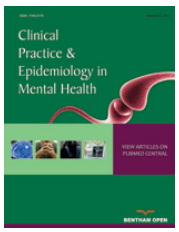

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REVIEW ARTICLE

Microcytic Anaemia as Susceptibility Factors in Bipolar Spectrum Disorders: Review of the Literature, Replication Survey, and Co-Segregation within Families

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Abstract:

Background:

Potential interactions between mood disorders and microcytic anaemias have been suggested by case reports, surveys of haematological parameters in psychiatric populations, and surveys of psychiatric morbidity in thalassaemic carriers.

Objectives:

a) To review published studies.

b) To study the prevalence of microcytic anaemia in a sample of Sardinian outpatients with recurrent mood disorders.

c) To check whether mood disorders and microcytic anaemia co-segregate within families.

Methods:

We extracted data on blood count and serum iron concentrations from the records of patients admitted between January 1st, 2001 and December 31st, 2016, to our clinic for mood disorders. Moreover, we studied siblings of subjects with both major mood disorders (according to Research Diagnostic Criteria) and heterozygous thalassaemia (according to Mean Corpuscular Volume, serum iron, and haemoglobin A_2 concentrations). Siblings affected with a major mood disorder were examined for haematological concordance with the proband (reduced MCV and/or increased HbA_2 in case of heterozygous β-thalassaemia, or presence of gene deletions in case of α-thalassaemia).

Results:

Microcytic anaemia was highly prevalent (81/337 = 24.0%) among outpatients with mood disorders. Starting from 30 probands with heterozygous ß-thalassaemia, concordance for reduced MCV and/or increased HbA_2 was found in 78% (35/45) of affected siblings. Starting from 3 probands with heterozygous α-thalassaemia, only one of the 5 affected siblings carried four α-globin functional genes.

Conclusion:

Based on the review of the literature, the high prevalence of microcytic anaemia in outpatients, and the concordance between affected siblings, we can conclude that a role of heterozygous thalassaemias is highly probable. Future studies are required to establish the relevance of heterozygous thalassaemias and evaluate the magnitude of the effect, possibly using a molecular diagnosis also in the case of heterozygous β-thalassaemia.

Keywords: Alpha-thalassemia, Anemia, Beta-thalassemia, Blood cell count, Erythrocyte indices, Hemoglobin A2, Mood disorders.

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1. INTRODUCTION

Over the past three decades, potential interactions between mood disorders and microcytic anaemias (and in particular-heterozygous thalassaemias) have been suggested by a series of observations, ranging from case reports to surveys of haematological parameters in psychiatric populations or surveys of psychiatric morbidity in thalassaemic carriers. The hypothesis of a direct role has been supported by several studies: however, the robustness of results varied from labile hints to much more robust proofs. Here we provide a comprehensive review of published studies, including very intriguing recent results. Moreover, we present some new data

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from our own population of Sardinian patients aiming at establishing whether:

a) The high prevalence of microcytic anaemia (up to 24%) can be replicated in a new sample of Sardinian patients with recurrent mood disorders;

b) Mood disorders and microcytic anaemia co-segregate within families;

1.1. Review of the Literature

1.1.1. Case Reports

Different groups from various countries reported co-occurrence of psychiatric disorders and heterozygous thalassaemias (Table **1**). Several reports suggested co-segregation of the blood condition and the psychiatric disorder within families (notes in Table **1**).

Most case reports regarded patients affected by bipolar disorder and identified as ß-thalassaemic carriers by common screening methods such as blood count and analysis of haemoglobin A_2 (Hb A_2) concentration. However, recurrent depression [7] or chronic psychosis [9] were also involved.

Many relatives with the major affective disorder were reported in haematological concordance with the probands, but the information was not invariably based on direct examination.

There was only one report involving heterozygous α-thalassaemia [6]. In this case, the following points are worth mentioning: a) the genetic mutation was characterized molecularly; b) haematological and psychiatric phenotype concordance was reported in both proband's parents; c) the severity of the psychiatric disorder was witnessed by four cases of suicide in the family.

1.1.2. Surveys of Microcytic Anaemias and/or Heterozygous Thalassaemias in Psychiatric Populations

The prevalence of microcytic anaemias and/or heterozygous thalassaemias was studied in psychiatric populations from various countries (Table **2**).

Sardinian [11, 13] and Argentinean [12] studies were prompted by the observation of an unexpectedly high prevalence of microcytic anaemia among patients suffering from specific subgroups of mood disorders, who were then furtherly studied with common screening methods of heterozygous β-thalassaemia.

Table 1. Case reports of heterozygous thalassaemias in patients with major psychiatric disorders.

