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KIR genotype and haplotype repertoire in Kuwaiti healthy donors, hematopoietic cell transplant recipients and healthy family members

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The gene complex located on chromosome 19q13.4 encodes the Killer-cell Immunoglobulin-like Receptors (*KIR*s), which exhibit remarkable polymorphism in both gene content and sequences. Further, the repertoire of *KIR* genes varies within and between populations, creating a diverse pool of *KIR* genotypes. This study was carried out to characterize *KIR* genotypes and haplotypes among 379 Arab Kuwaiti individuals including 60 subjects from 20 trio families, 49 hematopoietic cell transplantation (HCT) recipients and 270 healthy Kuwaiti volunteer HCT donors. *KIR* Genotyping was performed by a combination of reverse sequence specific oligonucleotide probes (rSSO) and/or Real Time PCR. The frequencies of *KIR* genes in 270 healthy Kuwaiti volunteer donors were compared to previously reported frequencies in other populations. In addition, we compared the differences in *KIR* repertoire of patients and healthy donors to investigate the reproducibility of previously reported significant differences between patients with hematological malignancies and healthy donors. The observed frequencies in our cohort volunteer HCT donors was comparable to those reported in neighboring Arab populations. The activating genes *KIR2DS1, KIR2DS5* and *KIR3DS1* and the inhibitory gene *KIR2DL5* were significantly more frequent in patients compared to healthy donors, however, none of the previously reported differences were reproducible in our Kuwaiti cohort. This report is the first description of *KIR* gene carrier frequency and haplotype characterization in a fairly large cohort of the Kuwaiti population, which may have implications in *KIR* based HCT donor selection strategies.

KEYWORDS
genotypes, haplotypes, *KIR*, Kuwaiti population

1 | INTRODUCTION

The killer-cell immunoglobulin-like receptors (*KIR*s) are a gene complex located on the long arm of chromosome 19 (19q13.4).[1] Genetic polymorphisms are a hallmark of *KIR* immunogenetics concerning gene content, sequence polymorphism, and copy number variation.[2] This *KIR* gene variability yields a remarkable diversity of natural killer (NK) cells based on which *KIR* variants are expressed at the cell surface.[3,4] Association among *KIR*

and corresponding ligands on target cells result in activating or inhibitory signals that moderate NK cell function.[5,6] This regulation is a major immune effector mechanism against cancer and other diseases.[7,8]

HLA genotype, together with *KIR* genotype/haplotype, influence the course of hematopoietic cell transplantation (HCT) and some autoimmune disorders.[9] *KIRs* well-known binding partners are Class I HLAs. The modulation of NK cell activation or inhibition may therefore be anticipated in response to any alteration in the *KIR* binding site or the HLA binding site that changes their affinity to one another. These inhibiting and activating *KIR*-HLA interactions closely regulate the cytotoxic and cytokine-secreting activities of NK cells. The host cells become potential targets for NK cells due to the downregulation of HLA class I molecules in infected cells.[10,11] It is possible that *KIR* gene variation and *KIR*-HLA association affect resistance and susceptibility to the pathogenesis of many diseases, such as infectious diseases and autoimmune/inflammatory disorders, through modulation of NK activation, cytotoxicity, and cytokine release, given the role of *KIRs* in the immune response and their extensive genomic diversity.[12] Specific *KIR* genes are associated with differential HCT clinical outcomes, particularly relapse of malignancy.[13,14] Furthermore, based on *KIR*– ligand reactivity adoptive therapy has been used to treat relapse of malignancy.[15] Several studies have reported that the presence of donor-activating *KIR* and/or weaker inhibitory receptor–ligand combinations reduces the risk of relapse, translating into improved overall survival.[16–19] In the paper of Sugioka and co-workers, the *KIR* genes resulting significantly different between patients and controls are *KIR2DS1, KIR2DS2, KIR2DS3, KIR 2DL2* and *KIR2DL5. KIR3DS1* seems to be not significantly different.[20]

The extensive *KIR* polymorphisms rivals that of the HLA family. The diversity in *KIR* genes arises mainly from (1) allelic polymorphism (SNP and Insertion/deletions) of individual *KIR* alleles at each locus, (2) differences in gene content among different *KIR* haplotypes, and (3) copy number variation among individual *KIR* genes.[21–26] The presence or absence of each *KIR* genes differs among and within populations, generating a distinct pool of *KIR* genotypes. Different haplotypes are derived by duplication and deletion of these genes.[2] The A haplotype contains a canonical constellation of -five genes (*KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4, KIR2DP1*) including the framework genes and contains fewer or no activator genes compared to haplotype B. Haplotype A is the most prevalent haplotype in most studied populations. Group B haplotypes show significant heterogeneity in gene content and a multiplicity of activating genes.[25–27] Full-length sequencing shows that the *KIR* gene arrangement on each haplotype includes a flanking *KIR3DL3* at the centromeric end, *KIR3DL2* at the telomeric end, and *KIR2DL4* and *KIR3DP1* in the middle.[26,28,29] All subjects can be classified as having one of the following two *KIR* genotypes: AA, signifying homozygous for group A haplotypes; or Bx, that comprises either AB heterozygous or BB homozygous group B haplotypes.[30] Different centromeric (cA01, cB01, cB02, and cB03) and telomeric (tA01, tB01) motifs have been identified in *KIR* haplotypes. Each motif has a specific gene content and arrangement.[3,21,31–34] Several additional haplotypes have been reported in studies of other populations, albeit uncommon.[35–37]

