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To cite this version:
Gwenaëlle Goude, Domingo Salazar-garcía, Robert Power, Maïté Rivollat, Lionel Gourichon, et al.. New insights on Neolithic food and mobility patterns in Mediterranean coastal populations. American Journal of Physical Anthropology, 2020, 10.1002/ajpa.24089. hal-02874873

HAL Id: hal-02874873
https://hal.science/hal-02874873
Submitted on 24 Nov 2021

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New insights on Neolithic food and mobility patterns in Mediterranean coastal populations

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Funding information ETICALP programme; Institut Danone France/ Fondation pour la Recherche Médicale; project INTERACT, Grant/Award Number: ANR-17-FRAL-0010

This article was published online on 18 June 2020. An error was subsequently identified in the figures order. This notice is included in the online to indicate that have been corrected 27 June 2020.

Abstract

Objectives: The aims of this research are to explore the diet, mobility, social organization, and environmental exploitation patterns of early Mediterranean farmers, particularly the role of marine and plant resources in these foodways. In addition, this work strives to document possible gendered patterns of behavior linked to the neolithization of this ecologically rich area. To achieve this, a set of multiproxy analyses (isotopic analyses, dental calculus, microremains analysis, ancient DNA) were performed on an exceptional deposit (n = 61) of human remains from the Les Bréguières site (France), dating to the transition of the sixth to the fifth millennium BCE.

Materials and Methods: The samples used in this study were excavated from the Les Bréguières site (Mougins, Alpes-Maritimes, France), located along the southeastern Mediterranean coastline of France. Stable isotope analyses (C, N) on bone collagen (17 coxal bones, 35 craniofacial elements) were performed as a means to infer protein intake during tissue development. Sulfur isotope ratios were used as indicators of geographical and environmental points of origin. The study of ancient dental calculus helped document the consumption of plants. Strontium isotope analysis on tooth enamel (n = 56) was conducted to infer human provenance and territorial mobility. Finally, ancient DNA analysis was performed to study maternal versus paternal diversity within this Neolithic group (n = 30).

Results: Stable isotope ratios for human bones range from −20.3 to −18.1‰ for C, from 8.9 to 11.1‰ for N and from 6.4 to 15‰ for S. Domestic animal data range
from $-22.0$ to $-20.2\%$ for C, from $4.1$ to $6.9\%$ for N, and from $10.2$ to $12.5\%$ for S. Human enamel $^{87}$Sr/$^{86}$Sr range from 0.7081 to 0.7102, slightly wider than the animal range (between 0.7087 and 0.7096). Starch and phytolith microremains were recovered as well as other types of remains (e.g., hairs, diatoms, fungal spores). Starch grains include Triticaceae type and phytolith includes dicotyledons and monocot types aspanicoid grasses. Mitochondrial DNA characterized eight different maternal lineages: H1, H3, HV (5.26%), J (10.53%), J1, K, T (5.2%), and U5 (10.53%) but no sample yielded reproducible Y chromosome SNPs, preventing paternal lineage characterization.

Discussion: Carbon and nitrogen stable isotope ratios indicate a consumption of protein by humans mainly focused on terrestrial animals and possible exploitation of marine resources for one male and one undetermined adult. Sulfur stable isotope ratios allowed distinguishing groups with different geographical origins, including two females possibly more exposed to the sea spray effect. While strontium isotope data do not indicate different origins for the individuals, mitochondrial lineage diversity from petrous bone DNA suggests the burial includes genetically differentiated groups or a group practicing patrilocality. Moreover, the diversity of plant microremains recorded in dental calculus provide the first evidence that the groups of Les Bréguières consumed a wide breadth of plant foods (as cereals and wild taxa) that required access to diverse environments. This transdisciplinary research paves the way for new perspectives and highlights the relevance for novel research of contexts (whether recently discovered or in museum collections) excavated near shorelines, due to the richness of the biodiversity and the wide range of edible resources available.

KEYWORDS
ancient DNA, dental calculus, marine resources, radiocarbon date, stable isotopes

1 INTRODUCTION

The Neolithic is the final periodic division of the Stone Age, which embodies the advent and development of plant cultivation and animal domestication; it also marks a new step in cultural and economic changes that led to profound biological and social modifications in human societies. Studying the diversity of dietary practices as well as their social implications is a relevant way to understand the relationships between the environment and the potential status of individuals within a society. Among these changes, the exploitation of the environment (domestic vs. wild; animal vs. plant; aquatic vs. terrestrial) and the respective social position of females and males are of importance in understanding the rise of new precursory markers of demographic expansion and economic development. The identity of females in Neolithic societies is commonly debated within the context of gender-based division of labor and contributions to subsistence. Ethnological comparisons (Umezaki et al., 2016), modeling of technical-economic systems (Hansen, Jensen, & Skovsgaard, 2015) and osteological analyses (Holden & Mace, 1999) put forward different arguments for the possible roles of each sex (Cohen, 2016). One of the most common hypotheses, sex-based division of task and food acquisition, argues that differential access to resources is determined by sexual division of labor, with males consuming greater amounts of meat and females consuming greater amounts of plant foods (Berbesque, Marlowe, & Crittenden, 2011). Beyond this possible dichotomy, differences in food consumption between females and males have been explored through stable isotope ratios obtained on Neolithic human bone (Goude et al., 2013; Rey et al., 2019; Rey, Goude, & Rottier, 2017). Carbon and nitrogen stable isotope data indicate either a significant difference in animal protein consumption between males and females (Rey et al., 2017) or a wider range of ratios recorded in female bones. The latter may have been linked to the diversity of food consumed and/or mobility and visited environments (Goude et al., 2013).

