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Presence of "ACKR1/DARC null" polymorphism in Arabs from Jisr az-Zarqa with benign ethnic neutropenia

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INTRODUCTION

Neutropenia is defined as a reduction in circulating polymorphonuclear cells, with an absolute neutrophil count (ANC) < 1.5×10^9 [1]. Benign ethnic neutropenia (BEN) is the most common form of neutropenia worldwide and yet its definition remains obscure ranging from an ANC <1.5×10^9–<2.5×10^9 [2,3]. This relatively low ANC does not predispose to infections and has been observed in various ethnic groups, including Africans, Afro-Caribbeans, Israeli Bedouins, Ethiopian and Yemenite Jews[2,4], Jordanians[5], and natives of the United Arab Emirates[6]. The inheritance pattern of BEN remains complex, in people of Sudanese or Arabic origin an autosomal dominant or codominant manner has been suggested[6,7]. Alternatively, in people of African descent an autosomal recessive inheritance pattern with a strong association to the null Duffy genotype has been implicated[8]. The Duffy blood group chemokine receptor gene (*DARC*, also known as *ACKR1*) is located on chromosome 1q22.23[9]. Analysis of the *ACKR1* gene promoter has revealed a polymorphic change (T → C) known as rs2814778 in one of the putative GATA binding sites upstream of the gene transcription initiation site that leads to a nonfunctional allele[10]. Therefore, while the Duffy antigen is expressed in the wild-type (T/T) and heterozygous phenotype (T/C) it is completely lacking in the homozygous state (C/C) designated ACKR1-null allele[11]. The ACKR1-null allele has been shown to play a role in inflammation, infection (reduced susceptibility to malaria infections[12]), and even a complex role in cancer (including triggering cell senescence, suppressing metastasis[13], and increasing likelihood of neutropenic fever following chemotherapy[14]).

Jisr az-Zarqa (Arabic for "bridge over the blue") is an Israeli Arab town on Israel's northern Mediterranean coastal plain. Jisr az-Zarqa has a history of 500 years and holds a population with absolute Muslim-Arab inhabitants[15]. During the Otteman era Jisr az-Zarqa was colonized by founding families, such as the Jurban family coming from the Jordan Valley, the Amash family from Qadum in Samaria, the Najar family from Al Arish or Egypt, the Shihab family from the Hauran, and the Twatcha and Um Bashi familes from Sudan (Bar-Cohen, A., 2001, The relationship between environmental conditions and traditional rural settlement and agrarian conditions in menashe plateau before 1948, unpublished M.A. thesis, Bar-Ilan University). Since this town is relatively isolated from other Arab communities and continuously practices endogamy, recessive and dominant alleles are likely to be enriched. The prevalence of BEN among individuals from Arab descent has been previously described, but the genetic etiology has yet to be fully addressed. In this study, we determined the presence of BEN and the ACKR1-null allele within a relatively socially closed Arab-Muslim community in Israel.