KIR frequency and genotype data, particularly in less studied non-white populations, may provide a greater understanding of these genes and the mechanisms involved in NK-mediated immune responses.[38] Arab populations are situated geographically in the midst of populations of African and Asian ancestry.[39] Kuwait lies in the northern end of eastern Arabia, at the north Western tip of the Arabian Gulf. The Arabian Peninsula, which sits at the nexus of Africa, Europe, and Asia, was an early destination for human migration out of Africa[40,41] and became a crossroads of early trading routes.[42] The Kuwaiti population comprises people who settled from different regions of the Arabian Peninsula, namely Persia and Saudi Arabia, and including the Bedouins.[43] Consequently, the genetic diversity of the Kuwaiti population has arisen from admixture of "city dwelling" Saudi Arabian, "tent-dwelling" Bedouin, Persians and African ancestries increasing the genetic heterogeneity.[44,45]

Family studies are the gold standard for studying segregation of haplotypes when sufficient numbers of family members are available and informative for haplotype segregation.[26,29] Studying *KIR* genotyping in families has been also used to compare frequency of individual *KIR* genes among patients and their healthy donor family members. Sugioka et al observed in a study of a Brazilian population that specific inhibitory and activating genes had different frequencies in healthy donor family members compared to patients and suggested that susceptibility to leukemia could be influenced partly by *KIR* receptors.[20]

The aim of this study is to (1) characterize *KIR* haplotypes in Kuwaiti subjects by haplotype segregation in families and compare haplotype frequencies with published data from other populations,[36,46] (2) compare the frequency of the *KIR* genes among a cohort of HCT recipients and healthy Kuwaiti volunteer donors from the Kuwaiti unrelated donor registry (3) compare the frequency of the *KIR* genes in healthy Kuwaiti volunteer donors to published frequencies in other populations,[36,47–65] and (4) investigate the generalizability of the observed differences in *KIR* gene carrier frequency among patients with hematological malignancies and healthy donors in

the Brazilian population were generalizable to other populations such as Kuwaitis.[20] Findings of this study may guide future research investigating the importance of the *KIR* genes and their associations with particular diseases and potentially *KIR* based HCT donor selection strategies.

2 | MATERIALS AND METHODS

2.1 | Study population

A total of 379 Arab Kuwaiti individuals were enrolled. Informed consent was obtained prior to sample collection. This group included 60 participants from 20 trio families (consisting of a child and their biological parents), 49 HCT recipients (patients) as shown in Supplementary Table 1 and 270 healthy Kuwaiti volunteer donors from the Kuwait unrelated donor registry. Family members were included in the study to aid prediction of the most probable *KIR* haplotypes by segregation. Two hundred and seventy healthy Kuwait volunteer HCT donors were used to estimate *KIR* gene carrier frequency and to conduct comparisons with frequencies reported in other population. 49 HCT recipients were included to analyze the differences in *KIR* gene carrier frequencies among Kuwaiti patients and healthy HCT donors in comparison to previously reported differences in comparable frequencies in a Brazilian study.

2.2 | *KIR* genotyping methods

KIR typing was performed for all study participants using the PCR-SSO method (*KIR* SSO Genotyping Test; One Lambda, Inc., a Thermo Fisher Company, Canoga Park, CA). Data analysis was performed using HLA Fusion version 4.1 software (One Lambda Inc., a Thermo Fisher Company, Canoga Park, CA). This kit identifies 16 *KIR* genes including *KIR3DL1, KIR2DL1, KIR2DL3, KIR2DS4, KIR2DL2, KIR2DL5, KIR3DS1, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR2DL4, KIR3DL2, KIR3DL3, KIR2DP1*, and *KIR3DP1*.

In addition, *KIR* genotyping for the 60 family members was performed also using a real-time PCR based method with a commercial *KIR* genotyping kit (Linkage Biosciences, a Thermo Fisher Company, Canoga Park, CA). This kit identifies 17 *KIR* genes, including *KIR2DL5A* and *KIR2DL5B* and distinguishes full-length and deleted forms of *KIR3DP1* and *KIR2DS4*. We performed genotype analysis and reporting with SureTyperTM software (Linkage Biosciences, a Thermo Fisher Company, Canoga Park, CA).

2.3 | Genotype and haplotype assignment for all samples

We determined the observed *KIR* gene carrier frequencies by the ratio of the number of gene present within the population to the total number of individuals in the population. We divided the *KIR* genotypes into two groups, AA and B/x, for the entire study cohort. In a sample, detection of one of the *KIR* B haplotype-defining loci (*KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5*, or *KIR3DS1*) indicated that the genotype consists of at least one B haplotype. These samples were designated B/x. *KIR* genotyping results for each individual were assigned a genotype number as defined in the Allele Frequency Net Database (AFND), publicly available at http://www.allelefrequencies.net/.[66] In addition, for participants whose family data were available, we predicted the probable haplotype combinations.

