Previously Unidentified Single Nucleotide Polymorphisms in HIV/AIDS Cases Associate with Clinical Parameters and Disease Progression

Vladimir V. Anokhin,1,2 Liliia B. Bakhteeva,3 Gulshat R. Khasanova,1,2 Svetlana F. Khaiboullina,4,5 Ekaterina V. Martynova,4 Richard L. Tillett,6 Karen A. Schlauch,6 Vincent C. Lombardi,4,6 and Albert A. Rizvanov4

1Kazan State Medical University, 49 Butlerova St., Kazan 420012, Russia
2Republican Clinical Hospital for Infectious Disease named after Agafonov, 83 Prospect Pobedy St, Kazan 420140, Russia
3Republican Center for the Prevention and Fighting of AIDS and Infectious Diseases, Ministry of Health of the Republic of Tatarstan, 2a Vishevkogo St., Kazan 420097, Russia
4Kazan Federal University, 18 Kremlyovskaya St., Kazan 420008, Russia
5Department of Biochemistry, University of Nevada, Reno, 1664 N. Virginia Street MS 0330, Reno, NV 89557, USA
6Nevada Center for Biomedical Research, University of Nevada, Reno, 1664 N. Virginia Street MS 0552, Reno, NV 89557, USA

Correspondence should be addressed to Vincent C. Lombardi; vlombardi@medicine.nevada.edu and Albert A. Rizvanov; albert.rizvanov@kpfu.ru

Received 19 August 2016; Accepted 8 November 2016

Academic Editor: Guochun Jiang

Copyright © 2016 Vladimir V. Anokhin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The genetic background of an individual plays an important role in the progression of HIV infection to AIDS. Identifying previously unknown or uncharacterized single nucleotide polymorphisms (SNPs) that associate with disease progression may reveal important therapeutic targets and provide a greater understanding of disease pathogenesis. In the present study, we employed ultra-high multiplex PCR on an Ion Torrent next-generation sequencing platform to sequence 23 innate immune genes from 94 individuals with HIV/AIDS. This data was used to identify potential associations of SNPs with clinical parameters and disease progression. SNPs that associated with an increased viral load were identified in the genes for the interleukin 15 receptor (IL15RA), toll-like receptor 7 (TLR7), tripartite motif-containing protein 5 (TRIM5), and two killer-cell immunoglobulin-like receptors (KIR2DL1 and KIR2DL3). Additionally, SNPs that associated with progression from HIV infection to AIDS were identified in two 2’-5’-oligoadenylate synthetase genes (OAS2 and OAS3). In contrast, other SNPs identified in OAS2 and OAS3 genes, as well as in the TRIM5 and KIR2DS4 genes, were associated with a slower progression of disease. Taken together, our data demonstrates the utility of ultra-high multiplex PCR in identifying polymorphisms of potential clinical significance and further identifies SNPs that may play a role in HIV pathogenesis.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a devastating disease caused by the human immunodeficiency virus (HIV). AIDS is defined as having a CD4+ T cell count below 200 cells per μL or displaying specific clinical presentations in association with an HIV infection [1]. On average, most individuals infected with HIV will progress to AIDS within 10 years if antiretroviral therapy is not administered.

It is evident that the clinical manifestations of HIV infection vary in different individuals. Previous studies have reported that these differences are not dependent on gender, mode of infection, or age [2]. Therefore, genetic variations in genes that control immune responses have been suggested to play a role in the progression of HIV or lack thereof [3, 4]. Indeed, innate immunity plays an important role in controlling HIV replication [5]. Upregulation of type I interferon (IFN) is essential to restrain HIV replication,
especially at the early stages of infection [6]. The activation of IFNα expression occurs primarily through the engagement of toll-like receptors (TLR) 7 and 9 by single stranded RNA or CpG DNA, respectively (reviewed by [7]). It has been demonstrated that single nucleotide polymorphisms (SNPs) in TLRs are associated with an increased susceptibility to infection [8–11] and are involved in different progressions of HIV/AIDS, although a consensus has not been reached. For example, Papadopoulos et al. demonstrated an association between a TLR4 polymorphism and increased risk of opportunistic infection in subjects with advanced HIV-1 infection [8]. However, a subsequent study by Soriano-Sarabia et al. could not confirm these results, although these investigators did identify a polymorphism in TLR9 that was associated with a lower CD4 count and a higher viral load in HIV-positive cases [12].

Numerous reports describe impaired immunomodulatory and cytotoxic activity of natural killer (NK) cells in HIV-1-infected individuals [5,13–15]. Additionally, HIV infections are often characterized by increased expression of inhibitory receptors and downregulation of activating receptors [16,17]. Killer-cell immunoglobulin-like receptors (KIRs) regulate NK activation status. Interestingly, NK cells expressing the activating KIR3DS1 inhibit HIV-1 replication in target cells [18]. Therefore, it could be suggested that differential expression of various KIRs may impact progression and outcome of HIV infection. Although the presence of some SNPs can alter the expression and function of KIRs, our knowledge regarding polymorphism of KIRs in HIV subjects remains limited.

