


A Multidisciplinary Approach to Neolithic Life Reconstruction

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Abstract The expansion of Neolithic stable isotope studies in France now allows distinct regional population-scale food patterns to be linked to both local environment influences and specific economic choices. Carbon and nitrogen isotope values of more than 500 humans and of animal samples also permit hypotheses on sex-biased human provenance. To advance population scale research, we here present the first study that draws together carbon (C), nitrogen (N), sulphur (S) and strontium (Sr), dental calculus, aDNA, and palaeoparasitology analysis to infer intra-population patterns of diet and provenance in a Middle Neolithic population from Le Vigneau 2 (human = 40; fauna = 12; 4720–4350 cal. BC) from north-western France. The data of the different studies, such as palaeoparasitology to detect diet and hygiene, CNS isotopes and dental calculus analysis to examine dietary staples, Sr and S isotopes to discriminate non-locals, and aDNA to detect maternal (mtDNA) versus paternal lineages (Y chromosome), were compared to anthropological information of sex and age. Collagen isotope data suggest a similar diet for all individuals except for one child. The provenance isotopic studies suggest no clear differences between sexes, suggesting both males and females used the territory in a similar pattern and had access to foods from the same environments.

Keywords Dietary reconstruction · Human provenance · Isotopes · Dental calculus · Palaeoparasitology · Ancient DNA

Introduction

Regional Background

Over the last 15 years, the diet of individuals and populations from the Neolithic period in France has been studied with stable isotope analyses on human remains. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope data have been used to define distinct regional food patterns linked to both local environment and specific economic choices, which has led to hypotheses of differential mobility of females during the Middle Neolithic (*ca.* 4500–3900 cal BC) (Goude 2007; Goude et al. 2013). Carbon and nitrogen stable isotope ratios of livestock vary due to local climate and substratum (*e.g.* temperature, precipitation, forest cover, N availability in soil, soil pH). ‘These point to a gradual change of values (from southern to northern latitudes) that impact the signals of environment and food choice (Goude and Fontugne 2016), thereby suggesting that in France, Middle Neolithic human groups have a significant intake of animal protein from terrestrial ecosystems, but also highlighting intra- and inter-group variability. Isotopic profiles from several individuals, mainly located in the western part of the French Mediterranean, have aligned more toward agriculture with minimal animal protein consumption (Herrscher and Le Bras-Goude 2010; Le Bras-Goude et al. 2009). High animal protein intake was commonly recorded in central (Goude et al. 2013) and northern France (Rey et al. 2017). Although no ichthyological remains were found, aquatic resources are an isotopically detectable part of human diet during the Middle Neolithic, but high freshwater protein diets have only been detected in a few individuals, and this is still in dispute (Rey et al. 2017). As demonstrated in other European regions, marine resources appear neglected at the beginning of the Neolithic (Richards and Schulting 2003; Salazar-García et al. 2018). However, recent data from

Southern France re-evaluated such hypotheses; stable isotope data on a few individuals support the consumption of marine protein (Provost et al. 2017), and are compatible with previous work performed in the region on shells and fish remains (e.g. Cade 1998; Desse-Berset and Desse 1999). In other Western European regions, and unlike the Early Neolithic, the Middle Neolithic shows a wider variety of subsistence patterns (e.g. Salazar-García et al. 2016). In northern France at the sites of Gurgy and Pontcharaud (Fig. 1), it has been argued that stable isotope variation between males and females shows food specificities and/or of different geographic origin (e.g. wider range of resources exploited by female) (Goude et al. 2013; Rey et al. 2017). In southern France, in addition to carbon and nitrogen data, preliminary strontium isotope analysis ($^{87}\text{Sr}/^{86}\text{Sr}$) supports the hypothesis of different mobility patterns (Goude et al. 2012). These results can be linked to an economic system (i.e. more mobile pastoralists in the Garonne area versus less mobile agriculturalists in the Languedoc area) in agreement with archaeological (e.g. presence of milling material) and burial (domestic versus funerary) observations (Loison and Schmitt 2009; Tchérémissinoff et al. 2005). The quantity and preservation of human remains allows researchers to study the biological and social effects of Middle Neolithic agropastoralism intensification. In particular, the question of variation in protein intake between groups and within groups suggests a link between access to local resources and mobility/origin of individuals.

The Archaeological Site of Le Vigneau 2

The necropolis site of Le Vigneau 2 (Pussigny, department of Indre-et-Loire, Centre Val de Loire region; Fig. 1), excavated in 2013, was an opportunity to investigate human diet and mobility owing to the location (area not previously studied with biochemical methods) and biological and economic records available. The site is established on a White Tuffeau substrate, a micaceous Turonian chalk commonly used for local construction, ca. 4 km from the current path of the La Vienne stream. Several features associated with funeral activities were discovered during a development-led archaeology campaign along the new TGV line Tours–Bordeaux. These features are located on a south-western slope of a dry valley, near a portal tomb on the valley floor. The earliest phase of the site consist of 102 graves dated to the Middle Neolithic (4720–4350 cal BC), and include a dog's burial. They belong to the Chambon culture according to the associated goods, especially the pottery, and provide direct dates from human bone collagen ^{14}C (Coutelas et al. 2015). Ninety-two graves contained a single individual, and ten others contained two individuals. The graves were clustered in three groups probably reflecting socio-economical aspects rather than a geographical distribution: (a) a sophisticated internal arrangement such as a cist or wooden frame, corresponding to the 'richest' with most of the grave goods, (b) an average 'family' graveyard, and (c) simple pits with graves of children and females in a pit without any grave goods. Ovis remains were also present as funeral offerings, with up to three bones per an individual grave. This Middle Neolithic graveyard was interpreted as a cemetery of shepherds, buried with their domestic animals, lithic tools for butchering and skinning activities, arrowheads for hunting, and some ceramics for more domestic purposes (Coutelas et al. 2015).

To reconstruct subsistence strategies and provenance from the Middle Neolithic population of Le Vigneau 2, we carried out a multi-proxy study: bone collagen carbon