2.4 | *KIR* haplotype assignment for families

KIR haplotypes were assigned by segregation analysis in the 20 families including the child and both biological parents. The most probable *KIR* haplotypes were predicted according to the following assumptions: (1) all haplotypes consisting of *KIR3DL3*, *KIR3DP1, KIR2DL4*, and *KIR3DL2*; (2) haplotypes consisting of either *KIR2DL2* or *KIR2DL3*, but not both; (3) haplotypes contained either *KIR3DL1 or KIR3DS1*, but not both; (4) haplotypes consisting of either *KIR3DP1 or KIR3DP1* variant (*3DP1*v), but not both; (5) haplotypes containing full-length *KIR3DP1* would be missing *KIR2DL1/KIR2DP1*; (6) *KIR2DS4*-containing haplotypes consisting of either *KIR2DS4F* or *KIR2DS4D*, but not both; and (7) group B haplotypes were characterized by the presence of one or more of *KIR2DL5*, *KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5*, and *KIR3DS1* and group A haplotypes by the absence of all of these genes. A set of published reference haplotypes was used to confirm that a pair of these haplotypes could explain each individual's genotype.[27,67,68]

2.5 | Statistical analysis

The *KIR* gene carrier frequency and haplotype frequency was characterized by direct counting of the respective number of specific *KIR* gene among the individuals in the studied population. Statistical analyses of the differences among different populations were determined by Fisher's exact test using EpiCalc 2000 v1.02 software (EpiCalc Software; Brixton Books, London). A two-tailed *p*-value of <0.05 was considered statistically significant. Visualization

of the comparison of *KIR* gene carrier frequencies among different populations was performed by principal component analysis (PCA) using Minitab Statistical Software (Minitab, LLC, State College, PA). The locus *KIR2DP1* was excluded from PCA due to missing values. To facilitate PCA, two missing values for *KIR2DL5* (UK Caucasian and Australian) and one missing value for *KIR2DS5* (Australian) were imputed using the normal/numeric model within the package mice (Multivariate Imputation by Chained Equations) V3.13.7 in R V4.0.4.[69]

3 | RESULTS

3.1 | Observed *KIR* gene carrier frequencies

A total of 16 *KIR* genes were identified in 270 healthy Kuwaiti volunteer donors. Of the non-framework *KIR* genes, the most frequent were the *KIR2DL1* inhibitory gene (97.4%) and the pseudogene *KIR2DP1* (97.4%). The activating genes *KIR2DS5, KIR2DS1, KIR3DS1, KIR2DS3*, and *KIR2DS2* were the least common in the Kuwaiti population (31.5%, 35.6%, 34.1%, 39.3%, and 57.4%, respectively), whereas the inhibitory *KIR* genes occurred at variable but high frequencies. Observed *KIR* gene carrier frequencies in the 270 healthy Kuwaiti volunteer donors are shown in Table 1.

In this study, we compared the observed *KIR* gene carrier frequencies with those in published studies of different populations (Table 2). The framework genes *KIR2DL4, KIR3DL2, KIR3DL3*, and *KIR3DP1* were present at a frequency of 100% in tested entities and are excluded in Table 2. Figure 1 depicts the PCA clustering of different populations based on the *KIR* gene carrier frequencies and percentages of carrier frequencies of different *KIR* genes among different populations based on the first 2 principal components (PC1 and PC2, respectively). The first two principal components explain 64.4% of the variation in the data. The variables that positively correlate the most with the first principal component (PC1) are *KIR2DS2* (0.451), *KIR2DL2* (0.422), and *KIR2DL5* (0.398). The variable that negatively correlate the most with the first principal component (PC1) are *KIR2DL3* (−0.376). The variable that positively correlate the most with the second principal component (PC2) are *KIR2DS4* (0.445), *KIR2DL2* (0.422), and *KIR2DL5* (0.398). The variables that negatively correlate the most with the first principal component (PC2) are *KIR3DS1* (−0.472) and *KIR2DS1* (−0.452). Statistical significance (p-value) testing of the *KIR* gene carrier frequencies reported across these populations compared to those observed in our study are presented in Supplementary Table 2. All three principal components combined contributed to 80.2% of the

TABLE 1 Frequencies of *KIR* genes in Kuwaiti population ($N = 270$).

KIR genes	*2DS2*	*2DL2*	*2DL3*	*2DP1*	*2DL1*	*3DL1*	*2DS4*	*3DS1*	*2DS1*	*2DL5*	*2DS3*	*2DS5*	*3DL3*	*3DP1*	*2DL4*	*3DL2*
n	155	155	240	263	263	260	260	92	96	159	106	85	270	270	270	270
Carrier Frequency (%)	57.4	57.4	88.9	97.4	97.4	96.3	96.3	34.1	35.6	58.9	39.3	31.5	100	100	100	100

Abbreviations: *N*, Number of individuals included in the study; *n*, number of individuals positive for each gene.

TABLE 2 Observed *KIR* gene carrier frequencies (%) in Kuwaiti population and other previously published populations.

