Human Immunology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Analysis of CCR5 gene polymorphisms in 321 healthy Saudis using Next Generation Sequencing

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ARTICLE INFO

Article history:
Received 22 June 2016
Revised 20 February 2017
Accepted 5 March 2017
Available online xxxx

ABSTRACT

Aims: To investigate the extent of CCR5 polymorphism in the healthy Saudi population.
Method: A total of 321 healthy Saudi individuals were sequenced using the ion Ampliseq™ Exome kit (Life Technologies, USA) on genomic DNA following manufacturer's protocol. Whole Exome Sequencing (WES) reads were aligned to the human reference genome (hg19 build) with Torrent Suite Software (v5.0.2) and the variants were called using the Torrent Variant Caller plugin (v5.0) and imported into Ion Reporter Server (v5.0) for the annotation. CCR5 coding exons variants were filtered and checked against the NHLBI GO Exome Sequencing Project (NHLBI), NCBI Reference dbSNPs database, 1000 genomes and Exome Aggregation Consortium datasets (ExAC).
Results: A total of 475 variants were identified. Table 1 shows polymorphisms/mutations detected within exons that introduced an amino acid change, deletion or copy number variants (CNV). Three mutations are predicted to influence CCR5 function, including the 32 bp deletion (Rs333). Four polymorphisms were detected, plus two CNV.
Conclusions: This is the first report on sequencing the full CCR5 gene using NGS in the Saudi population. Here we demonstrate seven polymorphisms/mutations that were reported before. All were detected within very low frequency including the delta 32 mutation. However, we report for the first time copy number variants at two CCR5 gene locations; 45072265 and 38591712.

1. Introduction

The chemokine receptor (C–C motif) 5 (CCR5) is the receptor for RANTES, CCL3 (MIP 1α), CCL4 (MIP 1β) and CCL3L1 [1,2]. It plays a major role in immune response. It was also found to act as a co-receptor for HIV entry into CD4 positive T lymphocytes [3].

Some people were found to lack the expression of CCR5 protein, due to a 32 bp deletion (delta 32) in the coding region of the gene. Homozygosity for the delta 32 allele appear to confer protection from HIV infection [4–6].

Studies on the frequency of the delta 32 allele found a discrepancy between north and south hemispheres, with higher allele frequency in the north and lower frequency in the south, reaching to complete absence in certain ethnic groups [7,8].

Early studies of the genetic polymorphisms within the CCR5 coding region revealed a polymorphic gene [9]. Many polymorphisms were described, majority of which were rare [3]. Mutations within the CCR5 were found to be associated with many inflammatory and autoimmune diseases [10–13].

The primary purpose of WES study was to catalogue all identified exomes variants within special building database that will be compared later with disease related variations find among affected individuals. Therefore, it will be impropriate mentioning all identified variants here in this manuscript. Future reporting of all identified genes variants would be beneficiary in separate publication. Here, in this study, we aimed to investigate the extent of polymorphisms/ variants within the CCR5 exomes only in a large cohort of healthy Saudis that might have implications in HIV or other diseases.

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http://dx.doi.org/10.1016/j.humimm.2017.03.003
0198-8859/

2. Methodology

Available genomic DNA on a total of 321 healthy Saudi individuals were subjected to Whole exome sequencing (WES). Informed consent was obtained from all individuals involved in this study after NGHA IRB ethical approval. Libraries were carried out on the genomic DNA using the ion Ampliseq™ Exome kit and quantified by qPCR. The enriched libraries were prepared on Ion Chef System (Life Technologies, USA) following manufacturer's protocol. Each library was sequenced on an Ion Proton instrument (Life Technologies, USA) using one ION PI chip kit V3 BC providing >95% of amplicons covered with at least 100× and an average base coverage depth that ranged 85–130.

Bioinformatics base calling, filtering low quality reads, adapter removing followed alignment against human reference genome (hg19 build) performed in Torrent Suite Software (v5.0.2), the variants were called using the Torrent Variant Caller plugin (v5.0) and imported into Ion Reporter Server (v5.0) for the annotation. Gene variants located within exomes, introns, predictive splice sites and UTRs were identified with less than 1% minor allele frequency of (MAF). Functional exomes variants were also identified as frameshift, synonymous, misssense, nonsense and stoploss.

In this WES study, >97% of Consensus Coding Sequences (CCDSs) including 5 bp padding around exons of CCR5 were covered then identified variants were checked against the NHLBI GO Exome Sequencing Project (NHLBI) (http://evs.gs.washington.edu/EVS/), NCBI Reference dbSNPs database (ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/), 1000 genomes (http://www.1000genomes.org/data#DataAvailable) and Exome Aggregation Consortium datasets (ExAC) (http://exac.broadinstitute.org/).

3. Results

A total of 475 variants were found. Table 1 shows polymorphisms/mutations were identified within exons that introduced an amino acid change, deletion or copy number variant but not introns and UTRs (data not shown). Six of the nine were single nucleotide polymorphisms, two of them were predicted to influence CCR5 protein function. One was indel mutation that included a 32 bp deletion (Rs333) known as the delta 32 polymorphism which leads to a premature stop codon. Two copy number variants (deletion) were also detected.

Out of the nine described polymorphisms (Table 1), three lead to no expression of the CCR5 gene, namely Rs333, CNV1 and CNV 2. Two mutations are predicted to affect protein function; c.164T>A p.Leu55QGlu (rs1799863) and c.192G>A p.Met64Iso (G/A). While the rest three are predicted as polymorphisms.

Two copy number variants were described for the first time, a 1.87 Mb and 8.3 Mb deletion. These were at positions 45072265 and 38591712 on chromosome 3. Both encompass the CCR5 gene thus leading to no expression of the gene.