Authors	Country	Proband's Origin	Blood Diagnosis	Notes
Joffe *et al*. (1986) [1]	Canada	Italian woman with bipolar disorder	Heterozygous β-thalassaemia	Two of three relatives across two generations had both major mood disorder and blood disorder (not studied directly)
Singh and Maguire (1988) [2]	Eire	Italian woman with bipolar disorder	Heterozygous β-thalassaemia	Brother with bipolar disorder but haematologically normal
Harada *et al* (1995) [3]	Japan	Two-generation Japanese family with bipolar disorder	Heterozygous β-thalassaemia	Co-occurrence evidenced in three affected family members. According to the pedigree structure, co-occurrence could be inferred in a fourth relative, given the rarity of thalassaemia in Japan
Brett and Dunn (1998) [4]	Australia	Italian woman with bipolar disorder	Heterozygous β-thalassaemia	Co-occurrence in one studied sister and referred in one son
Di Clemente *et al*. (2004) [5]	Luxembourg and Switzerland	One Antillean woman and one Portuguese woman with bipolar disorder	Heterozygous β-thalassaemia	Both probands' fathers with bipolar disorder, co-occurrence reported in one, haematological status not available for the other
Damsa *et al* (2005) [6]	Switzerland	One woman, native of Reunion Island, with bipolar disorder	Type 2 α-thalassaemia (absence of two α-genes)	Bipolar disorder and suicide in both parents, one brother, and one paternal uncle. Recurrent major depression in an additional brother. The only available haematological data regarded the moderate chronic anaemia reported in both parents
Borras and Constant (2007) [7]	Belgium and Switzerland	One Belgian woman with severe recurrent depression	Heterozygous β-thalassaemia	Co-occurrence of heterozygous β-thalassaemia and recurrent major depression in the proband's father and two paternal uncles
Kosehasanogullari *et al*. (2007) [8]	Turkey	One Turkish woman with bipolar disorder	Heterozygous β-thalassaemia	Multiple medical comorbidities and neuroleptic malignant syndrome caused by a combination of risperidone and lithium
Borras and Huguelet (2008) [9]	Switzerland	One Angolan woman and one Belgian man hospitalized for exacerbation of paranoid schizophrenia	Heterozygous β-thalassaemia	Co-occurrence of heterozygous β-thalassaemia and diagnosis of schizophrenia in one brother, one paternal uncle and one paternal cousin (from the Angolan family), and in father and one paternal uncle (from the Belgian family)

Table 2. Surveys of microcytic anaemias in psychiatric populations.

Authors	Country	Population studied	Blood diagnosis	Prevalence	Notes; *P*; OR (95% Confidence Interval)
Scherer and Eberle (1988) [10]	Germany	15 Mediterranean women hospitalised for depression	Heterozygous β-thalassaemia	5/15 = 33%	Muscular weakness and bone pain in: β-thalassaemia heterozygotes 4/5 = 80% versus Non-thalassaemics, 0/10 = 0%; *P* = 0.004; OR = 63.00 (2.13 –1861.17)
Bocchetta and Del Zompo (1990) [11]	Sardinia	180 psychiatric outpatients	Heterozygous β-thalassaemia	32/180 = 17.8%	Bipolar, 26/116 =22.4% (including Bipolar Schizoaffective, 14/45 = 31.1%) versus Unipolar, 5/55 = 9.1%; *P* = 0.036; OR = 2.89 (1.04 – 7.99)
Ciprian-Ollivier *et al*. (1991) [12]	Argentina	104 psychiatric patients	Microcytic anaemia	16/104 = 15.4%	Recurrent mood disorders, 14/79 = 17.7% versus Other disorders (schizophrenia, anxiety, *etc*.), 2/25 = 8%; *P* = 0.346; OR = 2.48 (0.52 – 11.74)
			Heterozygous β-thalassaemia	5/104 = 4.8%	Bipolar, 4/51 = 7.8%, Other (schizophrenia, anxiety, *etc*.), 1/53 = 1.9%; *P* = 0.201; OR = 4.43 (0.48 – 41.02)
Bocchetta (2005) [13]	Sardinia	1014 psychiatric outpatients (*including those from the 1990 survey)	Microcytic anaemia	234/1014 = 23.1%	Bipolar, 183/732 = 25.0% versus Other, 51/282 = 18.1%; *P* = 0.02; OR = 1.51 (1.07 – 2.13) Bipolar included Manic Schizoaffective disorder, predominantly affective subtype, 74/219 = 33.8%
			Heterozygous β-thalassaemia	148/1014 = 14.6% (estimate)	Bipolar, 120/732 = 16.4% Other, 28/282 = 9.9%; *P* = 0.01; OR = 1.78 (1.15 – 2.75) Bipolar included Manic Schizoaffective disorder, predominantly affective subtype, 49/219 = 22.4%
Hosseini *et al*. (2008) [14]	Mazandaran (Iran)	110 bipolar patients	Microcytic anaemia (MCV<75 μ^3)	11/110 = 10.0%	Controls with no psychiatric disorders, 12/118 = 10.1%; *P* = 1.00; OR = 0.98 (0.41 – 2.33)
			Heterozygous β-thalassaemia	9/110 = 8.2%	Controls with no psychiatric disorders, 5/118 = 4.2%; *P* = 0.27; OR = 2.01 (0.65 – 6.21)

1.1.3. Surveys of Psychiatric Morbidity in Thalassaemic Carriers

We provide (Table **3**) a summary of the results about psychiatric morbidity surveyed in populations of subjects who were: a) obligate carriers of ß-thalassaemia (parents of children with Cooley's anaemia) [16, 19]; b) potential carriers of ß-thalassaemia (siblings of children with Cooley's anaemia) [15]; c) ß-thalassaemia heterozygotes based on screening [18, 20, 21] or molecular methods [17].

Table 3. Surveys of psychiatric morbidity in thalassaemic carriers.

