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Establishment of Lymphoblastoid Cell Lines from Iranian Multiple Sclerosis Patients: A Renewable Genetic Resource

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Abstract

Background: Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disorder of the central nervous system with a complex interplay of genetic and environmental factors. The availability of high-quality, renewable sources of genomic DNA is crucial for advancing genetic and molecular investigations. This study aimed to establish a biobank of Epstein–Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) derived from Iranian patients with MS.

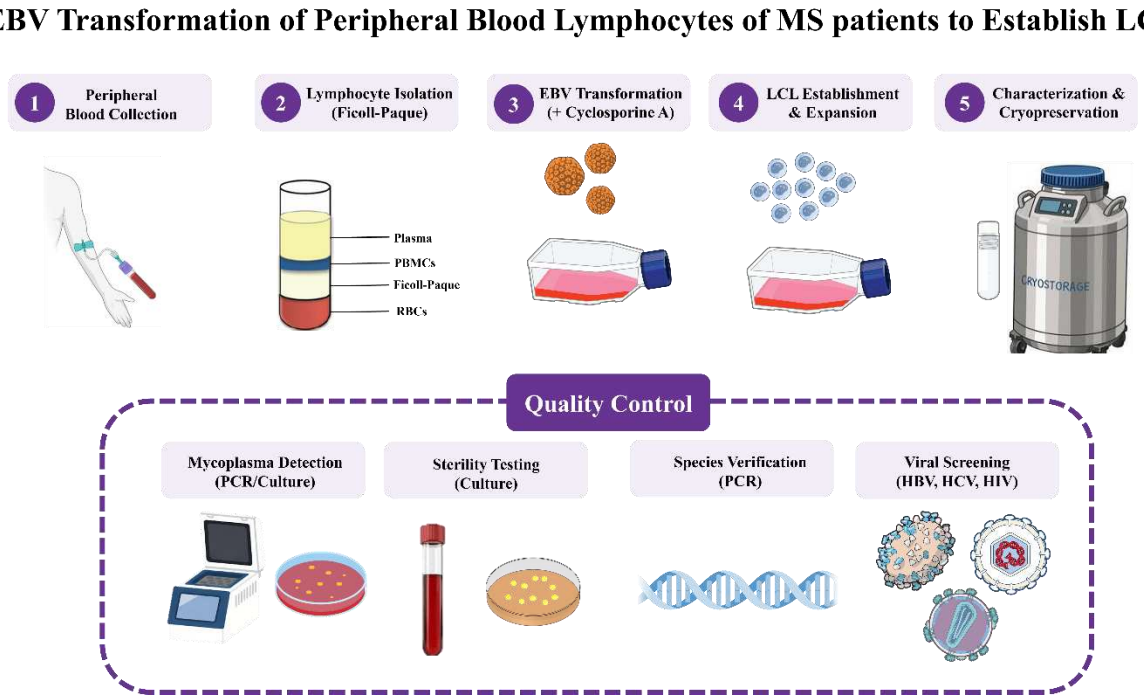
Methods: Peripheral blood samples were collected from 72 clinically confirmed MS patients following informed consent. Peripheral blood lymphocytes were isolated using Ficoll-Paque density gradient centrifugation and immortalized through EBV transformation using supernatant derived from the B95-8 cell line. Successfully established LCLs were expanded, characterized, and cryopreserved according to standard biobanking procedures. Quality control assessments included mycoplasma screening by Hoechst staining, PCR, and culture-based methods, bacterial and fungal sterility testing, species authentication using mitochondrial COI PCR, and viral screening for HBV, HCV, HIV, and EBV.

Results: Stable LCLs were successfully established from 48 of the 72 collected samples, corresponding to an overall success rate of 66.7%. All established cell lines were negative for mycoplasma, bacterial, and fungal contamination. Species authentication confirmed the human origin of all cell lines, and viral screening showed no evidence of HBV, HCV, or HIV infection. The generated LCLs were systematically documented, cryopreserved, and stored in the vapor phase of liquid nitrogen with comprehensive associated metadata.