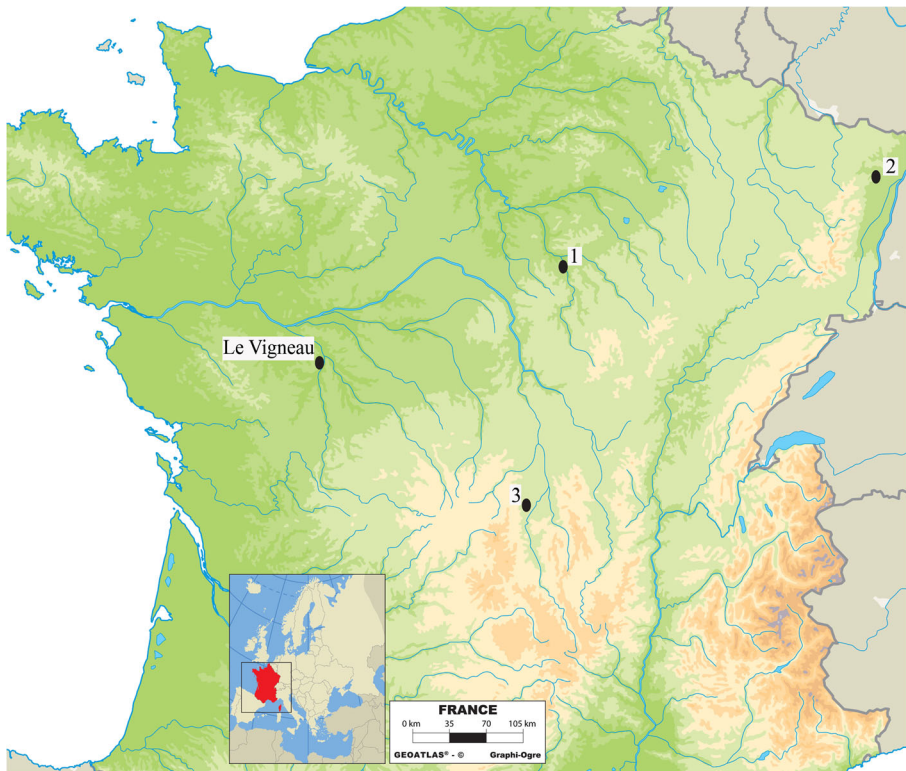


Fig. 1 Location of the site and sites mentioned in the text for comparison: 1 — Gurgy, Paris basin area (Rey et al. 2017), 2 — Gougenheim, Alsace area (Goude et al. 2015), 3 — Pontcharaud, Auvergne area (Goude et al. 2013)

(C), nitrogen (N), and sulphur (S) stable isotope ratio analysis, palaeoparasitological study, microremains from dental calculus study, aDNA tests, and teeth enamel Sr isotope ratio analysis. This represents the first time that all of these different approaches are carried out together, which considerably strengthening our knowledge of first farmers' dietary behaviour, while building life histories of individuals, particularly of male *versus* females. The potential of such multi-proxy comparison for future investigations in the region will also be evaluated.

Bioarchaeological Materials and Methods

Anthropological and Archaeozoological Remains

The Le Vigneau 2 archaeological necropolis yielded 102 tombs with 112 individuals including adults and juveniles. Osteological and funerary feature descriptions were performed *in situ* during the excavation, but a few traits were only identified during laboratory work (details on individuals sampled for this study can be checked at supp. Mat. 1). When possible, we performed sex diagnosis of coxal bone by using morphometric and probabilistic methods (Bruzek 2002; Murail et al. 2005). Among the

68 adults excavated in the necropolis, 26 had preserved coxal bones and were found to be 14 females and seven males. Adults' age was estimated from coxal bones too (Schmitt 2005), and juveniles' age was estimated from both tooth growth pattern (Moorrees et al. 1963) and bone maturation (Scheuer and Black 2000). Sixty-eight adults and 37 juveniles were identified, but the collection had no individuals from 15 to 19 years, and three aged less than 1 year. Other biological features were also identified, such as teeth non-metric traits (Scott and Turner 1997). Forty percent of the individuals present shovel shape incisors, found in five double burials that could be argued to belong to family groups. Calculus deposits and dental caries were identified on several individuals (Dobney and Brothwell 1987; S. W. Hillson 2001), but in general the impact of these pathologies on the human group from Le Vigneau 2 was low. Animal remains were only found in a specific context, *i.e.* they are buried alone (dogs) or associated with the deceased, even in the same content. Only a few species were identified, mainly sheep including new-born specimens (Barone 1999). The lambs are mainly found as funerary deposit in female tombs (one of them even had three contemporary offerings), but the low ratio of human gender determination does not allow us to associate all fauna species with human gender. Additionally, one dog was buried individually; a burial of a child had a young squid buried with it, and wild animal remains were only identified as grave goods: two perforated pendants in a split canine of a boar, a complete one, and a bear tooth (Coutelas et al. 2015). Absence of consumed animals makes it difficult to interpret the economic and dietary information usually discussed from archaeological faunal remains.

Stable Isotope Ratios from Bone Collagen

Bone collagen is a protein in which the chemical composition originates mainly from dietary protein (*e.g.* Ambrose and Norr 1993); therefore, its analysis will give information on protein consumption. It is also important to take into consideration when interpreting collagen data that bone remodeling during the life of the individual, involving phases of bone resorption and bone formation, and regulated by hormonal and local factors, can influence the resulting values (Hill and Orth 1998). Bone-remodeling velocity is dependent on sex, age, and genetic characteristics (Han et al. 1997). The remodeling of growing sub-adults is faster than that of adults (Valentin 2003), implying that chemical components of food and environments are registered faster in younger individuals, and that bone composition reflects a shorter time span for juveniles than for adults (*ca.* 15–20 years for adults) (Hedges et al. 2007).

Carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) have been used widely in studying the Neolithic (*e.g.* Salazar-García et al. 2018). In bone collagen, carbon and nitrogen isotope ratios help to detect the environment from which food resources are coming (*e.g.* terrestrial versus aquatic) and the proportion of animal protein in the individual diet (*e.g.* DeNiro and Epstein 1978; van der Merwe 1982; Schoeninger and DeNiro 1984; Bocherens and Drucker 2003). The interpretation of aquatic resource consumption should be made with caution if stable isotope values are not clearly from a marine source, as fish from estuarine or brackish waters can have lower nitrogen isotopic values than expected (Salazar-García et al. 2014). Applied to different past human communities, this method has allowed researchers to track the Neolithisation dietary transformation (*e.g.* Richards and Schulting 2003), to differentiate female and male

dietary practices (Ambrose et al. 2003), or to detect specific social status (Prowse et al. 2005). More recently, sulphur isotope analysis ($\delta^{34}\text{S}$) was recognized as another useful tool in complementing carbon and nitrogen (Nehlich 2015), particularly to document the provenience of individuals (Richards et al. 2001; Vika 2009) and the potential consumption of marine or freshwater resources (Nehlich et al. 2010). The combination of these three isotopic ratios in bone collagen has shown its relevance to study the potential access to freshwater resources (Drucker et al. 2016), as well as cultural behaviors (de Beccdelievre et al. 2015; Nehlich et al. 2011).

The preservation of osteological material at Le Vigneau 2 site allowed sampling of 40 humans (11 females, six males, 13 non-sexed adults, and six juveniles > 4 years old) and remains from 12 animals (ten lambs, one adult sheep, and one dog) for carbon and nitrogen isotope ratio analysis. From this initial corpus, 34 humans and 12 animal remains successfully provided enough collagen to get sulphur isotope ratios (*cf.* suppl. Mat. 1). Collagen was extracted according to a combination of Longin (1971), Bocherens (1992) and Richards and Hedges (1999) methodologies at the UMR 7269 LAMPEA laboratory in Aix-en-Provence (France). After demineralization of bone in HCl (0.5 M, 5 °C), samples are rinsed in H₂O_d and soaked in NaOH (20 h, room temperature). Collagen pieces are then rinsed in H₂O_d and solubilized in weak acid (HCl pH 2; 48h, 70 °C). Solubilized collagen is then filtered with an EzeerFilter® device, and the filtered residue is frozen (−65 °C, a few hours) and freeze-dried. Between 0.90 and 1.10 mg of freeze-dried collagen is loaded separately into aluminum tin capsules for carbon and nitrogen, and *ca.* 10 mg for sulphur (plus vanadium pentoxide catalyst). Elemental composition and stable isotope ratios are analyzed by EA-IRMS (Europa scientific elemental analyzer) and 20–20 IRMS (Iso-Analytical Ltd. Crewe, UK). Laboratory standards used are calibrated against the IAEA international standard for all measurements; measurement error is 0.1‰ for carbon and nitrogen and 0.2‰ for sulphur.

