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Report on the D32 CCR5 variant in the Sudanese Shagia tribe

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ABSTRACT The focus on small isolated populations provides important insights into the factors affecting the distribution of inheritable traits. Here, we present a report on the distribution of the CCR5 Δ32 mutation in the so far unstudied innate Sudanese population of Shagia people. The genetic material (buccal swabs) was collected from 125 individuals living in three African villages, Abu Haraz, Shibabit and El Higiena. The DNA was extracted, the polymorphic site PCR-amplified with a pair of specific primers flanking the Δ32 CCR5 mutation and reaction products electrophoretically separated in agarose gel. In the Abu Haraz and Shibabit villages, all investigated individuals were found to be homozygous for the wildtype of the receptor, while in El Higiena village one wt/D32 homozygote was identified with the remaining individuals homozygous for non-mutated CCR5. The frequency for the Δ32 CCR5 allele was 0,4%, with Δ32/wt genotype frequency of 0,8%. This is the first report on the presence of the Δ32 CCR5 allele not only in the genetically isolated Shagia tribe but also in the region of the Sudan.

KEY WORDS: HIV, mutation, Sudan

Human chemokine receptor 5 (CCR5), specific for C-C chemokine 5 (CCL5), is coded by the single exon open reading frame (ORF) and is considered the major coreceptor in HIV infection. It has been discovered that the deletion of the 32 base pairs within the coding region of the *CCR5* gene is related to the loss of a part

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of its transmembrane domain which is the reason for the natural resistance to HIV infection among Δ32 homozygotes [COHEN 1996, FAUCI 1996, SAMSON *et al*. 1996]. It is also noteworthy that among individuals bearing the allele, clinical progression of HIV infection is significantly slower, with an approximately 5 year delay in advancing to AIDS. It must also be noted that individuals bearing the mutated Δ32 allele do not present with major immunological defects, with the only known adverse effect being the higher risk of symptomatic infection with the Western Nile virus [GLASS *et al.* 2006].

The Δ32 *CCR5* allele probably originates from a single mutational event which occurred in the North of Europe in recent history, probably some 2000-3500 years ago, with subsequent positive selection of the allele related to the epidemics of infectious diseases. The most probable selective pressures included both widespread infection with *Yersinia pestis* or smallpox, which was responsible for reduction of the population size. [GALVANI and NOVEMBRE 2005]. The allele is the most common in the north of Europe in the region of the Baltic and North Seas, with a gradual decrease of frequency to the south. It is less common or virtually nonexistent in the genetically distinct populations of Asia and Africa. Heterozygous individuals have been identified in local populations in Egypt and Syria. In these isolated populations and local tribes the mutation is extremely rare. No mutation has been identified in the ethnic groups from the other neighboring country – Ethiopia [KANTOR and GERSHONI 1999]. To the authors' knowledge there is no published data on the prevalence of the mutation in population of Sudan.

We have isolated DNA from the population sample of Shagia people, inhabiting–ethnically uniform villages in Sudan, 800 km south from Egypt in the region of the fourth cataract. This tribe inhabits both banks of the river Nile, north of Korti to the third Cataract and in the region of Bayuda desert. The population of Shagia people, being innate and isolated, provides a unique opportunity to gain insight into evolutional and migration-related processes at the molecular level. This tribe is currently being transferred from its previous habitat to the prepared settlements where other, ethnically divergent tribes will also be placed. As the unique quality of being highly uniform, both anthropologically and genetically, may be lost in the future, the scientific value of the genetic material collected in this population seems immense.

The aim of this study was to assess the presence of the Δ32 *CCR5* allele in the sample representative of the Shagia tribe and to compare the results with the previously published data for other populations.

Materials and methods

Subjects

During the expedition organised by Archaeological Museum in Gdansk, Poland, managed by Henryk Paner, we have investigated the group of 125 individuals from three villages Abu Haraz (57 inhabitants, 32 female, 25 male), Shibabit (42 inhabitants 27 female, 15 male) and El Higiena (26 inhabitants, 12 female, 14 male). In total, 73 (58,4%)

participants in the study were female, and 52 male (41,6%).

Formal consent for the study was obtained from national Corporation for Antiquities and Museums of Sudan Republic, while the consent to collect, store and analyse individual samples was collected orally from all participants, under the supervision of the local interpreter.

Genotyping

Buccal swabs for subsequent DNA extraction were collected from all individuals who consented to participate in the study. Collected swabs were carefully dried in a separate area in order to avoid contamination. For DNA extraction BuccalAmp Dna Extraction Kit (Epicentre, Madison, USA) was used. Extraction was performed according to the manufacturer's protocol. DNA was re-suspended in TRIS-EDTA buffer (QIAgen, Hilden, Germany) and stored in 4°C for further analyses. To assess the presence of Δ32 *CCR5* mutation, PCR technique was used with the specific primer pair flanking the polymorphic site using PCR kit by "DNA – GDAŃSK II", Poland. The PCR was performed using Mastercycler Gradient device (Eppendorf, Germany), with the 41 μl of the final mixture containing 5 μl of genomic DNA, 39 μl of Mastermix, 1 μl of Delta 2 polymerase, 5 μl dNTP) Exact dNTP, polymerase concentrations and primer sequence are available from the producer (on-line access at *www.dnagda.com*). PCR conditions were: initial denaturation – 120 seconds (96 °C), followed by 40 cycles of: denaturation – 30 seconds (96 °C), primer annealing for 30 seconds (55 °C) and extension for 30 seconds (72 °C). Final elongation was performed for 120 seconds in 72 °C. Amplicons were separated by means of electrophoresis in 2% agarose gel, stained with ethidium bromide, visualized in UV light (Transilluminator 4000, Stratagene, La Jolla, USA) and recorded with DS-34 Polaroid Direct Screen Camera.