	Inhibitory KIR					Activating KIR						Pseudo gene
Populations	**2DL1**	**2DL2**	**2DL3**	**2DL5**	**3DL1**	**2DS1**	**2DS2**	**2DS3**	**2DS4**	**2DS5**	**3DS1**	**2DP1**
Kuwait (Present Study) $N = 270$	97.4	57.4	88.9	58.9	96.3	35.6	57.4	39.3	96.3	31.5	35.6	97.4
Saudi $n = 114$ (Omar et al., 2016)	88.6*	58.7	82.4	58.7	92.9	30.7	56.1	44.7	90.3*	28.9	21.0*	99.1
Iranian $n = 200$ (Hiby et al., 2010)	96.5	56.5	86.5	61.5	91.5*	45.5*	57.5	38	91.5*	40	44.5	96.5
Omani $n = 99$ (Williams et al., 2004)	98	50.5	87.9	59.6	96	32.3	49.5	30.3	94.9	39.4	29.3	98
Lebanese $n = 120$ (Rayes et al., 2008)	99.2	59.2	88.3	58.3	95.8	40.8	59.2	37.5	95	30.8	35.8	NT
Palestinian $n = 105$ (Norman et al., 2001)	83*	62	85	63	88*	44	64	37	88*	27	39	NT
Southern Turkey $n = 200$ (Ozturk et al., 2012)	97	53	80*	58	91*	38	53	33	92	39	42	96
Tunisian $n = 267$ (Meriem et al., 2015)	100*	58	94	54	99	27*	50	41	97	25	31	100*
Morocco $n = 67$ (Hollenbach et al., 2010)	95.5	70.1	73.1*	67.2	100	25.4	65.7	52.2	100	32.8	25	100
West African $n = 62$ (Denis et al., 2005)	79*	52	85	52	98	23	45	19*	97	24	13*	NT
Senegalese $n = 118$ (Denis et al., 2005)	100	55	100*	52	99	13*	42*	24*	100	30	4*	NT
Italian $n = 217$ (Bontadini et al., 2006)	95	53	88	33*	96	36	53	33	89*	28	35	NT
US Caucasian $n = 195$ (Du et al., 2007)	96.9	49.2	88.7	52.8	94.9	37.4	49.7	28.2*	94.9	35.9	39.5	97.9
UK Caucasian $n = 136$ (Norman et al., 2001)	91*	49	92	NT	97	45	51	24*	96	32	42	NT
Australian $n = 147$ (Witt et al., 1999)	86*	52	88	NT	94	50*	51	28*	92	NT	37	NT
Mexico Mestizo $n = 86$ (Gutierrez-Rodriguez et al., 2006)	100	43*	100*	49	99	42	44*	17*	97.6	40	42	97
S. Asian $n = 108$ (Norman et al., 2002)	82*	64	83	74*	88*	55*	69*	27*	81*	37	44	NT
Mongolian $n = 90$ (Jiang et al., 2013)	93.3	30*	93.3	46.7	95.6	47.8	33.3*	13.3*	93.3	34.4	36.7	96.7
Chinese Han $n = 104$ (Jiang et al., 2005)	99	17*	99*	34.6*	99.2	34.3	17.3*	12.5*	94.2	23.1	32.7	99
Tujia Ethnic $n = 124$ (Wang et al., 2016)	64*	12*	93	35*	98	27	9*	15*	61*	30	36	98
Korean $n = 154$ (Whang et al., 2005)	99.4	14.3*	99.4*	38.3*	94.2	37.7	16.9*	16.2*	94.2	26.6	36.4	100
Japanese $n = 41$ (Yawata et al., 2002)	100	15*	100*	39*	97	35	15*	15*	97	25	30	100
Brazilian $n = 136$ (Sugioka et al., 2016)	96	55	87	53	95	43	57	33	95	33	39	96

Note: NT: not tested. Observed carrier frequencies for Kuwaiti population and 22 previously published populations.
*Statistically significant *p*-values (at <0.05 level).

variance with the first, second and third principal components contributed 40.7%, 23.8%, and 15.7% of the variance, respectively.

The frequencies of *KIR2DL1, KIR2DP1, KIR2DS4* and *KIR3DL1* were above 95% in our population. Considering published frequencies in other populations, *KIR* gene carrier frequencies in this Kuwaiti population were most similar to that among Caucasian populations, particularly neighboring Arab populations like Lebanese, Omani, Saudi and Tunisians.

3.2 | *KIR* genotypes

The *KIR* genotypes of the 270 healthy donors were all reported previously in the Allele Frequency Net database (AFND), and the corresponding AFND genotype number is shown in Figure 2. In addition, Figure 2 shows the numbers of individuals with each genotype and the percentage distribution. Depending on the presence of 16 *KIR* genes, we identified 27 different *KIR* genotypes in Kuwaitis, which suggests considerable *KIR* diversity in this population. Of the 270 healthy donors, 85 had an AA genotype (31.5%), and 185 were B/x genotype (68.5%). This result clearly indicates the dominance of the Bx genotype over the AA genotype in this Kuwaiti population. Of interest, among participants with the AA genotype, all 85 individuals also had the AFND genotype ID 1 (31.5%), comprising nine *KIR* genes (*KIR2DL1, KIR2DL3, KIR3DL1, KIR2DS4, KIR2DP1*, and the four framework *KIR* genes). A total of 41 individuals and 24 individuals had the most frequent genotypes in the B/x group (15.2%, AFND genotype ID 5, and 8.9%, AFND genotype ID 2, respectively). In this study, Genotype 6 that consists of all the inhibitory and activating *KIR* genes was observed at a frequency of 6.7%.

