



Molecular Detection of Chemokine Ligand 3 Like-1 (CCL3L1, Mip-1 α p or LD78 β) Gene Copy Number Amongst HIV-1 Positive Sudanese Patients in Khartoum, Sudan



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Background



- Chemokines have been shown to play a pivotal role in disease susceptibility and progression, and this has been seen of recent in HIV infection.
- There is additional evidence that gene copy number variation of these chemokines influences phenotypic variation in HIV-1 infection.
- One of the chemokines, *CCL3L1*, Chemokine ligand 3-like 1 which is encoded by a variable copy-number gene in humans and other primates, binds to several pro-inflammatory cytokine receptors, including chemokine-receptor 5 (CCR5), which is being used by the R5 strains of the human immunodeficiency virus -1 to gain entry into the CD4 cells; thereby preventing this strain to some extent from infecting the host cell via CCR5 co-receptor.
- There is evidence that individuals with more copies of *CCL3L1* (CCR5 ligand) than the median number of copies of their population have been found to be less susceptible to HIV-1 infection.

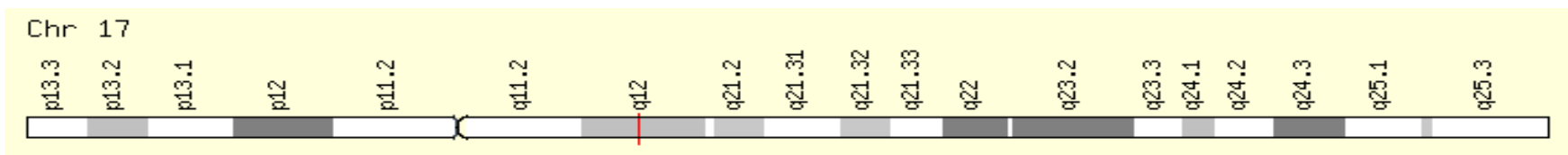


Fig 1. *CCL3L1* gene location on Chromosome 17



CCL3L1 (Mip-1alpha) blocking R5-HIV from entering CD4 cells

CCL3L1 gene dose and CCR5 appear to have a synergistic or interactive effects, being consistent with the notion that CCR5 genotypes also influence HIV/AIDS, often in a population specific manner.