Strontium Isotope Ratios from Teeth Enamel

Strontium isotopic ratio ($^{87}\text{Sr}/^{86}\text{Sr}$) analysis of skeletal material is a common method for detecting provenance and mobility among past humans (*e.g.* Strauss et al. 2015; Sarasketa-Gartzia et al. 2018; Villalba-Mouco et al. 2018). Since radiogenic isotope ^{87}Sr forms by radioactive decay from rubidium (^{87}Rb), the $^{87}\text{Sr}/^{86}\text{Sr}$ signature of a specific location is determined by the underlying bedrock age and its Rb content. Older geological formations such as granite rocks have higher $^{87}\text{Sr}/^{86}\text{Sr}$ values than younger geological formations such as volcanic rock. Strontium enters ecosystems and mammal tissues without fractionation (Faure and Powell 1972; Graustein 1989), being a specific geological strontium signature incorporated into body hard tissues by substituting calcium (Ericson 1985). The strontium is ultimately derived from the Sr of the bedrock, soils, and water where individuals were living when the teeth were formed, as they have incorporated the Sr mainly through food but also water (Bentley 2006).

Among skeletal tissues, tooth enamel is the preferred substrate, since it is resistant to diagenesis from the burial environment (Budd et al. 2000; Hoppe et al. 2003). Enamel is highly mineralized (96%), mainly composed of apatite, and has no turnover during life (Nanci 2013). Therefore, tooth enamel stores information from its formation during childhood (Humphrey et al. 2008). This means that it is potentially possible to identify

various changes occurring during infancy and childhood such as birth, breastfeeding, weaning, provenance, and territorial mobility (e.g. Humphrey et al. 2008). Specifically, in the case of provenance and territorial mobility it is useful to compare teeth from a single individual that reflect different moments of its life. For example, by comparing a P2/M2 with an M3, it is possible to detect differences in $^{87}\text{Sr}/^{86}\text{Sr}$ if the individual lived during childhood (P2/M2) in a geological substrate different to that in which it lived during early adulthood (M3). Furthermore, the analysis of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in tooth enamel from a large number of individuals from one population can tag possible non-locals. However, and ideally, in order to retrieve more detailed information on territorial mobility, bioavailable Sr mapping is necessary (Price et al. 2002; Evans et al. 2010). Unfortunately, the prohibitive cost of this type of analysis makes it difficult to carry out local detailed bioavailable Sr mapping.

From Le Vigneau 2, we selected 31 individuals (ten females, six males, 14 non-sexed adults and one individual aged between 10 and 14 years old) for Sr isotope ratio analysis on enamel. We made sure that we sampled two teeth per individual: one that mineralized during an early stage of life (P2 or M2; childhood) and one that mineralized later during early adulthood (M3). We defined tooth crown age formation using the London atlas of human tooth development, specifically the median data published with the beginning of the crown starting at Coc (cups outline complete) and the end of the crown finishing at Crc (crown completed) (S. J. AlQahtani et al. 2010): P4 crown growing between 3.5 and 7.5 years old, and M2 crown growing between 4.5 and 8 years old. The M3 growth pattern is variable (e.g. Engstrom et al. 1983; Liversidge 2008; Tuteja et al. 2012); here we consider that, generally but not exclusively, the crown grows between 8.5 and 17.5 years of age, even though eruption can appear in later adulthood stages or not happen at all (cf. supplementary material 2).

Sample preparation and analysis was carried out directly in dedicated facilities at the Department of Geology of the University of Cape Town (South Africa), as described herein. Enamel samples were taken longitudinally to average the time formation of the entire dental piece. Prior to analysis, enamel surfaces were cleaned by abrasion, rinsed, and ultrasonicated for 20 min in MilliQ water. Diamond drill bits were cleaned with ethanol and ultrasonicated in MilliQ water between samples to avoid cross-contamination (Budd et al. 2000). After this, ca. 20 mg of cleaned enamel sample was digested with 2 mL of distilled 65% HNO_3 in a closed Teflon beaker placed on a hotplate at 140 °C for an hour. Digested samples were then dried and dissolved again in 1.5 ml of 2 M distilled HNO_3 . These redissolved samples were centrifuged at 4000 rpm for 20 min, and the resulting supernatant was later used for strontium solution separation chemistry. A separate fraction for each sample was used to calculate the Sr concentration; ^{88}Sr intensity (V) regression equation was built with the SRM987 standard from the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Strontium atoms were isolated with 200 μl of Eichrom Sr Spec resin loaded in 2 ml Bio-Spin Disposable Chromatography Bio-Rad Columns following the method described in Pin et al. (1994). The separated strontium fraction for each sample was dried down, dissolved in 2 ml 0.2% distilled HNO_3 , and diluted to 200 ppb for isotope analysis. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were measured using a NuPlasma HR multicollector inductively-coupled-plasma mass spectrometer (MC-ICP-MS). Sample analyses were referenced to bracketing analyses of SRM987, using a $^{87}\text{Sr}/^{86}\text{Sr}$ reference value of

0.710255 from the NIST. All strontium isotope data are corrected for isobaric rubidium interference at 87 amu using the measured signal for ^{85}Rb and the natural $^{85}\text{Rb}/^{87}\text{Rb}$ ratio. Instrumental mass fractionation was corrected using the measured $^{86}\text{Sr}/^{88}\text{Sr}$ ratio, the exponential law, and a true $^{86}\text{Sr}/^{88}\text{Sr}$ value of 0.1194. Results for repeated analyses of an in-house carbonate standard processed and measured with the batches of samples in this study ($^{87}\text{Sr}/^{86}\text{Sr} = 0.708936$; 2 sigma 0.000041; $n = 33$) are in agreement with long-term results for this in-house standard ($^{87}\text{Sr}/^{86}\text{Sr}$; 0.708915; 2 sigma 0.000047; $n = 125$).

Dental Calculus Microremains

Dental calculus — oral plaque that has been hardened by salivary calcium phosphate — is an increasingly important material used for several techniques in the fields of prehistory and archaeology. Dental calculus is predominantly composed of mineralized plaque biofilm. This material has been shown to contain environmental and dietary remains such as starch grains, phytoliths, lipids, proteins, and DNA from plant and animal foods. Once environmental and dietary remains have been detected, in many cases they can be identified molecularly or morphologically to the plant or animal taxa (family, genus, and sometimes species) that produced them (*e.g.* Armitage 1975; Henry and Piperno 2008; Warinner et al. 2014; Power et al. 2015a). As dental calculus is a stable context that is believed to be sealed from taphonomy, environmental and dietary remains are believed to be relatively isolated from taphonomic processes. Dental calculus allows a unique insight into diet, including foods underrepresented by conventional approaches and also other types of materials that leave the mouth after being masticated (Power et al. 2015b).

For this study, 11 samples of dental calculus were taken (sup. Mat. 3) and then later processed at the Plant Foods in Hominin Dietary Ecology laboratory in the Max Planck Institute for Evolutionary Anthropology. After weighing, we added 25 μl of 10% hydrochloric acid to the calculus samples for 0.5 to 3 h. The samples were then centrifuged at 1691 \times g (Heraeus MEGAFUGE 16 with a microcentrifuge rotor) for 10 min, and then about 100 μl of supernatant was decanted and replaced with distilled water. This was repeated three times to remove the hydrochloric acid. After the second decanting, they were refilled with a 25% glycerine solution. We examined each slide under brightfield and cross-polarized light on a Zeiss Axioscope microscope at 400 \times magnification. To address the possibility of contamination, processing was done in a lab subject to a weekly regime of laboratory cleaning in addition to sediment and blank slide testing (see more specifically Power et al. 2015b, Power et al. 2016).