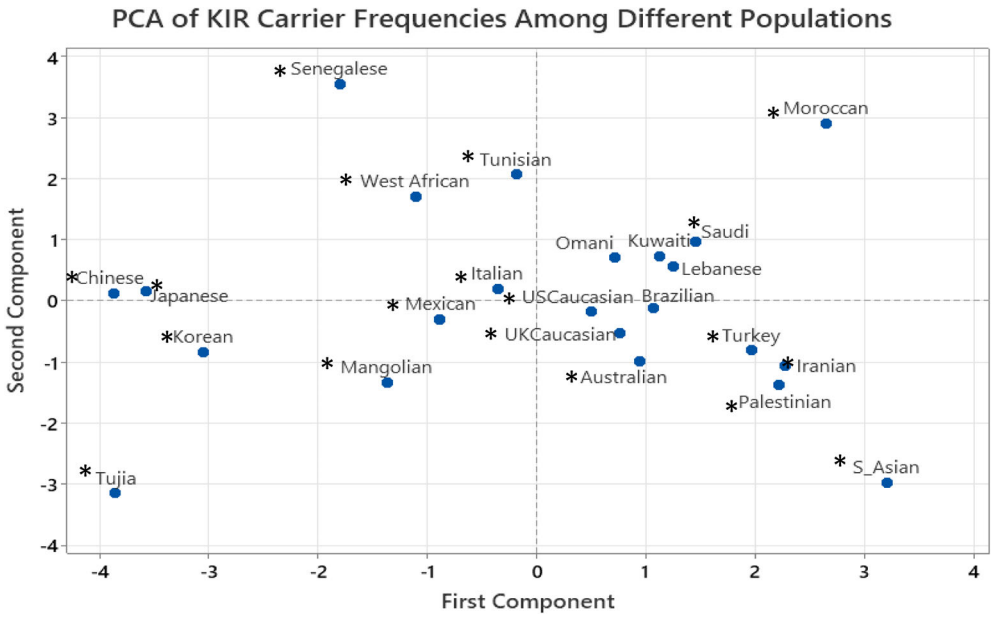

FIGURE 1 PCA representation of the clustering of different populations (listed in Table 2) based on the *KIR* gene carrier frequencies. *Statistically significant *p*-values (at <0.05 level).

		Centromeric genes					Telomeric genes				Cen or Tel genes			Framework genes					
Genotype	Genotype ID	2DS2	2DL2	2DL3	2DP1	2DL1	3DL1	2DS4	3DS1	2DS1	2DL5	2DS3	2DS5	3DL3	3DP1	2DL4	3DL2	Number of Individuals N=270	% of individuals
AA	1																	85	31.5
BX	5																	41	15.2
	2																	24	8.9
	4																	22	8.1
	6																	18	6.7
	3																	16	5.9
	71																	15	5.6
	9																	6	2.2
	70																	6	2.2
	7																	5	1.9
	8																	4	1.5
	13																	4	1.5
	73																	4	1.5
	72																	3	1.1
	76																	3	1.1
	68																	2	0.7
	90																	2	0.7
	11																	1	0.4
	21																	1	0.4
	25																	1	0.4
	27																	1	0.4
	69																	1	0.4
	81																	1	0.4
	91																	1	0.4
	97																	1	0.4
	188																	1	0.4
	382																	1	0.4

FIGURE 2 *KIR* locus profiles in the Kuwaiti population ($N = 270$). *N*, Number of individuals included in the study. Filled boxes indicate presence of a *KIR* gene and open boxes indicate absence of the gene. Genotype ID were referred to genotype classification according to http://www.allelefrequencies.net/

3.3 | *KIR* haplotypes

We performed haplotype segregation studies in 20 trio Kuwaiti families (60 individuals). Each family consisted of a trio including child and both parents. Based on the data, we predicted seven unique pairings of centromeric and telomeric *KIR* gene motifs. We investigated the deletion versus full subtypes of *KIR2DS4* and *KIR3DP1* in the 20 families studied (Supplementary Table 3: Haplotype segregation among 20 trio Kuwaiti families). In addition, family haplotype segregation suggested that one family member donor (father) might have an insertion of 5 genes block (*KIR3DP1-KIR2DL4-KIR3DS1-KIR2DL5A-KIR2DS3*) into the tA01 motif (depicted in Figure 3 as cB02|tA01-ins5). In assigning haplotypes with insertion, we relied on the linkages between the centromere motifs and *KIR3DP1* group in fully sequenced *KIR* haplotypes published elsewhere.[70,71] These linkages include, *KIR3DP1* (F; full ex1-ex5) being linked with *KIR2DS2- KIR2DL2* motif (cB02; *3DL3-2DS2-2DL2-3DP1*(F)), *KIR3DP1* (D; exon2 deletion) being linked with *KIR2DP1- KIR2DL1* motif (cA01 and cB01) and cB03 (*3DL3-2DL3-2DL5-2DS3/5-2DP1-2DL1*) is linked with *KIR3DP1* (F; full ex1-ex5). Since this subject has a *KIR3DP1* (F) and cB02, the haplotype was assigned as cB02|tA01-insertion (*3DP1-2DL4-2DL5A-2DS3*) without *KIR2DP1- KIR2DL1*. By the exception of the above patterns, cB02|tA01-insertion (*3DP1-2DL4-2DL5A-2DS3-2DP1-2DL1*) can be also possible. However, this observation needs to be interpreted with caution since neither of the 2 *KIR* genotyping methods we used interrogated gene copy number. Figure 3 depicts the predicted *KIR* haplotypes that we observed. For calculating frequencies of different haplotypes in the families, we excluded the children and calculated percentages based on the 80 haplotypes identified in the 40 parents to avoid potential over representation of some haplotypes. Most participants (61.3%) had the haplotype A–specific *KIR* gene motifs cA01 and tA01. In group B, the most frequent B–specific haplotype frequencies were combinations of cB01 and tA01 (17.5%) and of cA01 and tB01 (11.3%). A number of studies have considered the haplotype cB01|tA01 to be split into 2 subhaplotypes based on carrying *KIR2DS3* versus *KIR2DS5*.[37,72,73] 13 of the 14 cB01 | tA01 haplotypes in our study carried *KIR2DS3* and only one haplotype carried *KIR2DS5*. The combinations of cB01–tB01 and cB03–tA01 were least frequent (2.5% and 1.3%, respectively). Table 3 shows results of frequency comparisons among the predicted *KIR* haplotypes in the different populations. We observed that haplotype cB01|tA01 was significantly more common in the Kuwaiti population compared to Chinese, European American, Asian, and CAU populations ($p < 0.05$).