Conclusion: This study represents the first reported biobank of EBV-transformed LCLs established specifically from Iranian patients with MS. This renewable and well-characterized resource provides a valuable source of genomic DNA for future genetic association studies, biomarker discovery, and functional genomic investigations of MS in the Iranian population. The established cell lines are available through the Iranian Biological Resource Centre (IBRC) for qualified research purposes.

Keywords: Biobanking; Lymphoblastoid cell lines; Multiple sclerosis; Epstein-Barr virus; Genetic resource; Iran

Graphical Abstract



Introduction

Approximately 1.89 million people worldwide suffer from multiple sclerosis (MS), and over 62,000 new cases were reported in 2021. The prevalence has been steadily rising over the last three decades and is estimated to be 23.9 cases per 100,000 people worldwide [1]. Iran has a moderate-to-high burden of MS in the Middle East, with 50–80 cases per 100,000 people with an estimated 70,000–90,000 affected people [2, 3]. MS is about twice as common in women as in men and usually manifests between the ages of 20 and 40 [4]. Regardless of treatment, 85% of patients initially have relapsing-remitting multiple sclerosis (RRMS), and more than half develop a progressive disease course within 10–15 years [5]. The accumulation of chronically demyelinated lesions due to insufficient endogenous remyelination is a hallmark of progressive MS. Neurodegeneration is eventually caused by persistent axonal injury within these lesions, which results in the progressive accumulation of neurological impairment [6]. Complex interactions between genetic susceptibility and environmental exposures give rise to the pathogenesis of MS [7]. More than 200 susceptibility loci have been found through genome-wide association studies (GWAS), with the human leukocyte antigen (HLA) region—specifically, the HLA-DRB115:01* allele—consistently showing the strongest genetic association [8, 9]. However, the lack of adequate characterization of the genetic architecture of MS in non-European populations, such as Iranians, underscores the necessity of population-specific genetic resources [10]. High-quality, high-molecular-weight genomic DNA is essential for genetic and molecular investigations. Even though freshly collected peripheral blood offers an instant source of genomic DNA, repeated sample collection is frequently unfeasible, unethical, and impractical, especially in longitudinal studies or research on rare genetic variations [11]. Lymphoblastoid cell lines (LCLs) transformed by the Epstein-Barr virus (EBV) offer an almost limitless and renewable source of genomic DNA while maintaining the genetic background of the donor with high fidelity [12]. Peripheral blood B lymphocytes could immortalized by EBV to generate these cell lines, which allow for ongoing cell division without significantly changing the donor genome [13]. LCLs have been widely used in the development of HLA reference panels [14], genetic linkage analyses [15], transcriptomic studies [16], and as standardized control materials for molecular assays [17] due to their genetic stability and long-term availability. Furthermore, by promoting repeatable and cooperative

research, disease-specific LCL biobanks have emerged as important tools for studying uncommon inherited disorders and familial diseases [18].

Despite Iran's comparatively high MS prevalence, there is currently no centralized biobank of EBV-transformed LCLs from Iranian MS patients. Additionally, DNA samples are usually kept in private laboratory collections that are not easily accessible, which limits data exchange, multicenter collaboration, and independent verification of research results. In order to provide a sustainable and well-characterized genetic resource for upcoming translational and genomic research, this study aimed to establish a publicly accessible biobank of EBV-transformed LCLs from Iranian MS patients in compliance with international biobanking standards.

Materials and Methods

Ethical Approval and Patient Recruitment

The Royan Institute's Ethics Committee at the Academic Center for Education, Culture, and Research (ACECR), Tehran, Iran, reviewed and approved the study protocol (Approval No. IR.ACECR.ROYAN.REC.1397.24). All study procedures were carried out in compliance with the Declaration of Helsinki's guiding principles. Before peripheral blood was drawn, each participant provided written informed consent. The establishment of LCLs, genetic analyses, long-term biobanking, and the distribution of biological materials to qualified researchers for use in future studies were all approved as part of the consent process.

Sample Collection

Using sodium heparin Vacutainer tubes, peripheral blood (8 mL) was collected from patients with MS who had been clinically diagnosed. The updated McDonald criteria were used to diagnose every patient [19]. The study did not include people who had active infections or who had been tested positive for HIV, HBV, and HCV. Each participant's age, sex, and MS subtype (relapsing-remitting, primary progressive, or secondary progressive) were noted along with their clinical and demographic details. Blood samples were collected, transported to the lab at 4–8°C, and processed in 4 hours. For HBV, HCV, and HIV serological screening, a 200-µL serum aliquot was separated. Furthermore, a 200-µL sample of whole blood was kept for the extraction of genomic DNA. The remaining sample was utilized to isolate peripheral blood lymphocytes.

