Bone 132 (2020) 115180

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Skeletal maturation in relation to ethnic background in children of school age: The Generation R Study

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ARTICLE INFO

Keywords:
Skeletal age
Ethnic differences
Maturation
Pediatric cohort

ABSTRACT

Ethnicity is a well-established determinant of pediatric maturity, but the underlying genetic and environmental contributions to these ethnic differences are poorly comprehended. We aimed to evaluate the influence of ethnicity on skeletal age (SA), an assessment of pediatric maturation widely used in clinical settings. We included children from the Generation R Study, a multiethnic population-based pregnancy cohort, assessed at a mean age of 9.78 (± 0.33) years. SA was evaluated by a trained observer on hand DXA scans using the Greulich and Pyle method. Ethnic background was defined as geographic ancestry (questionnaire-based assessment) (N = 5325) and genetic ancestry (based on admixture analysis) (N = 3413). Associations between the ethnic background and SA were investigated separately in boys and girls, using linear regression models adjusted for age, height and BMI. Based on geographic ancestry, 84% of the children were classified as European, 6% as Asian and 10% as African. Children of European background had on average younger SA than those of Asian or African descent. Asian boys had 0.46 (95% CI 0.26–0.66, p-value < 0.0001) and African boys 0.36 years (95% CI 0.20–0.53, p-value < 0.0001) older SA as compared to European boys. Similarly, Asian girls showed 0.64 (95% CI 0.51–0.77, p-value < 0.0001) and African girls 0.38 years (95% CI 0.27–0.48, p-value < 0.0001) older SA as compared to European girls. A similar pattern was observed in the analysis with genetically-defined ancestry. Furthermore, an increase in the proportion of Asian or African component was associated with older SA in both boys (log[Non-European/European]proportion = 0.10, 95% CI 0.06–0.13, p-value < 0.0001) and girls (log [Non-European/European]proportion = 0.06, 95% CI 0.04–0.08, p-value < 0.0001). In summary, children of Asian and African backgrounds have on average older SA as compared to children of European descent, partially explained by a genetic component.

1. Introduction

The determination and understanding of normal growth and maturation in children are crucial from a medical and a psychosocial perspective [1]. Maturation is widely assessed through the examination of the development of carpal and metacarpal bones from the non-dominant hand to determine the skeletal age (SA). The Greulich and Pyle atlas method [2], compiled from European children and recognized as a referent standard to determine SA, is used in the field of pediatric radiology as the main tool for the assessment of individual skeletal maturation [3].

Deviation of SA from chronological age is decisive for pediatricians to diagnose and follow growth disorders, and to assess the effectiveness of any applied treatment [4–7]. An inaccurate SA assessment may result in an erroneous diagnosis and inadequate treatment of growth disorders, carrying severe and sometimes irreversible consequences [5–7].

During the developmental process, the skeleton increases in both bone size and density until the end of adolescence. This process is finalized after the closure of growth plates. Understanding the process of skeletal maturation may lead to the development of strategies aiming to assure that the child reaches optimal peak bone mass [8]. The International Society of Clinical Densitometry (ISCD) has pointed out the need to study the origin of ethnic differences in skeletal maturation, as a crucial step to improve the assessment of pediatric bone health [8].

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https://doi.org/10.1016/j.bone.2019.115180
Received 24 September 2019; Received in revised form 13 November 2019; Accepted 27 November 2019
Available online 28 November 2019
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The relative faster skeletal maturation of children from African ancestry has been attributed to factors such as body weight, composition and earlier onset of puberty [9]. However, these factors only partially explain ethnic differences in SA. Furthermore, there are few studies assessing differences in SA from the perspective of genetically-determined ancestry [9], or performed in a population comprising individuals from the three main ethnic continental definitions [10].

Therefore, we aimed to assess ethnic differences in SA within the Generation R Study, an optimal setting to investigate the influence of ancestry on skeletal maturation, as it comprises a population-based collection of children of diverse ethnic background (reclassified into European, Asian and African ancestry), within a similar age range and living in a restricted geographical area. Further, by employing both a geographic and a genetic definition of ancestry, we aimed to disentangle the genetic and environmental influences of the ethnic background on SA.