Palaeoparasitology

Standard palaeoparasitological analyses were conducted to retrieve eggs of gastrointestinal parasites, with the aim of accessing the health status of the population, and providing data on the lifestyle (*e.g.* diet, hygiene) of the individuals. Ten sediment samples taken from under the pelvis of the skeletons were prepared following the three-step RHM protocol (Rehydration-Homogenization-Microsieving), as recommended in Dufour and Le Bailly (2013). Sample analyses were performed using light microscopy (Olympus BX-51) with magnifications between $\times 100$ and $\times 600$ (UMR 6249 Chrono-environnement, France).

Ancient DNA

Ancient DNA analysis was performed in order to assess maternal (mitochondrial DNA) vs. paternal (Y chromosome) lineages, aiming to reconstruct matrilineal or patrilineal systems. Ancient DNA analyses were not anticipated before the excavation, so Le Vigneau 2 individuals were not excavated with aDNA precautionary care. Since petrous bones were targeted to obtain maximal aDNA recovery, only three individuals delivering enough preserved petrous bone were submitted to palaeogenetic analyses (*cf.* supplementary material 1). The petrous bones sampled were systematically decontaminated, *i.e.* scraped, cleaned with bleach, and subsequently exposed to UV radiation for 20 min on each side. All established aDNA guidelines were then followed to minimize contamination during all subsequent steps of analyses conducted in the aDNA facilities of the UMR PACEA (Bordeaux University). Fine-textured powder was collected from the inner part of the petrous bone by grinding it with an engraving cutter burr attached to a Dremel® drill. Powder was decontaminated through bleach incubation (15 min incubation with rotation at room temperature in 1 ml of 0.5% sodium hypochlorite solution) and then washed 3 times with 1 ml water to remove residual bleach. Powder was then incubated overnight in lysis buffer (0.5 M EDTA, pH 8, 25 mg/ml proteinase K, and 0.5% N-Lauryl sarkosyl). The procedure of Allentoft et al. (2015), which uses the MinElute kit from Qiagen, was then followed to extract the DNA. A combination of 18 mitochondrial and 10 Y chromosome SNPs, permitting the characterization of major maternal and paternal lineages known in European populations, were typed through one multiplex using MALDI-TOF MS-based SNP genotyping (iPLEXTM Gold technology, Sequenom, Inc., San Diego, CA, USA). All primers used for these experiments and procedure details are available in Rivollat et al. (2015). The mitochondrial first hypervariable region (HVR-I, nps 16,024–16,380) was targeted using four overlapping fragments (HVR-Ia/b/c/d), following the procedures described in Rivollat et al. (2015), to determine the maternal haplotypes of the individuals. Samples were assigned to mitochondrial haplogroups and haplotypes using the combined information of HVR-I and coding region variation, following the phylogenetic classification updated by van Oven and Kayser (2009) (PhyloTree Build 17; <http://www.phylotree.org>).

Results and Discussions

Diet

Ancient diet was assessed in this study by comparing results from stable isotope, calculus, and palaeoparasitological analysis. These different methodologies have the advantage of exploiting various bioarchaeological materials (bone, teeth calculus, burial sediment) to increase the probability (i) of detecting food behaviour signals, and (ii) of providing evidence of a wide range of food sources. All 52 bone collagen samples provide both elemental composition and C:N elemental quality control ratios compatible with the range we are using (DeNiro 1985; van Klinken 1999) (supp. Mat. 1). Faunal carbon and nitrogen stable isotope ratios are normally used to establish a reference baseline for the local environment and chronological period (*e.g.* Goude and

Fontugne 2016). In the case of Le Vigneau 2, several fauna species were found in the burials (young sheep dominating the assemblage), some of which appear symbolically deposited and therefore challenging the interpretation of human data. The isotopic data of the newborn lambs ($\delta^{13}\text{C}$: -22.8 to -21.5‰ ; $\delta^{15}\text{N}$: 7.1 to 7.3‰ ; $n = 4$) are consistent with the adult sheep individual (18 months; $\delta^{13}\text{C}$: -21.4‰ ; $\delta^{15}\text{N}$: 6.7‰). Both carbon and nitrogen values of these domestic specimens are consistent with herbivore data commonly recorded in northern France for C_3 terrestrial environments (Goude and Fontugne 2016). The range of values of the other lambs (3 and 9 months) is wider ($\delta^{13}\text{C}$: -23.4 to -21.4‰ ; $\delta^{15}\text{N}$: 4.7 to 7.4‰ ; $n = 6$). For these age categories, we would have expected higher nitrogen ratios due to milk consumption (Balasse et al. 1997), or at least similar values (already weaned) to those of adult and neonates. However, part of the animals of these age categories show lower nitrogen values (Fig. 2). To investigate this observation, a specific zooarchaeological study would help (see e.g. Balasse et al. 2002; Balasse 2003). In any case, for this human-focused study we will simply argue that this group of fauna is potentially from weaned specimens that might have consumed plants/fodder with low $\delta^{15}\text{N}$ values, such as legumes such as lucerne, clover, vetch. or lupine (Virginia and Delwiche 1982). The case of the dog (lower $\delta^{15}\text{N}$ than the human group) indicates similarities with several Middle Neolithic sites, and could reflect a consumption of human refuse with less animal protein than human food (Goude and Fontugne 2016).

Human data highlights limited isotopic variability independent of the sex, age, or other biological (tooth wear, calculus presence) and archaeological (ornaments, presence of shell) criteria: $\delta^{13}\text{C}$: -21.2 to -20.1‰ ; $\delta^{15}\text{N}$: 9.1 to 11.9‰ ; $n = 40$ (supp. Mat. 1). These isotope values show that humans based their diet on terrestrial resources. Except for a juvenile individual, the rest of the human group also shows a higher trophic position than that of the fauna studied ($\Delta^{13}\text{C}$: 1.8‰ ; $\Delta^{15}\text{N}$: 3.1‰), indicating a significant consumption of animal protein (Fig. 2). These results agree with preliminary observations proposed by the zooarchaeological study, highlighting an economy turned toward pastoralism and sheep herding instead of agriculture.

An immature individual (LV H28), aged ca. 6 years old, shows a higher $\delta^{15}\text{N}$ ($+1.9\text{‰}$) compared with the rest of the human group. In general, the juvenile groups of 3–5 and 6–9 years old show a range of data slightly wider ($\Delta^{13}\text{C}$: 1.1‰ ; $\Delta^{15}\text{N}$: 2.5‰ ; $n = 6$) than the adolescents and adults. The biological characteristics of these age categories, such as their generally low body mass index (egg, Rolland-Cachera et al. 1991; Cole et al. 2000; Martinson et al. 2015), could be one of the explanations for the variability observed, knowing that growth and physiological factors can have an impact on protein synthesis (e.g. de Luca et al. 2012). The bone and skull remains of the immature LV H28 were not well-preserved enough to provide health status data, so physiological/pathological hypothesis can be suspected but not supported by any evidence. Another explanation for these values, from the dietary point of view, could be that the immature consumed resources with high $\delta^{15}\text{N}$, such as meat of other terrestrial animals not consumed by the rest of the population (e.g. pig, commonly recorded in other northern Neolithic sites) and/or freshwater fish. None of these resources was found on the site during the excavation, but it should be kept in mind that the funerary context may misrepresent the economical practices from the population buried at the funerary site.