Lymphocyte Isolation

Ficoll-Paque (PAN-Biotech, Germany) density gradient centrifugation was employed to isolate peripheral blood mononuclear cells (PBMCs). Briefly, 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (100 U/mL; Gibco, USA) were added to 3 mL of peripheral blood that had been diluted 1:1 with RPMI-1640 medium (Gibco, USA). In a 15-mL conical centrifuge tube, the diluted blood was layered over 3 mL of Ficoll-Paque. The tube was then centrifuged at 400 × g for 20 minutes at room temperature with the brake disengaged. The PBMC layer at the plasma–Ficoll interface was collected after centrifugation, twice washed with RPMI-1640 containing 10% FBS (200 × g for 10 min), and then resuspended in complete culture medium. Using a Neubauer hemocytometer and the 0.4% trypan blue exclusion assay, cell viability and total cell count were assessed.

EBV Production

The EBV was obtained from the B95-8 marmoset B-cell line (IBRC C10050; Iranian Biological Resource Centre, Tehran, Iran). RPMI-1640 medium supplemented with 10% FBS and 2 mM L-glutamine (Gibco, USA) was used to maintain cells at 37°C in a humidified incubator with 5% CO_2. The culture supernatant was collected and centrifuged at 200 × g for 7 minutes to eliminate cellular debris after the culture had reached the proper cell density. Subsequently, the supernatant was run through a sterile 0. 22-µm membrane filter. The filtered EBV-containing supernatant was aliquoted and kept at -80°C until needed.

EBV Transformation

Freshly isolated PBMCs (2–3 × 10^6 cells) were resuspended in 5–7 mL of complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1× penicillin–streptomycin. After the cell suspension was put into T25 culture flasks, 5–10 mL of filtered supernatant containing EBV was added. For a full day, cultures were kept at 37°C in a humidified environment with 5% CO_2. The medium was then replaced with a new, antibiotic-free RPMI-1640 medium. Every 2-3 days, cells were observed for morphological changes linked to EBV-mediated transformation, such as cellular aggregation, clump formation, and the development of larger, asymmetrically shaped lymphoblastoid cells. While successfully transformed lymphoblastoid cells showed prolonged proliferation and expansion, non-transformed lymphocytes usually underwent apoptosis within 10–14 days.

Expansion and Cryopreservation

Transformed LCLs were cultured at 37°C in a humidified environment with 5% CO2 in complete RPMI-1640 medium supplemented with 10% FBS and 2 mM L-glutamine, without antibiotics. Every 3-4 days, cultures were subcultured by moving the cells into new medium while keeping the density at 3–5 × 10^5

cells/mL. Cells were collected for cryopreservation by centrifuging them at 200 × g for 7 minutes. They were then resuspended in a freezing medium that contained 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) and 90% FBS. A concentration of 5×10^6 cells per vial was used when aliquoting cells into cryovials. The corresponding cell line identifier, passage number, cell concentration, and cryopreservation date were labeled on each cryovial. The vials underwent controlled-rate cooling, first kept at -20°C for an hour and then kept at -75°C for a month while quality control evaluations were finished. Cryovials were moved to the liquid nitrogen vapor phase (−196°C) for long-term storage after quality verification.

Quality Control

Mycoplasma Detection

Three complementary detection techniques were used to assess the mycoplasma contamination. First, a quick screening technique called Hoechst staining was used. The cells were grown on sterile coverslips, stained with Hoechst 33258 (Sigma-Aldrich, USA), and fixed with methanol. Extranuclear fluorescence, a sign of mycoplasma infection, was examined using fluorescence microscopy (Olympus BX51, China). Second, 7 common Mycoplasma species, including M. orale, M. hyorhinis, M. arginini, M. fermentans, M. hominis, M. laidlawii, and M. salivarium, were detected through molecular screening using multiplex PCR (Bio-Rad, USA) targeting the 16S rRNA gene [20]. Third, culture-based testing was conducted by inoculating cell culture supernatants into pleuropneumonia-like organism (PPLO) broth (BD Difco, USA), which was then incubated for 7-14 days at 37°C in a humidified environment with 5% CO_2. For evaluating microbial growth, the cultures were then subcultured onto PPLO agar (BD Difco, USA). M. orale was used as the positive control, while sterile culture medium served as the negative control.