3.4 | Comparisons of *KIR* gene and genotype frequencies among different subsets in the Kuwaiti and Brazilian cohorts

The characteristics of the 49 HCT recipients (51% men; $n = 25$) by diagnosis and sex are presented in Supplementary Table 1 (General characteristics of HCT recipients) and *KIR* genotyping data is provided in Supplementary Table 4 (*KIR* genotyping data of 49 HCT patients). A comparison of *KIR* gene carrier frequencies among Kuwaiti healthy donors ($n = 270$) and their Brazilian counterpart ($n = 136$) is shown in Supplementary Table 5. There were no significant differences in the carrier frequency of any *KIR* gene between the two donor groups. Likewise, we carried out a similar comparison among

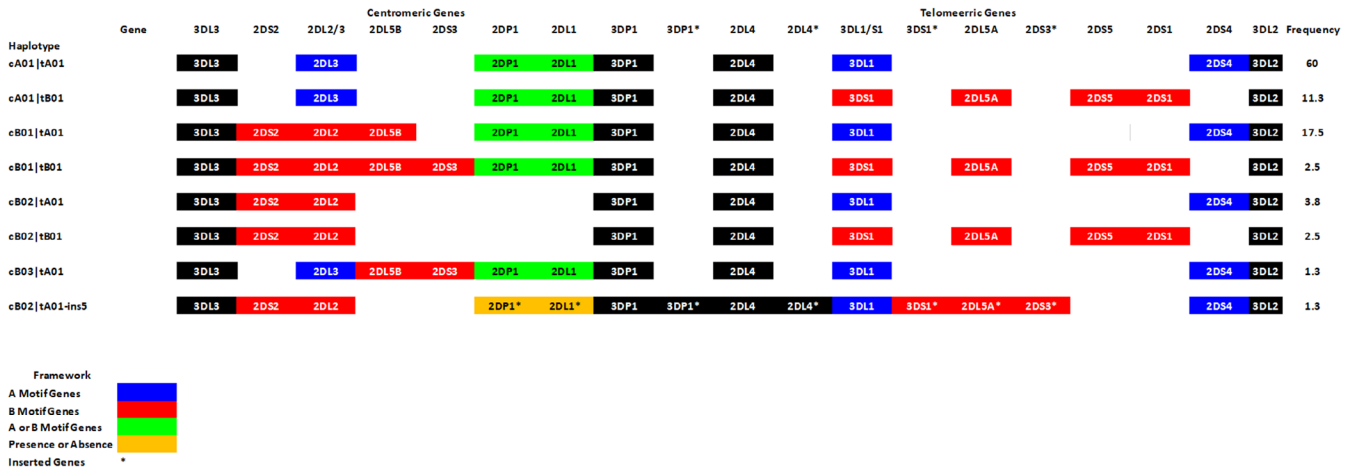

FIGURE 3 Schematic representation of the probable *KIR* haplotypes observed in 20 Kuwaiti families consisting of centromeric and telomeric motifs.

TABLE 3 *KIR* haplotype frequencies in different population.

Frequency (%)							
Full Haplotype	**Present study $N = 40$**	**Chinese population $N = 204$ (Bao et al., 2013)**	**European American $N = 506$ (Vierra Green et al., 2012)**	**ASI $N = 96$ (Bao et al., 2013)**	**CAU $N = 96$ (Bao et al., 2013)**	**AFA $N = 96$ (Bao et al., 2013)**	**HIS $N = 96$ (Bao et al., 2013)**
cA01|tA01	61.3	72.4	54.1	63.5	62.5	54.2	61.5
cB01|tA01	**17.5**	1.48	7.2	2.08	4.2	14.6	8.3
*p-value		**<0.000004**	**0.01284**	**0.00164**	**0.0122**	0.7936	0.1418
cA01|tB01	11.3	16.8	9.5	15.6	9.4	5.2	15.6
cB02|tA01	3.8	4.9	14.3	5.2	15.6	6.3	8.3
cB02|tB01	2.5	1.5	2.8	6.3	1.04	2.1	4.2

Note: Full haplotypes observed in 40 Kuwaiti subjects (determined by haplotype segregation in 20 families) were compared with haplotypes observed in Chinese, European, Asian (ASI), Caucasian (CAU), African American (AFA) and Hispanic (HIS). The haplotype cB01|tA01 was significantly more common in the Kuwaiti population. In calculating frequencies in Kuwaiti population, cB01|tA01 include cB01|tA01-2DS3 and cB01|tA01-2DS5.
*Statistically significant p-values (at <0.05 level) are indicated in bold.