The eleven samples of calculus analyzed produced a variety of microremains including starches, phytoliths, calcium oxalate, and fungal and invertebrate remains.

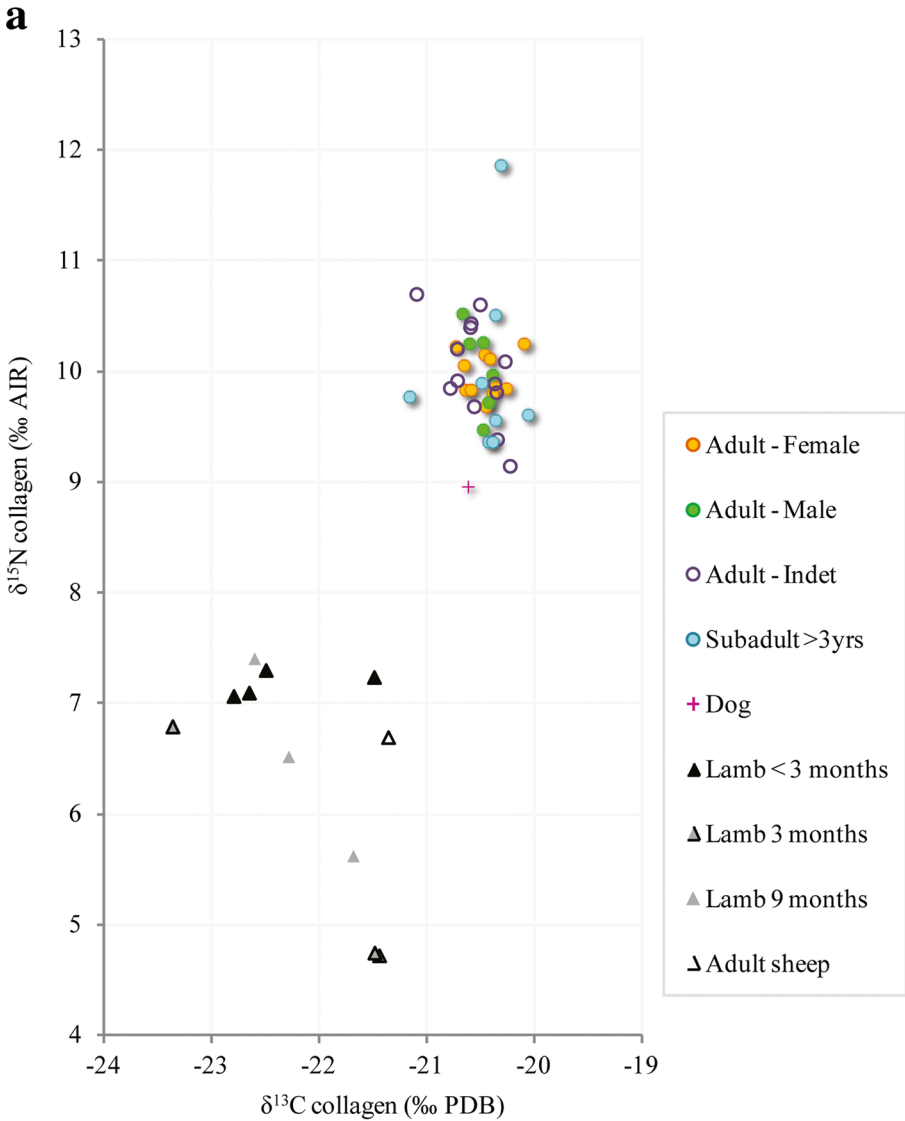


Fig. 2 a and b. Bone collagen stable isotope ratios

The remains derive from Triticeae seed, an unidentified non-Triticeae starchy plant, a form of leafy vegetable, fungi, mites, micro-charcoal, and various other non-dietary particles. As this technique is an emerging method, there is no Neolithic population to compare results with; however, the samples suggest a relatively homogenous diet. If assemblages are compared with reference populations from other earlier European foraging populations (Henry 2010; Power et al. 2016), Le Vigneau 2 shows on average a higher intensity in plant foods than Chalcolithic calculus per mg (Fig. 3; Power et al. 2014) but lower than Magdalenian samples (Henry et al. 2014; Power et al. 2015a). In terms of diversity, Le Vigneau 2 has the least diversity, although the small sample size

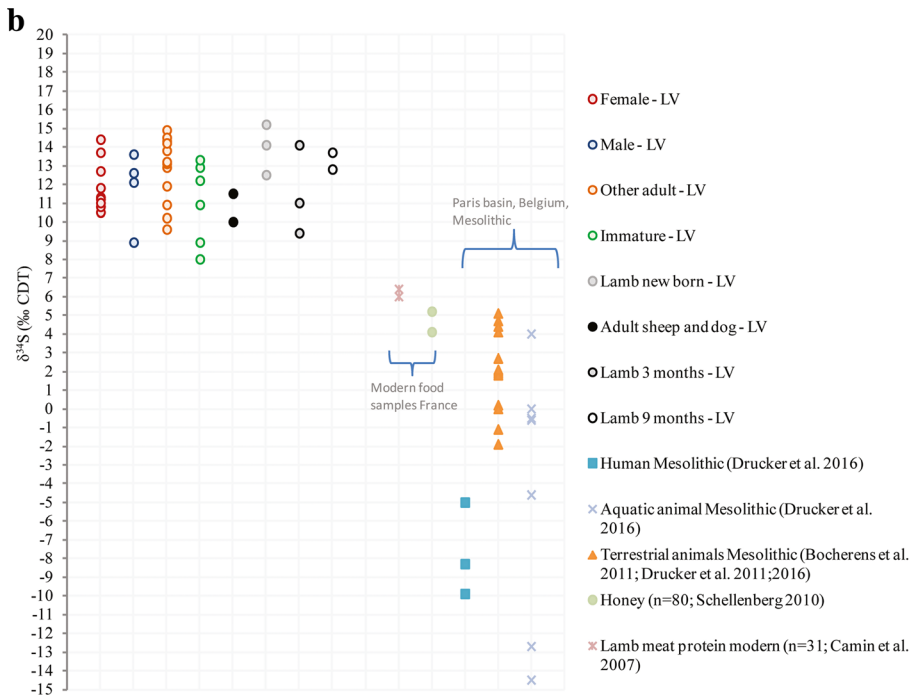


Fig. 2 (continued)

must be kept in mind (Fig. 3). The relative homogeneity observed in Le Vigneau 2 samples is consistent with a consumption pattern focused on few species, probably associated to agricultural practices, although wild species probably were consumed too. Palaeoparasitological microscopy did not allow the detection of parasite eggs. The abundance of mineral particles and the absence of organic matter in the prepared samples suggests taphonomic processes are responsible for the lack of results.

Mobility and Provenance

In order to assess both provenance and territorial mobility from these populations, we coupled sulphur stable isotope with strontium isotope analysis. The first gives information on geography and proximity to the coastline, while the second give information about the association of humans to specific geologies and can help discern locals from non-locals. We also carried out aDNA analysis to get information on ancestry from the individuals studied.