Bacterial and Fungal Sterility

Culture supernatants were inoculated into Tryptone Soya Broth (TSB; Fluka, Sigma-Aldrich, USA) and Thioglycolate Medium (Merck, Germany), respectively, to examine for aerobic and anaerobic bacterial contamination. After 14 days of incubation at 22°C and 32°C respectively, the inoculated media were checked every week for signs of microbial growth, as indicated by medium turbidity.

Species Verification

A conserved region of the mitochondrial cytochrome C oxidase subunit I (COI) gene was amplified by PCR using universal primers to confirm species identity [21]. The criterion for species verification was the expected amplicon pattern for human genomic DNA. As an internal control, PCR amplification of a

conserved region of the 18S rRNA gene was carried out concurrently to verify the success of DNA amplification.

Viral Screening

The QIAamp DNA Mini Kit and QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) for DNA and RNA isolation, respectively, were used to extract viral nucleic acids. Conventional PCR was used to detect HBV, HIV, and EBV DNA, while reverse transcription PCR (RT-PCR) was used to detect HCV RNA. The corresponding artus® RG PCR Kits (QIAGEN GmbH, Hilden, Germany) were used for all assays in accordance with the manufacturer's instructions.

Statistical Analysis

GraphPad Prism (version 8.0; GraphPad Software, San Diego, CA, USA) was used for statistical analyses. When appropriate, the chi-square test was used to analyze categorical variables and the independent t-test was used to compare continuous variables. The primer sequences, corresponding target genes, expected amplicon sizes, and relevant references are presented in Table 1.

Table 1. Primer sequences used for the molecular screening of viral pathogens, Mycoplasma contamination, and species authentication.

Target/ Pathogen	Primer	Primer sequence (5′–3′)	Expected amplicon size (bp)	Reference
HIV	Forward	ATAATCCACCTATCCCAGTAGGAGAAAT	144	[22]
	Reverse	TTTGGTCCTTGTCTTATGTCCAGAATGC		
HCV	Forward	GCCATGGCGTTAGTATGAGT	259	[23]
	Reverse	GTGCACGGTCTACGAGACCT		
HBV	Forward	GTGCACTTCGCTTCACCTCT	300	[24]
	Reverse	AGCTTGGAGGCTTGAACAGT		

EBV	Forward	GGCGCACCTGGAGGTGGTCC	220	[25]
	Reverse	TTTCCAGCAGAGTCGCTAGG		
COI	Forward	TAGACATCGTACTACACGACACG	391	[26]
	Reverse	TCCAGGTTTATGGAGGGTTC		
Mycoplasma	Forward 1	CGCCTGAGTAGTACGTTCGC	500	[27]
	Forward 2	CGCCTGAGTAGTACGTACGC		
	Forward 3	TGCCTGAGTAGTACATTCGC		
	Forward 4	TGCCTGGGTAGTACATTCGC		
	Forward 5	CGCCTGGGTAGTACATTCGC		
	Forward 6	CGCCTGAGTAGTATGCTCGC		
	Reverse 1	GCGGTGTGTACAAGACCCGA		
	Reverse 2	GCGGTGTGTACAAAACCCGA		
	Reverse 3	GCGGTGTGTACAAACCCCGA		

Results

Patient Characteristics

Peripheral blood samples were collected from 72 patients with MS; 51 of them were female and 21 were male, resulting in a female-to-male ratio of 2.6:1. Participants' ages ranged from 18 to 69 years, with a mean of 37.9 ± 11.0 years. Every patient who was enrolled had RRMS. The successful

and unsuccessful LCL establishment groups did not differ significantly in terms of age ($p = 0.38$, independent t-test) or sex distribution ($p = 0.27$, chi-square test), as shown in Table 2. These results imply that the outcome of LCL establishment was unrelated to demographic characteristics.

