1 in the first row (a-c), ind2 in the second row (d-f) and ind3 in the third row (g-i). Shown are the dentinoenamel junction in colour with transparent enamel surface. a,d,g, Labial perspective. b,e,h, Lingual perspective. c,f,i, Occlusal perspective.
Supplementary Fig. 2: Size comparison of the maxillary right first deciduous incisor (i1) of ind1 and ind2 from registered µCT data. 

a, Labial surface of the enamel of ind1 showing degree of degradation. 

b, Lingual surface of the enamel of ind2; surface of ind1 is shown in white. 

c, Distal enamel surface of ind2 with slice through ind1. 

d,e,f, Dentinoenamel junction of ind1 (red) and ind2 (blue) from labial, lingual and distal direction. Enamel surface is shown in white. 

g, Slice through ind1 showing faint neonatal line (NNL) (➤). 

h, Overlay of ind1 and ind2. NNL (➤) on ind1 aligns with enamel surface on ind2. 

i, slice through ind2 at same position as panel g.
Supplementary Fig. 3: Tooth measurements on μCT scans. A, Enamel surface, dentinoenamel junction (DEJ) and dentine surface. B, DEJ without and with smoothing. C, Landmarks at the crown rim with fitted cervical plane. D, Crown height (CH) and diameters in buccolingual (BL) and mesiodistal (MD) direction.
Supplementary Fig. 4: Micrographs of the upper lateral incisors (i2) Histology of ind1. (a,d), Histology of ind2. (b,e), Histology of ind3. (c,f). Magnified histological details are depicted below (d,e,f). Neonatal lines (arrowheads) are visible in ind1. (d) and ind3. (f) but missing in ind2. (e).
Supplementary Fig. 5: Prism length measurements on μCT scans. a, Mandibular i2 of ind1, overview showing position of measurements on a cutting plane in buccolingual direction through the dentine horn in the middle of the tooth. b, Mandibular i2 of ind1, virtual section displayed as volume rendering; without and with measurements. c, Mandibular i2 of ind2, virtual section displayed as volume rendering, without and with measurements. d, Maxillar i1 of ind1, virtual section displayed as volume rendering; without and with measurements. e, Maxillar i1 of ind2, virtual section displayed as volume rendering, without and with measurements. Neonatal line in red and accentuated lines in white.
**Supplementary Fig. 6:** Histologic images and sections of elemental ratios, and statistical evaluation of chemical imaging. A, B, C, Histologic image and $^{27}$Al/$^{138}$Ba ratio sections for individuals ind1, ind2, and ind3, respectively. D, E, F, Histologic image and $^{27}$Al/$^{43}$Ca ratio sections for individuals ind1, ind2, and ind3, respectively. G, H, I, Histologic image and $^{138}$Ba/$^{31}$P ratio sections for individuals ind1, ind2, and ind3, respectively. J, K, L, Histologic image and $^{31}$P/$^{43}$Ca ratio sections for individuals ind1, ind2, and ind3, respectively. M, N, O, Histologic image and $^{88}$Sr/$^{138}$Ba ratio sections for individuals ind1, ind2, and ind3, respectively. P, Q, R, Histologic image and $^{88}$Sr/$^{43}$Ca ratio sections for individuals ind1, ind2, and ind3, respectively.
Supplementary Fig. 7: Histologic and microscopic images of ind3. A Histologic image of ind3 with indication arrows for neonatal-line (NNL) and post-NNL stress line (SL). B Microscopic image past laser ablation of ind3 with indication arrows for neonatal line (NNL) and post-NNL stress line (SL).
**Supplementary Table 1**: Metric dimensions of the right maxillary second deciduous incisor of the Upper Palaeolithic Krems-Wachtberg infants: individual 1 (ind1), individual 2 (ind2) and individual 3 (ind3). Thickness and volume measurements are limited to the most apical 3 mm of the teeth.

| Extent (mm) | ind1  | ind2  | ind3  |
|-------------|-------|-------|-------|
| Mesial-Distal | 5.418 | 5.316 | 6.367 |
| Buccal-Lingual | 4.038 | 3.292 | 4.813 |
| Crown Height | 4.858 | 4.594 | 6.013 |
| Thickness (mm) | Dentin | 0.375 | 0.324 | 0.514 |
| | Enamel   | 0.385 | 0.322 | 0.570 |
| Volume (mm³) | Dentin | 5.401 | 4.461 | 7.519 |
| | Enamel   | 9.663 | 7.289 | 16.012 |
**Supplementary Table 2**: Results of age estimation applying the regression equations of Birch and Dean (2014). Upper and lower incisors were analysed using histologic specimens or µCT scans. Measurements of enamel prism length between Crown initiation (Ci), Accentuated Line (AL1), Neonatal line (NNL) and Crown end (Ce) are presented in micrometre. Results include age at death for all individuals and postnatal survival time for ind1 and ind3.

|       | i1 maxilla | i2 maxilla | i2 maxilla histology | i2 mandible | i1 maxilla | i2 maxilla | i2 maxilla histology | i2 mandible |
|-------|------------|------------|----------------------|-------------|------------|------------|----------------------|-------------|
|       | µCT        | µCT        | histology            | µCT         | µCT        | µCT        | histology            | µCT         |
| ind1  |            |            |                      |             |            |            |                      |             |
|       | 300        | 280        | 525                  | 240         | 290        | 280        | 216                  | 220         |
|       | 290        | 250        | 170                  | 250         | 290        | 240        | 262                  | 290         |
|       | 150        | 170        | 178                  | 150         |           |            |                      |             |
|       |            |            |                      |             |            |            |                      |             |
|       |            |            |                      |             |            |            |                      |             |
| ind2  |            |            |                      |             |            |            |                      |             |
|       | 290        | 280        | 216                  | 220         | 290        | 280        | 262                  | 290         |
| ind3  |            |            |                      |             |            |            |                      |             |
|       | 650        | 660        | 673                  | 671         | 320        | 320        | 339                  | 327         |
|       | 320        | 320        | 339                  | 327         |            |            |                      |             |
|       | 150        | 170        | 178                  | 150         |            |            |                      |             |