A significant body of evidence suggests that the innate immune system plays an important role in susceptibility to HIV infection and disease progression. For this reason, genetic polymorphisms in genes of innate immune pathways may also influence the course of the disease. In the present study, we focused our attention on several key genes involved in regulation of the innate immune response. We employed ultra-high multiplex PCR on an Ion Torrent next-generation sequencing platform to sequence 23 innate immune genes of 94 individuals with HIV/AIDS. These data were then used to identify potential associations of SNPs with clinical parameters and disease progression. Of the 649 SNPs identified in this study, SNPs associated with viral load were identified in the following genes at the respective chromosomal positions: *IL15RA* (rs2229135; chr10:5995052), *TLR7* (rs179008, chrX:12903659), *TRIM5* (rs11601507; chr11:5701074), *KIR2DL1* (rs77397437; chr19:55286864), and *KIR2DL3* (chr19:55251049). Further analysis revealed that the C/T genotype of *IL15RA* (rs2229135; chr10:5995052), the A/T genotype of *TLR7* (rs179008; chrX:12903659), the A/C genotype of *KIR2DL1* (rs77397437; chr19:55286864), the A/G genotype of *KIR2DL3* (chr19:55251049), and the C/A genotype of *TRIM5* (rs11601507; chr11:570074) were associated with increased viral load in HIV cases. Furthermore, two SNPs; one in OAS2 (rs2072137; chr12:133440921) and the other in OAS3 (chr12:13376388), were identified that associated with progression of HIV infection. Additionally, three SNPs associated with disease progression included *TRIM5* (rs11038628; chr11:56889460), *KIR2DS4* (chr19:55358734) as well as the SNP in *IL15RA* (rs2229135; chr10:5995052). These data further support the role of the innate immune response in maintaining viral persistence and disease progression in HIV-infected individuals.

### 2. Materials and Methods

#### 2.1. Subjects. Ninety-four cases (34 female and 60 male) hospitalized at the Republican Center for AIDS Prophylaxis and Prevention, Republic of Tatarstan, were enrolled in this study. Diagnosis of HIV infection was established based on presence of anti-HIV antibodies using ELISA and western blot methods. Cheek swabs were collected from all HIV-infected cases for genetic analysis. The Institutional Review Board of the Kazan Federal University approved this study and informed consent was obtained from each study subject according to the guidelines approved under this protocol (article 20, Federal Law “Protection of Health Right of Citizens of Russian Federation” N323-FZ, 11.21.2011). The clinical characteristics of HIV cases are summarized in Table 1.

#### 2.2. Ampliseq Analysis. Genomic DNA was isolated using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A DNA sample from each subject was used for sequencing by Ampliseq ultra-high multiplex PCR (IonAmpliseq Library kit; Life Technologies, Carlsbad, CA). Custom primer sets were designed using the Ion Ampliseq Designer tool (Life Technologies). The respective genes sequenced in this study are summarized in

| Characteristics | Age (years) | Male/female | Mode of transmission | Clinical stage of the disease (CDC) |
|-----------------|------------|-------------|----------------------|-----------------------------------|
|                 | 35.6       | 36/60       |                      |                                    |
| Mode of transmission | Sexual contact | 41       | IV drug user | 55         |
| HIV RNA viral load >250 copies/ml | yes | 57 | No | 39 |
| CD4 cell count/ml | 413.9 | 39925.9 | 53 | 46 |
Table 2: Genes selected.

| Gene   | Function                                                                 |
|--------|--------------------------------------------------------------------------|
| 1       | OAS1 Induced by INF, essential innate immune response protein, member of the 2-5A synthase family |
| 2       | OAS2 Induced by INF, essential innate immune response protein, member of the 2-5A synthase family |
| 3       | OAS3 Induced by INF, essential innate immune response protein, member of the 2-5A synthase family |
| 4       | IL15RA Binds specifically to IL15 (1993, *Mammalian Genome* 4 (8): 435-9)  |
| 5       | TLR3 Pathogen pattern recognition receptor; binds to dsRNA               |
| 6       | TLR4 Pathogen pattern recognition receptor; binds to LPS                 |
| 7       | TLR7 Pathogen pattern recognition receptor; binds to ssRNA               |
| 8       | TLR8 Pathogen pattern recognition receptor; binds to ssRNA               |
| 9       | TLR9 Pathogen pattern recognition receptor; binds to DNA                 |
| 10      | KIR2DL1 Inhibitory receptor, HLA-C alleles ligand                        |
| 11      | KIR2DL3 Inhibitory receptor, HLA-C alleles (HLA-Cw1, HLA-Cw3, and HLA-Cw7) ligands |
| 12      | KIR2DL4 Inhibitory receptor, expressed in endosome, HLA-Cw ligand (Front Immunol. 2012 Aug 20; 3:258. doi: 10.3389/fimmu.2012.00258. eCollection 2012) |
| 13      | KIR2DS4 Activation receptor; HLA-Cw4 ligand                             |
| 14      | KIR3DL1 Inhibitory receptor; HLA Bw4 ligand                             |
| 15      | KIR3DL2 Inhibitory receptor; HLA-A ligand                               |
| 16      | KIR3DL3 Inhibitory receptor, ligand unknown (Korean J Hematol. 2011 Dec; 46(4): 216-28. Doi) |
| 17      | IRF7 Activates transcription of INF; exclusively expressed in lymphoid tissue |
| 18      | TRIM5 Retrovirus restriction factor; binds to virus capsid and presents uncoating |
| 19      | TRIM21 Involved into intracellular antibody mediated proteolysis         |
| 20      | RANSEL Interferon-induced ribonuclease; degrade RNA cellular and viral   |
| 21      | MYD88 Adapter protein involved in the Toll-like receptor and IL-1 receptor signaling pathway |
| 22      | TRIF TLR adaptor protein                                                |
| 23      | TRAF6 Member of the TNF receptor associated factor; TLR adaptor protein |