Mobility is also an important component of dietary patterns and environment exploitation. Studies, based on strontium isotopic data ($^{87}$Sr/$^{86}$Sr), have demonstrated higher female mobility among Neolithic communities in northern Europe (German/Alsace regions) that may be linked to patrilocal residence rules (Bentley et al., 2012). Recent $^{87}$Sr/$^{86}$Sr data from northwestern France (Touraine area; site of Le Vigneau) found that females tended to move between childhood and adolescence, while at the same time carbon and nitrogen isotopic
parameters have, for example, been considered for a Corsican consideration because these features provide regular access to the shoreline morphology (e.g., cliffs, marshes) are important parameters to than Les Bréguières. The short distance to the sea as well as land-resources; however, most of the sites were located at a higher altitude demonstrated a subsistence economy geared toward terrestrial Neolithic sites in the area (review in Goude & Fontugne, 2016) have relation in the Mediterranean area. Previous analyses of Early and Middle Neolithic sites in Liguria demonstrate a reduced consumption of maritime resources, despite shoreline proximity (Goude, Binder, & Del Lucchese, 2011–2013; Goude. Dori, Sparacello, Stамиni, & Varalli, 2020; Mannino, Talano, Goude, & Richards, 2018; Salazar-García et al., 2014).

Considering the importance (size, location, sepulchral mode, and preservation) and the chronocultural attribution of the human remains recovered from Les Bréguières (cf. site description in the materials and method section), this collection was included within a multidisciplinary project (IDF/FRM 2016–2017 program; http://lampea.cnrs.fr/spip.php?article3473) to explore diet, mobility and social organization during the Early Neolithic. The work carried on Les Bréguières endeavors to new data on two main issues: (a) the work will explore diet in relation to mobility and social organization with specific reference to the role of maritime and plant resource consumption; (b) evidence for sex-specific variation in dietary behavior.

2 | MATERIALS AND METHODS

2.1 | Les Bréguières

The Les Bréguières site is located along the Mediterranean coastline of southeastern France, near the Alpes-Maritimes mountains. The site is located in a quarry and was discovered and excavated by Maurice Sechter in 1965–1966 (Figure 1a). The site is a sepulchral gallery (Figure 1b) and, at the time of discovery, an appropriate excavation method for human remains was not employed. However, the material was carefully removed, packed, and stored and it was possible to proceed with new analyses within the framework of a formal bioarchaeological research program (ETICALP, dir. D. Binder 2007–2014). Part of the site, including the initial entrance used during the Neolithic, was destroyed by the quarry activities and only a portion of the site was excavated.

2.2 | The human remains and associated materials

The area excavated yielded approximately 61 individuals found in a collective burial. The site, despite partial destruction, is one of the oldest of this type dated to the Neolithic in the north-western Mediterranean and the oldest in the south of France. Human remains at this site were conmingled. Infants were not among these materials. Sex could be determined from several os coxae (n = 19), and these bones also made it possible to estimate the age in a few selected individuals (n = 13). Pre-adult remains were recovered from the site, but age has not been estimated in these individuals (SI 1a and 1b; Provost et al., 2017). Very few artifacts (lithic tools, faunal remains and worked seashells) were associated with the human deposits and few pottery
shards were found in the deposits postdating the burial. Caprine (mainly sheep) and suids dominate the faunal assemblage; a few dog and cattle elements were also identified as well as wild species (including wild boar, fox, hare, and golden eagle). Cut-marks found on some caprine, suid, and bovid bones suggest they result from butchery and consumption activities. These findings must be interpreted with caution as Les Bréguières is a funerary deposit and no contemporary dwelling has been found nearby. Moreover, because of the limited field documentation, the original relationship between the animal and the human bones remains poorly understood.

2.3 | Chronology of the deposit and special features of the site in the regional context

A first series of 10 direct AMS measurements placed the site’s use as of collective burial between the end of the sixth and the beginning of the fifth millennium BCE (Provost et al., 2017). This has shed new light on the variability of funerary practices during the late Impresso-Cardial (IC) and Square-Mouthed Pottery culture (SMP) transition. Most of the burials of this period are isolated within domestic or herding sites, generally occurring as single burials or in a few cases as groups of small sets of several individuals (Zemour et al., 2016). Deposits of over 10 individuals are rare, for example, Orco Feglino—Arma dell’Aquila in Liguria (Bigi & Starnini, 2018), Cabrières—Baume Bourbon in Occitania, La Garrotxa—Cova de l’Avellaner in Catalonia, including cannibalized remains from Salernes—Baume Fontbrégoua in Provence and Ambérieu-en-Bugey—grotte du Gardon in Dauphiné (Zemour et al., 2016). In addition, regardless of the number of individuals, the funerary practices are associated with a large variety of subsistence activities at these sites. In this context, the reassessment of the Les Bréguières assemblage shows a contrasting situation, since it indicates that formal cemeteries situated away from habitation sites do exist as early as the IC period. The special configuration of such sites, here in a narrow fault, as well as the scarcity of associated goods, probably explains the low visibility of these locales.

The configuration of the site highlights some biases in the general appraisal of funerary practices and raises the issue of the reliability of palaeodemographic modeling within the Mediterranean Neolithic context (Bocquet-Appel & Bar-Yosef, 2008). In order to make the chronological range of the funerary occupation more accurate, another set of direct dates were estimated. Finally, 25 samples already selected from 23 human skulls for combined isotopic and paleogenetic studies, and from two animal and one plant remains have been dated; two skulls (BR H20_3236 and BRG H04_3386) were dated twice (SI2). Bayesian modeling was performed through ChronoModel v.2.0.18 (Lanos, Philippe, Lanos, & Dufresne, 2016) and IntCal.13 curves (Reimer et al., 2013). Out of the 23 dated skulls, 19 have adequate documentation which places these remains within the sequence described by the original field observations. This places the remains between layers C1 at the top and C8 at the bottom. For these 19 skulls, as well as for the three animal or plant remains attributed to the archaeological layers, it is possible to apply constraints in a Bayesian perspective (Figure 2). For the remaining four skulls, it appears that all are included in the chronological range given by the stratified ones. Using the modes a posteriori as representations of the beginning (BMAP) and end (EMAP) of the different phases (Binder et al., 2017), the final result (SI2) shows that most of the dated human remains (24/25) have been deposited between ca. 5,250 and 4,600 BCE. This range of dates covers distinct cultural episodes without indication of significant gaps. The earliest deposits from phase C8_C6 (four individuals) and C5 (seven individuals), from BMAP 5210 to EMAP 4875 BCE, postdate the Early Cardial (ca. 5,500-5,250 BCE) period which follows the pioneer Impressa settlements in the Tyrrenian side (ca. 5,800-5,500 BCE) (Binder, Lanos,
et al., 2017). This corresponds to a period of intense cultural turnover. Indeed, this temporal range includes Classical/Late Cardial mainly in western and central Provence, the earliest aspects of the Pre-Chassey in central Provence and aspects relating to the SMP Transition in eastern Provence and Liguria. The latter are probably under the influence of Neolithic groups from northeastern Italy, that is, Vho and Fiorano (Binder, Battentier, Delhon, & Sénépart, 2017; Binder, Lepère, & Maggi, 2008; Starnini, Biagi, & Mazzucco, 2018). The deposits from phases C4 (three individuals) and C3_C2 (eight individuals), from BMAP 4850 to EMAP 4430 BCE, cover the range of the Early SMP, that is, Finale-Quinzano, in Liguria and a large part of the Pre-Chassey culture (Binder et al., 2008).