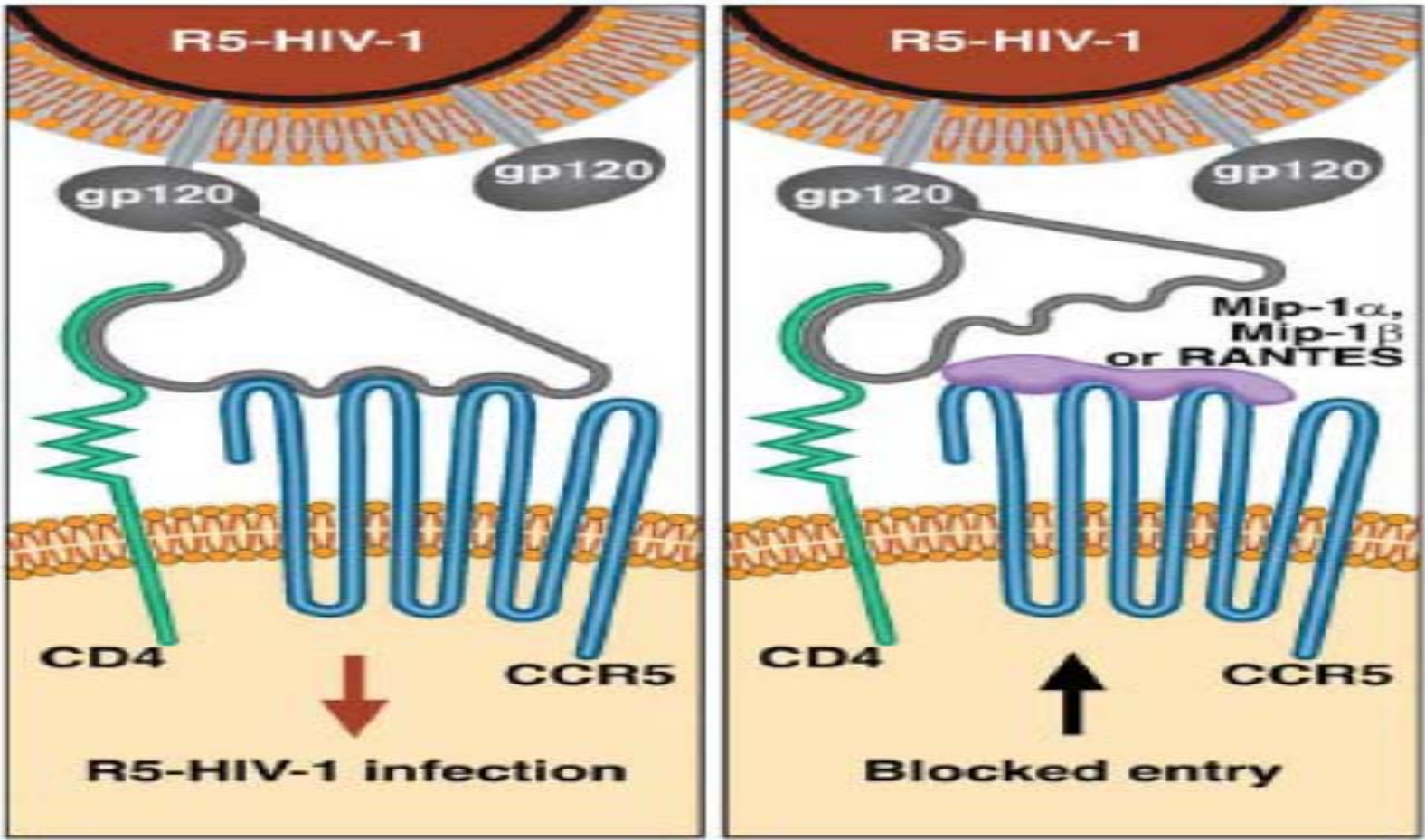


Fig. 2



Objectives



- Given the increased number of HIV cases being seen in the Sudan of recent we decided to study and possibly estimate the median copy number of CCL3L1 gene within the Sudanese population and compare it to that of Sudanese patients currently infected by the human immunodeficiency virus.



Methods



- A case-control study was conducted where we measured CCL3L1 gene copy numbers per diploid genome (pdg) in 76 Sudanese individuals (36 HIV-1 +ve and 40 HIV-ve) from different regions of the Sudan using the *Stratagene MxP 3000* real-time PCR and SYBR Green assay after extracting genomic DNA from whole blood.
- Generated a standard curve in order to calculate the CCL3L1 copy number using beta-globin (*HBB*) as the house-keeping gene.
- The copy number of CCL3L1 is the ratio of the starting template quantity for CCL3L1 to the starting template quantity for *HBB* multiplied by two.
- We also measured the CD4 count for all these 36 patients.



Ethnic and Geographic Origins of Participants



Fig. 4

Geographic Origin	Tribe	Number of participants HIV+	Number of participants HIV-
South	Dinka, Nuba, Shiluk, Acholi, Nuer, Madi, Baria	10	12
North Central	Shaigia, Ga'alin, Danagla, Nubian, Hawara, Betaheen, Gimoae, Halfa, Egemab, Bideria	14	21
West	Fur, Masselit, Hausa, Baggara, Kababish	9	4
East	Beja	3	3
Total		36	40



Results



- The median CCL3L1 copy number per diploid genome amongst Sudanese was found to be 3, and this was also found to be the same (i.e. 3) in both the Nilo-Saharan and Afro-Asiatic speaking groups, with a case-control Kruskal-Wallis test p -value of 0.001, a 2-tailed t -test p -value of 0.004 ($p < 0.05$) with a 95% confidence interval of between -2.13 and -0.43.
- Individuals with a lower CCL3L1 copy number compared to the population-specific median number were over represented among the HIV-1 positive subjects compared to the HIV-1 negative subjects.



Results (*cont'd*)



- More than 70% of the HIV infected individuals had an estimated CCL3L1 copy number lower than 3 and majority in stage III of the disease, and less than 30% had a copy number of CCL3L1 greater or equals to 3 with an associated better prognosis.
- We found four copies in HIV-negative Sudanese from the Southern and Western regions, two copies from the North/Central and Eastern regions, and three copies in HIV-negative Nilo-Saharan and Afro-Asiatic speaking groups.



Discussion & Conclusions



- These data suggest that Sudanese with CCL3L1 copy numbers lower than the median population (ethnic or linguistic)-specific copy number per diploid genome have a higher chance of acquiring HIV-1 infection if exposed to the virus.
- From these observations it could be suggested that infection with HIV-1 can exert a negative selective pressure on individuals with lower copy numbers, therefore, depending on the strength of this effect in the general population, it could change the population-specific distribution of CCL3L1 copy number with time.



Future Perspectives



- Increase the sample size.
- Possibly correlate progression of HIV disease and CCL3L1 copy number in this cohort.
- Finally to possibly clone more copies of CCL3L1 gene and insert into the genome.