**Supplementary Table 3**: Results of the N- and C-isotope ratios.

| Provenience | Sample ID | weight | d\(^{15}\)N\(_{AIR}\) | d\(^{13}\)C\(_{VPDB}\) | %N | %C | C:N |
|-------------|-----------|--------|------------------------|------------------------|----|----|-----|
| Austria; Krems-Wachtberg; ind3 | 150025 | 0.980 | 12.6 | -19.9 | 2.7 | 7.8 | 3.30 |
| Austria; Krems-Wachtberg; ind1; inv. No. 151425+151435 | 151566 | 0.900 | 11.9 | -20.5 | 2.3 | 6.6 | 3.36 |
| Austria; Krems-Wachtberg; ind2; inv. No. 151368+151461 | 151567 | 1.140 | -1.6 | -24.1 | 0.2 | 0.9 | 5.60** |

* error on all reported measurements is 0.2
** elevated C:N ratio indicates the isotopic measurements are unreliable.
**Supplementary Table 4:** Operating conditions of the laser ablation ICP-QMS setup.

| **Operating conditions** |  |
|--------------------------|--|
| **LA parameters (New Wave NWR 193)** |  |
| laser wavelength | 193 nm |
| spot diameter | 20 µm |
| line spacing | 0 µm |
| scan speed | 10 µm/s |
| wash time between lines | 20 s |
| frequency | 10 Hz |
| laser energy | 60 % |
| carrier gas | He |
| carrier gas flow rate | 0.9 L min⁻¹ |
| **ICP-QMS parameters (Perkin Elmer NexION 350D)** |  |
| ICP RF power | 1300 W |
| gas | Ar |
| auxiliary gas flow rate | 0.75 L min⁻¹ |
| plasma gas flow rate | 15 L min⁻¹ |
| sampler cone | nickel |
| skimmer cone | nickel |
| detector mode | dual |
| measured isotopes | \(^{27}\)Al, \(^{31}\)P, \(^{138}\)Ba, \(^{43}\)Ca, \(^{88}\)Sr ind1 and ind2: additionally \(^{57}\)Fe |
| integration time | 0.176 s |
| dwell time | 30 ms |
**Supplementary Table 5:** Statistical evaluation of chemical imaging. $^{138}$Ba/$^{43}$Ca ratios (cps/cps) and Ba mass fraction ($\mu$g g$^{-1}$) given as percentiles (5%, 25%, 75%, 95%, median, mean and standard deviation) in post-SL enamel, post-NNL enamel, pre-NNL enamel and dentin for ind1, ind2 and ind3.

|        | 138Ba/43Ca (cps/cps) |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
|--------|-----------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|        | Percentile 05 | Percentile 25 | Median | Percentile 75 | Percentile 95 | Mean | SD | Percentile 05 | Percentile 25 | Median | Percentile 75 | Percentile 95 | Mean | SD |
| ind1   | post-NNL enamel  | 0.42     | 0.47     | 0.53     | 0.59     | 0.68     | 0.54     | 0.08     | 207      | 234     | 262     | 291     | 335     | 352     | 264     | 42     |
|        | pre-NNL-SL enamel | 0.31     | 0.36     | 0.40     | 0.45     | 0.52     | 0.40     | 0.07     | 151      | 175     | 197     | 221     | 256     | 287     | 199     | 33     |
|        | pre-NNL enamel   | 0.25     | 0.30     | 0.34     | 0.39     | 0.46     | 0.35     | 0.07     | 123      | 148     | 168     | 190     | 226     | 247     | 171     | 32     |
|        | dentin          | 0.38     | 0.48     | 0.55     | 0.63     | 0.80     | 0.59     | 0.42     | 188      | 235     | 270     | 310     | 396     | 436     | 291     | 207    |
| ind2   | pre-NNL-SL enamel | 0.31     | 0.37     | 0.42     | 0.48     | 0.60     | 0.43     | 0.09     | 152      | 181     | 208     | 236     | 295     | 323     | 212     | 43     |
|        | pre-NNL enamel   | 0.24     | 0.30     | 0.33     | 0.38     | 0.45     | 0.34     | 0.07     | 119      | 146     | 163     | 185     | 224     | 250     | 167     | 33     |
|        | dentin          | 0.43     | 0.50     | 0.57     | 0.66     | 0.82     | 0.79     | 3.13     | 210      | 248     | 282     | 324     | 402     | 476     | 387     | 1540   |
| ind3   | post-NNL-SL enamel | 0.14     | 0.17     | 0.19     | 0.22     | 0.27     | 0.20     | 0.05     | 70       | 82      | 93      | 107     | 131     | 143     | 96      | 23     |
|        | post-NNL enamel  | 0.18     | 0.22     | 0.26     | 0.29     | 0.34     | 0.26     | 0.05     | 87       | 110     | 127     | 142     | 169     | 184     | 127     | 25     |
|        | pre-NNL enamel   | 0.21     | 0.27     | 0.30     | 0.35     | 0.31     | 0.27     | 0.04     | 103      | 120     | 133     | 149     | 173     | 190     | 135     | 22     |
|        | dentin          | 0.29     | 0.33     | 0.37     | 0.43     | 0.54     | 0.39     | 0.08     | 141      | 162     | 183     | 211     | 266     | 290     | 190     | 39     |