To the Editor,

Prolactin (PRL) is a protein hormone mainly secreted by lactotrophs in the pituitary. Three main PRL isoforms have been so far identified: monomeric PRL (23 kDa) – the most active form-, the dimer of monomeric form -big PRL (50–60 kDa)-, and the big-big or macroprolactin (mPRL) (150–170 kDa), a complex of monomeric PRL with anti-PRL autoantibodies, devoid of significant biological activity [1].

Predominance of mPRL in the serum is known as macroprolactinemia. It is a benign condition but may lead to misdiagnosis of hyperprolactinemia (hPRL), as currently all immunoassays for PRL cross-react with mPRL [1, 2]. The diagnosis of macroprolactinemia has been based on low recovery of total PRL after polyethylene glycol (PEG) precipitation (with cut-offs between <40% and <60%), and reported as mPRL positive [1, 3]. However, this approach may fail to distinguish patients with hPRL, which is completely accounted for by the presence of mPRL from real hPRL individuals, who, regardless of their post-PEG recovery, have elevated bioactive monomeric PRL. It is now advocated to classify cases as true macroprolactinemia, those individuals with low post-PEG recovery associated to a normal monomeric PRL, and as hPRL when there is excess of monomeric PRL, and to report post-PEG monomeric PRL with an assay specific reference range [2, 4, 5].

Not looking for the presence of true mPRL as a cause for hPRL may result in further and unnecessary diagnostic interventions and inadequate therapeutic management.

The development of autoantibodies against PRL occurs more frequently in patients with hPRL than among the general population. Thus, most protocols recommend screening for mPRL in all hPRL patients [1, 2, 4–7].

PRL is also produced in extra-pituitary sites, including cells of the immune system. Elevated serum PRL levels are known to interfere with B-cell tolerance induction and has been associated with autoimmune diseases [1]. It is well accepted that genetic diversity contributes to autoimmune susceptibility, and thereby underlies ethnic differences in the presentation of autoimmune diseases, often clustering in certain geographic areas [8].

In this retrospective study, we aimed to evaluate whether increased prevalence of mPRL serum levels, assessed by PEG precipitation, varies among subjects from different geographical regions of origin. Data from June 2016 to January 2020 were collected from the database of the laboratory. All female patients with hPRL for whom a PEG precipitation test had been performed and the country was recorded, were included in the study. Study patients were grouped according to the World Health Organization (WHO) Member States regions (African Region [AFR], Region of the Americas [AMR], South-East Asia Region [SEAR], European Region [EUR], Eastern Mediterranean Region [EMR], and Western Pacific Region [WPR]) (https://www.who.int/es/about/who-we-are/regional-offices. Accessed on January 21, 2021). Our hospital is part of the public healthcare network, accessible to all citizens regardless of their nationality or income.

The protocol used to screen for macroprolactinemia is described elsewhere [6]. Total and post-PEG PRL were determined by immunoassay (Abbott Architect, Chicago, IL, USA). A diagnosis of true macroprolactinemia was done when the patient had a post-PEG recovery ≤50% associated
to post-PEG monomeric PRL within our own reference range (4.0–20.4 μg/L), calculated according to Beltran et al. [7].

Three thousand and twenty-eight patients from different countries of origin were included. Most patients from the EUR were born in Spain, thus we first carried out a comparison between Spanish individuals (n=2,248) and those from other European countries (n=81) and found no significant differences in age, total PRL, positive mPRL, true mPRL, and hPRL (data not shown).

Demographic and biochemical data of the studied population grouped according to the WHO regions is shown in Table 1. Patients from the EMR were younger than those from the EUR, AMR, AFR, and SEAR; maximum median age differences among groups were less than 10 years. No inter-group differences were found in total PRL concentrations, however, prevalence of true mPRL in patients from the AMR was significantly higher in comparison to those from the EUR, EMR, and WPR, and showed no difference with those from the AFR. Percentage of true mPRL in patients from the EUR was higher than in those from the EUR (Figure 1). Consistently, the proportion of true hPRL was lower in people from the AMR in comparison to individuals from the EUR, EMR and WPR, whereas true hPRL was lower in people from the AFR in comparison to those from the EUR.

