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Trabecular bone microarchitecture analysis, a way for an early detection of genetic dwarfism? Case study of a dwarf mother's offspring

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ABSTRACT

A 66 year-old woman with a disproportionate dwarfism and who bore seven children was discovered at the Middenbeemster archaeological site (The Netherlands). Three are perinates and show no macroscopic or radiological evidence for a FGFR3 mutation causing hypo-or achondroplasia. This mutation induces dysfunction of the growth cartilage, leading to abnormalities in the development of trabecular bone. Because the mutation is autosomal dominant, these perinates have a 50% risk of having been affected. This study determines whether trabecular bone microarchitecture (TBMA) analysis is useful for detecting genetic dwarfism. Proximal metaphyses of humeri were µCT-scanned with a resolution of 7–12 µm. Three volumes of interest were segmented from each bone with TIVMI© software. The TBMA was quantified in BoneJ© using six parameters on which a multivariate analysis was then performed. Two of the Middenbeemster perinates show a quantitatively different TBMA organization. These results and the family's medical history suggest a diagnosis of genetic dwarfism for this two perinates. This study provides evidence to support the efficacy of µCT for diagnosing early-stage bone disease.

1. Introduction

Hypochondroplasia and achondroplasia result from an autosomal dominant FGFR 3 (*fibroblast growth factor receptor 3)* mutation. The typical dysmorphology is less pronounced in hypo- than in achondroplasia. Nevertheless, in both cases, a child has a 50% risk of inheriting a mutated allele from an affected parent (Cremin and Beighton 1978). For the purposes of the analyses conducted here, the two disorders will be subsumed within the term genetic dwarfism (GD) until the discussion. FGFR3 mutations induce a dysfunction of endochondral ossification occurring at the growth plates, resulting in short limbs and a shortened stature (Horton et al., 2007). Clinical diagnosis can be difficult in newborns, especially for individuals with less severe phenotypes, as is typical for hypochondroplasia. The altered growth may not be obvious before late childhood or adolescence (Nicoletti et al., 1988).

An adult female with a form of disproportionate dwarfism was discovered at the post-Medieval archaeological site of Middenbeemster in The Netherlands (Fig. 1). Diagnosis was based on strong signs consistent with this type of genetic dwarfism. Her upper and lower limbs are shortened and show a rhizomelic pattern with bones having a thickened cortical bone, rugose and robust muscle attachment sites. Her bowed tibias (shorter than fibulas) are consistent with a *genu varum;* and posterior bowing of humerus and radius, with a reduced extension/rotation of elbow joints. Additional signs are found as a skull with frontal and parietal bossing, an oval foramen magnum and a protruding mandible, a *coxa vara* and vertebrae with shortened pedicles and small neural foramen (Waters-Rist and Hoogland, 2013). According to public records, she bore seven children and died in 1863 at the age of 66. Five of her children were buried at the same cemetery and were analyzed for evidence of inherited dwarfism (Waters-Rist and Hoogland, 2013). The morphology of the mother, a 21-year-old son and a 10 year-old daughter are consistent with GD. The remaining three children are perinates: public records note two were stillborn and hence were not named preventing us from identifying their sex while the third is a

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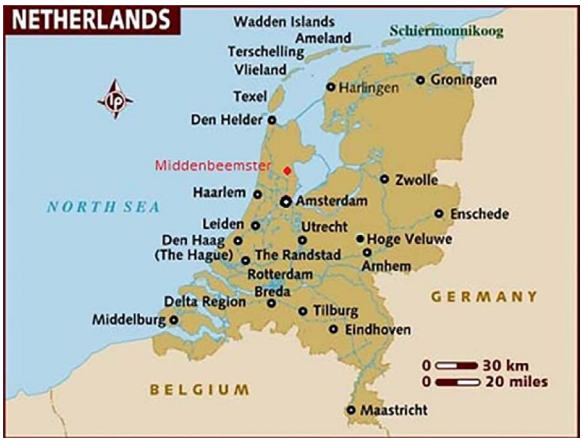

Fig. 1. Location of Middenbeemster in Netherlands.

three-day-old female. These perinates present neither specific macroscopic evidence nor radiological evidence for an FGFR3 mutation. However, at such a young age, even if they carry the mutation, bone changes may not be observable yet.

Trabecular bone is ossified endochondrally at the epiphyseal growth plates, so any dysfunction of the growth plate cartilage should also be observable as trabecular bone microarchitecture (TBMA) abnormalities. Therefore, we hypothesize that TBMA analysis could provide evidence of a pathological condition useful in the present case for a diagnosis of GD for one or more of the Middenbeemster perinates.