Kuwaiti patients ($n = 49$) in our cohort and a Brazilian patients ($n = 39$) as shown in Supplementary Table 6.[20] We found that the frequencies of activating genes *KIR2DS3* (49%), *KIR3DS1* (57%), *KIR2DS1* (59%), and *KIR2DS2* (57%) as well as of the inhibitory genes *KIR2DL2* (59%) and *KIR2DL5* (80%) were significantly higher in our patient cohort compared to the Brazilian patient population *KIR2DS3* (13%), *KIR3DS1* (26%), *KIR2DS1* (15%), *KIR2DS2* (31%), *KIR2DL2* (23%), *KIR2DL5* (28%), with p values of 0.0008, 0.006, 0.00008, 0.0243, 0.0015, and 0.000004, respectively. Frequencies of the remaining inhibiting and activating *KIR* genes did not vary between patients from both the populations. We compared the frequencies of individual *KIR* gene between Kuwaiti patients ($n = 49$) and healthy donors ($n = 270$) (Supplementary Table 7). The activating genes *KIR2DS1* ($p = 0.003$), *KIR2DS5* ($p = 0.03$), and *KIR3DS1* ($p = 0.004$) and the inhibitory gene *KIR2DL5* ($p = 0.010$) were significantly more frequent in patients when compared to healthy donors.

4 | DISCUSSION

To our knowledge, this study represents the first investigation of *KIR* genotypes and haplotypes in a Kuwaiti population, with a sizable cohort of 379 individuals including members of 20 families.

We compared frequencies of *KIR* genes in 270 healthy Kuwaiti volunteer donors to earlier reported frequencies in neighboring Arab populations, Caucasians, African and other populations. Such comparisons may be used to determine the genetic relationships among populations from different geographic areas. In the study population, the frequencies of the *KIR* genes varied from 31.5% (*KIR2DS5*) to 97.4% (*KIR2DP1* and *KIR2DL1*) and were similar to those identified in Lebanese, Tunisian, and Iranian populations.[51,55,60] The clustering of different populations based on the *KIR* gene carrier frequencies as indicated by PCA suggests that the carrier frequencies in the Kuwaiti population is most similar to published frequencies observed in Lebanese, Omani and Saudi followed by US Caucasian populations.[49,58,60,63] This similarity might be at least in part due to historic immigration and intermarriages among individuals of these neighboring populations. Saudi Arabian, Iranian, and other Arabian Peninsular immigrants predominate the population of Kuwait. The settlements and subsequent admixtures have shaped the genetics of Kuwait.[44] Even though Kuwait resembles their geographical neighbors, a significant difference in *KIR2DL1* and *KIR3DS1* is observed in apparently close populations like Saudi due to the differences in the genetic background. These significant differences between some *KIR*-gene frequencies of the Saudi and Kuwaiti populations could also be explained by the unexpected low frequencies of *KIR2DL1, KIR2DS4* and *KIR3DS1* which are discordant from those observed in the same study for genes tightly linked to the former (*KIR2DP1, KIR3DL1* and *KIR2DS5*, respectively). Therefore, they might be explained by some issue in their genotyping technique or, less likely, by presence of many unusual haplotypes.

The framework genes (*KIR2DL4, KIR3DL2, KIR3DL3*, and *KIR3DP1*) were found in all tested subjects. However, the inhibitory and activating *KIR* genes showed carrier frequencies that differed among individuals of different subsets of our cohort. Compared to Kuwaiti healthy controls, Kuwaiti patients showed higher carrier frequency of

haplotype B *KIR* genes *KIR2DS1*, *KIR2DS5*, *KIR3DS1*, *KIR2DL5*. These findings are consistent with the postulated contribution of genetic variation in hematological diseases. *KIR* genes regulate susceptibility to viral infections, autoimmune disorders, and hematological malignancies. Traditional statistical techniques are used in molecular epidemiology investigations to find links between *KIR* genes and disease. Compared to healthy donors, patients with hematological malignancies had a higher prevalence of *KIR2DL2*.[74] The potential immunologic mechanisms underlying some *KIR* genes' higher prevalence in hematologic patients compared to healthy individuals. In contrast to people without known pathology, patients with AA and ALL were significantly more susceptible to the *KIR* genes (*KIR2DL5, KIR2DS1, KIR2DS3*, and *KIR3DS1* for AA), and AML and MDS did not appear to be similarly affected. AML and MDS were not significantly correlated with other *KIR* genes, such as *KIR2DL1-KIR2DL3*, *KIR3DL1-KIR3DL3*, *KIR2DS2*, *KIR2DS4*, and two pseudogenes (*KIR2DP1* and *KIR3DP1*).[75] Another study found that as compared to healthy people, MDS patients had lower frequencies of *KIR2DL3* and greater frequencies of *KIR2DS5*, respectively.[76] According to research findings, there was no correlation between acute AML and CML, however there was an increase in the frequency of activation of the *KIR* genes *KIR2DS1*, *KIR3DS1*, and *KIR2DS3* in standard risk patients compared to high risk patients.[10,77,78] However, our results are not consistent with those of a study comparing individual *KIR* genes among patients with hematological disorders and their healthy donor family members in a Brazilian population.[20] In the Brazilian study, inhibitory genes *KIR2DL2* and *KIR2DL5* and activating genes *KIR2DS1, KIR2DS2* and *KIR2DS3* were more frequent in healthy donor family members than in patients. Consequently, the authors suggested that susceptibility to leukemia could be influenced partly by *KIR* receptors. We investigated whether these observations are generalizable to other populations and could be reproduced in the Kuwaiti population. Interestingly enough, in spite of geographic distance and ancestral diversity between the Kuwaiti and Brazilian populations, there were no significant differences in the carrier frequency of any *KIR* gene among healthy donors of both populations. However, when we compared Kuwaiti patients with hematological diseases and their Brazilian counterparts, haplotype B genes *KIR2DS1, KIR2DS2, KIR2DS3, KIR3DS1, KIR2DL2* and *KIR2DL5* were significantly higher in the Kuwaiti patients.[20] The remaining *KIR* genes were not significantly different. Nevertheless, none of the observed differences in the Brazilian study in *KIR* gene carrier frequencies among patients and healthy donors could be reproduced in the Kuwaiti population. To the contrary, in our studies, *KIR2DS1* and *KIR2DL5* were more frequent in Kuwaiti patients and *KIR2DL2, KIR2DS2* and *KIR2DS3* were not significantly different. This lack of reproducibility in our study that includes more subjects with fairly comparable *KIR* gene carrier frequencies among healthy Kuwaiti and Brazilian subjects does not support the generalizability of the findings in the Brazilian study.