To date, there are no studies in which prevalence in distinct geographical regions has been assessed using the same method (same PEG precipitation protocol and immunoassay) in a single laboratory. Reported mPRL prevalence in hPRL patients are highly variable [2–5, 7], attributed to different factors, including used immunoassays (antibodies from different immunoassays have different cross-reactivity for mPRL), variable cut-offs to define positive mPRL, different criterions to report post-PEG results, and the criterions applied to screen the population (selected or unselected). These factors hinder the comparison of prevalence reported in different studies.

We observed significant variations in positive mPRL and true mPRL prevalence among the different geographical areas of origin using the same mPRL screening

Table 1: Demographic and biochemical data of the whole study population and per world regions according to the World Health Organization division (European Region, Eastern Mediterranean Region, Region of the Americas, African Region, Western Pacific Region, and South–East Asia Region).

|                      | Total population | European Region | East Mediterranean Region | Region of the Americas | African Region | Western Pacific Region | South–East Asia Region | p-Value |
|----------------------|------------------|-----------------|---------------------------|------------------------|---------------|-----------------------|-----------------------|---------|
| N                    | 3,028            | 2,329           | 316                       | 239                    | 66            | 43                    | 35                    |         |
| Age (years)          | 33–42            | 35              | 28.4a                     | 34                     | 33            | 29                    | 28                    | <0.001c |
| Total prolactin, μg/L | 36.9             | 36.9            | 35.5                      | 38.1                   | 39.9          | 40.9                  | 37 NS                 |         |
|                      | (30.3–50.9)      | (30.2–51)       | (30–45.7)                 | (29.8–55.7)            | (32.5–54.8)   | (32.2–50.9)           | (30.8–62.9)           |         |
| Macroprolactin: recovery ≤ 50% | 11.6% | 10.0%           | 10.4%                     | 25.9%                  | 22.7%         | 11.6%                 | 14.3%                 | <0.001b |
| PRL-post PEG recovery, % | 77.2 | 77.6a           | 75.3                      | 74.6                   | 75.1          | 76.8                  | 75.9                  | <0.001c |
|                      | (70.1–82)        | (71.1–82.2)     | (67.8–80.1)               | (49.3–81.5)            | (59.1–81.2)   | (74–81.6)             | (68.7–82.1)           |         |
| PRL-post PEG <50% recovery, % | 30.6 | 30.6            | 35.7                      | 29.1                   | 34.8          | 22.5                  | 23.2 NS               |         |
|                      | (19.9–41.4)      | (19.2–41.6)     | (27–44.8)                 | (18.2–39.6)            | (29.7–38.9)   | (15.6–22.5)           | (18.6–47.4)           |         |
| MP within the reference range | 17.6% | 15.9%           | 19.9%                     | 29.7%                  | 22.7%         | 16.3%                 | 19.9%                 | <0.001b |
| True macroprolactinemia: recovery ≤50% and MP within the reference range | 11% | 9.5%            | 9.6%                      | 23.6%                  | 19%           | 12.2%                 | 15.2%                 | <0.001b |
|                      | 307              | 206             | 27                        | 52                     | 12            | 5                     | 5                     |         |
| True hyperprolactinemia: MP above the reference range | 82.4% | 84.1%           | 80.1%                     | 70.3%                  | 77.3%         | 83.7%                 | 80.0%                 | <0.001b |

