Acta Genet Med Gamellol 30:67-75 (1981)
The Mendel Institute/Alan R. Liss, Inc.

Received 25 August 1980
Final 12 March 1981

The Inheritance of Plasma and Red Blood Cell Magnesium and Zinc Levels Studied From Twin and Family Data

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The variability of magnesium and zinc concentrations in plasma and erythrocytes was investigated by twin and family studies. Twins were sampled in two distinct ways and in two different West-European regions. In one of the samples, a distinction was made between twins living together and twins living apart. Two series of families were studied, one in a homogeneous environment, the other in a more diverse environment. Samples were compared by variance analysis. The results show 1) that genetic variability is significant for red blood cell (RBC) magnesium and zinc, minor for plasma magnesium and absent for plasma zinc; 2) that the family environment affects the extent of resemblance between twins and between siblings more for plasma levels than for RBC levels of magnesium and zinc. Furthermore, the intercorrelation analysis suggests that the genetic regulation systems of RBC magnesium and zinc are different, whereas some of the environmental regulation systems of plasma magnesium and zinc are the same. Biological interpretations are brought forward and discussed.

Key words: Magnesium levels, Zinc levels, Genetic variance, Family environment, Twin and family studies

INTRODUCTION

The assumption that genetic factors explain a large part of the variability of red blood cell (RBC) and plasma magnesium levels has been initially supported by wide differences between various populations [15, 16, 18, 20]. These differences may be found even when populations are transplanted [16, 18]. Moreover, the contrast between high interindividual and low intraindividual variability of RBC magnesium and zinc led us to the same genetic hypothesis [3, 19]. The preliminary results of a twin investigation showed that RBC magnesium and zinc levels present a much closer resemblance between monozygotic (MZ) than between dizygotic (DZ) twin partners. This is less so for plasma magnesium and does not apply to plasma zinc, potassium, and iron [4, 5].

The present report aims to provide further results collected during two investigations differing both by the sampling and the geographic origin of twins. Furthermore, environmental effects are analyzed by comparing twins living together and twins living apart. Finally,

0001-5660/81/3001-0067$03.00

the twin data are compared with data from two family studies that also differ with regard to sampling, geographic origin, and the degree of homogeneity among family circles. Some assumptions are discussed concerning how genetic control may act on blood mineral regulation.

POPULATIONS AND METHODS

Twin data come from two different samples (Table 1):

1) Twins living in and around Paris. This sample consists of 82 MZ pairs (28 male and 54 female) and 22 DZ pairs (six male and 16 female), based on the analysis of 14 genetic marker systems (performed by M. Moullec and A. Muller, Centre National de Transfusion Sanguine). The MZ sample is further divided into twins living together (MZT, 27 pairs) and twins having lived apart for over one year (MZA, 14 pairs). These twins range in age from 17 to 54 years.
2) Twins living in and around Brussels. Zygosity determination is based on the analysis of about 25 genetic marker systems [6]. The age range is lower than in sample one (17–26 years), so that the number of MZA paris in this sample is too small to be considered.

The family data were collected in the course of two different studies (Table 1):

1) Families living in and around Paris. These families consist of parents and several children (mean: 3.9; variance: 3.12). The primary purpose of this investigation was to widen a preliminary study showing a link between HLA and magnesium metabolism [17].
2) Families settled in a village community of a Pyrenean valley. These families are characterized by the homogeneity of their environmental and their level of inbreeding, which has been studied since the 18th century [9]. Each family includes several children (mean: 1.6; variance: 1.35).

In each group, the individual values have been standardized by the following transformation: $x' = (x-m)/s$, x being the observed value and m and s, the mean and the standard deviation of the corresponding sex group. Thereby, no further distinction will be made

TABLE 1. Summary of Data Collection for Twin Studies and Family Studies

Twin studies
- a) Paris (1)
  - Monozygotic twins (MZ) n = 82 pairs
    - Living together (MZT) N = 54 pairs
    - Living apart (MZA) N = 28 pairs
  - Dizygotic twins (DZ) N = 22 pairs
- b) Brussells (1, 2)
  - Monozygotic twins (MZ) N = 94 pairs
  - Dizygotic twins (DZ) N = 56 pairs

Family studies
- a) Paris (1,3), 44 families
- b) Pyrenean community (1, 4, 5), 42 families

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We gratefully acknowledge the collaboration of the blood donors and the invaluable assistance in collecting the data given by Constans (4), J. Dausset (3), P. Fernet (4), G. Franck-Riquer (1), J. Jacquard (INED, Paris), L. Jakobi (5), M. Moullec and A. Muller (CNTS, Paris), and J. Roullier (3).

in this work between male and female. Besides, preliminary research has shown no significant effect of sex on resemblance between MZ or DZ twins [5].

