



Population Genetics of ABO and Rh Blood Groups in the Al-Jabal Al-Akhdar Region, Libya: A Study of Hardy–Weinberg Equilibrium

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ABSTRACT

This study presents a population genetic analysis of the ABO and Rh blood group systems in the Al-Jabal Al-Akhdar region of Libya. The population's genetic structure was investigated by testing the Hardy–Weinberg equilibrium (HWE). Chi-square test compared observed ABO and Rh blood group counts with HWE-expected counts. The entire population's observed ABO blood-group phenotypes significantly deviated from HWE ($p < 0.001$). Significant deviations were observed in Al-Bayda, Shahat, Qandula, Maraweh, Al-Haniya, and Al-Qubah, suggesting potential evolutionary influences. In contrast, Omer-Al Mukhtar, Al-Faydia, Al-Bayadah, and Derna did not differ significantly from HWE for ABO, indicating these subpopulations are not experiencing strong evolutionary pressures that alter allele frequencies. Observed Rh phenotypes across the entire population similarly exhibited a significant deviation from HWE ($p < 0.001$). Omer-Al Mukhtar, Qandula, Maraweh, Al-Haniya, and Al-Faydia showed no significant deviation, whereas Al-Bayda, Shahat, Derna, Al-Bayadah, and Al-Qubah did. These results offer initial insights into the evolutionary and genetic structure of the Al-Jabal Al-Akhdar population.

الوراثة السكانية لفصائل الدم ABO والعامل الريزي Rh في منطقة الجبل الأخضر، ليبيا: دراسة لتوازن هاردي- واينبرغ

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الكلمات المفتاحية:

فصائل الدم.
التكرار الأليلي.
توازن هاردي- واينبرغ.
العامل الريزي.
علم الوراثة السكانية.

المخلص

تقدم هذه الدراسة تحليلاً وراثياً سكانياً لنظامي فصائل الدم ABO و Rh في منطقة الجبل الأخضر، ليبيا، تم التحقق من التركيب الجيني للسكان عن طريق اختبار توازن هاردي-واينبرغ (HWE)، وقارن اختبار مربع كاي أعداد فصائل الدم ABO و Rh المرصودة مع الأعداد المتوقعة وفقاً لتوازن هاردي-واينبرغ. وبشكل عام، انحرفت الأنماط الظاهرية لفصائل الدم المرصودة انحرافاً كبيراً عن توازن هاردي-واينبرغ ($p < 0.001$). ولوحظت انحرافات كبيرة في البيضاء، وشحات، ودرنة، وقندولة، ومروة، والحنية، والقبة، مما يشير إلى تأثيرات تطورية محتملة. في المقابل، لم تختلف أصناف عمر المختار، والفايديّة، والبياضة اختلافاً معنوياً عن توازن هاردي-واينبرغ بالنسبة لنظام فصائل الدم ABO مما يشير إلى أن هذه المجموعات الفرعية لا تتعرض لضغوط تطورية قوية تغير ترددات الأليلات. أما بالنسبة لنظام فصائل الدم Rh، فقد انحرفت الأنماط الظاهرية الملاحظة أيضاً عن توازن هاردي-واينبرغ بشكل عام (قيمة $p < 0.001$). لم تُظهر عينات عمر المختار، وقندولة، ومروة، والفيدية أي انحراف يُعتد به إحصائياً، بينما أظهرت عينات البيضاء، وشحات، ودرنة، والبياضة، والحنية، والقبة انحرافاً يُعتد به إحصائياً. تُقدم هذه النتائج رؤى مبدئية حول البنية التطورية والجينية لسكان الجبل الأخضر.

1. Introduction

The study of blood group polymorphisms provides valuable insights into the genetic structure and evolutionary history of human populations [1]. The ABO and Rh blood group systems are widely used as genetic markers in population genetic studies due to their polymorphic nature and simple mode of inheritance [2]. Human blood

groups are genetically determined and exhibit varying degrees of polymorphism. The prevalence of specific blood groups differs widely between populations [3].