2. Methods

2.1. Study population

The Generation R Study, a multiethnic population-based prenatal cohort, was established at the Erasmus University Medical Center in Rotterdam. A total of 9778 mothers, with expected delivery between April 2002 and 2006, gave consent to be included in the study. The aim of the Generation R Study is to investigate early genetic and environmental influences on pediatric growth, development and health [11]. All measurements used to address the current research question(s) were collected in the third cohort visit (mean age of 9.78 ± 0.33 years). Details about blood sampling and genotyping of the Generation R Study can be found elsewhere [12,13]. This study was conducted in agreement with the guidelines of the Helsinki Declaration and approved by The Medical Ethic Committee of the Erasmus Medical Centre (MEC-2012-165), with the informed consent obtained for all participating children. The study included participants with complete information, comprising 5325 children with geographic- and 3413 children with genetic ancestry ($N_{baseline}$ = 5686). A flow chart of the study population is presented in Supplementary Fig. 1.

2.2. Demographic and anthropometric measurements

Information about sex and birth date of all participating children was collected from medical records and registries. Child's age was calculated at the DXA examination visit date. Personnel trained at the Erasmus research center measured child's height with a Harpenden stadiometer (Holtain Limited, Dyfed, UK) and weight with a SECA scale (Almere, The Netherlands). Body mass index (BMI) was derived as the division of weight (kg) by height squared (m^2).

2.3. Geographic ancestry

Geographic ancestry was determined using questionnaire-based assessment of country of parents' origin (following the classification of Statistics Netherlands) [14]. If one parent was born abroad, the child's ethnicity was determined according to that parent. If both parents were born abroad, the child was classified according to the mother's ancestry. Further, children were divided into three main ethnic groups: 1) European: including Dutch, Turkish, North African, Oceanic, American and other European; 2) Asian: including Surinamese-Hindu, Indonesian and other Asian; and 3) African: including Surinamese-Creole, Sub-Saharan African, Cape Verdean, Antillean and other African [15].