2.4 | Sample selection

Sample preparation is described in detail in SI 1a and 3 to 5.

The multiple burial context is usually not favorable to develop such an approach, but bone selections permitted the investigation of the different issues. The minimum number of individuals of the human collection was established with the femur, determining at least 61 individuals, including 35 adults and 26 pre-adults (Provost et al., 2017). Even if providing the greatest number of individuals, the femur was not included in our sampling selection, as not informative regarding the sex determination and not efficient for aDNA and strontium analyses. Two anatomical elements were thus selected: (a) coxal bone (n = 17) and (b) skull associated with mandible and/or maxilla (n = 19). For coxal bone selection, only remains providing reliable sex determination (>99%; Murail, Bruzek, Houët, & Cunha, 2005) were used. This includes eight male and nine females (Provost et al., 2017; SI 1). The internal face of the ilium part was sampled for all coxal bones, by using small Dremel® like saw and cutting device (diam 10 mm). Bone chunk is used for CNS stable isotope analysis to discriminate, if applicable, male vs. female diet and mobility. For skull selection, only skulls in

FIGURE 2  Model of phases built for Les Bréguières AMS dates. The constraints are only applied on groups of events with available information on relative stratigraphic position (in brown); the chronological range of samples with unspecified stratigraphic location is colored in gray. Light colors correspond to the density of probability of phase beginnings, darker ones to phase ends.
association with the mandible and/or maxilla were selected. This sample includes 19 adults (18 + 1 previously sampled in Provost et al., 2017) and 17 immature skulls (SI 1). This sampling procedure permits access to carbon, nitrogen, and sulfur stable isotope analysis, petrous bone for aDNA, teeth for calculus microremains study and teeth for $^{87}\text{Sr}/^{86}\text{Sr}$ analysis. Only one individual (Br 19) provided anatomical elements in connection, which permitted extraction from both cranial and postcranial elements. For the other samples, there remains the risk of duplicating results from the same individual; however, in a first step, coxal and skull samples are independently studied. To complete, several animal species (domestic and wild: SI 1) were sampled for bone stable isotope analysis (C, N, S), for tooth enamel $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and for calculus, to provide local environmental baselines.

### 2.5 Methods

Stable isotope analysis of carbon, nitrogen, and sulfur (C, N, S) was performed to infer protein intake during tissue development (Ambrose & Norr, 1993; Szulc, Seeman, & Delmas, 2000; Valentín, 2003). Carbon isotope ratios allow discussing the environment in which food items were gathered-hunted and/or cultivated-bred. Nitrogen isotope ratios also allow environmental discrimination and anthropic management of soils, but mainly provide additional information on the trophic level of individuals (more methodological details are available in Bogaard, Heaton, Poulton, & Merbach, 2007; Hedges & Reynard, 2007; Herrscher & Goude, 2015; Richards & Hedges, 1999; Webb et al., 2017). Sulfur isotope ratios are commonly used as a geographical marker and help differentiate between exploited environments (marine vs. terrestrial vs. freshwater; Nehlich, 2009), if local isotopic baselines are properly provided (Nehlich, 2015; Zazzo, Monahan, Moloney, Green, & Schmidt, 2011). In inland contexts, the combination of these isotopes in human bone permits identification of food consumption from different ecological areas (Goude & Fontugne, 2016) and the consumption of freshwater resources (Drucker et al., 2018). These data additionally provide information on the consumption of marine resources and/or the closeness to the sea in coastal areas (Lelli et al., 2012; Virginia & Delwiche, 1982); foods from estuarine environments can also be detected (Eerkens, Byrd, Spero, & Fritschi, 2013; Salazar-García et al., 2014).

Dental calculus is oral plaque that has been mineralized by saliva-deposited calcium phosphate. This ubiquitous oral disease can often be found on ancient human and animal teeth. Ancient dental calculus is a useful supplement to dietary reconstruction because it can contain embedded dietary information including starch grains, phytoliths, lipids, proteins and DNA from plant and animal foods (Bovadajian et al., 2016; Dobney & Brothwell, 1986, 1988; Power, Salazar-García, Wittig, & Henry, 2014; Power et al., 2018; Warinner et al., 2014). Although dental calculus presents a highly stochastic record (Leonard, Vashro, O’Connell, & Henry, 2015; Power, Salazar-García, Wittig, Freiberg, & Henry, 2015), it is uniquely valuable because it offers direct information on food consumed and on substances that entered the mouth such as fibers, medicine and even airborne dust (Armitage, 1975; Hardy et al., 2009; Henry & Piperno, 2008; Leonard et al., 2015; Power et al., 2014, 2015, 2016; Warinner et al., 2014).

For this study, 24 samples of human dental calculus were analyzed, with six samples including faunal dental calculus and archaeological sediment as controls. Each tooth in the study was evaluated and sampled for dental calculus at the CEPAM Laboratory (Nice, France) (SI 4). Most teeth had dental calculus deposits and further description of methods are available in SI 4.