The Hardy-Weinberg equilibrium (HWE) is a fundamental principle in population genetics that provides a baseline for detecting evolutionary changes. The Hardy–Weinberg principle is the foremost

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analytical tool in both theoretical and applied studies of population and quantitative genetics [4]. The Hardy–Weinberg principle asserts that in a population with random mating and without mutation, migration, or selection on a gene, genotype frequencies are simply related to allele frequencies. When no outside forces act, the population remains in this equilibrium [5]. Deviations from HWE can indicate the action of evolutionary forces such as natural selection, genetic drift, gene flow, and non-random mating [6]. It was reported that the Hardy–Weinberg principle can be used to estimate blood-group frequencies in the Libyan population and to compare them with those of other populations [7].

1.1. literature Review

In a study conducted in Libya with a sample size of 527 individuals, researchers reported that the population of Sebha was in Hardy–Weinberg genetic equilibrium. This finding indicates that, at least for the genetic loci examined in that investigation, the Sebha population did not show evidence of being influenced by the primary evolutionary forces: natural selection, genetic drift, gene flow, or non-random mating during the period and within the scope sampled [7]. By contrast, research based on blood donors at the Central Blood Bank in Benghazi revealed a different pattern: those investigators observed a statistically significant deviation from Hardy–Weinberg equilibrium for the ABO blood group system, suggesting that one or more evolutionary or sampling factors (for example selection bias among donors, population substructure, non-random mating, migration, or genotyping error) may be acting on ABO allele frequencies in that donor pool; importantly, the same study reported that the Rh blood group system did not deviate from Hardy–Weinberg expectations, implying stability for Rh in that sample [8]. A separate, more recent study at the Central Blood Bank in Benghazi, which examined donor data collected over the three years from 2018 to 2020, documented year-to-year variation in their findings [9]. Those temporal differences could reflect changes in donor demographics, shifts in sampling strategy, public health campaigns, or technical and methodological differences across years; together, these studies underscore that population genetic patterns can differ substantially depending on location, sample composition, blood-group system assessed, and temporal context.

A study of the Pakistani population found no statistically significant difference between the observed and expected frequencies of the ABO and Rh blood systems, suggesting the population follows the Hardy–Weinberg equilibrium [10].

Africa demonstrated compatibility with the Hardy–Weinberg equilibrium. In Nigeria, the distribution of ABO and Rhesus blood types among 318,940 and 280,514 individuals, respectively, aligned with Hardy–Weinberg expectations [11]. In Bote Town, Ethiopia, blood samples from 392 of 775 students showed no significant difference between observed and expected frequencies for both the ABO and Rh systems [12]. Additionally, in Northwest Ethiopia, an analysis of 6,471 subjects found no statistically significant differences between observed and expected allele and genotype frequencies [13]. In Ogbomoso, southwestern Nigeria, 7,653 individuals were tested for ABO blood groups; the distributions closely conformed to Hardy–Weinberg expectations [14]. In contrast, a study in Ilorin, North-Central Nigeria, examining 9,643 individuals found that ABO blood groups deviated significantly from expected Hardy–Weinberg frequencies [15].

This research aims to investigate the population genetics of Libya’s Al-Jabal Al-Akhdar region by analyzing the ABO and Rh blood group systems, with a focus on testing the HWE to assess evolutionary dynamics and genetic differentiation in the population. Ultimately, the study seeks to deepen understanding of the region’s genetic landscape and contribute baseline data for future anthropological and evolutionary research.

2. Materials and Methods

2.1. Field of Research

Al-Jabal Al-Akhdar, also known as Green Mountain, is situated north of 32 degrees latitude, between Benghazi and Tubruq. This region spans 7,800 km² and reaches an elevation of 900 meters, as documented [16].