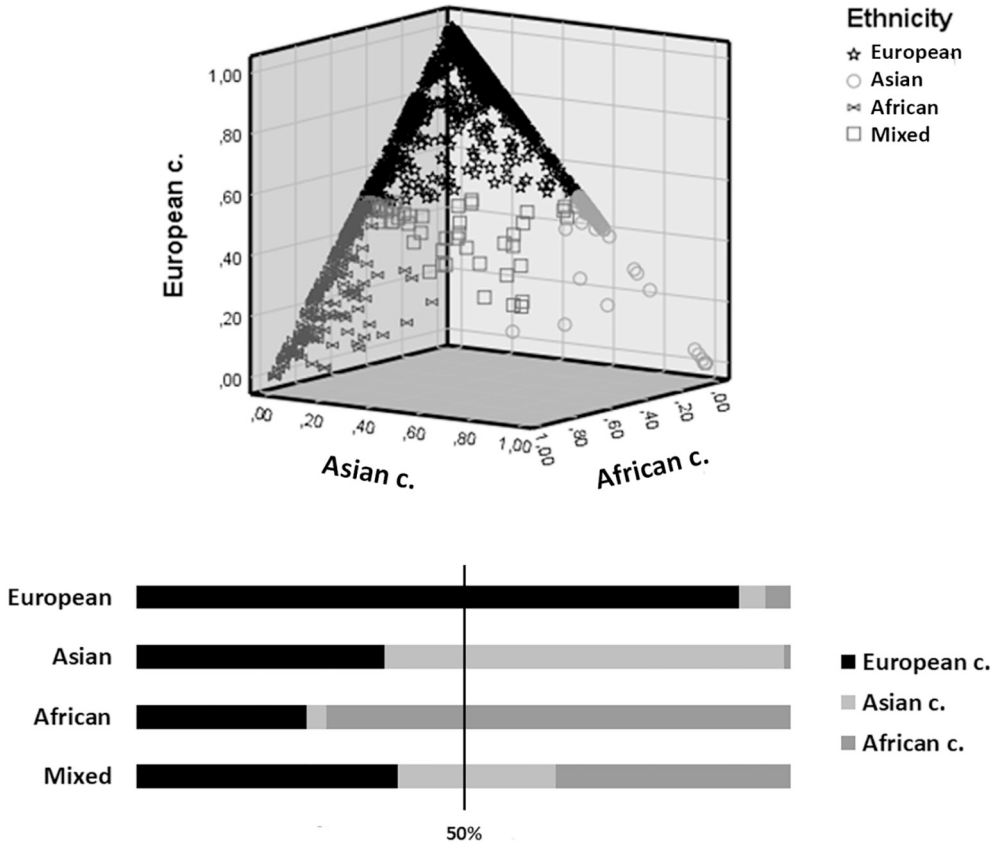

Fig. 1. Genetic determination of ancestry (N = 3413). Children are classified as European, Asian or African based on the highest proportion of estimated ancestral component (c) (> 50%) (presented on x, y and z axes) or mixed if none of ancestral components reached 50%.

2.4. Genetic ancestry

ADMIXTURE software [16], modeling the probabilities of observed genotypes and based on ancestral proportions and population allele frequencies, was used in the determination of genetic ancestry for the subset of children with genome-wide genotyping data available (N = 3413). Participants were classified as having European, Asian or African ancestry based on the highest proportion of ancestral component (> 50%), while those who did not reach 50% for any ancestral component (N = 48), were classified as of mixed ancestry (Fig. 1) [17,18].

2.5. Skeletal age

DXA-scans of the left hand were obtained by a trained investigator using an iDXA densitometer (General Electric, formerly Lunar Corp., Madison, WI, USA). Scanning was performed in a supine position with the left hand placed flat on the table for 66 s. Starting scan point was defined at two-finger widths below the radiocarpal articulation. Obtained images included all hand bones, the wrist and distal part of radius and ulna. SA was obtained through the comparison of the maturity indicators estimated on hand DXA-scans with the standardized references provided in the Greulich and Pyle atlas [19]. One trained observer performed the SA assessment in all children using enCORE V. 13.60 (Madison, WI, USA) and the optimal zoom. A randomly selected subsample of 150 DXA-scans was blindly re-evaluated by the same observer to assess intra-observer reliability demonstrating high consistency (intra-class correlation 89%).

2.6. Statistical analyses

All analyses were performed separately in boys and girls, given the already described sexual dimorphism underlying skeletal maturation [2]. Association between SA and ancestry of the children, both geographically and genetically determined, was evaluated using a linear regression model adjusted for age, height and BMI. Genetically determined ancestry was additionally analyzed following a compositional data approach. A log-ratio transformation [20] was used to assess if the proportion of Non-European (Asian or African) component of ancestry, as compared with the proportion of European component - set as the reference - affects SA (log[Non-European/European]proportion). In the sensitivity analyses, relative skeletal age (RSA) was used as an outcome (RSA - difference between skeletal and chronological age), excluding chronological age from the adjustment. SPSS Statistics version 21 (IBM Corp, New York, USA) was used for all statistical analyses, with statistical significance set at p-value ≤ 0.05.

3. Results

This study included 5325 children (2616 boys, 49.1%) at a mean age of 9.78 (± 0.33) years with available SA assessment, geographic ancestry information, and anthropometric measurements (Supplementary Fig. 1). Based on the geographic ancestry definition, 4445 (83.5%) children were classified as European, 549 (10.3%) as African and 331 (6.2%) as Asian. For the analysis using genetically-determined ancestry, we included 3413 children (1671 boys, 49%) at a mean age of 9.79 (± 0.33) years. Following this definition, 3019 (88.5%) children were classified as European, 226 (6.6%) as African, 120 (3,5%) as Asian and 48 (1.4%) as of mixed ancestral background.

Significant differences were observed in all baseline characteristics across the three ethnic groups (p-value < 0.0001). African children presented as older, taller and heavier than children of European and Asian background (Table 1). Likewise, African children showed the oldest SA as compared to the two other ancestral groups (p-value < 0.0001) (Table 1). To a great extent, these differences were mirrored in the sex-stratified analyses (Supplementary Table 1) and also in the analysis employing genetically-determined ancestry classification (Supplementary Table 2). Lastly, girls had older SA than boys across all ancestral groups (mean difference$_{_Europeans}$: 0.25, 95% CI 0.17–0.32, p-value < 0.0001; mean difference$_{_Asians}$: 0.40, 95% CI 0.10–0.70, p-value = 0.010 and mean difference$_{_Africans}$: 0.45, 95% CI 0.22–0.68, p-value = 0.0001).