Table 2. Comparison of demographic characteristics between successful and failed LCL establishments.

Variable	Successful (n=48)	Failed (n=24)	Test Statistic	P-value
Age (years), Mean ± SD	37.9 ± 11.0	35.8 ± 8.6	$t = 0.89$ (df = 57)	0.38
Sex (Female/Male), n	36 / 12	15 / 9	$\chi^2 = 1.21$ (df = 1)	0.27

LCL Establishment and Expansion

Stable LCLs were successfully established from 48 of the 72 peripheral blood samples that were processed, yielding an overall success rate of 66.7%. The remaining samples were eliminated due to microbial contamination, poor sample quality (mainly blood clotting upon receipt), or EBV-mediated transformation failure despite of suitable culture conditions.

Dense cellular aggregates, enlarged lymphoblastoid cells with irregular morphology and cytoplasmic projections, and sustained cell proliferation were the hallmarks of successful transformation, which became apparent 10–14 days after infection. On the other hand, during the first 10 days of culture, non-transformed lymphocytes gradually lost viability and experienced cell death. Before being cryopreserved, established LCLs were expanded for 3-5 passages. The population doubling time was between 24-48 hours, which is in line with the growth characteristics of EBV-transformed LCLs that were anticipated. Figure 1 indicates microscopic images of MS LCLs.

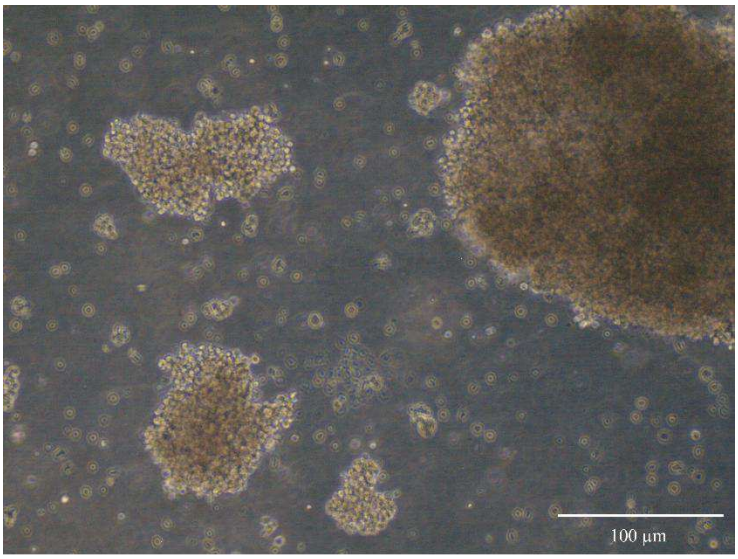


Figure 1. Microscopic images of MS LCLs at 20× objective magnification. Scale bar represent approximately 100 µm. The scale bar was estimated based on theoretical calculation using the known sensor dimensions of the camera and the 1.2× magnification of the microscope adapter (Olympus E330-ADU1.2X). A stage micrometer was not available at the time of imaging.

Cryopreservation

A total of 48 established LCLs were successfully cryopreserved; each cell line produced an average of 4 cryovials. The IBRC bank identification (e.g., IBRC C11000), sample code, de-identified patient demographic data, dates of sample collection and cryopreservation, cell count per vial, and quality control results were all methodically recorded in a searchable Excel-based database. Additionally, the demographic and clinical characteristics of all enrolled patients, along with the outcome of LCL establishment for each sample, are summarized in Supplementary Table 1.

Quality Control Results

Mycoplasma detection: Hoechst staining revealed no extranuclear fluorescence in any of the 48 cell lines, indicating the absence of mycoplasma contamination. These findings were further confirmed by both culture-based assays and multiplex PCR-based screening. The positive controls showed successful PCR amplification and microbial growth, while the negative controls showed neither growth nor an amplification signal. Figure 2 shows multiplex PCR-based screening for Mycoplasma contamination in 10 out of the 48 established LCLs. All 48 cell lines tested negative; the remaining samples are not shown.