Table 2. The library after purification, equalization, and the template prep was processed for the Ion 318 Chip on the Ion OneTouch system (Thermo Fisher Scientific, Waltham, MA). Final sequencing was conducted using the Ion PGM Next-Generation Sequencing Platform (Thermo Fisher Scientific, Waltham, MA).

Reads from Ampliseq amplicon sequencing were aligned to the reference human genome (NCBI37/hg19) using the Bowtie2 alignment tool [19]. Single nucleotide variants were called at each amplicon using SAMtools and BCFtools [20]. Possible genotypes and their likelihoods were computed at each position with SAMtools mpileup, from which variants and genotypes were called using BCFtools [19].

3. Statistical Analysis

Six hundred forty-nine polymorphisms were identified across genotypes of 94 of the HIV samples using Ampliseq sequencing technology. Analyses of identified polymorphisms revealed 261 SNPs exhibited a minor allele frequency of 3.5% [94 HIV samples + CF1 + CFS2 + USCTR = 160]. Next, 261 SNPs were examined for association with clinical parameters including sCD14, viral load, and progressor status.

To identify association between genotype and sCD14 or viral load measures, a simple linear model approach was performed between each clinical parameter and the genotypes of the HIV samples. These p values were then adjusted using a multiple testing correction (false discovery rate) [21], and any adjusted p values with p < 0.05 were considered statistically significant.

Simple Chi-squared tests were performed to establish any relation between the genotypes of the 94 HIV samples and their progressor status. The p values of the 261 Chi-squared tests were not adjusted.

4. Results

4.1. Clinical Presentation of HIV Cases. Diagnosis of HIV infection was established by detection of anti-HIV antibodies using ELISA and western blot methods. The following criteria were used for patient enrollment: (a) detection of anti-HIV antibody; (b) CD4 counts ≥ 350 cell/μL at the time of enrollment; and (c) absence of opportunistic infections at the time of enrollment. Subjects were followed up for five years after enrollment to determine progression of the disease.

A total of 94 subjects were enrolled in this study (Table 1): 34 female (36.2%) and 60 male (63.8%) HIV-infected cases with an average age 35.6 years. Mode of transmission included 39 sexual contact cases (41.5%) and 55 IV drug users cases (58.5%). Enrolled cases were at clinical stage 3 (30 cases; 31.9%), stage 4A (19 cases; 20.2%), and stage 4B (45 cases; 47.9%). Thirty-nine cases (41.5%) received antiviral treatment, while 55 cases (58.5%) remained without virus-specific therapy. Antiviral treatment included nucleoside analogs,
nonnucleoside reverse transcriptase inhibitors, and protease inhibitors. Average HIV RNA viral load was 39925.9 copies/mL; 55 cases (58.5%) had a viral load higher than 250 copies/mL and the remaining 39 cases (41.5%) had an undetectable viral load or less than 250 copies/mL.

Based on CD4 counts and the rate of their decline, all cases were separated into two groups: group 1 “typical progressors” and group 2 “slow progressors.” Group 1, typical progressors, was characterized by a decline of CD4 counts < 350 cells/µL annually with overall CD4 counts < 350 cells/µL during the five years of observation [22–24]. During this five-year period, subjects in group 1 lost on average 250 cells/µL CD4 cells. Group 2, slow progressors, was characterized by <50 cells/µL annual decline in CD4 counts with overall CD4 counts > 350 cells/µL. Group 1 included 55 cases (58.5%), while group 2 contained 39 cases (41.5%).