Authors	Country	Population studied	Blood diagnosis	Notes	*P*; OR (95% Confidence Interval)
Labropoulou and Beratis (1995) [15]	Greece	71 non-thalassaemic siblings of subjects with Cooley's anaemia (mean age = 12 years);	Not specified, but 2/3 expected to be β-thalassaemia heterozygotes	Any psychiatric disorder in: Siblings of thalassaemics: age 5-10 years, 3/20 = 15%; age 10-19 years, 27/51 = 53%; Controls: age 5-10 years, 4/18 = 22%; age 10-19 years, 15/53 = 28%	0.57 (age 5-10 years); OR = 0.62 (0.12 – 3.23) 0.01 (age 10-19 years); OR = 2.85 (1.27 – 6.42)
Rao *et al*. (2004) [16]	India	19 mothers and 11 fathers of children with Cooley's anaemia	Obligate β-thalassaemia heterozygotes	Depression in 21/30 = 70%; No controls	n.a.
Lykeridou *et al*. (2004) [17]	Greece	159 women with heterozygous β-thalassaemia undergoing chorionic villus sampling (spouses were carriers too)	Heterozygous β-thalassaemia	Clinically relevant depression in: β-thalassaemia heterozygotes, 16/159 = 10.1%; Women undergoing karyotyping for risk of trisomy, 7/150 = 4.7%; Women undergoing a routine first trimester scan, 1/309 = 0.3%	0.0842 (vs risk of trisomy); OR = 2.29 (0.91 – 5.72) 0.0001 (vs routine scan); OR = 34.5 (4.53 – 262.40)

(Table 3) contd.....

Authors	Country	Population studied	Blood diagnosis	Notes	*P*; OR (95% Confidence Interval)
Marvasti *et al*. (2006) [18]	Iran	208 β-thalassaemia heterozygotes (mean age = 24 years)	Heterozygous β-thalassaemia	Mild to severe depression in: β-thalassaemia heterozygotes, 98/208 = 47.1%; Controls 87/200 = 43.5%	0.49; OR = 1.16 (0.78 – 1.71)
Sharghi *et al*. (2006) [19]	Iran	98 mothers of children with β-thalassaemia major	Obligate β-thalassaemia heterozygotes	Beck Depression Inventory >18 in: Mothers of thalassaemics, 50/98 = 51%; Controls, 31/99 = 31.4%	0.006; OR = 2.28 (1.28 – 4.08)
Keşkek *et al*. (2013) [20]	Turkey	53 β-thalassaemia heterozygotes (mean age = 39 years)	Heterozygous β-thalassaemia	Abnormal (>7) score in the Hamilton Depression Rating Scale: β-thalassaemia heterozygotes, 43/53 = 81%; Controls, 22/53 = 41.5%. Severe/very severe (>18) score in the Hamilton Depression Rating Scale: β-thalassaemia heterozygotes, 17/53 = 32%; Controls, 1/53 = 1.9%.	0.0001 (HDRS>7); OR = 7.93 (3.13 – 20.08) 0.0001 (HDRS>18); OR = 24.56 (3.13 – 192.88)
Graffeo *et al*. (2018) [21]	Sicily	4943 β-thalassaemia heterozygotes	Heterozygous β-thalassaemia	Odds Ratio for hospitalisation for mood disorders between 4943 β-thalassaemia heterozygotes and 21,063 controls = 2.08 (95% Confidence Interval = 1.15-3.75)	0.015 (Logistic penalised regression model)

The initial aim of some of these studies was to evaluate the burden of being a relative of children with severe disease (Cooley's anaemia). However, the collected cohort could also be seen as a sample of carriers of ß-thalassaemia who had undergone a psychiatric assessment. Given the nature of the ascertainment, researchers focused on depressive symptoms, the most plausible to be found in families of severely ill children [15, 16, 19] or at risk of giving birth to ill children [17]. More recent surveys were based on populations screened for heterozygous β-thalassaemia [18, 20, 21].

2. NEW DATA

2.1. Replication Survey of Microcytic Anaemia

2.1.1. Aims

The most intriguing conclusion of our previous surveys [11, 13] was that up to 24% of patients admitted to our outpatient unit between 1980 and 2000 showed microcytic anaemia. Therefore, it appeared logical to repeat the survey by studying the patients admitted to our outpatient unit between 2001 and 2016.

2.1.2. Methods

Since 1980 our unit has been one of the reference facilities for the management of lithium and related treatments of mood disorders. On admission, detailed information is obtained from the patient and other available sources (such as relatives and referring clinicians) concerning demographic characteristics and lifetime medical and psychiatric history. Moreover, all available records regarding previous prescriptions or hospitalizations are examined. Psychiatric diagnosis is made according to modified Research Diagnostic Criteria (RDC) [22]. For the purpose of this study, we extracted data on routine laboratory tests from the clinical records. Blood count and serum iron concentrations were available for 337 patients consecutively admitted between January 1^{st,} 2001, and December 31^{st,} 2016.

All subjects gave their informed consent to participate in the study. The study was performed in accordance with the Declaration of Helsinki. Data were made anonymous and used only for statistical evaluation.