PRL, prolactin; PEG, polyethylene glycol; MP, monomeric prolactin; NS, non-significant. Quantitative results are presented as medians (25–75 percentiles). *Significance corresponds to differences between WHO Regions. **Chi-squared test among WHO regions. ***Kruskal-Wallis test among WHO regions. Statistical significance for pairwise comparisons between continuous values (Mann–Whitney U test) or categorical values (Chi-squared test). (to limit type I error rate in multiple pairwise comparisons, p value was set at 0.01 after the post hoc Bonferroni correction). \( p < 0.001 \), vs. Europe and vs. Region of the Americas; \( p = 0.008 \) vs. African region; \( p = 0.005 \) vs. South–East Asian Region. \( p < 0.001 \), vs. European Region and vs. Eastern Mediterranean Region. \( p = 0.007 \) vs. Europe. \( p < 0.001 \), vs. Europe and vs. Region of the Americas. \( p < 0.001 \), vs. European region; \( p = 0.002 \) vs. Eastern Mediterranean Region. \( p < 0.001 \), vs. European region and vs. Eastern Mediterranean Region.
criterion and methodology. The highest prevalence of true mPRL was observed in patients from the AMR followed by patients from the AFR. Our results concur with those of a recent meta-analysis on mPRL prevalence that included 67 studies from 27 countries [9]. There are large variations among studies from different geographical regions, with the highest prevalence reported for countries from the AMR (pooled prevalence: 29.1%) and the AFR (pooled prevalence: 30.4%), whereas in the EUR (17.5%), EMR (13.9%), WPR (12.6%), and SEAR (12.7%) prevalence was significantly lower. High prevalence of mPRL in patients from the AMR has also been reported by Gibney [2] in prevalence comparisons carried out with patients from Latin America [10, 11] against subjects from European countries.

Furthermore, we also observed high prevalence in the AFR, with no difference respect to the AMR, which concurs with the data from the only two studies with populations from the AFR reported in a meta-analysis by Che Soh [9]. Despite the heterogeneity among studies from EUR countries, median prevalence for the EUR [9] was almost half of that reported for studies with individuals from the AMR and the AFR. In our study, using the same immunoassay and PEG-protocol, prevalence in patients from the EUR was around half of that of patients from the AMR and AFR. The lower prevalence of true mPRL found in our study in patients from the EUR concurs with other studies with patients from the EUR [2, 4, 6, 9].

Limitations of this study include the assumption that the country of birth implies the existence of a common ethnicity among individuals from that country of origin, which cannot be guaranteed. However, the large number of patients included in the study (n=3,028) may outweigh possible exceptions. Moreover, the lack of clinical data to verify whether the presence of lower concentrations of monomeric PRL, was associated with lower clinical symptoms attributable to hPRL.

In conclusion, in our study, prevalence of macroprolactinemia, either expressed as low PRL post-PEG recovery or including the presence of bioactive monomeric PRL within our laboratory specific reference range, differs among geographical areas of origin between individuals living in the same geographical region, suggesting an ethnic predisposition to the disease as occurs with other autoimmune diseases.

Our findings reinforce the relevance of including monomeric PRL concentrations in the macroprolactin lab report with the appropriate reference intervals when assessing mPRL. For equal PRL levels, the percentage of true mPRL may vary among populations from different geographical areas.

Figure 1: Prevalence of true macroprolactin in the whole population and for each World Health Organization Region.

| Region                        | Prevalence of True Macroprolactin (%) |
|-------------------------------|---------------------------------------|
| European Region               | 9.5                                   |
| Eastern Mediterranean Region   | 9.6                                   |
| Region of the Americas        | 23.6                                  |
| African Region                | 19                                    |
| Western Pacific Region        | 12.2                                  |
| South-East Asia Region        | 15.2                                  |
| Total population              | 11.0                                  |
Acknowledgments: The authors would like to thank Dainora Jaloveckas (Ciencia Traducida) for providing editing assistance.

Research funding: None declared.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Signed informed consent was not necessary since all patients were anonymous and no additional analyses were made on their samples.

Ethical approval: The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practices and, was approved by the Ethics Committee of our Hospital.

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2021-1124).