Ethnic differences in SA were still evident after adjustment for age, height and BMI in both sexes. Boys of European background had 0.46 years younger SA as compared to boys of Asian background (95% CI 0.26–0.66, p-value < 0.0001), and 0.36 years younger SA in comparison to African boys (95% CI 0.20–0.53, p-value < 0.0001). Likewise, girls of European background had 0.64 years younger SA as compared to girls of Asian background (95% CI 0.51–0.77, p-value < 0.0001), while they had 0.38 years younger SA in comparison to African girls (95% CI 0.27–0.48, p-value < 0.0001).

Conclusions drawn from the genetically derived ancestry (excluding children with mixed ancestry), mirrored the ones drawn from the geographic ancestry. European boys had 0.76 years younger SA as compared to Asian (95% CI 0.40–1.11, p-value < 0.0001) and 0.48 years younger SA as compared to African boys (95% CI 0.24–0.73, p-value < 0.0001). Also, European girls had 0.79 years younger SA as compared to Asian (95% CI 0.60–0.99, p-value < 0.0001) and 0.30 years younger SA as compared to African girls (95% CI 0.14–0.46, p-value = 0.0002). Furthermore, increase in proportion of Asian or African (Non-European) ancestry was associated with older SA in both boys (Per log[Non-European/European]proportion: 0.10, 95% CI 0.06–0.13, p-value < 0.0001) and girls (Per log[Non-European/European]proportions: 0.06, 95% CI 0.04–0.08, p-value < 0.0001). An overview of ethnic differences in SA is presented in Fig. 2.

Sensitivity analyses using RSA as an outcome supported the same conclusions (Supplementary Table 3).

4. Discussion

The current study assessed ethnic differences in skeletal maturation in 5325 children from the Generation R Study with a mean age of 9.78 years. Children of Asian and African background have on average an older SA than children of European descent, in line with the contention that children of non-European background reach skeletal maturation earlier. These findings are not explained by differences in body

Table 1
Baseline characteristics of the population across geographic ancestral groups.

N = 5325	Europeans (4445) Mean (SD)	Asians (331) Mean (SD)	Africans (549) Mean (SD)	ANOVA p-value
Age (years)	9.78 (0.32)	9.78 (0.32)	9.84 (0.39)	< **0.0001**
Height (m)	1.416 (0.06)	1.403 (0.07)	1.424 (0.08)	< **0.0001**
Weight (kg)	35.09 (6.87)	34.84 (7.52)	38.34 (9.61)	< **0.0001**
BMI (kg/m^2)	17.41 (2.62)	17.58 (2.92)	18.71 (3.40)	< **0.0001**
SA (years)	9.33 (1.27)	9.81 (1.39)	9.92 (1.35)	< **0.0001**

N - sample size, SD - standard deviation, BMI - body mass index.
Significant p-values (p-value ≤ 0.05)- presented in bold.