MATERIALS AND METHODS

The index cases included two children aged between 2 and 18 years from separate families living in Jis az-Zarqa (herein referend to as child A and child B). These patients were originally referred to the Hemato-Oncology Center at Sanz Medical Center—Laniado Hospital for further testing, following low neutrophil counts <1.5 K/μL seen in a routine complete blood count. Blood samples were collected from both index cases and 32 first-degree family members (parents, offspring, and siblings) aged 2–60 years ($n = 34$). Neutrophils were counted (normal range neutrophil count 2.0–8.0 K/μL) and genomic DNA was prepared. Our genetic analysis consisted of three generations with a history of consanguineous marriage. The pedigree chart for child A is described in Fig. 1. The polymorphic site in the ACKR1 gene, rs2814778, was genotyped on both alleles using the "allelic discrimination" method. For this, the SNP probes and primers were designed using the "Assays by Design" software version 2.0 [Applied Biosystems (ABI), Foster City, CA] and the available GenBank sequence (AF055992) for the promoter region of ACKR1, which contains the polymorphic site. The sequences of the two probes corresponding to the ACKR1-null (FY−) and ACKR1 (FY+) alleles were: 5′-FAMCCAAGGTAAGAGCC-3′ and 5′-VIC-CTTCCAAG ATAAGAGCC-3′, respectively. The sequences of the flanking primers were forward: 5′-CTGATGGCCCTCATTAGTCCTT-3′ and reverse: 5′-GCTGGGACGGCTGTCA-3′. The assays were performed using the ABI-7900 sequence detection system. For quality control and validation, 25 samples were Sanger sequenced (ABI-3500 Genetic Analyser, ABI) to verify the SNP genotyping. Genomic DNA was prepared for 30 out of 34 family members. The study's data was analyzed using the independent *t* test. Categorical data are reported as frequency (*n*) and percent (%), and numerical data as mean and standard deviation (SD) or median [interquartile range, IQR]. Binary logistic regression was used to examine correlation between neutrophil count to rs2814778 polymorphism. Statistical significance was set as a two-tailed *p* value of <0.05. Statistical data analysis was performed with IBM SPSS Statistics (IBM SPSS Statistics v25.0., Armonk, NY: IBM Corp.). All procedures were performed in accordance with the requirements of good clinical practice and the Israeli Ministry of Health regulations for the conduct of clinical studies, Helisknki 0055-20LD.

RESULTS

The mean age of the studied cohort was 26.1 years (SD = 17.4) with equal representation of sexes (17 males and 17 females). Genetic material for analysis was available for 30 out of 34 individuals. Twenty-six individuals were found to carry the ACKR1-null genotype (homozygosity C/C) and four individuals were identified as heterozygotes (T/C). The homozygote T/T was not

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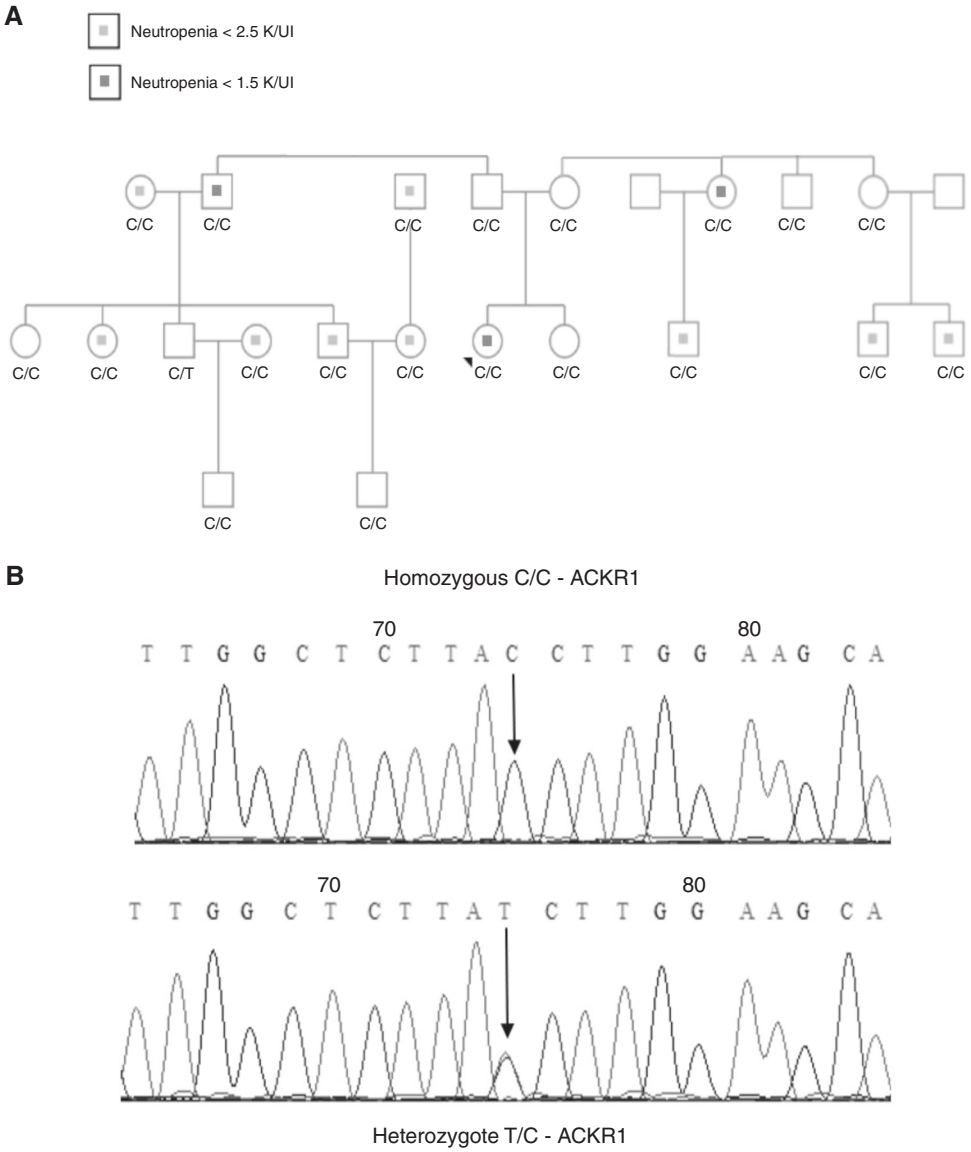