2.2. Research Design and Context

The study was conducted on a sample of 3363 individuals from various towns in the Al-Jabal Al-Akhdar region of Libya. The ABO and Rh blood groups were recorded from families for two consecutive generations (parents and offspring). It was used only on randomly selected unrelated families to satisfy independence assumptions. The genotypes of the ABO and Rh blood groups were defined using the pedigree analysis. The allele frequencies for the ABO and Rh systems were calculated using the following formulas, based on the observed phenotype frequencies.

ABO system:

$$r = \frac{oo + \frac{1}{2}Ao + \frac{1}{2}Bo}{Total} \quad p = \frac{AA + \frac{1}{2}Ao + \frac{1}{2}AB}{Total}$$

$$q = \frac{BB + \frac{1}{2}BO + \frac{1}{2}AB}{Total}$$

Rh system:

$$D = \frac{DD + \frac{1}{2}Dd}{Total} \quad d = \frac{dd + \frac{1}{2}Dd}{Total}$$

The allelic frequencies of the ABO and Rh blood groups were used to define the expected value under the assumption of Hardy–Weinberg equilibrium (HW) (Table 1).

$$p + q + r = 1$$

Table 1: Applying the Hardy-Weinberg principle to the allele frequencies of the ABO blood group.

Phenotypes	Genotypes	Genotypes (HW)	phenotypes (HW)
A	AA	p ²	p ² +2pr
	AO	2pr	
B	BB	q ²	q ² +2qr
	BO	2qr	
AB	AB	2pq	2pq
O	OO	r ²	r ²
Rh +	DD	p ²	p ² +2pq
	Dd	2pq	
Rh-	dd	q ²	q ²

2.3. Statistical Analysis:

The data were analyzed with Minitab Version 20.3. Hardy–Weinberg equilibrium was assessed using a chi-square test. The Hardy–Weinberg equilibrium (HWE) was assessed for both the ABO and Rh blood group systems by comparing the observed phenotype count with the expected count. A p-value of less than 0.05 was considered statistically significant, indicating a deviation from HWE.

3. Results

Across the whole population, the observed phenotypes of ABO blood groups differ from the expected under Hardy–Weinberg, revealing a significant deviation (p-value = 0.000*). On the other hand, Omer-Al Mukhtar, AL-faydia, AL-bayadah, and Derna blood type numbers do not differ from the expected under Hardy–Weinberg, p-value = 0.131, 0.280, 0.178, and 0.058, respectively. However, Al-Bayda, Shahat, Qandula, Maraweh, AL Haniya, and Al-Qubah blood type numbers differ from the expected under Hardy–Weinberg, p-value =0.000*, 0.002*, 0.023*, 0.027*, 0.032*, and 0.001* respectively (Table 2) (Figure 1).

Throughout the entire population, the observed Rh types differ from the expected under Hardy–Weinberg, revealing a significant deviation (p-value = 0.000*). On the other hand, Omer-Al Mukhtar, Qandula, Maraweh, AL-Faydia, and Al Haniya numbers do not differ from the expected under Hardy–Weinberg, p-value = 0.306, 0.195, 0.128, 0.190, and 0.057, respectively. However, Al-Bayda, Shahat, Derna, Al-Bayadah, Al-Qubah, Rh type numbers differ from the expected under Hardy–Weinberg, p-value =0.000*, 0.000*, 0.000*, 0.022*, and 0.003* respectively (Table 3) (Figure 2).

Table 2: Chi-Square test for Observed ABO blood types type with expected under the Hardy-Weinberg.