2.1.3. Results

The proportion of cases with an MCV lower than 80 μ^3which were admitted to the outpatient unit between 2001 and 2016 are shown in Table **4**. Data are compared with those from our previous survey [13], which concerned patients admitted to the same unit between 1980 and 2000. In both samples, Manic Schizoaffective disorder was the subgroup with the highest proportion of reduced MCV. In the current survey, we found another high prevalence category that had not specifically been envisaged in our previous survey. It is represented by patients that, although included among those with a diagnosis of Recurrent Major Depression, are characterized by clinical depression superimposed on a lifelong hyperthymic temperament. It was defined as "bipolar IV" (hyperthymic depression) by Akiskal and Pinto [23].

Table 4. Reduced Mean Corpuscular Volume (MCV) in outpatients with mood disorders: new Survey (N = 337) and previous survey (N = 952) [10] for comparison.

Psychiatric Diagnosis	Current Survey		Previous Survey [10]	
	Total number	MCV<80 μ^3 N (%)	Total number	MCV<80 μ^3 N (%)
Manic Schizoaffective	43	17 (39.5)	288	88 (30.6)
Bipolar with Mania	109	17 (15.6)	269	59 (21.9)
Bipolar with Hypomania	97	24 (24.7)	175	36 (20.6)
Depressive Schizoaffective	12	3 (25.0)	96	19 (19.8)
Recurrent Major Depression	34	9 (26.5)	124	24 (19.4)
Hyperthymic Depression*	32	11 (34%)	-	-
TOTAL	337	81 (24.0)	952	226 (23.7)

*clinical depression superimposed on a lifelong hyperthymic temperament, so-called "bipolar IV" according to Akiskal and Pinto [23]. Not diagnosed in the previous survey.

Table 5. Haemoglobin concentrations in patients with normal or reduced Mean Corpuscular Volume (MCV).

Subgroup	Mean haemoglobing/dL (± SD)	*P*
Women with MCV ≥ 80 μ^3 (N = 136)	13.2 (± 0.8)	<0.0001
Women with MCV < 80 μ^3 (N = 54)	11.4 (± 1.2)	
Men with MCV ≥ 80 μ^3 (N = 101)	14.8 (± 1.2)	<0.0001
Men with MCV < 80 μ^3 (N = 25)	13.2 (± 1.2)	

Patients with iron deficiency were excluded from calculations.

As expected, subjects of both sexes with MCV < 80 μ^3 had significantly lower haemoglobin concentrations (Table **5**).

2.2. Co-segregation in Relatives

2.2.1. Aims

Given a large number of patients with heterozygous thalassaemias identified during our surveys [11, 13], we were prompted to study potential co-segregation in family members in order to extend the limited observations from case reports in the literature.

2.2.2. Methods

We studied affected siblings starting from patients (probands) with reduced MCV, normal serum iron concentration, and RDC diagnosis of major mood disorder, either bipolar (Bipolar I, Bipolar II, or Manic Schizoaffective) or unipolar (Major Depressive or Depressive Schizoaffective), ascertained during our first surveys of microcytic anaemia [11, 13]. Probands were first characterized for heterozygous thalassaemias by analysing their haemoglobin A_2 (Hb A_2) concentration. Analyses were repeated after iron therapy in case of iron deficiency. Affected sibs were those with a diagnosis of major mood disorder (Bipolar I, Bipolar II, or Manic Schizoaffective) or unipolar (Major Depressive or Depressive Schizoaffective) disorders. Incapacitation in the major life role during depressive episodes was considered mandatory. Psychiatric diagnosis of affected siblings was based on any available source (direct interview, clinical charts, history of psychiatric medication). The diagnosis was made blind to the subject's haematological status. The latter was established on the basis of blood counts, serum iron concentration, and/or haemoglobin electrophoresis (or chromatography). Probands and affected siblings with microcytic anaemia, normal iron, and normal HbA_2 concentration were sent to Ospedale Microcitemico "Antonio Cao" – Azienda di Rilievo Nazionale ed Alta Specializzazione "Giuseppe Brotzu", Cagliari, to be further characterised molecularly for α-thalassaemia. Genomic DNA was isolated from whole blood using standard methods. GAP-PCR and digestion of α-globin genes, previously amplified, with the specific restriction enzymes NcoI and HphI were performed to screen all samples for the most common α-thalassemia deletion in Sardinia (−α3.7 NG_000006.1: g.34164_37967del3804;alfa 2 NcoI (T->C) HBA2:c.2T>C;alfa 2 IVSI (-5 nt) donor HphI HBA2:c.95+2_95+6delTGAGG). No further procedure was necessary because all the three studied sibships were found positive for such a mutation.

2.2.3. Results

2.2.3.1. Heterozygous ß-thalassaemia

Starting from the 30 probands with heterozygous ß-thalassaemia shown in Table **6**, concordance for reduced MCV and/or increased HbA_2 was found in 78% (35/45) of siblings with a diagnosis of major mood disorder. The affected siblings consisted of 26 sisters and 19 brothers. RDC diagnoses were the following: Manic Schizoaffective = 8; Bipolar with Mania = 6; Bipolarwith Hypomania = 8; Depressive Schizoaffective = 3; Major Depression (with hospitalization and/or incapacitation in the major life role) = 20. MCV was available for 36 siblings, averaging 66.3 μ^3 (range 57-79 μ^3) among concordant siblings, and 89.4 μ^3 (range 84-98 μ^3) among discordant siblings. HbA_2 concentration was available for 23 siblings, averaging 5.42% (range 3.76-6.47%) among concordant siblings, and 2.62% (range 1.79-2.55%) among discordant siblings.