We assessed S isotopic values from 46 collagen samples. Of them, 41 exhibited good preservation indicators (%S, C:S, N:S) (Nehlich and Richards 2009); the remaining five (two animals and three humans) are excluded from the interpretation (supplementary material 1). Animal sulphur isotope values vary by age (Fig. 2b): adult sheep, dog, and two lambs of 3 months show the lowest values (from 9.4 to 11.5‰), while the rest of the lambs show values ranging from 12.5 to 15.2‰. Moreover, there is a significant correlation ($p < 0.05$; Pearson correlation; Statistica 9.1 ®) between $\delta^{34}\text{S}$

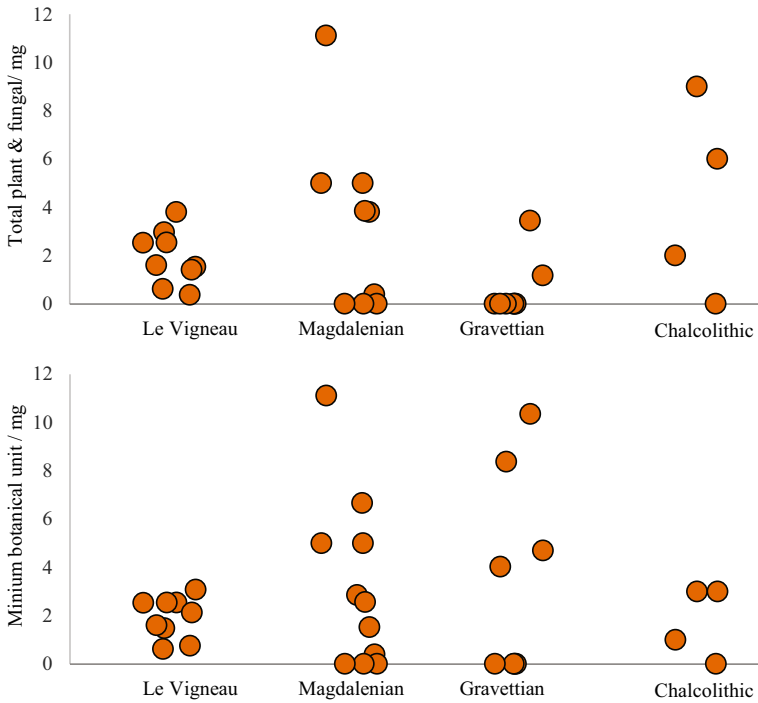


Fig. 3 Le Vigneau 2 total numbers of plant microremains (*top*) and minimum botanical unit per mg (*bottom*) with previously published comparison data from Magdalenian, Gravettian, and Chalcolithic populations. Minimum botanical unit is measure of the lowest number plant taxa or parts represented (Power et al. 2018). Magdalenian = Abri Pataud, La Madeleine and El Mirón (Henry et al. 2014; Power et al. 2015a), Gravettian = Předmostí, Dolní Věstonice and Pavlov (Henry 2010; Power et al. 2016), Chalcolithic = Camino del Molino (Power et al. 2014)

and $\delta^{15}\text{N}$ for all ovis samples; the animals with lowest nitrogen values also show the lowest sulphur isotopic ratios, which could be related to different breeding/feeding areas, and is perhaps explained by animal trade.

For humans, the sulphur isotope ratios from 8.0 to 14.9‰, with slight distribution variations according to sex and age, but with no significant statistical difference. Most of the human values fall within the onsite fauna range. When this data set is compared with published data from Mesolithic sites and modern food samples (only data available) from elsewhere in France (Camin et al. 2007; Schellenberg et al. 2010; Drucker et al. 2016), we observe higher ratios than what was known up to now in this inland context (Fig. 2b). The local geology (<http://infoterre.brgm.fr/viewer/MainTileForward.do#>) does not provide any clue as to why the existence of this high $\delta^{34}\text{S}$ is recorded in most of the archaeological samples. High $\delta^{34}\text{S}$ ratios can be found in geological contexts rich in gypsum or barite (O. Nehlich 2009), or in coastal environments. The Le Vigneau 2 site is located *ca.* 192 km from the Atlantic coast, *ca.* 150 km away from main gypsum deposits of the Paris basin, and *ca.* 90 km away from the closest barite deposit still exploited today (Chaillac). The presence of nearby agricultural lands and the potential use of modern chemical fertilizers could be an argument for local sulphur pollution in soils. However, neither %S, C:S nor N:S ratios (still in the range of accepted values) are statistically

correlated together, nor the $\delta^{34}\text{S}$ to the burial depth (supplementary materials 1). In such a context, the interpretation of archaeological sulphur isotope ratios must be cautious. Both animal and human present important $\delta^{34}\text{S}$ variability, reflecting either a wide local range of $\delta^{34}\text{S}$ in soils or a significant trading network of this group across different territories with different sulphur isotopic compositions.

Strontium isotope ratios were obtained from 61 teeth from 31 individuals, and range from 0.708 to 0.714 (supplementary materials 2). The $^{87}\text{Sr}/^{86}\text{Sr}$ database of continental France (Willmes et al. 2014) was used to assess bioavailable Sr from the region. The spots in it measured ($n = 3$; 20 to 27 km away from the Le Vigneau 2 site) indicate variability among plant samples (from 0.708 to 0.714; <http://80.69.77.150/>), with no specific spatial gradient. These plant values are similar to the archaeological human ratios from this study. This high variability in the broader region, together with the lack of a specific mapping of the immediate surrounding of the site, makes it difficult to define territorial mobility. However, provenance can be approached by analyzing the data from the actual population analyzed by comparing M2 values from all individuals, and individual life histories can be approached by individually comparing M2 values to M3 values. Overall, the variation observed (at both inter- and intra-individual levels) is not homogeneous, and could be the result of different behaviors, provenance, or territorial mobility.

In the human group, the M3 mean ± 2 sd of the $^{87}\text{Sr}/^{86}\text{Sr}$ is of 0.7098 ± 0.0029 , and the M2/P4 mean ± 2 sd of the $^{87}\text{Sr}/^{86}\text{Sr}$ is of 0.7101 ± 0.0037 . The two are similar, and would define the local range where most of the population lived. At an intra-individual level, the difference of $^{87}\text{Sr}/^{86}\text{Sr}$ between early adulthood (M3) and childhood (M2/P4) is small (≤ 0.001 ; $n = 6$) for males, and small as well but wider for females ($\Delta^{87}\text{Sr}/^{86}\text{Sr}$ from $|0.000|$ to $|0.004|$; $n = 10$) when compared to the rest of the group (supplementary materials 2; Fig. 4). A noteworthy pattern observed is that in the case of the males, M3 values are always higher than M2/P4 values, while for females M3 values are most of the time lower than the M2/P4 values (Fig. 4), suggesting perhaps a pattern in which males and females came from different areas or consumed different resources from the overall region. In any case, the Sr variation recorded between early adulthood and childhood is not statistically significant when comparing male/female/unsexed individuals ($p > 0.05$; non-parametric U Mann–Whitney test, Statistica 9.1 ©). As a curiosity, the site delivered several double burials with individuals showing shovel shape incisors and thus a potential family group. One of the double burials has Sr data available for both individuals (H11 and H12; Fig. 4), showing that both could have spent childhood and early adulthood in the same environment, potentially away from the site.