4.2. Ampliseq Analysis. Twenty-three genes were selected for Ampliseq analysis (Table 2). The selected genes were chosen as mediators of innate immune responses and their corresponding intracellular pathways. Using the Ampliseq approach, complete sequences of each gene of interest were obtained allowing the identification of known, as well as previously unidentified SNPs. A total of 649 SNPs were obtained allowing the identification of known, as well as previously unidentified SNPs. A total of 649 SNPs were available for 59 HIV cases. Further, screening of genetic variants in selected genes led to the discovery of a 32 bp deletion (CCR5Δ32) in the C-C chemokine receptor type 5 (CCR5) gene, which codes for a coreceptor used by the virus to enter the host cells [29, 30]. It has been demonstrated that the CCR5Δ32 genotype delays HIV progression in heterozygous individuals and prevents infection in homozygous individuals [31, 32]. Discovery of the CCR5Δ32 genotype provided compelling evidence to support the role of host genetic factors in the pathogenesis of HIV and AIDS. Since then, many studies have been conducted

4.3. Correlation between viral load and SNPs. Analyses were then performed to identify potential correlations between SNPs and disease progression (Table 4). OAS2 (rs2072137; chr12:113440921) and OAS3 (chr12:113376388) SNPs showed significant correlation with disease progression. Also, OAS3 A/A genotype (chr12:113376388) was associated with slow disease progression, while genotypes G/G and G/A were linked to “typical” disease progression. Additionally, the OAS2 T/C and C/C genotypes (rs2072137; chr12:113440921) were associated with “typical” disease progression, while the T/T genotype was associated with higher frequency in the slow progressors. Lastly, our analysis revealed a higher frequency of TRIM5 C/T genotype (rs11038628; chr15:6688940), the KIR2DS4 G/A and A/A genotypes (chr19:55358734), and IL15RA (rs8177743; chr10:5995052; C/T genotype in the “typical” progressors.

Table 3: Correlation between viral load and SNPs.

| Gene   | Chromosome | Position   | Reference genotype | SNP Seq | Number | p value |
|--------|------------|------------|--------------------|---------|--------|---------|
| IL15RA | 10         | 5995052    | C                  | CT      | 6      | 3.36E–06 |
| KIR2DL1| 19         | 55286864   | A                  | AC      | 5      | 0.01    |
| KIR2DL3| 19         | 55251049   | A                  | AG      | 8      | 0.04    |
| TLR7   | X          | 12903659   | A                  | TT      | 5      | 0.02    |
| TRIM5  | 11         | 5701074    | C                  | CA      | 6      | 0.04    |

5. Discussion

Without antiretroviral therapy, the majority of those infected with HIV develop uncontrolled viremia and undergo progressive immune impairment ultimately leading to AIDS within three years of their diagnosis [24, 25]. However, a small number of HIV-infected individuals are characterized by a slow progression of HIV infection where CD4 counts are maintained above 350 cells/µL without the development of AIDS for decades [26–28]. Several mechanisms have been suggested to explain this phenomenon including viral mutation, immune response, and host genetic factors. Since the virus relies heavily on the host’s cellular machinery for replication, initial studies have focused on the host’s genetic factors in an effort to explain these observations. For instance, screening of genetic variants in selected genes led to the discovery of a 32 bp deletion (CCR5Δ32) in the C-C chemokine receptor type 5 (CCR5) gene, which codes for a coreceptor used by the virus to enter the host cells [29, 30]. It has been demonstrated that the CCR5Δ32 genotype delays HIV progression in heterozygous individuals and prevents infection in homozygous individuals [31, 32]. Discovery of the CCR5Δ32 genotype provided compelling evidence to support the role of host genetic factors in the pathogenesis of HIV and AIDS. Since then, many studies have been conducted
to identify additional genetic markers that influence disease progression.

Using a combination of analyses including genome-wide association studies (GWAS), many SNPs have been identified that associate with different forms of HIV progression. For example, it has been shown that long-term nonprogressors have a higher frequency of the HLA B allele that associate with different forms of HIV progression. GWAS, many SNPs have been identified to identify additional genetic markers that influence disease progression.

Table 4: Correlation between disease progression and SNPs.

| Gene   | Chromosome | Position | Reference genotype | SNP Seq number | p value |
|--------|------------|----------|--------------------|----------------|---------|
| TRIM5  | 11         | 5688940  | C                  | CT             | 15      | 0.1005 |
|        |            |          |                    | CC             | 80      |        |
| OAS2   | 12         | 113440921| T                  | AA             | 7       | 0.01936|
|        |            |          |                    | GA             | 6       | 0.04115|
|        |            |          |                    | GG             | 82      |        |
| OAS3   | 12         | 113376388| G                  | GA             | 62      | 0.09112|
|        |            |          |                    | GG             | 33      |        |
| KIR2DS4| 19         | 55358734 | G                  | GA             | 62      | 0.09112|
|        |            |          |                    | GG             | 33      |        |
| IL15RA | 10         | 5995052  | C                  | CT             | 6       | 0.07974|
|        |            |          |                    | CC             | 89      |        |