Strontium (Sr) isotope analysis on tooth enamel ($n = 56$) was performed to infer human provenance and territorial mobility (Strauss et al., 2015). The Sr isotope ratio used for this analysis depends on the proportions of $^{87}\text{Sr}$ and $^{86}\text{Sr}$ isotopes found at different geological areas. Strontium enters ecosystems through weathering rocks, through the soil and into the food chain. It eventually gets incorporated into mammal tissues such as enamel by substituting calcium, all with minimal isotopic fractionation (Ercison, 1985; Faure & Powell, 1972; Graustein, 1989). As living organisms incorporate Sr mainly through food but also water, this means that the Sr in enamel is ultimately derived from the Sr of the bedrocks, soils and water where individuals were living when the teeth were formed (Bentley, 2006). The $^{87}\text{Sr}/^{86}\text{Sr}$ signature of a specific location, which varies between 0.700 and 0.750 on Earth, is determined by the underlying bedrock age and its content of rubidium (Rb). Older geological formations like granite rocks have higher $^{87}\text{Sr}/^{86}\text{Sr}$ values than younger geological formations such as volcanic rock because radiogenic isotope $^{87}\text{Sr}$ forms by radioactive decay from rubidium ($^{87}\text{Rb}$). Effects such as weathering, hydrological cycles, biopurification and diagenesis will also affect the ultimate bioavailable Sr ratio from a specific location and should consequently be considered when designing the geological sampling strategy and discussing the interpretation (Bentley, 2006; Slovak & Paytan, 2013).

Tooth enamel, a highly mineralized (96%) tissue mainly composed of apatite, is the preferred substrate for biogeochemical analysis as it is more resistant to diagenesis than the burial environment than other skeletal tissues (Budd et al., 2000; Hoppe et al., 2003). Because enamel has no turnover during life (Nanci, 2013), an inedible isotopic record of infant and childhood diet is captured in this tissue (Humphrey et al., 2008). Due to the incorporation of bioavailable Sr values in humans during enamel mineralization, isotopic data can be used to provide information on provenance and territorial mobility based on the idea of matching the isotopic signatures from an individual to the biologically-available signature at a suspected location of origin. Furthermore, it is potentially possible to identify other changes occurring during infancy and childhood such as birth, breastfeeding, and weaning (Humphrey et al., 2008). When studying provenance and territorial mobility it is useful to compare teeth from a same individual that reflect different moments of its life, such as by comparing a P2/M2 with an M3 to assess through $^{87}\text{Sr}/^{86}\text{Sr}$ if changes in geological substrate occurred between childhood and early adulthood. Population-wise, the analysis of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in tooth enamel from a large number of individuals from a single population can also be useful to tag possible nonlocals. In order to be able to retrieve more detailed information on territorial mobility, bioavailable Sr
mapping is necessary (Evans, Chenery, & Montgomery, 2012; Price, Bentley, Lüning, Gronenborn, & Wahl, 2001), but its elevated cost makes difficult the detailed mapping of bioavailable Sr in the study region. Sample preparation for Sr analysis is provided in SI 1a.

Finally, ancient DNA (aDNA) analysis was performed to infer maternal vs. paternal diversity in the Les Bréguières group. A total of 30 samples derived from preserved petrous bone were submitted for DNA analyses that targeted uniparental markers, that is, mitochondrial DNA (mtDNA), for the characterization of maternal lineages and Y chromosome for paternal lineages (see details in S15; Mendisco et al., 2011). Because the human remains were excavated without decontamination methods, bones were systematically decontaminated, that is, scraped, cleaned with bleach and subsequently exposed to UV radiation for 20 min on each side (Boessenkool et al., 2017). All procedure details concerning DNA isolation and mtDNA/Y chromosome SNPs (Single Nucleotide Polymorphisms) analyses are available in Rivollat et al. (2015) and in SI 5. The dataset compiled by Beau et al. (2017) and grouping mitochondrial data available for Mesolithic to Late Neolithic populations (ca. from 14,500 to 2,600 cal. BCE) was used to explore the relationships of the Les Bréguières group with other ancient populations. The dataset was divided into 16 chronological, geographical, and cultural groups for the sake of clarity (see details in Beau et al., 2017). Classical PCA based on haplogroup frequencies was performed in Past version 3.14 software (Hammer, Harper, & Ryan, 2001) using major European and Near Eastern haplogroups (see Rivollat et al., 2015 for details).

When appropriate, statistic comparisons were performed by using nonparametric tests (Mann-Whitney U test, median test and Kruskal-Wallis test; Statistica 9.1®) due to the small sample size.

3 | RESULTS

3.1 | Carbon and nitrogen stable isotopes

Complete datasets are provided in detail in SI 1b and 2 to 6.

Animal values indicate different ranges according to the species (Figure 3; SI 1b). Domestic caprine, Bos sp. and Sus sp. data range from −22.0 to −20.2‰ for C (n = 11), from 4.1 to 6.9‰ for N (n = 11) and from 10.2 to 12.5‰ for S (n = 7). Values recorded for C and N are in the known variability of these species for the period and the region (Goude & Fontugne, 2016). Sulfur ratios are in the variability of data recorded for prehistoric animal remains located in the Mediterranean and Adriatic areas (Lelli et al., 2012; Varalli, Moggi-Cecchi, Moroni, & Goude, 2016). Stable isotope ratios for human coxal bones and juvenile skulls range from −20.1 to −18.1‰ for C (Δ13C 2‰; n = 34), from 8.9 to 11.1‰ for N (Δ15N 2.2‰; n = 34) and from 6.4 to 14.6‰ for S (Δ34S 8.2‰; n = 33). Stable isotope ratios for human adult skulls range from −20.3 to −18.9‰ for C (Δ13C 1.4‰; n = 19), from 9.0 to 10.9‰ for N (Δ15N 1.9‰; n = 19), and from 9.3 to 15.0‰ for S (Δ34S 5.7‰; n = 18). The mean difference between animal species (caprine, Bos sp., Sus sp. and deer) and human coxal, as well as juvenile samples, is 0.9‰ for C and 4.3‰ for N (Figure 4).