2. Material and methods

2.1. Material

Humeri of the three perinates from Middenbeemster were analyzed and compared to the humeri of two additional individuals of comparable age who were included as controls for normal development. These control individuals did not suffer from diseases known to affect the endochondral growth process and do not demonstrate any macroscopic signs of other diseases. They come from known-age and –sex French skeletal collections of the Normal Anatomy Institute, Strasbourg University (Rampont, 1994) and of the *Musée de l'Homme*, Paris[1] (Table 1). For conservation purposes, only humeri from the left side were used in the study.

Table 1
General information about studied bones (F: female, I: indeterminate).

Collections/origin	Specimen number	Age at death	Sex	Humeral length (cm)	Micro-CT device used
Musée de l'Homme	25962	*Fetus 7 months in utero*	F	5.9	v|tome|x 240 L
Strasbourg	embr.98	0	F	6.52	BIR ACTIS 225/300
Middenbeemster	V0485	0	I	6.81	GE nanotom
	V0486	0	I	6.15	
	V0884	3 days	F	6.17	

Table 2
µCT acquisitions parameters.

Device location	BIR ACTIS 225/300	v|tome|x 240 L	GE nanotom
	MPI Evolutionary Anthropology, Leipzig (Germany)	UMS 2700 OMI, Paris (France)	UMS 3626 PLACAMAT, Bordeaux University (France)
Acquisition parameters			
Voltage (kVp)	130	85	66
Current (µA)	60	190	350
Number of projections	2500	2600	2000
Frame averaging	3	3	3
exposure time (ms)	200	500	500
Rotation (°)	360	360	360
Filter	0,25 mm Brass	–	–
Resolution (µm)	7.3	8.3	12

2.2. Methods

2.2.1. µCT-scan acquisitions

In archaeological sciences, micro-computed tomography (µCT) is one of the best alternatives to histomorphometry for studying bone microarchitecture, as it provides access to histological details but is neither destructive nor invasive (Coqueugniot et al., 2015; Immel et al., 2016). All µCT-scans were performed with a resolution ranging from 7 to 12 µm, depending on the specimen and the machine used (Table 2).

2.2.2. Volumes of interest (VOI) selection

For each humerus, three VOIs were selected from µCT-scans with TIVMI® software.[2] TIVMI® is based on the HMH (Half-Maximum Height) algorithm (Spoor et al., 1993) extended to 3D (Dutailly et al., 2009), which provides more precise volumetric reconstructions than algorithms implemented in other software (Guyomarc'h et al., 2012). From the 3D volumetric reconstructions of each proximal metaphysis, we developed a semi-automated protocol to select VOIs precisely (Fig. 2), as variation in VOI location can affect measurement variation (Kivell et al., 2011). Our selection protocol was based on the metaphyseal border (MB), defined as the line delineating cortical bone from growth cartilage (Fig. 2a). The MB is the only macroscopic structure visible during the entire growth period of the proximal humerus, and it is easily observed in perinates. Consequently, we were able to extract anatomically homologous regions (Fig. 2d). VOIs were located on the central longitudinal axis of the bone. Each VOI is a cube with a height equal to 3% of the humeral length. Scaling the VOIs in this manner helps mitigate size biases (Burrows et al., 2010). Finally, VOIs were segmented (Fig. 2e) and the binary images exported to be measured.

2.2.3. µCT variables and analysis

VOIs were imported into ImageJ to measure with the BoneJ 1.4.2 plugin (Doube et al., 2010) the following six trabecular bone variables as defined by histomorphometrists to characterize TBMA (Dempster et al., 2013; Parfitt et al., 1987). Bone volume fraction (BV/TV) is the proportion of a VOI that is bone tissue and is calculated as the number of voxels assigned to bone tissue by the segmentation divided by the total number of voxels. Trabecular thickness (Tb.Th) is the mean thickness of trabeculae inside the VOI and trabecular spacing (Tb.Sp) corresponds to the diameter of the maximum sphere filling the spaces between trabeculae. Tb.Th and Tb.Sp are calculated with the same algorithm implemented in BoneJ (Dougherty and Kunzelmann, 2007; Hildebrand and Rüegsegger, 1997). Connectivity density (Conn.D) highlights the complexity of the trabecular network by estimating the total number of trabeculae using a topological approach (Odgaard and Gundersen, 1993). The degree of anisotropy (DA) has a value from 0 (isotropic) to 1 (anisotropic) and expresses whether the trabeculae are oriented uniformly or preferentially, respectively. DA was calculated

[1] www.museedelhomme.fr/en/collections/biological-anthropology/modern-human-remains (information about the collection, 15-06-2017).

[2] Developed by Bruno Dutailly, UMR 5199 PACEA, free software dedicated to anthropological researches.