With regard to aDNA analysis and the information this reveals on genetic provenance, Table 1 presents the mitochondrial haplogroups (SNPs typing) retrieved from the human remains. SNPs typing made it possible to assign one individual (LVH3, male < 60 years old) to maternal lineage K (or derivatives), and another individual (LVH12) to lineage H (or derivatives), whereas the low number of SNPs recovered for the last sample (LVH26) did not make it possible to assign any haplogroup. No Y chromosome SNP, as well as no reproducible result for HVR-I sequences, could be obtained for any Le Vigneau 2 individual. Unfortunately, major DNA degradation prevents precise identification of the maternal and paternal lineages, and these two mitochondrial haplogroups do not allow any assessment about female mobility. However, we can note that maternal lineages characterized in the Le Vigneau 2 site are quite common in Neolithic farmer groups and fit within the French Middle Neolithic variability (from 14

to 25.5% for haplogroup K and from 7.9 to 40.9% for haplogroup H; Beau et al. 2017), including farmers from the Paris Basin (35% of H and 18.33% of K for the Gurgy site; Rivollat et al. 2015).

Human Behaviors

The data obtained about animal consumption (carbon and nitrogen isotope ratio, the record of animal remains) and the plant foods (calculus microremains) indicate an homogenous specialized diet. The collagen isotope ratios and the low variability of plant species identified indicate a diet dominated by animal resources, along with an economy centred on pastoralism rather than agriculture. The ritual deposition of lambs in female tombs strengthens this hypothesis. The fact that no female–male differences have been reported could be linked to the targeted activities on sheep breeding and exploitation of secondary products such as wool (Coutelas et al. 2015). If sheep constitute the main economic resource, males and females (even if performing differentiated activities, which is unknown) have access to the same animal products, in line with a less gender-based variability. In the absence of archaeological data on dwelling sites, our discussion was based on partial funerary observations and bioarchaeological analyses.

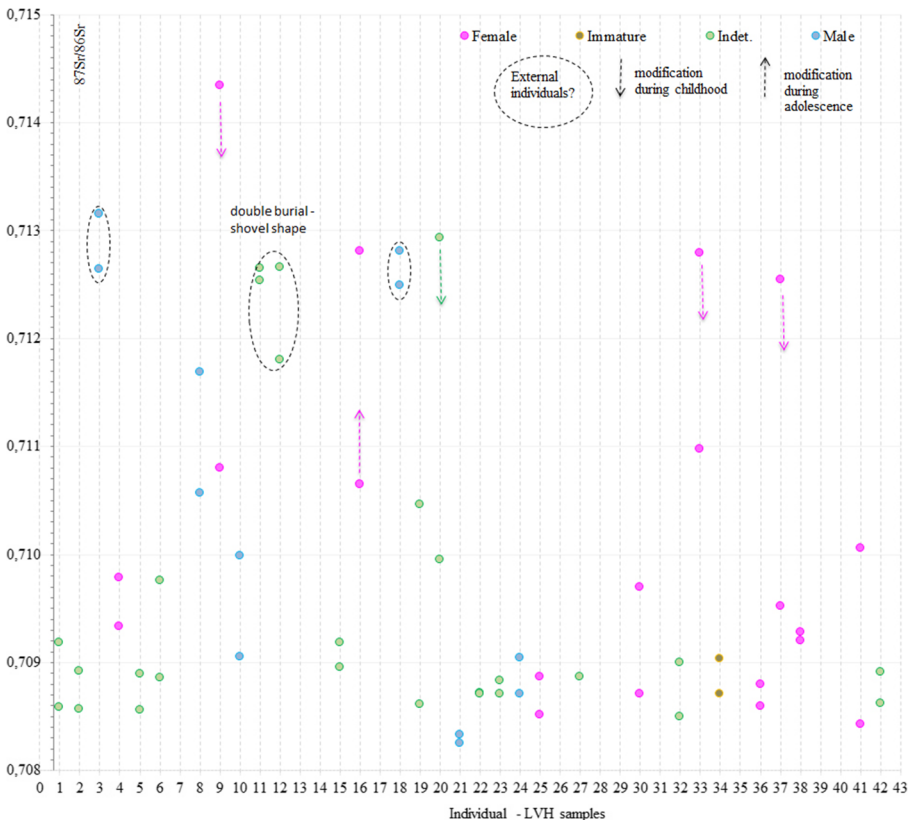


Fig. 4 Radiogenic strontium isotope ratios from human teeth

Table 1 Mitochondrial and Y chromosome SNPs recovered from Vigneau human remains. Vigneau 13: burial 1184 sample LVH 2.6, Vigneau 35: burial 1511 sample LVH3, Vigneau 85: burial 1028–06 sample LVH12

ndmtDNA SNPs															
M*10400	N*10873	N*10873N1a	I 10034	W 3505	X 6371	R*12705	HV*14766	H 2706	H1 3010	H3 6776	V 4580	J 12612	T 1888	U*11467	U4 11,332
13,780															
Vigneau 13	C	-	A	-	A	C	C	-	-	-	-	-	-	-	-
Vigneau 35	C	-	A	-	A	C	C	-	G	G	T	-	-	-	G
Vigneau 85	C	-	A	-	A	C	C	C	A	G	-	-	-	-	A

ndmtDNA SNPs																
Y chromosome SNPs																
SNPsmtDNA SNPs Haplogroups																
Haplogroups																
U5 13.617	K 10550	E L537	E1b1b	F M213	G M201	I M170	J M304	K M9	R M207	R1a M51	R1b M343	Y SNPs Haplogroups				
Vigneau 13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	nd
Vigneau 35	T	G	K	-	-	-	-	-	-	-	-	-	-	-	-	nd
Vigneau 85	T	A	H	-	-	-	-	-	-	-	-	-	-	-	-	nd

It is difficult to compare the whole dataset to other Middle Neolithic populations, as no previous study has combined these techniques. Despite this, the available stable isotope database (CN) on animal and human bone collagen from the centre and the north of France (Goude et al. 2015; Goude et al. 2013; Rey et al. 2017) allows partial regional comparison of dietary practices. Despite being near aquatic resources, the people of Le Vigneau 2 differ from other French and Iberian Middle Neolithic populations in that they did not exploit aquatic foods (Goude et al. 2013; Rey et al. 2017; Salazar-García et al. 2016) (Fig. 5). The immature outlier's values we found are probably more related to physiological phenomena than a specific diet; this period of child growth is poorly documented in stable isotope studies, even though medical literature warns about skeletal growth heterogeneity until pubertal age (Szulc et al. 2000). Compared to other regional sites (Fig. 5), Le Vigneau 2 sites are differentiated by (1) the low variability of carbon and nitrogen data and the absence of gender-based