Fig. 1 Pedigree and genetic variants. A Family pedigree of index case child A. Internal square symbols indicate affected individuals. Turquoise squares indicate neutropenia under 2.5 K/uL and purple squares indicate neutropenia under 1.5 K/uL. Variants in ACKR1 are presented below the pedigrees. Homozygous variants (ACKR1 null genotype) are presented as C/C in the pedigree. Homozygous variants are presented as C/T in the pedigree. **B** Sanger sequencing validation of the two detected ACKR1 genotypes in the studied family.

observed within the two families. The mean WBC count in our cohort was 5.9 K/μL (SD = 1.73), the median neutrophil count was 5.3 K/μL [IQR = 1.825] and the range was 3.6–11.2 K/μL. A statistically significant difference ($p < 0.001$) in WBC count was observed between the C/C and C/T subgroups (5.68 K/μL, SD = 1.57 vs. 8.35 K/μL, SD = 0.83, respectively). This correlates with previous findings and can be explained by predominantly low neutrophil counts[2].

Complete blood count values and patient characteristics of our study cohort are summarized in Table 1. The mean neutrophil count in our cohort was 2.35 K/μL, the median neutrophil count was 2.15 K/μL [IQR = 1.35], and the range was 0.9–4.9 K/μL. A total of 64.7% (22/34) of participants in this study displayed neutrophil counts <2.5 K/μL. This can be further subdivided into 35.2%(12/34) of participants with neutrophil counts between 2.5 and 1.5 K/μL, and 29.74%(10/34) with neutrophil counts under 1.5 K/μL. A total of 95% of the subjects with neutrophil counts under 2.5 K/μL and DNA sequencing were homozygous for the polymorphism C/C (17/18). Within the ACKR1-null genotype (C/C) subgroup, the mean neutrophil count was decreased (2.2 K/μL, SD = 0.73, the median neutrophil count was 2.15 K/μL [IQR = 1.25] and the range was 0.9–3.4 K/μL) even though 35% of individuals displayed normal neutrophil counts. Interestingly, this is ~3.5-fold more common in our cohort than previously described studies that reported ~8–9% normal neutrophil count in individuals with ACKR1-null genotype (C/C). A compensatory mutation occurring at a different site in the genome could partially remedy the effect of the original mutation through epistatic interactions. This type of genetic adaptation has been described in deleterious germline variants[3] and could explain the relatively less potent null Duffy phenotype we observed yet warrants further studies. The heterozygous (T/C) group displayed a normal range mean neutrophil count (3.8 K/μL, SD = 1.87), the median neutrophil count was 4.65 K/μL [IQR = 2.95], and the range was 1.–4.9 K/μL. A low neutrophil count was seen only within 25% (1/4) of individuals. Despite the general trend of decreased neutrophil count in the homozygous (C/C) group, it did not reach statistical significance. This may be a result of our small sample size and represents a limitation of the study. Nonetheless a correlation

Table 1. Patients characteristics according to ACKR1 genotype.