Sulfur data for animals and human bones are presented in Figure 5, as well as combined with published prehistoric regional data in Figure 6 (Lelli et al., 2012; Varalli et al., 2016). The local range was established by using available animal values for the site (caprine, Sus sp. and deer). Humans exhibit different ranges. Except for two females (Br 3723, Br 3696), the rest of the coxal bone values fall within the local range as do most of the juvenile skull values.

The adult skulls S isotope ratios present two distinct groups, with four individuals within the range of the values from coxal bones and juvenile samples, while the rest of the individuals have higher S ratios.
Adult and juvenile skulls significantly differed ($p < .01$; Mann–Whitney U test); juveniles providing lowest $\delta^{34}$S compared to adults. On this basis, it is possible to have individual compatibility between four adult skulls and the coxal bones and between two female coxal bones and the rest of the adult skulls. In such a context, and assuming there is no significant difference in bone turnover between the two anatomical structures (thus not marking different life periods; Fahy, Deter, Pitfield, Miszkiewicz, & Mahoney, 2017), a second step of the analysis explored isotope data by group and then by excluding the potential compatible samples: (a) 15 coxal bones (i.e., excluding the 2 females Br 3723 and Br 3696 with higher S values possibly belonging to the individuals represented by the skulls also with higher S values), (b) 17 juvenile skulls, and (c) 14 adult skulls (i.e., excluding the three skulls with lowest S values potentially compatible with coxal bone data, the duplicate of Br H19_0019, and Br H03_3386 bis with no S data). The comparison between the three groups (representing ca. 88% of the initial sampling) indicates a significant difference in N and S isotopic ratios. This difference is still observable when comparing only the adult samples (coxal bone vs. skull) and when comparing the juvenile to the adult skulls for S ($p < .01$; Mann–Whitney U test).

### 3.2 Dental calculus microremains

Plant consumption was identified in dental calculus from 15 individuals sampled (Figure 7). Samples of the exterior calculus taken from the decalcification rinses produced infrequent microremains. However, one rinse produced several hundreds of fungal spores. In three of the four samples, microremains were rarer than in the inner sample, except in H22_4239 which was an unusually low-mass sample. Across the samples, low numbers of starch and phytolith microremains were recovered (Figure 7; SI 4). Starch grains belong to five types, including one consistent with Triticeae, a hard endosperm starch producing seed (Figure 7). Grass phytoliths closely follow the morphology of grass cells and are readily identifiable using Twiss’s conventional guide for grasses, in contrast to phytoliths from dicotyledons (woody herbaceous or arboreal plants), which have irregular forms (Albert, Weiner, Bar-Yosef, & Meignen, 2000). These phytoliths were identified with the help of a variety of sources (Metcalfe & Chalk, 1950; Pearsall & Dinan, 1992; Piperno, 2006; Twiss, Suess, & Smith, 1969). The monocot types of phytoliths that were found included long-cell elongates, parallelepiped and trichomes. These species are indicative of grasses but undiagnostic of the type of grass or distinctions between domesticates or wild grasses. However, 13 short-cells including seven rondels deriving from pooid grasses and two bilobates from panicoide grasses were found. Grass types were identified in nine of the human dental calculus samples, while just six individuals show dicot types (H09_3356, H16_3252, H31_3218 and H29_3240). These included plate, jigsaw, globular rugulate/echinate, and other phytolith morphotypes indicative of dicots (Metcalfe & Chalk, 1950; Pearsall & Dinan, 1992). A variety of other microremains were found in small numbers, including unsilicified plant tissues from monocots, dicots and a conifer, calcium oxalates deriving from a plant, benthic (a shallow water type) diatom, fungal spores, mammal hairs, a feather fragment and microcharcoals.

### 3.3 Strontium isotope ratios

All Sr isotope data generated in this study is in SI 3. The concentration of Sr from all archaeological samples (both human and faunal) is consistent and therefore points to taphonomic robustness of the enamel and
discards confounding traceable diagenesis. Background data to define the local bioavailable Sr and with which to compare the human values studied have been obtained from both the IRHUM database (Willmes et al., 2014) and meso- and micro-fauna analyzed from the immediate surroundings of the site itself (Figure 8). Data from the fauna (European hare and hedgehog; $n = 7$) range between 0.7087 and 0.7096, showing only slightly higher values than topsoil and grass samples recorded in a nearby area (8 km north from the site) for which data are available in the IRHUM database (Willmes et al., 2014). All human enamel values range from 0.7081 to 0.7102 ($n = 32$). Except for one individual, H32_3260—which belongs to the earliest deposits—all human data plot within the defined local range (Figure 8). Furthermore, for those individuals from whom two different dental pieces were analyzed, almost no difference is observed between the different stages of the life cycle. The $^{87}\text{Sr}/^{86}\text{Sr}$ values of individual H32_3260 are only slightly higher than those of the local range obtained from fauna, topsoil and plants from the surrounding area of the site: values of 0.710187 (M2) and 0.709979 (M3). When calculating the mean of all human sample Sr isotope values from the population (0.708812 ± 0.000988; 2σ), both values still plot outside of the local range (0.707823–0.709800). Notwithstanding, given the current archaeological and anthropological elements available, this potential non-local individual does not present any further particulars to discuss.