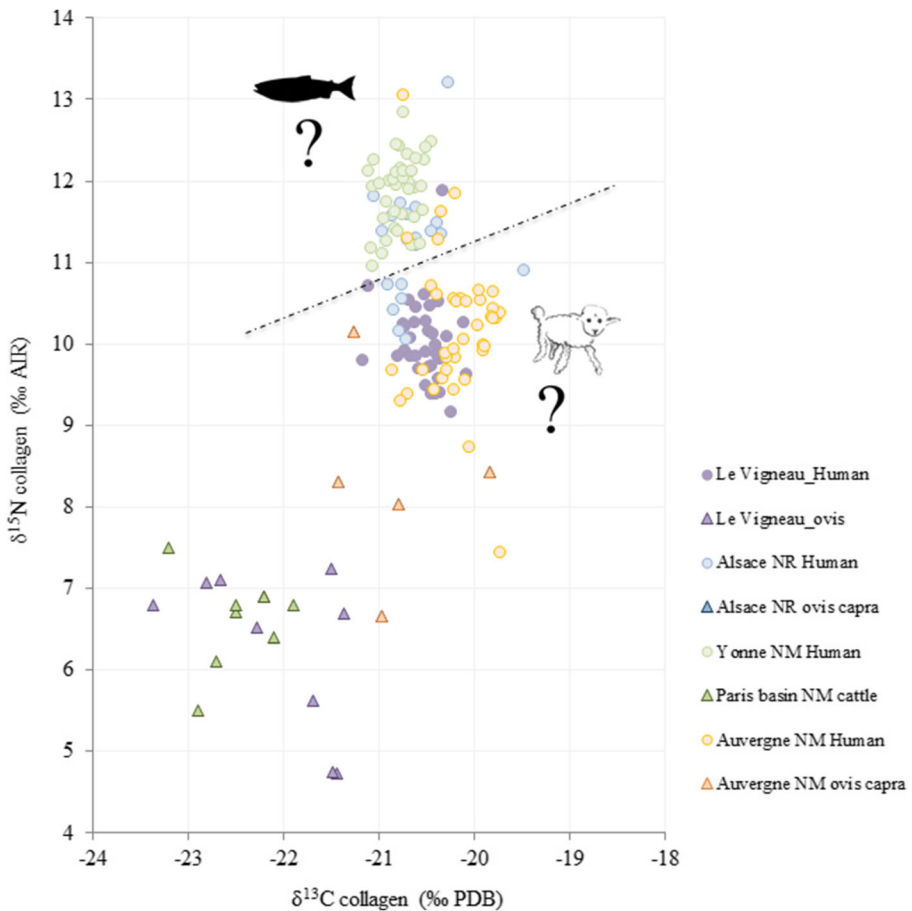


Fig. 5 Comparison of Le Vigneau 2 carbon and nitrogen ratios with those from other Middle Neolithic and Late Neolithic sites in Northern France (from Goude et al. 2013; Goude et al. 2015; Rey et al. 2017). *Dotted line* represents hypothetical delineation between individuals consuming mainly terrestrial resources (*below*), with various amount of animal protein intake and individuals including other resources (*above*) such as freshwater fish/young animal proteins in diet

difference, and (2) the low diversity of animal species potentially exploited. However, gender-based differences in stable isotope data on other Neolithic sites from the region do exist, as is the case at the Gurgy and Pontcharaud sites, and could indicate either different access to food resources (in terms of species preferentially exploited) and/or different mobility patterns (inferred from consumption of resources from different ecosystems and isotopic variability). On Gurgy and Pontcharaud funerary sites, several animal species were found (mainly pig, cattle, sheep, and goat), but sheep are not considered as the dominant resource in these areas, as mentioned by regional archaeozoological data (Bréhard 2011) and stable isotope analysis (Goude et al. 2013; Rey et al. 2017).

Discussion about territorial mobility patterns also suffers from the lack of studies and regional databases (Willmes et al. 2014). Previous works using Sr isotope analysis in southern French Middle Neolithic individuals from five sites (Goude et al. 2012) have highlighted a potential greater mobility for pastoralists than for agriculturalists. Furthermore, at the Middle Neolithic site of Pontcharaud, a patrilocal society in which adolescent females came from abroad into the study groups has been suggested, based on significant isotopic variability (CN) recorded in female bone collagen (Goude et al. 2013). Sr data from neighboring regions in Central Europe support the presence of patrilocal systems from the beginning of the Neolithic, as males indicate lower Sr variability compared to females (Bentley et al. 2012). As we have seen, this is not clear for the Le Vigneau 2 population, neither for carbon and nitrogen nor for strontium isotope data, which shows there is no strong trace of this purported patrilocal social structure in the Middle Neolithic of northern France. However, female Sr variability (0.71018 ± 0.00171 ; $\Delta = 0.00591$; $n = 20$) is greater than what was observed in Early Neolithic Alsatian sites, and agrees with the European patrilocal pattern proposed by authors (Bentley et al. 2012). Ethnographic evidence frequently shows that female exogamy is a common trait (e.g. Murdock 1967; Wrangham 1987), and publications refer to this pattern to highlight the role of female mobility for trade (Brown 2016). However, we should not simply blindly project ethnographic evidence into a deep prehistoric past that might have well had very different social structures and mind-sets to those we have recorded in living communities.

Conclusions and Perspectives

Recent years have seen significant expansion of archaeological science specialization. This broadening field is now targeting many facets of the lives of ancient people. Continuing a data-rich trajectory is important for advancing our knowledge about human societies. However, with the growing specialization available in archaeological science, there is a danger that large amounts of data are created that fail to solve the biggest questions about ancient human societies, due to the lack of optimally multidisciplinary research. It may not at a later stage always be possible to address uneven application of multiple disciplinarity. We show in this study how different techniques can be applied to target a singular research question. The goal of identifying intra-population variability in diet and mobility variation is still an elusive challenge in Neolithic archaeology, and we hope that our study provides a dataset that helps researchers appropriately address this challenge.

Conducting this specific multi-proxy study allowed us to identify different types of foods consumed by Neolithic human groups. Stable isotope data, archaeological artifacts, and anthropological and zooarchaeological information together support the hypothesis of an economy mainly focused on pastoralism, with no visible gender-related difference in terms of food consumption. Dental calculus analysis revealed the exploitation of wild plants, not observed before in Neolithic anthropological studies. The comparison with archaeological hunter–gatherer groups also made it possible to document the variability of plant species exploited, potentially reduced for these agropastoralists in comparison. We observe no food differences between males and females, and our isotopic provenance study suggests no strong trace of a patrilocal social structure, thus the use of the territory by this population is not clearly linked to gender. As a next step, the individuals from Le Vigneau 2 will be studied through dental wear to document whether specific economic activities were carried out (*e.g.* wool, fibre processing) and if males and females had comparable behaviors.

Our ambitious multi-proxy study encountered limitations, particularly for sensitive bioarchaeological material (mainly aDNA, parasite eggs), but further investigations can be considered in the future, such as to test the presence of the human pathogenic amoeba (*Entamoeba histolytica*) in the samples; palaeogenetics could also be performed to test the presence of other type of parasite markers (Côté et al. 2016; Le Bailly and Araujo 2016). We propose that S isotope data may also be able to corroborate palaeogenetic evidence of mobility, as well as providing new information on its timing. This highlights the necessity to further develop archaeological geographical databases and mapping of current soil use and modern potential contaminants. However, it seems necessary to continue such a multi-proxy approach to determine the best archaeological context for this kind of investigation.

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Authors Contribution The site was excavated under the supervision of AH and AC. Anthropological material was studied by JT. Isotope studies were conducted by GG, DCSG, and GA. Dental calculus was studied by RCP. Genetic analyses were conducted by MR, MFD, and MHP, and parasitological study was carried out by MLB. The core of manuscript writing and editing was done by GG, DCSG, and RCP. This pilot study is part of a wider pluridisciplinary project for which other complementary data will be compared particularly to document the place of women at the onset of farming. Archaeozoological material was studied and provided for analysis by Léa Roux. We thank Mariska Carvalho and Julie Power for proofreading.

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