Characteristics	T/C—heterozygous ($N = 4$)	C/C—homozygous ($N = 26$)	p Value
WBC count (K/μL)			**<0.001**
Mean (SD)	8.35 (0.83)	5.68 (1.57)	
Median (IQR)	8.20 (1.5)	5.20 (1.5)	
Range	7.5–9.5	3.6–11.2	
Neutrophil count (K/μL)			
Mean (SD)	3.80 (1.87)	2.20 (0.733)	
Median (IQR)	4.65 (3.00)	2.10 (1.20)	
Range	1.0–4.9	0.9–3.4	
Neutrophilic count, % (n/N) > 2.5 K/μL	75% (3/4)	34.6% (9/26)	
Relative neutropenia, % (n/N) between 2.5 and 1.5 K/μL	0% (0/4)	42.3% (11/26)	**<0.01**
Neutropenia, % (n/N) < 1.5 K/μL	25% (1/4)	23.1% (6/26)	
Hemoglobin count (g/dL)			
Mean (SD)	13.65 (2.83)	12.51 (1.88)	
Median (IQR)	12.80 (5.2)	12.40 (2.9)	
Range	11.4–17.6	9.1–17.1	
Platelets count (K/μL)			
Mean (SD)	244.25 (53.98)	258.46 (49.06)	
Median (IQR)	232 (101)	260 (60)	
Range	195–318	140–362	
Age (years)			
Mean (SD)	15.5 (13.9)	28.3 (17.6)	
Median (IQR)	16.0 (25)	27.0 (26)	
Range	2.0–28.0	2.0–60.0	
Gender, % (n/N)			
Female	75% (3/4)	46.15% (12/26)	
Male	25% (1/4)	53.85% (14/26)	

Statistically significant values are in bold.
SD standard deviation, *IQR* interquartile range, *WBC* white blood cell.

between neutrophil counts under 2.5 K/μL and the ACKR1-null genotype ($p < 0.01$) was established.

DISCUSSION

Our study describes the presence of the ACKR1 (rs2814778) polymorphism in Arabs from Jis Az Zarka and its correlation with BEN. Many of the inhabitants of Jis Az Zarka originate from other regions of the Middle East and North Africa. Since BEN has been previously described in these Arab populations, the presence of the rs2814778 SNP could be explained by a founder effect brought in by the original settling families in combination with large family sizes and a high prevalence of endogamy. Many healthy individuals with a low neutrophil count often undergo unnecessary investigations to exclude pathologic neutropenia (as was the case for the index cases described in this study). Higher awareness of physicians about BEN within specific populations could prevent unnecessary investigations and minimize management setbacks in neutropenic patients.

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AUTHOR CONTRIBUTIONS

Conceptualization: N.S., D.E., Y.B., and A.J.S.; data curation: M.Y.; formal analysis: Y.B.; investigation: D.E., A.J.S., and Y.B.; methodology: D.E, A.J.S., Y.B., and N.S.; project administration: N.S.; resources: M.Y.; supervision: N.E.; validation: D.E. and A.J.S.; visualization: D.E. and A.J.S.; writing—original draft preparation: D.E.; and writing—review and editing: D.E., A.J.S., and N.S.

COMPETING INTERESTS

The authors declare no competing interests.

CONSENT STATEMENT

Patient consent was obtained as required by our hospital's Helsinki committee.

ADDITIONAL INFORMATION

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