3.4 | Ancient DNA data

The aDNA analyses (SI 5) and the chronological modeling of mtDNA lineages distribution (SI 7) provided additional elements for discussion concerning the Les Bréguières group composition. From the 30 analyzed individuals (represented by petrous bones), complete and repeatable mitochondrial SNP profiles could be obtained for 20 individuals (66.6% success rate), permitting the characterization of eight different maternal lineages (haplogroups) for the Les Bréguières population (Table SNPs SI 5). Unfortunately, no samples yielded reproducible Y chromosome SNPs, preventing any paternal lineage characterization. Complete and authenticated HVS-I sequences proved difficult to obtain in most of the human remains analyzed. Such a classical palaeogenetic approach was, therefore, abandoned. Consequently, all analyses presented here were based on mitochondrial haplogroup (lineage) diversity. A significant diversity of
maternal lineages characterized the group of Les Bréguières, including H1, H3, HV, J, J1, K, T, and U5 lineages. The PCA based on aDNA haplogroup frequencies compiled for ancient European groups (see SI5 for details of the groups) showed that the maternal diversity of the Les Bréguières community (BRE) fits well with the known ancient European Neolithic population diversity (clearly distinguished from ancient hunter-gatherer groups; SI5). Moreover, it is worth noting that the population of Les Bréguières stands in an intermediate position between groups composed of Early Neolithic farmers from Central Europe and those from the Iberian Peninsula. This position is linked to (a) the frequencies of lineages HV (5.26%) and J (10.53%) in the BRE group which are close to those in Central European Neolithic groups (LBKT and LBK), (b) the low frequency of lineage T (5.2%) which is closer to the frequency found in Cardial groups from Iberia (CRD), and (c) the noticeable frequency of lineage U5 (typical hunter-gatherer lineage; 10.53%) that drives the BRE group closer to Middle/Late Neolithic groups from France (MNF, MICH), Central Europe (BEC), or Iberia (LNS).

4 | DISCUSSION

4.1 | Terrestrial versus marine environment exploitation

Isotopic data from all the coxal bone and juvenile skull compared with potential consumed animal species (caprine, Bos sp., Sus sp. and deer) indicate elevated reliance on terrestrial animal protein, as is often observed during the Neolithic in eastern Provence and Liguria (Goude et al., 2011–2013). However, one male (Br 3515) presents data consistent with the consumption of different proteins, probably marine resources (France, 1994; Schoeninger & De Niro, 1983). The correlation between C, N, and S isotopic values was not examined of all the coxal bones. Thus, the hypothesis of a diet turned toward marine resources is not favored to explain the specific δ³⁴S recorded in two female coxal bones (Figure 5). This data suggests these two women lived in a different place, perhaps more exposed to the sea spray effect, a factor known to add marine enriched S in soils, which is then reproduced in primary producers (Zazzo et al., 2011). Few juveniles (ca. one third of the immatures), and more particularly Br 3256 bis, present low S values, compatible with a more inland geographical origin (Figure 5).

CNS bone data were secondly considered along three new groupings excluding the potential compatible samples. The N and S isotopic ratio differences recorded could be due to different bone turnover, life phases, and element uptake for each of the three identified groups, each represented by a different anatomical element. This result leads to questions about the geographical origins and the use of the funerary space between 5,250 and 4,600 BCE. Different dietary patterns and/or the exploitation of different ecological habitats emerge, particularly for individuals represented by adult skulls (Figure 9). Some of these individuals show high N and S values (see SI1, individuals with δ³⁴S > 13.0‰) which are compatible with either...
the sea spray effect (Virginia & Delwiche, 1982) and/or consumption of $^{15}N/{^{34}}S$ enriched resources (possibly marine foods). This pattern seems slightly different to that of the male individual previously highlighted as having high C and N isotopic ratios (Br 3515). A comparison with other prehistoric human groups and coeval animal remains from eastern Provence and Liguria strengthens the specific trophic position of some of the Les Bréguières individuals (Figure 9; Pettitt, Richards, Maggi, & Formicola, 2003; Le Bras-Goude et al., 2006; Le Bras-Goude, Binder, Simon, & Richards, 2006; Le Bras-Goude, Binder, Zemour, & Richards, 2010; Müller, Le Bras-Goude, Buscaglia, & Reiche, 2009; Goude et al., 2011–2013). When the pattern is compared to that of an Upper Paleolithic individual and considering the wide range of values of marine fish and mammals in the Mediterranean and Adriatic (García-Guixé, Subirà, Marlasca, & Richards, 2010; Lightfoot et al., 2011; Pouydebat, 1997; Salazar-García et al., 2014), the consumption of marine food appears as a suitable hypothesis to explain the outlier individuals, in particular the male Br 3515 and Br H28_3220. For the other individuals, dietary habits are not evident as carbon isotope ratios do not correlate with a N and S increase. The comparison between the animal data (caprine, Bos sp.,...
Sus sp. and cervid) of Les Bréguières and other Liguro-provençal areas does not show a statistical difference; however, the same comparison for humans indicates a significant difference (excl. Outlier Br individuals) for both C and N. Even if significant, the difference recorded is small (+0.1‰ for C + 0.8‰ for N, in favor of the Les Bréguières individuals) which could be explained by different geographical origins and the influence of the sea spray effect on the data.

For further discussion, local marine resource datasets are needed, which for the moment are unavailable. Previous analyses in the area (Early and Middle Neolithic; review in Goude & Fontugne, 2016) confirmed a subsistence economy turned toward terrestrial resources. However, most of these sites are at a higher altitude than Les Bréguières. The new data from Les Bréguières bring to light, for the first time, a diversity in protein intake, including the general trend observed among local Neolithic human groups (consumption of terrestrial resources) and the peculiarity of two (or more?) individuals consuming marine foods. This result raises the question of the origin and social status of the buried individuals and the potential link with food consumption. Indeed, if only very few isotopic data indicate significant marine protein intake in coastal early farming communities so far, this could reflect purposeful burial for specialized individuals within caves around this region. The funerary distinctiveness of Les Bréguières (a collective burial) could be considered a mark of a “less selective” place, used by several generations with possibly different origins and cultural traditions.

Contrary to what is shown by the S isotope data, the apparent homogeneity of the Sr isotope values suggests that the Les Bréguières individuals sampled portray relatively low or non-existent territorial mobility (or an intralife mobility which is not visible through teeth Sr data). This contrasts with the main trend observed through the S stable isotope analysis. These findings suggest that S values observed could reflect diet instead of mobility, and in this case, foods could have traveled through a trading network instead of migratory practices. In any case, and as is usual in this type of approach, the lack of a detailed bioavailable mapping of the broader region limits interpretation. It is also possible that individuals with Sr values compatible with those of the area surrounding the burial migrated from regions with geological features and strontium values. Accordingly, the dataset would simply reflect the $^{87}\text{Sr}/^{86}\text{Sr}$ homogeneity of a larger territory.