In this study, we have analyzed the complete sequence of 23 innate immune genes from 94 individuals with HIV/AIDS. The genes studied were divided into three groups: one group pertained to NK cell function and included genes of KIR family as well as IL15RA. The second group encompassed genes coding for the intrinsic antiviral factors OAS, RNASEL, TLRs, and TRIMs. Finally, the third group included the downstream type I interferon response transcription factors TRIF, MYD88, TRAF6, and IRF7. The Ampliseq method employed in this study utilizes a high throughput sequencing approach to identify known as well as novel SNPs. Correlation analyses were performed between the 649 identified SNPs and clinical laboratory findings such as CD4 counts, anti-LPS antibody, anti-LPS glycolipid antibody, and viral load. Selection of clinical parameters was based on their role in disease progression and outcome. Changes in CD4 counts determine the course of HIV infection and establish a starting point for HAART [39]. Presence of anti-LPS and anti-LPS glycolipid antibodies reflect the integrity of gut epithelium, which is affected as HIV infection progresses [40]. Viral load is a diagnostic marker for HIV infection as well as being used to monitor disease progression and efficacy of HAART [41, 42].

We observed the following SNPs, at the respective chromosomal positions, to be associated with viral load: IL15RA (rs2229135; chr10:5995052), TLR7 (rs179008; chrX:12903659), TRIM5 (rs11601507; chr11:5701074), KIR2DL1 (rs77397437; chr19:55286864), and KIR2DL3 (chr19:55251049). The SNP rs2229135 of IL15RA occurs in the three prime untranslated region (3'-UTR) of the gene and thus does not result in an amino acid change. However, SNPs that occur in this region can influence translation efficiency, polyadenylation, localization, and mRNA stability of their respective gene [43, 44]. Given the role of IL15 signaling in NK cell function, altered translation of IL15RA may broadly impact NK-cell-mediated antiviral immunity. In contrast to the IL15RA polymorphism, the SNPs observed in TLR7 and TRIM5 both result in missense substitutions. The SNP rs179008 in TRL7 leads to a nonsynonymous Gln11Leu substitution within exon of the gene. Previous studies have reported this polymorphism is associated with chronic hepatitis C virus infection and systemic lupus erythematosus [45, 46]. Additionally, in support of a role for this polymorphism in HIV and in further support of our observations, Beima-Sofie et al. reported an association between a the TLR7 321 (rs179008) allele and time to mortality in female infants infected [47]. The SNP rs1601507 in TRIM5 leads to a nonsynonymous Val112Phe missense substitution; however, previous studies addressing the potential role for this SNP and HIV found no association [48, 49]. However, other SNPs identified in the TRIM5 report that some SNPs in this gene may be protective [50]. Notwithstanding, our data support the notion that cellular mechanisms involving TRIM5 and TLR7 are important in controlling HIV infection.

Two significant SNPs were identified in two KIR genes that also associated with viral load-KIR2DL1 (rs77397437; chr19:55286864) and KIR2DL3 (chr19:55251049). The rs77397437 SNP in KIR2DL1 leads to the synonymous substitution Pro206, while the previously nonannotated SNP KIR2DL3 (chr19:55251049) occurs in an intron. Although other studies have identified KIR associations with HIV infection, to the best of our knowledge, our data represents the first association with these specific polymorphisms and HIV viral load.

Also occurring within introns, two additional SNPs showed significance when analyzed in relation to disease progression-OAS2 (rs2072137; chr12:113440921) and OAS3
We observed the OAS2 T/C and C/C genotypes (rs2072137; chr12:113440921) to be associated with "typical" disease progression; however, the T/T genotype presented with higher frequency in the slow progressors. It is well documented that SNPs residing within introns, or those upstream or downstream of genes, also have the capacity to be causal [51–54]. In fact, in a recent study, Farh and colleagues utilized a fine-mapping algorithm to analyze GWAS data for 21 autoimmune diseases and reported that approximately 90% of all causal variants map to noncoding regions [55]. They further reported that only 10–20% of causal SNPs directly alter recognizable transcription factor binding motifs.

Our results also provide evidence of an association between various KIRs and HIV progression. A large body of evidence suggests a role for KIR3DS1 and its HLA ligand Bw4 in protection from HIV infection and delayed HIV progression [56, 57]. For instance, Alter et al. demonstrated that KIR3DS1+ NK cells are more potent in killing HIV-infected Bw4+80I+ CD4 T cells [18]. Additionally, an epidemiological study by Martin et al. revealed an association between slower HIV progression and the expression of KIR3DL1 [58]. It has been suggested that KIR3DL1 could play a role in "educating" NK cells, with higher expression of this KIR, promoting the generation of a larger pool of functionally competent NK cells, thus establishing a more vigorous response towards infection [59, 60]. Previous data pertaining to SNPs in KIRs has been limited to demonstrating that the variations in KIR2DS3 were associated with sustained viral response to pegylated-IFN and ribavirin treatment in HIV cases coinfection [59, 60].

In conclusion, we identified association between previously reported SNPs in IL15RA, TLR7, TRIM5, KIR2DL1, and OAS2 with maintenance of viral load and disease progression. However, further studies will be required to firmly establish the relationship these SNPs and the course of the disease.