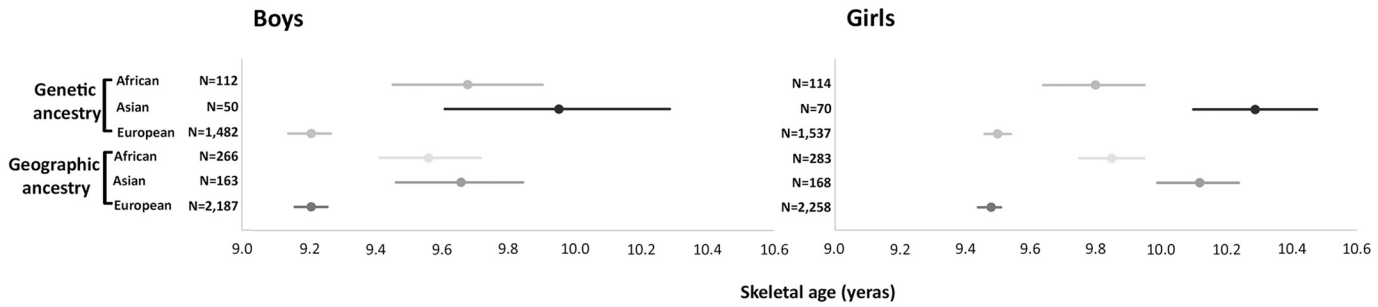

Fig. 2. Sex-stratified representation of skeletal age across different ethnic groups. Skeletal age (years) - estimated marginal means for skeletal age (adjusted for age, height and BMI) presented with 95% confidence interval.

height and/or BMI across ethnic backgrounds. With the analysis using a genetic assessment of ancestry, we showed that a genetic ancestral component partially accounts for differences in skeletal maturation. Also, our analyses indicate that ethnic differences in skeletal maturation are not sex specific.

Differences in skeletal maturation in children of distinct ethnic background have been reported previously [9,21,22]. McCormack et al. assessed the influence of ancestry on RSA in a healthy longitudinal cohort in the US including 1592 non-obese children in the age range of 5–17 years at study entry (4622 assessments, 19% African-American). Consistent with our findings, the authors reported older RSA in African-American as compared to non-African-American children [9], and older RSA in girls as compared to boys. Ethnic and sex disparities in skeletal maturation were also assessed in the "Birth to twenty" cohort, from South Africa, including 607 (66% black) children in the age range of 9 to 20 years at study entry [23]. In line with our findings, girls matured 1.9 years earlier than boys. Conversely, the authors reported no differences in skeletal maturational timing between black and white girls, while skeletal maturation was postponed for 7 months in black as compared to white boys [23]. Discrepancies in the observations from the South African cohort and our study could be explained by the different age range of both cohorts, the presence of a different degree of genetic admixture or overall health, and other environmental differences affecting skeletal maturation. Furthermore, the outcome was defined using bone maturity scores based on the Tanner-Whitehouse standards, originally developed in Scottish children [23,24]. This method was reported as more reproducible than the Greulich and Pyle method by Bull et al. [25]; whereas another study suggested the Greulich and Pyle method as the more appropriate one for use in clinical practice, showing adequate reproducibility and requiring significantly shorter time to be performed as compared to its concurrent [26]. Most importantly, the study by McCormack et al. has the drawback of grouping European and Asian children into the non-African group. This may result in the creation of one highly heterogeneous group with respect to both genetic and behavioral characteristics. Further, neither McCormack et al. or Cole et al. provided information about skeletal maturation in Asian as compared to European and/or African children [9,23]. In contrast, our study classified children into the three continental ancestry groups. To the best of our knowledge, there is only one other study that investigated SA across four different ethnicities: Asian, African, European and Hispanic background [10]. That study included children and adolescents between 1 and 18 years of age and found older RSA in Asian as compared to European children, in girls between 10 and 13 years and in boys of 11 and 15 years of age. However, the former study did not report differences in SA beyond this age range, nor differences between African and European children. These slightly different findings may be related to the statistical approach used by the authors - analysis of variance - with no adjustment for any of the anthropometric covariates.

BMI/overweight and pubertal timing have been postulated as the most relevant confounders when investigating differences in skeletal maturation [9,18,27–29]. Indeed, we observed higher BMI in children of African and/or Asian ancestry as compared to European children (Table 1). However, in our study population, adjustment for BMI in the association of SA and ethnicity resulted only in an average change of 2% in the effect estimates, indicating that BMI differences are unlikely to explain a considerable proportion of the ethnic differences in skeletal maturation. Nevertheless, BMI could play a more relevant role on SA in children of more advanced pubertal stage. Since the assessment of pubertal stage in our cohort was not performed, we could not account for it in our association models.

As a large fraction of the participants are third-generation immigrants, determination of ethnicity based on the country of parents' birth, does not fully capture the genetic background of the children [13]. Therefore, we evaluated skeletal maturation also in the context of genetic ancestry (Fig. 1). An increase in either Asian or African proportion of ancestry (as compared to the proportion of European ancestry) was associated with older SA. This is in line with the findings of McCormack et al. [9] reporting that an increase in the proportion of African admixture was associated with advanced skeletal maturation. Further, this is the first study to demonstrate advances in SA with the increase in the proportion of Asian admixture.