Table 6. Characteristics of probands with heterozygous β-thalassaemia and available affected siblings.

-		Heterozygous β-thalassaemia (N= 30)
-		-
Men/Women		11/19
Mean age (min-max)		45.6 (24-72)
RDC diagnosis		-
Manic Schizoaffective	14	
Bipolar with Mania	5	
Bipolar with Hypomania	6	
Depressive Schizoaffective	2	
Recurrent Major Depression	3	
Mean Corpuscular Volume, mean (min-max) μ^3		67.8 (58-79)
Haemoglobin A_2 (% concentration), mean (min-max)		5.40 (3.41-7.41)

Table 7. Characteristics of probands with heterozygous α-thalassaemia and available affected siblings.

Proband						Sib					
Sex	**Age**	**RDC diagnosis**	**MCV**	**HbA_2**	**Genotype**	**Sex**	**Age**	**RDC diagnosis**	**MCV**	**HbA_2**	**Genotype**
F	34	SA-M	73	2.36	- α / - α	F	29	SA-M	71	2.23	- α / - α
F	45	BP-1	75	2.53	- α / - α	F	49	UP	76	2.76	- α / - α
						F	41	BP-2	71	2.95	- α / - α
F	38	SA-D	67	2.14	- α / αα	F	35	SA-M	82	2.16	- α / αα
						F	19	UP	60	1.48	αα / αα

Based on results from another study [24], we point out that six of the ten discordant siblings carry the G6PD Mediterranean mutation, which was not necessarily shared with the proband. Another discordant sibling and the respective proband had a diagnosis of idiopathic unconjugated hyperbilirubinemia (Gilbert's syndrome).

2.2.3.2. Heterozygous α-thalassaemia

Phenotypes and genotypes of multiple sibships segregating for α-thalassaemia are shown in Table **7**.

All subjects with two α-globin functional genes (- α / - α) showed the α-thalassaemia phenotype. Subjects with three α-globin functional genes (- α / αα) showed either the normal or the α-thalassaemia phenotype. The subject with all four α-globin functional genes(αα / αα) showed a reduced MCV, but iron serum concentration was not available.

Other family members were reported to suffer from severe psychiatric disorders but were not available for direct examination (in several instances, members had committed suicide). The discordant sibling (αα / αα) belonged to a family with a history of severe psychiatric disorders (and suicides) in both the paternal and the maternal side.

3. DISCUSSION

3.1. Cases of Co-occurrence of Microcytic Anaemias and Mood Disorders: Chance Observation or Genetic Condition with Pleiotropic Effects?

The first clues suggesting a potential involvement of microcytic anaemias in psychiatric disorders date back to the 1980s. The Italian woman with bipolar affective disorder and thalassaemia minor was ascertained in Canada in 1986 [1]. Her family history of potential co-segregation of the mood and the blood disorders raised the hypothesis of linkage between the ß-globin gene and a gene for manic-depressive illness on chromosome 11p15. At that time, chromosome 11p15 had become popular in psychiatric genetics after the first linkage study in the Amish population [29, 30]. The subsequently published cases often reported co-segregation in affected relatives, and most of the authors hypothesised that linkage disequilibrium between globin genes and genes involved in neurotransmission was a possible explanation. They underscored, for example, that the ß-globin gene is closely linked to the genes encoding tyrosine hydroxylase or dopamine D4 receptors on chromosome 11p15. However, the quality of family data was limited in several of these early reports due to the lack of haematological information regarding some relatives, the lack of molecular diagnosis of the thalassaemic genes, and the heterogeneity of the psychiatric cases involved.

Moreover, given the high worldwide prevalence of mood disorders, co-occurrence with microcytic anaemias might be attributed to chance, especially in areas where thalassaemia genetic mutations are highly prevalent. Nevertheless, some observations remain very intriguing, such as the multiplex family ascertained in Japan [3], where thalassaemias are very rare.

3.2. Excessive Prevalence of Heterozygous β-thalassaemia in Bipolar Subgroups: Geographical Stratification?

Our first observation of the potential association between microcytic anaemias and bipolar spectrum disorder [11] was

serendipitous. We collected blood counts to calculate glucose-6-phosphate dehydrogenase (G6PD) activity in an early study using G6PD deficiency as a phenotype marker of X-linkage with manic-depressive illness [30]. We then noticed the disproportion of reduced MCV among some diagnostic subgroups, namely psychotic manias [11]. When we discussed our results with the late professor Antonio Cao, a distinguished expert in thalassaemia, he warned us about geographical stratification as a potentially misleading factor. Indeed, a wide variation in the prevalence of ß-thalassaemic carriers (3.8 to 38%) was found across Sardinian areas by the 1966's pioneer study of more than 6000 observations in 52 villages showing the same distribution once observed for malarial morbidity [31]. If mood disorders with psychotic and/or manic features are more prevalent for unknown reasons in once malarial areas in Sardinia, a spurious association with heterozygous thalassaemias might have derived in patients from our catchment area. A recent survey on 63,285 13- to 14-year-old students has found a more homogeneous prevalence distribution of ß-thalassaemic carriers (9.1 to 11.7%) across 13 Sardinian sub-regions [32]. Based on data of Hb A_2 concentration, we had estimated a 16.4% prevalence of heterozygous β-thalassaemia in bipolar subgroups from our previous survey [13], which is in any case higher than the 10.3% mean prevalence currently found in the general population. We are not able to compare data regarding prevalence of α-thalassaemic carriers because the 80 μ^3MCV cut-off we choose to define microcytic anaemias does not detect many carriers, particularly those with three α-globin functional genes (- α / αα). This may also be one of the reasons why there are fewer case reports in the literature and fewer multiplex sibships from our study, despite the much higher prevalence of α- than ß-thalassaemic carriers in the population (10 to 37% in the recent Sardinian survey [32]).