All described polymorphisms (Table 1) were very rare and occurred in heterozygous form. The most common polymorphism was rs1800945 which was found six people (0.94%). While four polymorphisms were found in one individual each; position 46414585 (G/A), position 46414467 (T/A) and CNV 1 and CNV 2. The rest three polymorphisms, including the delta 32 (RS.333) were found in two individuals each (Table 1).

4. Discussion

We have employed next generation sequencing technique to map out the extent of CCR5 gene polymorphisms in 321 healthy Saudi individuals that might have implications in HIV or other diseases. A total of nine polymorphisms were identified.

CCR5 delta 32 mutation was detected at a very low frequency in this Saudi cohort. This is consistent with a study by Abuelsaad et al. [14] and Jawdat et al. [15]. A study by Stephens et al. [7] suggested a gradient of frequency of this mutation from high in the European Caucasians to low in Mediterranean countries. CCR5 is a co-receptor for HIV, together with CD4, it facilitates HIV entry into target cell. Delta 32 CCR5 mutation affects the expression of CCR5 protein, thus render people resistant to HIV [16]. This study would be improved by assess CCR5 SNPs within HIV population of Saudis as well but this was not possible due to unavailability of many HIV patients in our hospital to address the issue of HIV resistance.

CNV in the CCR5 gene were described in two studies before, where they analyzed the full human genome [17,18]. Both of these studies found a gain in copy number, while here we identified two large DNA segments that were deleted; 1.87 Mb and 8.3 Mb. Thus leading to one chromosome copy of the CCR5 gene. Studies have found that one functional copy of the CCR5 influenced its function and susceptibility to HIV infection [16]. Each of the CNV described in Table 1 was found in one individual out of the 321 tested.

The most common polymorphism found in this study was Tyr339Phe. Boldt et al. investigated this polymorphism in different ethnic groups and found that the highest frequency was in Afro-Americans (0.026) while it was not detected in Afro-Brazalians, Euro-Brazalians Orientals and Hispanics[8]. Similar results were also observed by Carrington et al. [9]. The second most frequent polymorphism was Leu55Glu. This polymorphism was investigated before and found to be rare in many ethnic groups, including Afro-Americans (0.008%), Euro-Americans (0.041%), Hispanics (0.01%), it was not found in Chinese or Orientals[8]. The third polymorphism Val46Met was not described before, however, the 1000

Table 1
Polymorphisms/mutations detected in exons that introduced an amino acid change, deletion or copy number variants.

Ch#3 location	ID	Allele freq N (%)	Substitution	Zygosity Status	
46415409	rs1800945	6 (0.94)	c.1016A>T p.Tyr339Phe	AT	Polymorphism
46414557	rs1799863	3 (0.47)	c.164T>A p.Leu55Glu	AA	Pathogenic
46414529	rs41425744	2 (0.31)	c.136 G>A p.Val46Met	AG	Polymorphism
46414618	rs1800941	2 (0.31)	c.225T>C	TC	Polymorphism
46414943	INDEL Rs333	2 (0.31)	frameshift Deletion	×1	p.Ser185fs
46414585	G/A	1 (0.16)	c.192 G>A p.Met64Iso	AG	Pathogenic
46414467	T/A	1 (0.16)	c.74T>A p.Val25Glu	AT	Polymorphism
45072265	CNV 1	1 (0.16)	1.87 Mb CNV (deletion)	×1	1.87 Mb CNV involved a contiguous gene deletion that includes CCR5
38591712	CNV 2	1 (0.16)	8.3 Mb CNV (deletion)	×1	8.3 Mb CNV involved a contiguous gene deletion that includes CCR5

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human genomes browser revealed a very rare polymorphism in different ethnic groups for this polymorphism[19]. The c.225T>C polymorphism was analyzed by Picton et al. in two South African populations, they found it in South Africans (0.014%) but not in Caucasians [20], here we found the c.225T>C in 2 individuals out of 321 analyzed. MAF of some of the above variants were reported in the 1000 Genomes, NHLBI Exome Sequencing Projects studies and ExAC available at their corresponding website.

Both Met64Iso (http://exac.broadinstitute.org/variant/3-46414585-G-A), and Val25Glu (http://exac.broadinstitute.org/variant/3-46414467-T-A) were not described before suggesting that they are very rare mutations. Here we identified them in one individual each out of the 321 individuals analyzed.

Recently, Abuelsaad et al. [14] studied 135 healthy people from Taif (Western Province) and found five mutations, each one in different individual, Pro250Arg, Phe158Tyr, Glu227Asp, Met64Lys, Met49Iso. None of these mutations matched the ones described here.

It's worth mentioning that the primary purpose of WES in healthy individuals was to catalogue all identified exomes variants within special building database that will be used to compare later with disease related variations find among affected individuals. Therefore, it will be impropriate mentioning all identified variants here in this manuscript. Rather future reporting of all identified genes variants would be beneficiary in separate publication.

It has been shown in several mammals that the CCR5 genes present high levels of gene conversion with the chromosomally adjacent CCR2 [21–25]. Here we used stringency analysis parameters to reach high sensitivity, specificity and detection rates, parameters like minimum coverage 100 bp, minimum coverage on each strand 25 bp, maximum strand bias 0.95 to avoid false positive variants.

In conclusion, this report identified the polymorphisms/variants within the CCR5 exomes using NGS technology. In general all identified polymorphisms are rare and don't reach the level identified in other populations specially for the delta 32 polymorphism. The variation in the frequency of delta 32 polymorphism lead scientist to believe the it may evolved through environmental pressure of an agent such as a virus. This could have happened over many years, such environmental pressure does not seem to have happened in this part of the world.

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