The role of TRIM5 in controlling HIV replication is well established [62–64]. Previous studies have shown that the presence of SNPs in TRIM5 exon2 and linker regions attenuates its ability to control viral replication [65, 66]. Our data provides further support for a TRIM5 involvement in controlling HIV replication and disease progression. We identified two SNPs, (rs11601507; chr11:5701074) and (rs11038628; chr11:5688940), which have a greater frequency in HIV cases with high viral load and "typical" progression of the disease. Further studies of HIV cases with these SNPs may provide additional support for their potential application as biological markers of disease progression.

Perhaps our most intriguing observation was the association between viral load, disease progression, and IL15RA. IL15RA binds specifically to IL-15, which is required for the differentiation of NK cells, CD8+ lymphocytes, and memory CD8+ T cells [67, 68]. Importantly, Naora and Gougeon demonstrated that when used in vitro, IL-15 stimulates the proliferation of CD56+, CD16+, CD4+, and CD8+ T cells from HIV-infected individuals [69]. They also showed that IL-15 was more potent than IL-2 as a survival factor for CD56+ cells, and this effect was associated with upregulation of Bcl-2 expression. Taken together, these findings indicated that IL-15 plays a pivotal role in survival and proliferation of NK cells in control of HIV maintenance and disease propagation. Our SNP data with regard to the IL15RA identifies a potential mechanism for the regulation of NK cell activity in HIV-infected individuals, in addition to presence of KIR SNPs. However, these previously unreported observations would require further in vitro and clinical evaluation to confirm their involvement in HIV disease progression.

Our data on the association of SNPs in genes coding for OAS2 and OAS3 with disease progression represents an additional and potentially novel finding. The OAS enzymes are interferon-inducible proteins required for the activation of RNase L [70]. While they become activated by the transcription responsive region of HIV-1 mRNA, OAS activation is prevented by viral Tat binding to TAR [71–73]. Furthermore, RNase L inhibitor is activated in HIV-1-infected cells down-regulating the OAS/RNase L pathway [74]. Therefore, it is not surprising that OAS enzymes are activated in HIV-infected cells as part of innate antiviral response and the efficacy of antiviral defense will, in part, depend on the ability of infected cells to prevent OAS inhibition. Our data on association between HIV disease progression and SNPs in OAS genes provides additional evidence of a role for these genes in HIV progression; however, additional studies are needed to firmly establish importance of these SNPs in HIV pathogenesis.

The prevalence of a given allele, whether it is protective or causative, may contribute to the prevalence of a disease. The Global Minor Allele Frequency (MAF) of rs2229135 for IL15RA is 0.09 [75]. In contrast, the same allele has a frequency of 0.11 in African populations, 0.05 in East Asian populations, and 0.08 in European populations [75]. The MAP of rs2072137 for OAS2 has minor allele frequency in America, East Asia, and Europe of 0.48, 0.38, and 0.42, respectively, but only 0.06 in African populations [75]. Even more striking, the MAF of rs179008 for TLR7 is 0.12 and a minor allele frequency of 0.23 in European populations but is only 0.05 in Southern Asian populations and is virtually zero in East Asian populations [75]. Larger studies that investigate clinical presentations, such as disease progression, in concert with genetic screening, may provide further support for the role of these genes in the pathogenesis of HIV/AIDS.

In summary, we have identified multiple SNPs that associate with viral load in the following genes: IL15RA (rs2229135; chr10:5995052), TLR7 (rs179008; chrX:12903659), TRIM5 (rs11601507; chr11:5701074), KIR2DL1 (rs77397437; chr19:55286864), and KIR2DL3 (chr19:55251049). Furthermore, two SNPs, OAS2 (rs2072137; chr12:113440921) and OAS3 (chr12:113376388), were identified to associate with the progression of HIV infection. Finally, SNPs in three genes demonstrated associations with disease progression including TRIM5 (rs1038628; chr1:5688940), KIR2DS4 (chr19:55358734), and IL15RA (rs2229135; chr10:5995052). If confirmed by future studies, these data may reveal important therapeutic targets for vaccine development and provide a greater understanding of disease pathogenesis.

6. Conclusions

In conclusion, we identified association between previously unreported SNPs in IL15RA, TLR7, TRIM5, KIR2DL1, and
KIR2DL3 and an increased viral load. Additionally, novel SNPs that associated with progression from HIV infection to AIDS were identified in OAS2 and OAS3 genes. In contrast, other SNPs identified in OAS2 and OAS3 genes, as well as in the TRIM5 and KIR2DS4 genes, were associated with a slower progression of disease. Further studies will determine the significance of identified SNPs in pathogenesis of HIV infection and AIDS.

Competing Interests
The authors declare no conflict of interests.