We have limited our study and this discussion to the simplest cases because rarer haemoglobin mutations or complex phenotype interactions can go undetected by common screening methods.

Published surveys of microcytic anaemias and/or heterozygous thalassaemias in psychiatric populations (Table **2**) vary in terms of sample size, diagnostic methods, and choice of control subjects. However, we emphasise that they are invariably in the same direction, even if single studies were not invariably able to conclude for a significant association. We have calculated that the excessive prevalence of heterozygous ß-thalassaemia found in the Argentinean [12] and the Iranian [14] bipolar subgroups compared to controls (7.8% versus 1.9%; 8.2% versus 4.2%; respectively) would have attained statistical significance with larger (for example threefold) sample sizes. The authors reported an estimated prevalence of ß-thalassaemic carriers in the general population of 5% in Argentina [12] and 10% in Mazandaran, the northern province of Iran [14]. We maintain that the association has to be researched in areas with high prevalence. Thalassaemia genes are common in many populations of the Mediterranean, Sub-Saharan Africa, Middle East, India, China, and South-East Asian regions that have previously shown endemicity of malaria. On the contrary, surveys carried out in other countries will be less likely to reveal significant results. For example, in a population of 289 patients who were consecutively admitted to a public psychiatric hospital in California and screened for physical disorder, 13 cases (4.5%) were found with unspecified anaemia, but only one (1.1%) with thalassaemia [33]. Nevertheless, people with globin genetic mutations may end up anywhere given the increasingly mobile world, as witnessed by the variety of countries where cases of concurrent heterozygous thalassaemias and mood disorder were ascertained (Tables **1** and **2**).

3.3. The predisposing Factor for Psychotic Manias or Any Bipolar Spectrum Disorder, Including Unipolar Depression?

In our present replication, we confirmed that microcytic anaemia could be found in up to ¼ of Sardinian outpatients with recurrent mood disorders. Once more, manic schizoaffective disorder is the RDC diagnostic subgroup with the highest prevalence, but the new category of hyperthymic depression has emerged as potentially associated with the microcytic anaemia. The bipolar/non-bipolar distinction is not as clear-cut as previously assumed in our previous studies [11, 13]. For example, in the present survey, "typical" bipolar cases (RDC diagnosis of bipolar with mania) had a lower prevalence of microcytic anaemias (15.6%) compared to the remaining subgroups, including non-bipolar categories (Table **4**). One possibility is that patients referred to our clinics over the last 15 years differ from those included in our previous surveys in terms of the severity of depressive episodes. Indeed, microcytic anaemias may have a role also in non-bipolar cases. For example, one of the early reports about the small population of Mediterranean women hospitalised in Germany [10] found that heterozygous ß-thalassaemia was associated with peculiar symptoms (muscular weakness and bone pain) that may be prominent in severe depressive syndromes. Moreover, one of the case reports (Table **1**) regarded a Belgian woman with severe recurrent depression [7].

Our is a clinical pharmacology unit that might select patients with some characteristics that make them different from common psychiatric outpatient units. Lithium may be prescribed by any psychiatrist in the Cagliari area, but our unit has been one of the reference centres for the monitoring of lithium treatment since its introduction in Sardinia. There might have been a selection of patients responding to lithium during acute episodes or carrying high suicide risk. Our interest in genetic studies, dating back to the 1980s, has led us to adopt modified RDC criteria rather than DSM or ICD criteria. With regard to the thalassaemic trait, it is mostly asymptomatic, and patients are not aware of their presence unless they had undergone some screening procedure. We often detect it by simply examining routine blood counts. In any case, we do not choose a specific psychiatric treatment. However, we have learned that β-thalassaemia heterozygotes with Manic Schizoaffective Disorder appear to respond well to lithium alone or combined with low dose antipsychotics, whereas β-thalassaemia heterozygotes with predominant depressive episodes (Bipolar with Hypomania, Recurrent Major Depression, or Hyperthymic Depression) are often stabilised with lithium alone or combined with antidepressants. However, whenever possible, antidepressants are discontinued to prevent

switch to hypomania or cycle acceleration. The latter caution is particularly recommended to prevent mood instability in cases with Hyperthymic Depression, the so-called "bipolar IV" according to Akiskal and Pinto [23].