Selected samples, including the Δ32/wt *CCR5* heterozygous one, were genotyped for reference with the following primer pair flanking the polymorphic site:
ATAGGTACCTGGCTGTCGTCCAT (forward primer),
CCAGCCCCAAGATGACTATCT (reverse primer).

The confirmation PCR was performed using ABI 9700 device (Appliedbiosystems, USA), with the 20μl of the mixture containing 40 ng of genomic DNA, 10μl of Mastermix by MBI Fermentas, Lithuania (0,05u/μl of recombined *Taq* polimerase, in a reaction buffer containing 4mM $MgCl_2$ and 0,4 mM of each dNTP – dATP, dCTP, dGTP, dTTP) and 4pmol of each primer in the following conditions: initial denaturation – 5 mins (94 °C), followed by 37 cycles of: denaturation – 30 seconds (94 °C), primer annealing – 30 seconds (58 °C) and extension – 55 seconds (72 °C). Final elongation was performed for 10 minutes in 72 °C. Amplicons were separated by means of electrophoresis in 3% agarose gel, stained with ethidium bromide, visualized in UV light (Transilluminator 4000, Stratagene, La Jolla, USA) and recorded with DS-34 Polaroid Direct Screen Camera.

Results and Discussion

Genotyping revealed that all fifty-seven investigated individuals from Abu Haraz village were homozygous for the wild-

Table 1. Δ32 CCR5 mutation among Shagia people

	Δ32 genotypes (%)			Δ32 allele frequency (%)
	Wt/Wt	Wt/Δ32	Δ32/Δ32	
Abu Haraz (n=57)	100	0	0	0
Shibabit (n=42)	100	0	0	0
El Higiena (n=26)	96.25	3.85	0	1.92
Total	99.2	0.8	0	0.4

Table 2. Δ32 CCR5 allelic frequency in various populations

Population	Δ32 CCR5 allele frequency (%)	Reference
Shigia people	0.4	This study
Polish newborns	14.3	Unpublished own material
Lebanon	2.5	[Karam *et al*. 2004]
Crete	3.25	[Apostolakis *et al*. 2005]
African-Americans	2.0	[Kostrikis *et al*. 1999]
Egypt	0.6	[Salem and Batzwr 2007]
Africans	0	[Lu *et al.* 1999]
Turkey	2.18	[Degerli *et al*. 2005]
Thailand	0	[Nguyen *et al*. 2004]

type allele of the receptor. Similar situation was observed in Shibabit village, with no Δ32 mutation found among forty-two analysed samples. In El Higiena village, a single carrier of this mutation, a two year old girl was found, with all the remaining twenty six individuals homozygous for the non-mutated CCR5 (Table 1). The cumulative frequency of Δ32 CCR5 allele was 0,4%, with Δ32/wt genotype frequency of 0,8%.

Shagia people, a tribe of the Semitic origin, is known to live both a settled and nomadic way of life. Historically, they lived in the northern part of Sudan and mixed mostly with the people of the Nuba tribe. Nowadays the tribe has spread throughout the whole country. Subjects included in this study belong to an isolated population living in local, isolated villages, in the vicinity of the Nile, marrying within the tribe only.

Finding a single delta32 allele of the CCR5 gene in this population is in accordance with the previously published research by GALVANI and NOVEMBRE [2005] describing rarity of this allele in African populations (Table 2). In fact, it is interesting that the mutation was found among the Shagia people at all. It might be related to the nomadic way of life and the possibility of the recent European admixture.

Conclusions

This is the first report on the frequency of the Δ32 *CCR5* polymorphism not only in genetically isolated population of the Nomad Shagia tribe but also in Sudan.

This study shows the rarity of Δ32 *CCR5* allele in Sudan. The presence of a single allele in a genetically isolated population of Shagia people may be related to the origins of this tribe and its nomadic history with the possibility of the allele acquisition by admixture.

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Streszczenie

W trakcie ekspedycji naukowej w rejon obejmujący obszar IV katarakty na Nilu, między miastami Karima i Abu Hamad, w lutym 2005 r., zabezpieczono materiał genetyczny w postaci wymazów z nabłonka jamy ustnej od 125 osób z plemienia Shagia. Po przeprowadzeniu izolacji, DNA poddano amplifikacji metodą PCR przy użyciu pary specyficznych (flankujących) primerów. Produkty reakcji zostały rozdzielone elektroforetycznie na żelu agarozowym, w celu ujawnienia i identyfikacji ewentualnie istniejącej mutacji Δ32 CCR5.

Zidentyfikowano jedną heterozygotę wt/D32, podczas gdy pozostali przebadani osobnicy byli homozygotyczni w zakresie niezmutowanego CCR5 (tab. 1). Oporność na zakażenie wirusem HIV jest związana ze zmniejszeniem ilości funkcjonalnego receptora CCR-5 na powierzchni komórek, przez co wnikanie wirusa HIV odbywa się ze znacznie mniejszą efektywnością. Częstość występowania badanych polimorfizmów jest znacząco mniejsza na kontynencie afrykańskim niż w Europie (tab. 2). Homozygotyczność w zakresie allelu Δ32 niesie za sobą niemal całkowitą oporność na zakażenie wirusem. Zaprezentowane badania są unikatowe w skali afrykańskiej, szczególnie dla wybranej zamkniętej populacji i pozwalają uzyskać istotny wgląd w zmienność genetyczną tamtejszej ludności. Interesujący jest sam fakt odnalezienia omawianej mutacji wśród ludu Shagia. Może to być związane z wcześniej prowadzonym, koczowniczym trybem życia oraz ewentualną domieszką genów europejskich.