The *KIR* haplotype frequencies in the family study were analogous to the frequencies observed in an African population except for haplotype cA01|tB01, which was higher in our study.

We also observed that only the *KIR* AA genotype AFND ID 1 was more frequent in healthy Kuwaiti volunteer donors, which is a typical feature of Northeast Asian and European populations.[66] Genotype 5, the most common genotype of the B/x group observed, occurs more frequently in African populations. The high occurrence of genotype 5 in our population is likely associated with African ancestry. The Bx genotype is more frequent in the Kuwaiti population compared to the AA genotype. A total of 18 participants had all 16 *KIR* genes present.

Considering the growing body of literature supporting the association between specific *KIR* genes/*KIR*–ligand combinations and HCT outcomes, a better understanding of the immunobiology underlying this association could lead to development of more evidence-based and *KIR*-based donor selection and adoptive immune therapy strategies.[79–84] Our results show that *KIR* gene carrier frequencies observed in the Kuwaiti population are comparable to those reported in other neighboring Arab populations. On the other hand, *KIR* haplotype frequencies in Kuwaiti populations were most comparable to those reported in African populations.[46] These differences may reflect the mixed origin of our population. Interestingly, the haplotype cB01|tA01 was observed in the Kuwaiti population at a higher frequency than most populations in particular at a significantly higher frequency than those reported in Chinese, other Asian, European American, and other Caucasian populations. Studies have demonstrated that the activating *KIR* gene content haplotype cB01/tA01 is significantly enhanced in autism and the homologous HLA ligand (HLA-C1k) activates an activating gene (*KIR2DS2*) and inhibits two inhibitory genes (*KIR2DL2* & *KIR2DL3*) in this haplotype, enhancing natural killer (NK) cell killing.[85–87]

As shown from the determination of the significant differences for our study population versus others, the Senegalese, Australian, Mexico Mestizo, S. Asian, Chinese Han, Tujia Ethnic, Korean, and Japanese significantly segregated themselves across four to seven out of the 12 examined *KIR* genes frequencies. Furthermore, the Saudi, Iranian, Palestinian, Southern Turkey, Tunisian, Morocco, West African, Italian, US Caucasian, UK Caucasian, and Mongolian displayed significant differences in one to three

KIR genes. The Arab-related populations (Omani and Lebanese) and Brazilian showed no significant differences among the *KIR* genes. It is not surprising that the *KIR* gene frequencies observed in our study population are closer to those found in Arab populations from neighboring geographical regions with similar cultures and the same mother tongue.

The current findings shed some light on the *KIR* gene and haplotype frequencies in the Kuwaiti Arab population and how these patterns compare to other populations. This comparison could be particularly clinically relevant considering that in Kuwait, families tend to be relatively large, and most Kuwaiti patients have considerable healthy haploidentical relatives who could be potential HCT donors. Hence, *KIR*-based donor selection to enhance transplant outcomes in the Kuwaiti population is an area of significant interest. Our results are limited by a relatively small cohort size and that *KIR* genotyping was performed by gene presence/absence without copy number or allele level sequencing at each *KIR* locus, which might have overrepresented predicted haplotypes. However, this limitation are inherent in most methods of *KIR* haplotype assignment including family based and copy number based except when sequencing fully phased complete haplotype. In our study, all assigned haplotypes were predicted based on segregation from the family data. In addition, there are differences in the disease mix between the Brazilian and Kuwaiti studies even though most patients in both studies were AML and ALL. These differences may have contributed to differences in observed *KIR* gene frequencies in patients of the 2 studies. Finally, insertions in *KIR* haplotype motifs are based on best-fit prediction of observed segregation in families and needs to be interpreted with caution since neither of the 2 *KIR* genotyping methods we used interrogated gene copy number.

In conclusion, our data characterized *KIR* gene carrier frequencies and *KIR* haplotypes and indicated that Kuwaiti Arab *KIR* genotypes are diverse and distinct compared to other ethnic populations. The study of the distribution of *KIR* genes could potentially benefit in guiding selection of unrelated HCT donors in the Kuwaiti population.

AUTHOR CONTRIBUTIONS

Reem Ameen conceived and designed the analysis, Reem Ameen and Salem Al Shemmari collected the data and wrote the paper. Roshni Titus and Jeethu Anu Geo provided technical support and reviewing the data in the family study analysis and haplotype segregation. Daniel E. Geraghty and Chul-Woo Pyo contributed analysis tools. Medhat Askar conceptualized the study, reviewed the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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