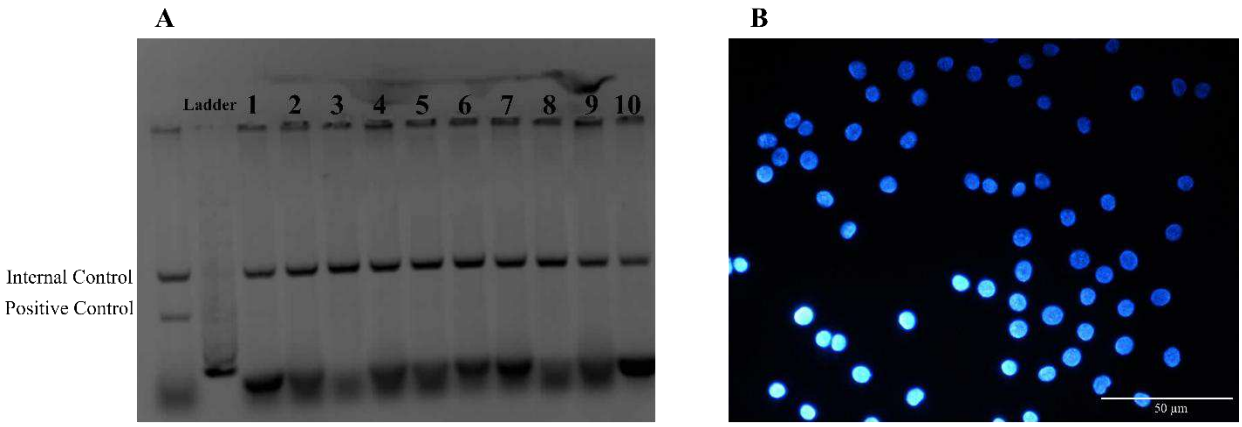


Figure 2. Assessment of Mycoplasma contamination and nuclear morphology in established LCLs. **(A)** Gel electrophoresis results showing the screening for Mycoplasma contamination. **(B)** Hoechst staining of LCLs illustrating nuclear morphology. The image was captured using an Olympus BX51 microscope equipped with a 40× objective lens, an Olympus DP72 camera, and a U-TV0.63XC adapter. Scale bar: 50 µm. Note: The scale bar was determined via theoretical calculation based on the known sensor dimensions of the DP72 camera (4140 × 3096 pixels) and the 0.63× adapter magnification, as a stage micrometer was not available during imaging.

Bacterial/fungal sterility: After a 14-day sterility test using TSB and thioglycolate media incubated at 22°C and 32°C, respectively, no bacterial or fungal contamination was found in any of the cryopreserved LCLs.

Species verification: Each of the 48 identified LCLs produced the anticipated 658-bp COI gene amplicon, confirming their human origin. None of the examined cell lines showed signs of cross-species contamination.

Viral screening: PCR and RT-PCR tests revealed that all donor blood samples were free of HIV, HBV, and HCV. On the other hand, EBV DNA was found in each sample, which is in line with the high rate of EBV transformation. The results also represented no active infection at the time of sample collection.

Discussion

In this work, 48 EBV-transformed LCLs from Iranian MS patients were successfully established and thoroughly characterized. The established cell lines' high post-thaw viability, stable proliferation, lack of microbial contamination, preserved DNA quality, and long-term stability during cryopreservation all supported their applicability for upcoming genomic and molecular

studies. Following the International Society for Biological and Environmental Repositories' (ISBER) best practice guidelines, all successfully established LCLs underwent stringent quality control [28]. The quality control panel verified that all lines were free of bacterial, fungal, and mycoplasma contamination; confirmed their human origin using mitochondrial COI PCR; and tested negative for blood-borne pathogens such as HIV, HBV, and HCV. These thorough quality control procedures are essential to guaranteeing that the dispersed cells are "fit-for-purpose" and won't introduce confounding variables into subsequent functional, genetic, or epigenetic assays. The ethical and practical difficulties of repeated blood collection are eliminated for genetic studies, such as genome-wide association studies (GWAS), whole-exome sequencing, and bisulfite pyrosequencing, when sufficient amounts of pristine DNA (≥20 µg per 10^7 cells) are obtained from a single expansion [29, 30]. Although long-term culture of LCLs has been shown to cause cumulative copy number variations (CNVs) and some epigenetic drifts [31], the field is still in agreement that the EBV genome persists extrachromosomally as an episome without integrating into the host genome, and the core genomic DNA sequence maintains high fidelity to the original donor across numerous passages. As a result, LCL-derived DNA is typically regarded as equivalent to DNA isolated directly from peripheral blood for the great majority of genotyping and sequencing applications [32]. Crucially, our long-term monitoring shows that even after about ten years of cryopreservation in liquid nitrogen vapor phase, both cell viability and DNA integrity remain consistently high, highlighting the robustness of our biobanking procedures and the dependability of this resource for long-term and retrospective research.