Acknowledgments
This study was supported, in part, by Russian Science Foundation Grant 15-14-00016. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities. Some of the experiments were conducted using the equipment of the Interdisciplinary Center for Collective Use of Kazan Federal University, supported by the Ministry of Education of Russia (1D RFMEFI59414X0003), Interdisciplinary Center for Analytical Microscopy and Pharmaceutical Research and Education Center, Kazan (Volga Region) Federal University, Kazan, Russia. An award from the National Institutes of Health (NIH) supported work in the National Institutes of Health (NIH) supported work conducted in the laboratory of Dr. Lombardi (Grant R01 AI078234). The authors thank the Nevada INBRE Bioinformatics Core for their help in analyzing the Ampliseq data.

References
[1] H. W. Murray, I. H. Godbold, K. B. Jurica, and R. B. Roberts, “Progression to AIDS in patients with lymphadenopathy or AIDS-related complex: reappraisal of risk and predictive factors,” The American Journal of Medicine, vol. 86, no. 5, pp. 533–538, 1989.
[2] J. F. Okulicz, V. C. Marconi, M. L. Landrum et al., “Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US department of defense HIV natural history study,” Journal of Infectious Diseases, vol. 200, no. 11, pp. 1714–1723, 2009.
[3] M. J. Dolan, H. Kulkarni, J. F. Camargo et al., “CCL3L1 and CCR5 influence cell-mediated immunity and affect HIV-AIDS pathogenesis via viral entry-independent mechanisms,” Nature Immunology, vol. 8, no. 12, pp. 1324–1336, 2007.
[4] S. K. Ahuja, H. Kulkarni, G. Catano et al., “CCL3L1-CCR5 genotype influences durability of immune recovery during antiretroviral therapy of HIV-1-infected individuals,” Nature Medicine, vol. 14, no. 4, pp. 413–420, 2008.
[5] C. Tomescu, S. Abdulhaq, and L. J. Montaner, “Evidence for the innate immune response as a correlate of protection in human immunodeficiency virus (HIV)-1 highly exposed seronegative subjects (HESN),” Clinical and Experimental Immunology, vol. 164, no. 2, pp. 158–169, 2011.
[6] K. M. Cheney and A. Mcknight, “Interferon-alpha mediates restriction of human immunodeficiency virus type-1 replication in primary human macrophages at an early stage of replication,” PLoS ONE, vol. 5, no. 10, Article ID e13521, 2010.
[7] V. C. Lombardi and S. F. Khaiboullina, “Plasmacytoid dendritic cells of the gut: relevance to immunity and pathology,” Clinical Immunology, vol. 153, no. 1, pp. 165–177, 2014.
[8] A. I. Papadopoulos, B. Ferwerda, A. Antoniadou et al., “Association of toll-like receptor 4 Asp299Gly and Thr399Le polymorphisms with increased infection risk in patients with advanced HIV-1 infection,” Clinical Infectious Diseases, vol. 51, no. 2, pp. 242–247, 2010.
[9] M. G. Netea, C. A. A. Van der Graaf, A. G. Vonk, I. Verschuere, J. W. M. Van der Meet, and B. J. Kullberg, “The role of toll-like receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis,” Journal of Infectious Diseases, vol. 185, no. 10, pp. 1483–1489, 2002.
[10] B. Ferwerda, M. B. McCall, S. Alonso et al., “TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans,” Proceedings of the National Academy of Sciences of the United States of America, vol. 104, no. 42, pp. 16645–16650, 2007.
[11] A. Carvalho, A. C. Pasqualotto, L. Pitzurra, L. Romani, D. W. Denning, and F. Rodrigues, “Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis,” Journal of Infectious Diseases, vol. 197, no. 4, pp. 618–621, 2008.
[12] N. Soriano-Sarabia, A. Vallejo, R. Ramirez-Lorca et al., “Influence of the Toll-like receptor 9 1635A/G polymorphism on the CD4 count, HIV viral load, and clinical progression,” Journal of Acquired Immune Deficiency Syndromes, vol. 49, no. 2, pp. 128–135, 2008.
[13] A. Vallejo, A. Valladares, B. De Felipe et al., “High thymic volume is associated with viral replication and immunologic impairment only early after HAART interruption in chronic HIV infection,” Viral Immunology, vol. 18, no. 4, pp. 740–746, 2005.
[14] M. L. LaBonte, P. F. McKay, and N. L. Letvin, “Evidence of NK cell dysfunction in SIV-infected rhesus monkeys: impairment of cytokine secretion and NKG2C/C2 expression,” European Journal of Immunology, vol. 36, no. 9, pp. 2424–2433, 2006.
[15] G. Alter, N. Teigen, B. T. Davis et al., “Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection,” Blood, vol. 106, no. 10, pp. 3366–3369, 2005.
[16] D. Mavilio, G. Lombardo, A. Kinter et al., “Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection,” Journal of Experimental Medicine, vol. 203, no. 10, pp. 2339–2350, 2006.
[17] A. De Maria, M. Fogli, P. Costa et al., “The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44),” European Journal of Immunology, vol. 33, no. 9, pp. 2410–2418, 2003.
[18] G. Alter, M. P. Martin, N. Teigen et al., “Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes,” Journal of Experimental Medicine, vol. 204, no. 12, pp. 3027–3036, 2007.
[19] B. Langmead and S. L. Salzberg, “Fast gapped-read alignment with Bowtie 2,” Nature Methods, vol. 9, no. 4, pp. 357–359, 2012.
[20] H. Li, B. Handsaker, A. Wysoker et al., “The sequence alignment/map format and SAMtools,” Bioinformatics, vol. 25, no. 16, pp. 2078–2079, 2009.
[21] Y. Benjamini and Y. Hochberg, “Controlling the false discovery rate: a practical and powerful approach to multiple testing,”
J. B. Margolick, A. Muñoz, D. Vlahov et al., “Changes in T-lymphocyte subsets in intravenous drug users with HIV-1 infection,” The Journal of the American Medical Association, vol. 267, no. 12, pp. 1631–1636, 1992.