### 4.2 Evidence for plant consumption diversity by Neolithic people

With the exception of those from isolated teeth, the assemblages of microremains from Les Bréguières are suggestive of diets with a significant contribution of cereal plant foods. There is also evidence of leafy dicot plants in the diets. Pooid grasses, such as wheat or barley, seem to have been predominant but phytoliths from panicoids were also recovered. Panicoid phytoliths have previously been found in early Neolithic sediments at Castellar—Pendimoun, a few km to the east (Delhon, Binder, Verdin, & Mazuy, 2020). This study could only find three taxa (all wild) that may have contributed such as phytoliths including *Setaria* spp., *Digitaria sanguinalis* or *Echinochloa crus-galli*. *Triticum* species may have been collected at Les Bréguières either intentionally for consumption or accidentally during harvesting of crop fields. Unfortunately, preservation is low for these highly diagnostic types, such as multicell husk phytoliths.

The archaeobotanical dataset available for the Early Neolithic in Provence is still limited but information on cultivated and collected wild plants has been gathered from several sites. It is well known that Neolithic farmers of the late IC of Southern France favored naked wheat (*Triticum aestivum/turgidum*) and naked barley (*Hordeum vulgare var. nudum*) (Marinval, 1993), while glume wheats, emmer (*T. dicoccum*) and einkorn (*T. monococcum*), were also emphasized, especially at the beginning of the Neolithic (Binder et al., 2020; Bouby, Durand, Rousselet, & Manen, 2018; De Vareilles et al.,...
The predominance of naked cereals in several sites from the end of the sixth mill. BCE could possibly explain the under-representation of multicell husk phytoliths, as glumes of naked cereals are easily eliminated by simple threshing, often immediately after the harvest. This explanation could also be offered at Le Vigneau as naked cereals were favored in the whole Chassey culture area until ca. 4,000 BCE (Martin et al., 2016). Alternatively, the under-representation of husk phytoliths could be explained by (a) the thorough processing of any kind of cereal and by removing most of the husk and (b) the poor preservation of these phytoliths due to local alkaline soil conditions.

4.3 The diversity of plants consumed may be linked to human mobility

Although there is not a wide variety of datasets to compare with, the Les Bréguières results are relatively richer than those from Le Vigneau (Goude et al., 2019). This could possibly reflect a more diversified diet at Les Bréguières regarding plant foods. It is especially true regarding the four individuals (H09_3356, H16_3252, H29_3240, H31_3218), who yielded the most diversified content in micromains, including phytolith evidence for the consumption of eudicotyledon plant products. It is worth mentioning that these individuals are adults presenting high $\delta^{13}$S values (SI 1b and 4), potentially coming from other places, maybe closer to the seashore, compared with the other individuals. Cultivated eudicotyledons are rather scarce in the archaeobotanical record. From a global point of view, carbonization favors the preservation of cereal remains compared with most of the dicotyledons; cereal processing can involve heating at different steps and caryopses preserve well when charred. Eudicotyledons are therefore often under-represented in charred archaeobotanical assemblages. Pulses have been occasionally encountered in Cardial sites from Provence (end of the sixth mill.), at la Font-aux-Pigeons (Châteauneuf-les-Martigues; Marinval, 1988) and la Baume Fontbré goua (Savard, 2000). Various wild eudicotyledon fruits and seeds were also probably eaten by Neolithic populations. Fruit remains were found at several Cardial sites in Provence, especially acorns (Quercus sp.) at Pendimoun, Grotte Lombard, Vieux Mounoi and Fontbré goua as well as several other tree and shrub species in this last site. Additionally, many wild resources, such as leaves, bulbs, roots, seeds from herbaceous plants, were also likely gathered but, due to preservation problems, can only be identified in rare favorable conditions (Behre, 2008).

Wild resources probably represented an important proportion of the eudicotyledons consumed by Neolithic people, but barely left any trace in most sites. The diversity of biotopes all rich in eudicotyledon plants, accessible at a short distance from Les Bréguières, from the seashore to mountain environments, probably favored the use of a high diversity of food resources. These results provide the first evidence that the groups of Les Bréguières consumed a wide breadth of plant foods that required access to diverse environments.

4.4 A social organization potentially ruled by a patrilocality system

The mitochondrial data obtained for the Les Bréguières group highlighted a maternal pool that fits well with the known ancient European Neolithic population diversity (clearly distinguished from ancient hunter-gatherer groups; SI5) and that stands in an intermediate position between groups composed of Early Neolithic farmers from Central Europe and the Iberian Peninsula. This intermediate position, between the more ancient and the contemporary farming groups associated with distinct neolithization migratory waves, raises questions of surrounding genetic affiliations between individuals at Les Bréguières and Neolithic migrations (Rivollat et al., 2015). The low sample size recovered from the Les Bréguières group and the current lack of genetic data for other Early/Middle Neolithic groups from southern France (or even Italy), however prevents any further interpretation. The noticeable mitochondrial diversity measured in the Les Bréguières group has been regularly observed in Neolithic farming groups (Lacan et al., 2011; Szécsényi-Nagy et al., 2015) and could be an indicator of a patrilocal residence rule. Evidently, Y chromosome data are necessary to directly test this hypothesis. Nevertheless, given the important chronological distribution of the individuals (over more than 30 generations), a more parsimonious but not exclusive explanation would be the use of the funerary site by maternally differentiated groups. This suggestion finds a notable echo in their dietary diversity at the site. Whatever the explanation, the Sr data indicate that individuals share homogenous Sr values compatible with those measured for the area surrounding the cave. That would mean (a) that, in the case of patrilocality, the females originated from geographically close communities and (b) that, with regards to the use of the funerary site by different groups, these groups originated from the same local territory. Through chronological modeling of mtDNA haplogroups an analysis of their distribution through time, that is, during the use of the burial, can be conducted (Figure SI2).