The statistical analysis applied to the twin data is a classical analysis of variance which partitions the total variance into between-pair (s_b^2) and within-pair (s_w^2) variance. The ratios of variances (F ratios) and intra-class correlation coefficients (r) are calculated too.

For the family data, the same variance analysis is applied to siblings. Furthermore, the regression coefficient of children on mid-parent has been computed by Kempthorne's method [22], taking into account all children of each family. A previous study [2] shows that no assortative mating exists for these variables, the mother's variance is similar to the father's, and there is no difference between the regression coefficients estimated from the offspring on the father only and the regression coefficients on the mother only.

Both members of each twin pair were tested in the morning of the same day, and all members of each family were tested in the morning of the same day (first sample) or in the same period of the year (second sample). Blood was taken from the inner elbow vein on lithium heparin. After homogenization, the blood sample was at once centrifuged (3000 rpm) for ten minutes in order to separate plasma and red cells. Red blood cells and plasma magnesium and zinc concentrations were determined by atomic absorption spectrophotometry. The sample conservation, the analytical procedure, and the reproducibility of the method are described by Franck-Riquer et al [10].

RESULTS

The results obtained in MZT and MZA twins are compared in Table 2. The data show that inter-pair variances are always significantly greater than intra-pair variances. Moreover, the resemblance estimated by the intra-pair variance, s_w^2, is significantly greater among MZT than among MZA twins. However, even in the MZAs likeness between cotwins remains very high, as shown by the intra-class correlation coefficients, the lower being equal to 0.52 for plasma zinc. Therefore environmental factors that begin to differ after the twins have been separated have some influence on the twins' RBC and plasma magnesium and, in a minor way, on their RBC zinc levels, without suppressing, however, the intra-pair likeness, which remains rather high.

Tables 3 and 4 compare MZ and DZ twins, respectively, in Paris and in Brussels. The The F ratios obtained when the inter-pair variances are compared to the intra-pair variances are significant for all variables in all groups, except the Paris DZ twins, the smallest group. Furthermore, whether in Paris or in Brussels, resemblance in DZ twins is always smaller than in MZ twins for RBC magnesium and zinc.

For plasma magnesium, the results are not clear, there being no concordance in either sample. This is in good keeping with the following facts: plasma magnesium is the variable that is the most sensitive to changes in the intra-pair environment; moreover, the proportion of twins living apart is higher among DZ twins (46%) than among MZ twins (34%). Therefore, if we compare DZ to MZA twins, the differences between MZ and DZ weaken (F = 1.73 instead of F = 3.15), in accordance with the results provided by the Brussels twin sample (F = 1.16).

For plasma zinc, the difference between MZ and DZ twin resemblances is significant only in the Paris sample. But in the DZ group of this sample and for this variable, the inter-pair variance is lower than the intra-pair variance. If we except this group, likeness for plasma zinc seems, however, to be constantly high, as well in MZ as in DZ twins, supporting the assumption that environmental factors play a decisive role in likeness in cotwins, living either together or apart. These environmental influences would therefore be imposed prior to the separation and their actions would have long-lasting effects.

TABLE 2. Results of Variance Analysis: MZ Twins Living Together and Apart †

		MZT[a]	s_b^2/s_w^2 ratio	MZA	s_b^2/s_w^2 ratio	F(MZA/MZT)
P Mg	s_b^2	2.287	15.88***	2.227	4.96**	0.97
	s_w^2	0.144		0.449		3.12**
	r	0.88		0.66		
	n	27		14		
RBC Mg	s_b^2	1.573	61.21***	2.392	32.50***	1.52
	s_w^2	0.026		0.074		2.86**
	r	0.97		0.94		
	n	27		14		
RBC Zn	s_b^2	2.240	22.72***	1.538	7.93***	0.69
	s_w^2	0.099		0.194		1.97
	r	0.92		0.78		
	n	27		14		
P Zn	s_b^2	1.866	13.39***	0.914	3.17*	0.49
	s_w^2	0.139		0.288		2.07
	r	0.86		0.52		
	n	24		14		

†Intrapair (s_w^2) and interpair (s_b^2) variances for plasma magnesium (P Mg) and zinc (P Zn), for red blood cell magnesium (RBC Mg) and zinc (RBC Zn) levels.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

The results of the family data analysis are shown in Table 5. There is agreement in both samples with regard to children's regressions on mid-parent and sibling correlations. Regression coefficients are all significantly different from zero and very high for RBC magnesium, RBC zinc, and plasma magnesium, but low for plasma zinc. Similar results are observed for the sibling correlations, which are slightly higher for RBC magnesium than for plasma magnesium. The only surprising result concerns the Pyrenean sample, the intra-sibling correlation being nil for RBC zinc, because of its particularly low inter-sibling variance, whereas its intra-sibling variance stays within normal range. Assuming the RBC zinc level to be mainly genetically determined, as indicated by our results, this discordance between the Paris and the Pyrenean families could be explained in terms of different gene frequencies. The reduction of the RBC zinc inter-individual variability could also result from the environmental homogeneity in the Pyrenean community. This is what has been observed for our three variables, which all present an inter-family diversity, with s_b^2 lower in the Pyrenean families than in the Paris ones. Then one would have to suppose that RBC zinc levels are particularly sensitive to environmental factors.

