Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Sharma A, Phillips AJ, Jüppner H. Hypoplastic metatarsals — beyond cosmesis. N Engl J Med 2015;373:2189-90. DOI: 10.1056/NEJMc1508520

Supplementary Appendix

Hypoplastic Metatarsals- Beyond Cosmesis

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Supplemental Methods and Results

The search for epigenetic *GNAS* changes and for an allelic loss at *GNAS* or *STX16* was performed by Multiplex Ligation-dependent Probe Amplification (MLPA) and Methylation-Sensitive MLPA (MS-MLPA), respectively (1) (MRC-Holland, Amsterdam, The Netherland). There was no evidence for a change in copy number, yet loss-of-methylation at *GNAS* exons A/B, XL, and AS, and gain-of-methylation at *GNAS* exon NESP. In contrast, both healthy sisters and the healthy mother showed no evidence for *GNAS* methylation abnormalities at either of the four investigated, differentially methylated regions; consequently, all three revealed no evidence for an abnormal regulation of calcium and phosphate homeostasis. Furthermore, our patient was heterozygous for six of eight investigated microsatellite markers (D20S86, 261P9-CA, 806M20-CA, 543J19-TTA, D20S171, and D20S93; analyses performed at Center for Human Genetic Research of the Massachusetts General Hospital) (1,2). At markers D20S100 and 907-rep2, she was homozygous, but showed no discordance with her mother. Taken together, these data excluded paternal uniparental isodisomy involving the long arm of chromosome 20 (patUPD20q) and they provided no evidence for a maternally inherited deletion involving the *GNAS* region. Moreover, one of the healthy sisters had inherited the same maternal allele for the telomeric portion of chromosome 20q, making it more likely that the patient is affected by a sporadic PHP1B variant that has not yet been defined at the molecular level (2).

Consents and DNA Collection

After obtaining written informed consent through our IRB approved protocol, blood samples were collected from the investigated family members for DNA extraction using established methods.

References

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2. Fernández-Rebollo E, Pérez de Nanclares G, Lecumberri B, Turan S, Anda E, Pérez de Nanclares G, Feig D, Nik-Zainal S, Bastepe M, Jüppner H. 2011 Exclusion of the *GNAS* locus in PHP-Ib patients with broad *GNAS* methylation changes: evidence for an autosomal recessive form of PHP-Ib? Journal of Bone and Mineral Research 26:1854-1863.