Since higher levels of adrenal androgens are associated with advances in skeletal maturation [30], ancestry-specific differences in SA could be explained by differences in hormonal regulation in children of different ethnic background. For instance, higher levels of dehydroepiandrosterone-sulfate (DHEA-S) together with insulin-like growth factor 1 (IGF-1) have been reported in healthy pre-pubertal girls (age range 7.5–9.0 years) of African-American origin as compared to European girls, before any other evidence of adrenarche [31]. In addition, the increase in African genetic admixture was associated with the increase in IGF-1 levels in pre-pubertal boys and girls (mean age 8 years) of African-American origin [32]. Likewise, advanced SA in girls as compared to boys, has been attributed to slightly elevated estrogen levels in girls prior to puberty [9,33,34]. Moreover, the accumulation of certain steroid hormones in congenital adrenal hyperplasia [35–37], has been linked to deviations from the physiological pace of skeletal maturation, further pointing to the essential role of hormonal levels during development. However, not only the hormonal status, but also its complex interaction with environmental factors, might play an important role in skeletal maturation [38].

In a previous study embedded in the Generation R Study, we have already investigated ethnic differences in maturation assessed through permanent teeth maturation - dental development (DD) - [18]. Despite a slightly different ethnic classification, in line with our current findings, DD was more advanced in Antillean and Surinamese-Creole children as compared to Dutch children. Furthermore, higher proportions of Asian or African ancestry were associated with advanced DD. This may suggest that overall biological maturation occurs later in the European children as compared with non-European ones.

Our study holds some limitations. In the third visit of the Generation R Study, neither puberty nor hormonal levels in the blood were

assessed, as specified above, and some children could have entered puberty resulting in increased hormonal secretion and faster pace of growth and development [39,40]. This could be determinant in the interpretation of our results, especially considering that it has been reported that children of African background enter puberty earlier than European children [41]. However, in the report from McCormack et al. [9], ethnic disparities in skeletal maturation were evident even after accounting for pubertal timing. Furthermore, it has been recently suggested by a meta-analysis that the Greulich and Pyle atlas method may be imprecise when applied to African females and Asian males [1], despite being the most commonly used method for SA assessment in both clinical and research settings [9]. Nevertheless, this precision issue is more likely to bias the determination of chronological age for forensic purposes across individuals, rather than the summary level estimates drawn in well-powered epidemiologic studies [1]. In addition, given the low number of non-European children included, conclusions should be interpreted with caution. Lastly, residual confounding cannot be discarded, despite our effort to include the most relevant confounders in our analyses.

Our study accounts also a number of strengths. To the best of our knowledge, this is the largest cohort of healthy children with a comprehensive determination of SA and available information on both geographic and genetic ethnicity. There is also an advantage of having study participants drawn from a similar age range and well-defined geographic area (i.e., living in Rotterdam), as environmental exposures (such as sociodemographic and lifestyle factors together with uniform access to health care) are expected to be less influential than in studies drawn across distinct populations. Also, with all study participants being examined at the Erasmus Medical Center, using the same devices and assessments, our cohort results in a relatively-well harmonized collection of pediatric data, suitable to investigate the influence of ancestry on different outcomes, including skeletal maturation.

5. Conclusion

Overall, children of Asian and African ancestry have on average older skeletal maturation as compared to European children. Furthermore, our study is pointing to a genetic origin of ethnic differences in skeletal maturation, providing insight into biologic differences in pediatric bone health. However, it is still to be determined to which extent the presented biological component can explain differences in the pace of maturation as compared to environmental (cultural) factors.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.115180.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Acknowledgements

We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam, involved in the Generation R Study.

Funding sources

The general design of Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organisation for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Dutch Ministry of Health, Welfare and Sport. Additional grants from the Netherlands Organisation for Health Research and Developmentsupported this study (ZonMw 907.00303, ZonMw 916.10159, ZonMw VIDI 016.136.361 and ZonMw VIDI 016.136.367 to F.R. and C.M-G).

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