https://doi.org/10.1017/S0001566000006632 Published online by Cambridge University Press

TABLE 3. Results of Variance Analysis: MZ and DZ Twins From Paris†

		MZ (Paris)	s_b^2/s_w^2 ratio	DZ (Paris)	s_b^2/s_w^2 ratio	$F_{(DZ/MZ)}$
P Mg	s_b^2	2.215	8.93**	2.029	2.60	0.92
	s_w^2	0.248		0.780		3.15*
	r	0.80		0.57		
	n	41		11		
RBC Mg	s_b^2	1.806	43.00**	1.277	1.84	0.71
	s_w^2	0.042		0.693		16.50**
	r	0.95		0.30		
	n	41		11		
RBC Zn	s_b^2	1.960	14.96**	1.082	1.07	0.55
	s_w^2	0.131		1.008		7.69**
	r	0.87		0.04		
	n	41		11		
P Zn	s_b^2	1.503	7.71**	0.743	0.93	0.49
	s_w^2	0.195		0.801		4.11**
	r	0.77		...		
	n	38		10		

†Intrapair (s_w^2) and interpair (s_b^2) variances for plasma magnesium (P Mg) and zinc (P Zn), for red blood cell magnesium (RBC Mg) and zinc (RBC Zn) levels.

*$P < 0.01$.

**$P < 0.001$.

The comparison between twin and family data indicates greater resemblance values for plasma magnesium in family studies than in twin studies. Twin data show that plasma magnesium levels have a very slight genetic variability, whereas regression coefficients, as well as full-sib correlations, are high for this variable. This suggests that the family environment produces a stronger resemblance for plasma magnesium than for the other variables.

DISCUSSION

The results seem to be quite coherent in spite of the diversity of both approach and samples. They demonstrate the existence of a high genetic variability in RBC magnesium and zinc, while genetic factors play a minor role in plasma magnesium and possibly plasma zinc diversity. Environmental influences are important mainly for plasma magnesium and zinc and are slight for RBC magnesium and zinc.

These twin and family studies are merely considered as a first step, and other investigations have to be done to specify how genetic factors act on the magnesium and zinc regulation. Yet, the present indications enable us to foresee which directions to follow for further research.

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Because there is a significant correlation between plasma magnesium and plasma zinc. (r = 0.26, see Table 6), common factors are presumed to influence their variability. These factors are probably of an environmental nature, the genetic variability being relatively small.

Because of the statistical independence observed between RBC magnesium and RBC zinc concentrations (r = 0.06, see Table 6), we assume the genetic factors involved in their respective regulation to be different and, therefore, we discuss them separately.

RBC Zinc Level

The zinc flow through the RBC membrane obeys a passive mechanism, at least in vitro [23]. The zinc gradient between intra- and extra-erythrocyte compartments reflects the respective proportions between linked protein zinc and free zinc in these compartments. Genetic anomalies in the zinc binding proteins, such as albumin, are well known to cause an abnormally high value of zinc concentration in plasma [26], without any evident alterations in the RBC zinc content. Furthermore, in the RBC, energy-dependent links between zinc and an unknown substance [25], which could be hemoglobin [14], are now established. Zinc regulation would then depend upon the binding level of intra- and extra-RBC zinc.

TABLE 4. Results of Variance Analysis: MZ and DZ Twins From Brussels†

		MZ	s_b^2/s_w^2 ratio	DZ	s_b^2/s_w^2 ratio	$F_{(DZ/MZ)}$
P Mg	s_b^2	2.846	6.00*	1.874	4.60*	0.66
	s_w^2	0.474		0.407		0.86
	r	0.71		0.64		
	n	47		28		
RBC Mg	s_b^2	2.057	18.40*	2.747	4.79*	1.34
	s_w^2	0.112		0.573		5.13*
	r	0.90		0.65		
	n	47		28		
RBC Zn	s_b^2	1.902	31.76*	2.844	5.81*	1.50
	s_w^2	0.060		0.490		8.18*
	r	0.94		0.71		
	n	47		28		
P Zn	s_b^2	1.608	7.76*	1.967	10.00*	1.22
	s_w^2	0.207		0.197		0.95
	r	0.77		0.82		
	n	42		28		

†Intrapair (s_w^2) and interpair (s_b^2) variances for plasma magnesium (P Mg) and zinc (P Zn), for red blood cell magnesium (RBC Mg) and zinc (RBC Zn) levels.