The individuals with haplogroups K and H1, which are better represented, respectively with seven and four individuals, can be observed throughout the site’s use, from ca. 5,160 to ca. 4,600 BCE (i.e., Late Cardial/Vho group and Early SMP), potentially indicating genetic continuity over time (Tables SI2). For the remaining mtDNA lineages, the number of individuals is unfortunately too small to discuss chronological distribution. For U5 and J1, which could appear even earlier than K and H1, stratigraphic constraints are lacking for half of the individuals, a fact that could explain the wider spread of probability densities. Only the T individual could have post-dated the others. No clear association could be observed between a specific period of use of the burial and maternal lineages. Nevertheless, the specific presence of lineages K and H1 (five and three individuals respectively) among clusters C2–C4, that is, between 4,840 and 4,650 BCE has to be pointed out. The maternal homogeneity encountered for this short period could correspond to individuals tied by kinship, being buried together. Finally, when comparing mitochondrial haplogroups to both groups determined by S isotopic ratio as (a) falling within the local range and (b) under the influence of the
seashore, it appears that each group has its own distribution of mitochondrial profiles, except for K, a typical Neolithic haplogroup, which is present in both groups. For unique haplogroups, this does not reveal any pattern. However, a pattern appears for H1 \((n = 4)\) and U5 \((n = 2)\) which are both in the group with a local signal. This could suggest an internal biological structure of the Les Bréguières group with two different dietary behaviors, which needs to be investigated in the near future. In sum, palaeogenetic results for the Les Bréguières group are only preliminary and future genome-wide analyses are necessary to provide more information on community composition and origin, residence rules or even diet/health questions that are documented through isotope analyses.

### 4.5 New insight on Mediterranean Neolithic behaviors

This study has demonstrated that question-driven sampling strategies for multiproxy methodologies can provide meaningful data from old and poorly documented osteological collections. The sampling and methodological strategies allowed documenting the objectives of the research. First, the necessary evidence to reevaluate the explanation on early farming diets was collected. The results demonstrate that the diversity of food resources consumed was probably related to the richness of local Mediterranean biodiversity. On the one hand, marine proteins were significant for certain individuals, supporting a reevaluation of the previously proposed patterns for dietary transition in Western Europe during neolithization. On the other hand, plants recovered in dental calculi demonstrated the use of domesticated species, such as barley and wheat, as well as some wild species, for example panicoids. However, for this last category, the input could be due to either food or contamination from the environment.

Secondly, this work provided new information on social organization. The funerary space at Les Bréguières was used by local and/or sublocal human groups during mainly four centuries. The hypothesis of a use by nonlocal people could be strengthened both by (a) the Sr stable isotope results that allow discrimination of individuals who originate from locations exposed to different environmental influences, such as sea spray, and (b) the noticeable maternal diversity measured for the Les Bréguières group. However, intra- and interindividual Sr isotopic results do not provide evidence for high territorial mobility.

Finally, despite the difficulties due to the sample bias (the material only comes from a collective burial) the issue of female versus male behaviors was revealed. This new study provides some elements about the question of female versus male behaviors within the early farmer communities. No clear sex-related dietary/mobility pattern is evidenced, but it is worth reminding that, among coxal bones studied, marine protein intake was recorded only in a male individual and specific sulfur isotope ratios were recorded only in female individuals. While some Neolithic human groups demonstrate gender-based protein intake (e.g., in the Paris Basin (Rey et al., 2019): females consumed different animal proteins; or Auvergne (Goude et al., 2013): wider variability of resources consumed by women), Mediterranean groups studied until now (this study; i.e., Salazar-García et al., 2018) do not show clear difference between male and female. However, gender-related mobility pattern seems to be a more common observation in many European places. Strontium data in northeastern France and central Europe already demonstrated a wider mobility operated by females (Bentley et al., 2012) and first aDNA mitochondrial results support this social organization mode (Rivollat et al., 2015). Result from this study also support this social organization through preliminary analysis of aDNA data and potentially also with S isotope ratios, which look to be a more efficient tool compared to Sr in this Mediterranean area. These new palaeogenomic data should increase the number of individuals for sex determination and allow for a discussion of male versus female behaviors. Finally, this transdisciplinary research paves the way for new perspectives, particularly the potential for novel research on remains excavated near shorelines, including newly excavated assemblages or those curated in museums.

### ACKNOWLEDGMENTS

This research was funded by Institut Danone France/Fondation pour la Recherche Médicale 2015 partnership (Women and diet at the beginning of farming, fifth–third millennium BC, France: a bioanthropological approach; Dir. G. Goude 2016–2017; http:// institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-iiie-millenaires-av-j-c-france-approche-bio-anthropologique/), ETICALP programme (Dir. D. Binder), and new dates have been obtained thanks to the ongoing project INTERACT (ANR-17-FRAL-0010, DFG-HA-5407/4–1; Dir. M.-F. Deguilhoux/W. Haak).

### AUTHOR CONTRIBUTIONS

Anthropological material was partially studied by S. Provost. Isotope studies were conducted by Gwenaelle Goude and Domingo C. Salazar-García; laboratory assistance was provided by A. Varalli and S. Bernardini. Dental calculus and archaeobotanical analyses were performed by Robert C. Power and Laurent Bouby. Genetic analyses were conducted by Maité Rivollat, Marie-France Deguilhoux, and Marie-Hélène Pemonge. Archaeozoological material was studied and provided for analysis by Lionel Gourichon and M. Vuillien. This pilot study is part of a wider multidisciplinary project which compares other complementary data in particular to document the place of women at the onset of farming. All authors contributed to writing the article under the coordination of GG. The article has been edited by a professional: https://karinagerdau.com/. We are also very grateful to the reviewers and editors for their relevant comments and fruitful corrections and editing which helped to deeply improve the article.

### DATA AVAILABILITY STATEMENT

The authors declare that the data supporting the findings of this study are all available within the article and its supporting information (SI) files.

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