3.4. Mood Disorders in Thalassaemic Carriers: From Early Clues to Current Proofs

Surveys of psychiatric morbidity in populations of β-thalassaemia heterozygotes (Table **3**) show that there may be a particular susceptibility to depressive symptoms. We choose to include in this review some studies on relatives of children with Cooley's anaemia who had undergone a psychiatric assessment because they represent samples of ß-thalassaemia carriers even if data were collected for another aim. The advantage is that no further molecular diagnosis is necessary. However, such studies were focused on depressive symptoms that are the most plausible to be found in families of severely ill children or at risk of giving birth to ill children. We can speculate that bipolarity did not emerge as often happens in non-clinical samples. Carta and Angst [34] already warned that epidemiological studies, especially when derived from interviews carried out by lay staff, which only reflect the patient's point of view, may miss the detection of hypomanic episodes. More recent surveys summarized in Table **3** started from subjects diagnosed with heterozygous ß-thalassaemia using common screening methods (mostly haemoglobin electrophoresis) [18, 20, 21].

The negative survey from Iran [18] was limited to depression and a relatively young age cohort. It found a small higher prevalence in the 208 β-thalassaemia heterozygotes (47.1%) compared to the 200 controls (43.5%). Given those rates, the statistical difference would have been achieved only with a much larger (*e.g*., ten-fold) sample.

On the contrary, the most striking evidence of the role of heterozygous thalassaemia as a predisposing factor to severe mood disorder is provided by the recent Sicilian study [21]. The aim was to follow-up β-thalassaemia heterozygotes (compared to a control group) by studying mortality and hospitalisations for various complications. The following causes of hospitalisation were investigated: arrhythmia, cholelithiasis, cirrhosis, diabetes, mood disorders, kidney diseases, and ischaemic cardiomyopathy. Among other results, there was an increased risk of hospitalisation for mood disorders of 2.08 (95% CI 1.15 to 3.75; $P = 0.015$) in β-thalassaemia heterozygotes. The Sicilian study has many advantages. The sample size was the largest studied to date, including almost 5000 subjects with heterozygous ß-thalassaemia. Another advantage in making bias less probable is that the authors do not appear to be aware of all previous studies associating heterozygous thalassaemias with psychiatric disorders. Indeed, psychiatric illness was just one of the potential health problems investigated, and psychiatric assessment was independent of the authors' judgement because it was based on data of subsequent hospitalisations. Finally, the two-fold odds ratio was just mentioned without any particular emphasis or any hypothesis on potential pathogenic mechanisms. One limitation is that no specific distinction was provided about hospitalisations for manic or depressive episodes.

3.5. Co-segregation in Relatives

Co-segregation in relatives is considered a robust method to rule out false associations due to geographical stratification. To date, the evidence regarding the role of thalassaemic genes in mood disorders cannot be considered as established. Early reports were based on limited numbers of probands and affected relatives. The latter were not studied directly in many cases, or their haematological phenotype was unknown. Nevertheless, some observations remain very intriguing, such as the multiplex family ascertained in Japan [3], where thalassaemias are very rare.

Our study of 30 probands and their 45 affected siblings adds some information. The 78% rate of phenotype concordance is striking even if not complete. Nevertheless, we must consider that 50% of siblings of thalassaemic carriers are expected in advance to be haematologically identical by descent with the proband, irrespective of their psychiatric status.

Apart from the three sibships segregating for the α-thalassemia deletion, we did not deepen the diagnosis at the molecular level. Molecular diagnosis is mandatory in the prevention of Cooley's anaemia because genetic compounds are expected to be frequent in Sardinia. For example, coinheritance of mutated ß and α globin can result in normal MCV, because ß and α chains are less unbalanced compared to simple heterozygotes. Therefore, we cannot exclude that some phenotypically-discordant affected siblings may represent false negatives. On the other hand, phenotypically-concordant siblings may carry different genes compared to the proband.

The predominant mutation of the ß-thalassaemia chromosomes in the Sardinian population is the codon 39 nonsense mutation, which accounts for 95.7% of cases [35]. On the contrary, the α-globin genotypes identified with thalassaemia screening in Sardinia are known to be complex [36, 37].

We would also like to underscore that some of the 10 discordant siblings carry additional mutations potentially involved in the pathogenesis of psychiatric disorders, such as G6PD deficiency [38, 39].

With regard to the concept of the bipolar spectrum, we considered 20 siblings with unipolar depression as affected. The bipolar/non-bipolar distinction has long been challenged by family studies of bipolar probands. Increased rates of depression are found in relatives of both bipolar and unipolar probands compared to normal probands when incapacitating episodes alone are considered [40]. This is the reason why we included incapacitation in the major life role during depressive episodes among criteria for affected siblings. It is noteworthy that one of the reported cases in the literature regarded a Belgian woman with severe recurrent depression and co-occurrence of heterozygous β-thalassaemia and recurrent major depression in the proband's father and two paternal uncles.

The heterogeneity of phenotypic presentation is not unusual in psychiatric genetics: in fact, examples exist of identified mutations that predispose to a variety of neurological or neuropsychiatric phenotypes [41]. Therefore, reports of the potential role of heterozygous thalassaemias in various

psychiatric illnesses as those summarized in Table **1** are not unexpected, including the Angolan woman and the Belgian man hospitalised in Switzerland for exacerbation of paranoid schizophrenia with co-segregation of heterozygous β-thalassaemia and schizophrenia in several relatives [9].

3.6. Potential Mechanisms

Since the first suggestion of a potential role of heterozygous thalassaemias in psychiatric disorders, various potential mechanisms have been hypothesised. Altinoz and Ince [42] have recently reviewed the emerging roles of haemoglobins in mental disorders, discussing thoroughly metabolical, genetical, and immunological aspects. We also suggest that increased concentrations of unconjugated hyperbilirubinemia are included, as these may be a shared characteristic of heterozygous thalassaemias, G6PD deficiency, and Gilbert's syndrome.