Towns	Allele Frequency	ABO	Obs	Exp (HWE)	X ²	P-Value
Al-Bayda	p 0.162	A	259	164	141.9	0.000*
	q 0.342	B	208	401		
	r 0.496	O	368	216		
		AB	44	98		
Shahat	p 0.236	A	207	193	14.70	0.002*
	q 0.258	B	211	214		
	r 0.506	O	195	167		
		AB	41	79		
Derna	p 0.275	A	214	211	7.488	0.058
	q 0.249	B	187	187		
	r 0.476	O	167	142		
		AB	58	86		
Omer-Al mukhtar	p 0.321	A	44	50	5.627	0.131
	q 0.17	B	32	24		
	r 0.509	O	36	30		
		AB	5	13		
Qandula	p 0.276	A	39	31	9.575	0.023*
	q 0.329	B	41	39		
	r 0.395	O	20	16		
		AB	5	19		
Maraweh	p 0.411	A	51	42	9.171	0.027*
	q 0.233	B	23	20		
	r 0.356	O	12	11		
		AB	4	17		
AL-Faydia	p 0.19	A	18	18	3.831	0.280
	q 0.225	B	25	23		
	r 0.585	O	27	24		
		AB	1	6		
ALBayadah	p 0.161	A	28	28	4.912	0.178
	q 0.342	B	63	69		
	r 0.497	O	52	38		
		AB	9	17		
AlHaniya	p 0.229	A	85	87	8.8	0.032*
	q 0.233	B	82	88		
	r 0.538	O	108	84		
		AB	15	31		
Al-Qubah	p 0.3	A	136	136	17.67	0.001*
	q 0.253	B	116	110		
	r 0.447	O	103	76		
		AB	24	57		
Entire Population	p 0.28	A	1116	1162	52.52	0.000*
	q 0.242	B	953	978		
	r 0.478	O	979	768		
		AB	315	456		

Note: Obs = Observed, Exp = Expected, X² = Chi-square test, *significant difference (p < 0.05).

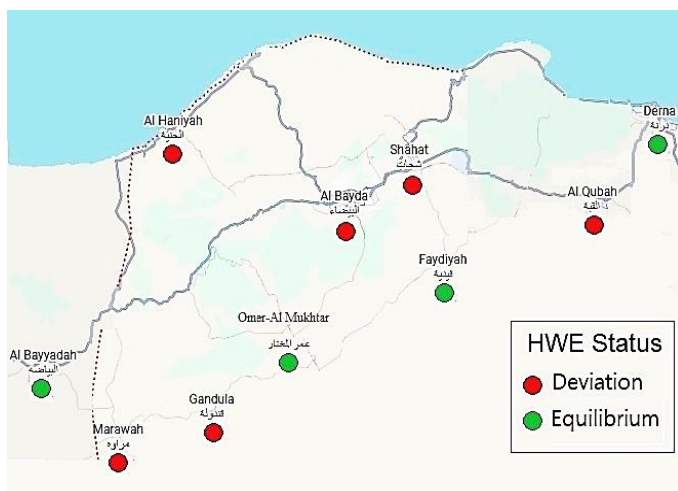


Fig. 1: Graphical representation of HWE status for ABO blood types across different towns in Al-Jabal Al-Akhdar.

Table 3: Chi-Square test for Observed Rh factors with expected under the Hardy-Weinberg.

Town	Allele Frequency	Rh	Obs	Exp (HWE)	X ²	P-Value
Al-Bayda	D 0.74	Rh+	755	821	390.42	0.000*
	d 0.26	Rh-	124	58		
Shahat	D 0.83	Rh+	603	635	15.456	0.000*
	d 0.17	Rh-	51	19		
Derna	D 0.75	Rh+	549	587	13.719	0.000*
	d 0.25	Rh-	77	39		
Omer-Al Mukhtar	D 0.70	Rh+	101	106	1.047	0.306
	d 0.30	Rh-	16	11		
Qandula	D 0.83	Rh+	98	102	1.680	0.195
	d 0.17	Rh-	7	3		
Maraweh	D 0.62	Rh+	69	77	2.321	0.128
	d 0.38	Rh-	21	13		
AL-Faydia	D 0.79	Rh+	64	68	1.721	0.190
	d 0.21	Rh-	7	3		
AL-Bayadah	D 0.78	Rh+	134	145	5.274	0.022*
	d 0.22	Rh-	18	7		
Al_Haniya	D 0.74	Rh+	258	271	3.633	0.057
	d 0.26	Rh-	32	19		
Al-Qubah	D 0.79	Rh+	341	362	8.645	0.003*
	d 0.21	Rh-	38	17		
Entire Population	D 0.77	Rh+	2972	3178	81.420	0.000*
	d 0.23	Rh-	391	184		

Note: Obs = Observed, Exp = Expected, X² = Chi-square test, *significant difference (p < 0.05).