By giving access to well-characterized LCLs derived from an understudied Middle Eastern population, this resource may help fill a significant gap in MS genetics research. The Iranian population, which is distinguished by a clear mixture of Caucasian, Central Asian, and Middle Eastern ancestries, is still comparatively underrepresented in current large-scale genetic studies, despite the fact that the great majority of MS genomic studies have concentrated on populations of European ancestry [33]. Iran, which has a population of over 90 million, has a medium to high risk of MS due to a 40.4 percent increase in MS prevalence between 1990 and 2015 [34, 35]. Thus, specialized genetic resources are desperately needed. Iranian researchers and foreign partners can perform replication studies, validate risk variants found in Western cohorts, and possibly find new susceptibility loci specific to this population with the help of this LCL bank. In the context of current international biobanks, our project enhances existing repositories rather than replaces them.

For example, the Norwegian MS Biobank mainly keeps blood, cerebrospinal fluid, and extracted DNA from living patients [36], whereas the UK MS Tissue Bank focuses on post-mortem central nervous system specimens [37]. Although they are geographically and genetically remote from the Middle Eastern population, the few LCL-focused collections, like the Australian MS LCL bank, have proven invaluable for functional genomics [38]. Thus, the creation of the Iranian MS LCL Biobank is a crucial step in expanding access to genetic resources and guaranteeing that non-European populations can benefit from biomarker discovery [35] and personalized medicine [39]. The comparison of important characteristics between international MS biobanks is summarized in Table 3.

Table 3. Comparative overview of international MS biobanks.

Feature	Iranian Biological Resource Center (IBRC)	Swiss MS Cohort (SMSC)	Norwegian MS Registry & Biobank	Australian MS Stem Biobank
Country/Region	Iran	Switzerland	Norway	Australia
Primary Sample Type	LCLs (lymphoblastoid cell lines)	Serum, plasma, whole blood (DNA), CSF, and MRI data	DNA, serum, CSF, and CNS tissue	iPSCs (induced pluripotent stem cells)
Main Objective	A sustainable, unlimited, high-quality source of genomic DNA for genetic and molecular studies in the Iranian population.	Understanding MS mechanisms through long-term observation of clinical, MRI, and biomarker parameters.	Systematic collection of clinical data and biological samples to identify risk factors and treatment markers.	A platform to study MS pathophysiology and conduct preclinical drug screening using human cell models.
Scale / Number of Samples	48 LCLs (from 72 patients)	2,178 patients, with over 18,000 documented visits	Over 90% of all MS patients in Norway have consented to inclusion	Multiple iPSC lines from people with and without MS
Unique Feature	The only specialized MS LCL bank in the Middle East.	One of the largest MS clinical research databases in Europe and North America.	A national registry linked to a biobank with high patient participation.	Uses stem cell technology for disease modeling and drug discovery.
Accessibility	Through IBRC with a standard MTA and a cost-recovery fee (details on https://ibrc.ir).	Through the SMSC for researchers, with a transparent application process.	By application, all researchers have access to data and samples.	Available to researchers on a cost-recovery basis for non-commercial research.