*$P < 0.001$.

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Moreover, Frithz and Ronquist [11] have recorded high RBC zinc concentrations in essential hypertensive patients. Since this type of hypertension is submitted primarily to a genetic control involving sodium and potassium transmembrane flow [12, 13], a possible relation between both observations is further supported by the fact that genetic factors seem to be involved in both cases: RBC zinc concentration and Na/K balance.

These observations lead us to forward the following hypothesis: the genetic factors involved in the RBC zinc regulation might control the binding between zinc and one or several specific proteins: this Zn-protein affinity could vary according to the energy cell level, the Na/K balance, and possibly, the structural and genetic modifications of those proteins.

Plasma and RBC Magnesium

The correlation between plasma magnesium and RBC magnesium on the same blood sample is 0.2 (in this report r = 0.17, see Table 6) whereas the correlation between RBC magnesium at a given time and plasma magnesium recorded a month earlier is significantly higher: r = 0.4 [1, 2]. Furthermore, Dunn and Walser [8] have shown that RBC magnesium level depends upon the plasma magnesium concentration at the time of erythropoiesis. Therefore, at least one genetic factor could be common to the regulation of both plasma and RBC magnesium. This factor would act on the balance of intra- and extra-cellular magnesium.

TABLE 5. Full-Sib Variance Analysis and Intra-Class Correlation Coefficients and Offspring on Mid-Parent Regression Coefficient

		Full-sib analysis of variance and correlation[a] ($r < \frac{1}{2} h^2$)				Child on mid-parent regression[b] ($b = h^2$)		
		s_b^2	s_w^2	F	r	n	b	s_b
Pyrenean Valley	P Mg	1.227	0.613	2.00*	0.29	63	0.66	0.17
	RBC Mg	1.735	0.778	2.23**	0.33	64	0.61	0.23
	RBC Zn	0.927	0.746	1.24	0.09	64	0.43	0.12
	P Zn	1.250	0.580	2.16**	0.32	64	0.36	0.16
		N = 105;	n = 42;	n_0 = 2.49				
		s_b^2	s_w^2	F	r	n	b	s_b
Paris	P Mg	1.917	0.704	2.72***	0.30	80	0.61	0.18
	RBC Mg	2.709	0.506	5.36***	0.52	80	0.86	0.14
	RBC Zn	2.472	0.617	4.01***	0.42	80	0.74	0.17
		N = 181;	n = 44;	n_0 = 4.10				

[a] r = correlation coefficient.

[b] b = standard deviation (s_b).

N = number of subjects.

n = number of family.

n_0 = weighted number of siblings by family.

*P < 0.05.

**P < 0.01.

***P < 0.001.

https://doi.org/10.1017/S0001566000006632 Published online by Cambridge University Press

TABLE 6. Inter-Correlation Coefficient Estimated† From Correlation Coefficients of Each Sample

	P Mg	RBC Mg	RBC Zn	P Zn
P Mg	1	0.17*	−0.02	0.26**
RBC Mg		1	0.06	0.01
RBC Zn			1	0.03

†After 2 – Fisher transformation; ddl > 450.

*P < 0.05.

**P < 0.001.

In the reticulocytes, the magnesium concentration is high. Then, as the erythrocytes get older, magnesium progressively leaves the cell, following an exponential curve to reach a steady state [27]. A genetic variability may also be observed at that level, in direct connection with the membrane permeability: as a matter of fact, in E coli, several mutations, genetically mapped, are known to modify the membrane transport and/or permeability to magnesium [24]. Furthermore, an inter-specific variability (including humans) of magnesium kinetics through the membrane has been demonstrated [21]. At present there is no biological system known to regulate magnesium flow through the membrane in man.

The RBC magnesium genetic variability reported in this paper, as well as in the literature mentioned above, suggests the occurrence in human erythrocytes of one or several genetic systems involved in the membrane transport and/or permeability to magnesium. These systems would exhibit a genetic diversity, as demonstrated for lithium [7] or for sodium and potassium [12, 13].

CONCLUSIONS

Twin and family studies on magnesium and zinc concentrations in erythrocytes lead us to concordant conclusions. These biological variables present an extremely high genetic variability, about the same as for stature in man, for instance. However, the genetic regulation mechanisms in RBC seem to be different for magnesium and for zinc, as suggested by their low inter-correlation. A study of the literature suggests a genetic diversity of the membrane transfer and/or permeability to magnesium, whereas a genetic diversity of the binding proteins seems to be involved in zinc regulation. Yet these assumptions are not exclusive. Moreover, the fact that there is a genetic regulation does not prevent intra-RBC magnesium and zinc concentrations from being sensitive to various environmental factors, such as appear following twin separation. However that may be, magnesium and zinc concentrations in erythrocytes are still less sensitive to family, non-genetic, long-term influences than are their plasma concentrations.

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