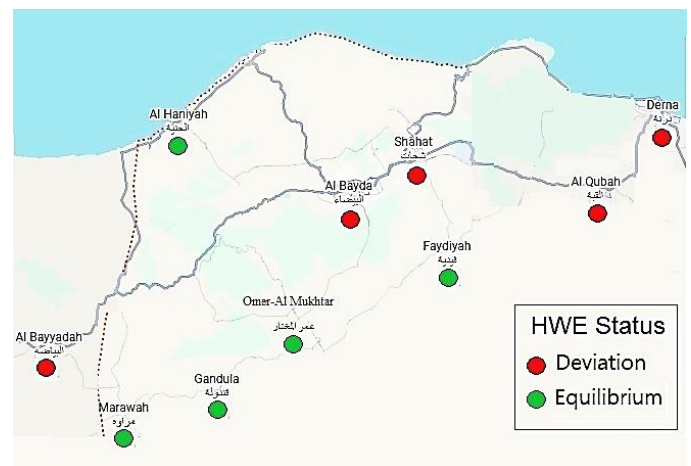


Fig. 2: Graphical representation of HWE status for the Rh factor across different towns in Al-Jabal Al-Akhdar.

4. Discussion

The study found a significant difference between observed ABO and Rh frequencies and those expected under the Hardy–Weinberg model for the entire population of Al-Jabal Al-Akhdar, suggesting influence from evolutionary forces (e.g., selection, mutation, drift, migration, or non-random mating) [17-18].

At the locality level, ABO frequencies in Al-Bayda, Shahat, Qandula, Maraweh, Al-Haniya, and Al-Qubah deviated significantly from Hardy–Weinberg expectations. Also, observed Rh frequencies in Al-Bayda, Shahat, Derna, Al-Bayadah, and Al-Qubah also showed significant deviation. In contrast, Omer-Al Mukhtar, Al-Faydia, Derna, and Al-Bayadah did not differ significantly for ABO, and Omer-Al Mukhtar, Qandula, Maraweh, and Al-Faydia did not differ significantly for Rh. These results suggest those subpopulations are not currently subject to strong evolutionary forces altering allele frequencies. These are consistent with what was reported in Sebha, Libya: no significant deviation from Hardy–Weinberg equilibrium [7]. In Africa, large Nigerian samples matched equilibrium for ABO and Rh [11]; Northwest Ethiopia and Southwest Nigeria showed no significant differences [13-15]; a Pakistani study found no significant differences for ABO and Rh [10]. Conversely, Lagos (Southwest Nigeria) and a 341-person Iraqi sample showed significant departures from equilibrium [10, 16-19].

Overall, the combined population departs significantly from Hardy–Weinberg expectations for both ABO and Rh, while several localities remain in equilibrium. Recurrent deviations across both systems in the

same localities suggest population structure, nonrandom mating, or demographic history as likely causes.

5. Conclusion

The entire population of Al Jabal Al Akhdar shows significant deviations from the Hardy–Weinberg equilibrium for both ABO and Rh blood groups, indicating the action of evolutionary forces or population structure. However, several localities remain in equilibrium, suggesting heterogeneity across the region. Repeated departures in the same localities for both systems (ABO and Rh) indicate nonrandom mating, subpopulation structure, or past demographic events as the likely drivers. Further targeted genetic and demographic studies are recommended to identify the specific causes and assess their public health and forensic implications.

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