The rate of successfully generated cell lines (48/72; 66.7 percent) is highly consistent to previously reported LCL biobanking initiatives, like the Australian MS Longitudinal Study Biobank, which

reported a similar success rate of 67.7 percent for MS patients [40]. Additionally, the observed success rate is within the 60–90% range that is frequently reported in the literature. Freshly processed samples usually have higher success rates (≥80%) than cryopreserved or poorly handled specimens [41]. Notably, despite the inherent difficulties of operating in a setting with limited resources, our use of fresh peripheral blood processed within 4 hours of collection probably helped us achieve an acceptable yield. Insufficient viable lymphocytes, microbial contamination (including bacterial, fungal, and mycoplasma), clotting upon delivery, and transformation failure of unknown causes, were the main causes of the 24 failed transformations. These results highlight two crucial pre-analytical elements that biobanking workflows frequently overlook. First, in order to maintain lymphocyte viability, sufficient blood volume (≥8 mL) and careful transportation at 4–8°C are crucial, especially in MS patients whose lymphocyte counts may have changed as a result of disease-modifying immunotherapies [42]. Second, it is imperative that aseptic technique be strictly followed during Ficoll-Paque separation and the subsequent culture setup. In order to optimize transformation efficiency in future expansions, we suggest using pre-collection screening for absolute lymphocyte counts and, when clinically acceptable, temporarily stopping immunosuppressive medications.

Despite its advantages, this study has a number of shortcomings that should be noted. The clinical and genetic heterogeneity of MS in Iran, which includes a variety of ethnic groups and distinct disease subtypes (relapsing-remitting, primary progressive, and secondary progressive), may not be adequately captured by the small sample size (n=48). We also acknowledge that we did not perform transcriptomic or proteomic profiling of these lines; these multi-omics analyses would significantly increase the resource's value for future systems biology approaches, even though they are outside the scope of this foundational biobanking study. The usefulness of this resource would be further enhanced by future research assessing transcriptomic stability [43], epigenetic profiles [44], and cellular responses to immune stimulation [45, 46].

Conclusion

We have effectively created the first biobank of EBV-transformed lymphoblastoid cell lines from multiple sclerosis patients in Iran. The Iranian Biological Resource Center now provides access to 48 cryopreserved, well-characterized, and quality-controlled LCLs. In order to support future genetic association studies, biomarker discovery, functional genomics, and personalized medicine

approaches in the Iranian population, this resource offers the Iranian and international research community a renewable source of high-quality genomic DNA from MS patients.

Abbreviations

ACECR; Academic Center for Education, Culture, and Research

CNS; Central nervous system

CNVs; Copy number variations

COI; Cytochrome C oxidase subunit I

CSF; Cerebrospinal fluid

DMSO; Dimethyl sulfoxide

EBV; Epstein–Barr virus

FBS; Fetal bovine serum

GWAS; Genome-wide association studies

HBV; Hepatitis B virus

HCV; Hepatitis C virus

HIV; Human Immunodeficiency Virus

HLA; Human leukocyte antigen

iPSCs; Induced pluripotent stem cells

ISBER; International Society for Biological and Environmental Repositories

LCLs; Lymphoblastoid cell lines

MS; Multiple sclerosis

PBMCs; Peripheral blood mononuclear cells

PPLO; Pleuropneumonia-like organism

RRMS; Relapsing-remitting multiple sclerosis

RT-PCR; Reverse transcription PCR

TSB; Tryptone Soya Broth

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Author Contributions

Conceptualization: Samaneh Mahmood-Aghdam, Mina Gholami, Alireza Ahmadi; **Supervision and Visualization:** Abdolreza Daneshvar Amoli, Seyed Abolhasan Shahzadeh Fazeli; **Writing – original draft:** Samaneh Mahmood-Aghdam, Alireza Ahmadi, Hedieh Rahmati; **Writing – review & editing:** Sepideh Ashouri Movassagh, Mansoureh Farhangniya, Seyed Abolhasan Shahzadeh Fazeli, Abdolreza Daneshvar Amoli; **Formal analysis and investigation:** Samaneh Mahmood-Aghdam, Mina Gholami, Alireza Ahmadi, Mehrnaz Izadpanah, Hedieh Rahmati.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

The study protocol was approved by the Ethics Committee of the Royan Institute-Academic Center for Education, Culture and Research, Tehran, Iran (Approval Code: IR.ACECR.ROYAN.REC.1397.24).

Informed Consent

Written informed consent was obtained from all individual participants included in the study.

Data Availability

The immortalized cell lines described in this study have been deposited at the Iranian Biological Resource Centre (IBRC) under specific accession numbers. All relevant data are included in this published article. Requests for samples should be directed to IBRC (https://www.ibrc.ir) and will be subject to a standard Material Transfer Agreement (MTA) and applicable handling fees.

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