3.6.1. Linkage Disequilibrium

Most early reports mentioned linkage disequilibrium between globin genes and genes involved in neurotransmission as a potential explanation, underscoring, for example, that the ß-globin gene is closely linked to the gene encoding tyrosine hydroxylase on chromosome 11p15, which was a popular candidate gene for bipolar disorder in the 1980s [29]. Similarly, the rarer reports involving heterozygous α-thalassaemia, suggested potential linkage disequilibrium of α-globin genes with candidate genes on chromosome 16p13 [42].

3.6.2. Anaemia

An alternative hypothesis is that microcytic anaemias can themselves represent susceptibility and/or modifying factors through some of their already known characteristics. For example, when we realized that microcytic anaemia might be found in around 25% of outpatients with recurrent mood disorders in Sardinia [12] or in up to 18% in Argentina [15], we were surprised by the lack of studies on microcytic anaemia in the psychiatric literature. In fact, there are several symptoms shared between anaemia and depression (fatigue, asthenia, somatic complaints, *etc*.). On the contrary, many studies have investigated macrocytic anaemia and the effects of vitamin B12 deficiency in the CNS [43]. Table **7** shows haemoglobin concentrations by sex and MCV in patients from the present survey.

3.6.3. Low Serum Cholesterol

Low serum cholesterol, which has long been known to be present in thalassaemic carriers [25, 26], is one of the potential mechanisms predisposing to mood disorders [27] and suicide, as first reviewed by Muldoon *et al*. [44].

We have confirmed in an unpublished study that cholesterol concentrations in patients with microcytic anaemia are often in the lowest quartiles of their sex and age categories. The proportion of patients with MCV < 80 μ^3 was significantly greater among those with cholesterol concentrations in the lowest quartile (63/203 = 31% in quartile I versus 128/580 = 22% in quartiles II to IV; $P = 0.0134$).

Prompted by the literature linking low cholesterol and suicide, Ghiam *et al*. [45] surveyed heterozygous ß-thalassaemia in a population of subjects who had attempted suicide in south Iran. They concluded negative results because the 8.9% prevalence they found in the 293 thalassaemic carriers did not differ significantly from the 6.7% found in 300 healthy controls ($P = 0.3$). However, keeping the same rates, the significance would have been attained with 5-fold sample size.

The most cited mechanism linking low cholesterol with suicide behaviour involves serotonergic transmission [46]. Cholesterol is low in thalassaemic carriers because inefficacious erythropoiesis enhances cholesterol requirement [47].

4. LIMITATIONS

The principal limitation of this study is regarding the lack of control groups that does not allow calculation of the magnitude of the effects. In the replication survey, we did not include a control group because it would have suffered from the potential geographical stratifications, as already discussed. Unfortunately, there are no data about the prevalence of unspecified microcytic anaemia in Sardinia because epidemiological studies had rather focused on the prevention of Cooley's anaemia that could not be based on reduced MCV alone. In the co-segregation study, concordance rates were supposed to exceed the expectation without calculating the magnitude of the effect. As mentioned above, 50% of siblings of ß-thalassaemic carriers are expected in advance to be haematologically concordant with the proband for Mendelian reasons if only one parent is a carrier too. However, the expected concordance for a ß-thalassaemic gene may be higher in high prevalence areas because both parents might be carriers too. Not to mention the complexity of expected phenotype concordance due to the presence of α-thalassaemic genes, which are even higher prevalent.

On the other hand, we did not include unaffected siblings as possible controls after we realised the difficulties in defining them incontrovertibly. Indeed, we found many cases of siblings with suspected bipolarity based on the history of abnormal behaviour, but who had never been diagnosed or treated. For the same reason, we preferred to focus only on severe cases of depression.

CONCLUSION

Based on the review of the literature, the confirmed high prevalence of microcytic anaemia in Sardinian outpatients with mood disorders, and the high concordance rates between affected siblings, we can conclude that a predisposing role of heterozygous thalassaemias in areas with high prevalence is highly probable. Given the severity of Cooley's anaemia, it is understandable why researchers have been mainly interested in patients with homozygous thalassaemias and their families to evaluate the magnitude of the effect from the psychiatric point of view. Future studies are required to establish the relevance of heterozygous thalassaemias and evaluate the magnitude of the effect, possibly using a molecular diagnosis also in the case of heterozygous β-thalassaemia.

AUTHORS' CONTRIBUTION

All authors have agreed on the final version of the manuscript and meet at least one of the following criteria (recommended by the ICMJE [http://www.icmje.org/recommendations/):

1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;

2. Drafting the article or revising it critically for important intellectual content.

AUTHORS' CONTRIBUTION

AB devised the study, participated in its design and coordination, and drafted the manuscript. CC and RA participated in the study design, literature search, diagnosis of mood disorders, and interpretation of data. MCS participated in the study design, literature search, molecular characterisation of α-thalassaemia, and interpretation of haematological data. All authors read and approved the final manuscript.

CONSENT FOR PUBLICATION

All patients signed informed consent for the publication of anonymous and aggregate data derived from their medical records.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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