M. D. Hughes, D. S. Stein, H. M. Gundacker, F. T. Valentine, J. P. Phair, and P. A. Volberding, “Within-subject variation in CD4 lymphocyte count in asymptomatic human immunodeficiency virus infection: implications for patient monitoring,” Journal of Infectious Diseases, vol. 169, no. 1, pp. 28–36, 1994.

A. Audigé, P. Taffé, M. Rickenbach et al., “Low postseroconversion CD4 count and rapid decrease of CD4 density identify HIV+ fast progressors,” AIDS Research and Human Retroviruses, vol. 26, no. 9, pp. 997–1005, 2010.

H. Farzadegan, D. R. Henrard, C. A. Kleeberger et al., “Virologic and serologic markers of rapid progression to AIDS after HIV-1 seroconversion,” Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, vol. 13, no. 5, pp. 448–455, 1996.

H. W. Sheppard, W. Lang, M. S. Ascher, E. Vittinghoff, and W. Winkelstein, “The characterization of non-progressors: long-term HIV-1 infection with stable CD4+ T-cell levels,” AIDS, vol. 7, no. 9, pp. 1159–1166, 1993.

M. Bakari, W. Urassa, F. Mhalu, G. Biberfeld, K. Pallangyo, and E. Sandström, “Slow progression of HIV-1 infection in a cohort of antiretroviral naïve hotel workers in Dar es Salaam, Tanzania as defined by their CD4 cell slopes,” Scandinavian Journal of Infectious Diseases, vol. 40, no. 5, pp. 407–413, 2008.

J. Fellay, K. V. Shianna, D. Ge et al., “A whole-genome association study of major determinants for host control of HIV-1,” Science, vol. 317, no. 5840, pp. 944–947, 2007.

R. Liu, W. A. Paxton, S. Choe et al., “Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection,” Cell, vol. 86, no. 3, pp. 367–377, 1996.

M. Carrington, M. Dean, M. P. Martin, and S. J. O’Brien, “Genetics of HIV-1 infection: chemokine receptor CR5 CR3 polymorphism and its consequences,” Human Molecular Genetics, vol. 8, no. 10, pp. 1939–1945, 1999.

C. Quillent, E. Oberlin, J. Braun et al., “HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CR5 gene,” Lancet, vol. 351, no. 9095, pp. 14–18, 1998.

G. J. Stewart, L. J. Ashton, R. A. Biti et al., “Increased frequency of CCR-5 delta 32 homozygotes among long-term non-progressors with HIV-1 infection. The Australian Long-Term Non-Progressor Study Group,” AIDS, vol. 11, no. 15, pp. 1833–1838, 1997.

F. Pereyra, X. Jia, P. J. McLaren, A. Telenti, P. I. W. De Bakker, and B. D. Walker, “The major genetic determinants of HIV-1 control affect HLA class I peptide presentation,” Science, vol. 330, no. 6010, pp. 1551–1557, 2010.

S. A. Migueles, M. S. Sabbaghian, W. L. Shupert et al., “HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors,” Proceedings of the National Academy of Sciences of the United States of America, vol. 97, no. 6, pp. 2709–2714, 2000.

M. Stern, K. Czaja, A. Rauch et al., “HLA-Bw4 identifies a population of HIV-infected patients with an increased capacity to control viral replication after structured treatment interruption,” HIV Medicine, vol. 13, no. 10, pp. 589–595, 2012.

S. Gaudieri, D. DeSantis, E. McKinnon et al., “Killer immunglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression,” Genes & Immunity, vol. 6, no. 8, pp. 683–690, 2005.

Y. Qi, M. P. Martin, X. Gao et al., “KIR/HLA pleiotropism: protection against both HIV and opportunistic infections,” PLoS Pathogens, vol. 2, no. 8, p. e79, 2006.

Y. Jiang, O. Chen, C. Cui et al., “KIR3DS1/L1 and HLA-Bw4-801 are associated with HIV disease progression among HIV typical progressors and long-term nonprogressors,” BMC Infectious Diseases, vol. 13, article 405, 2013.

A. C. Lepri, A. N. Phillips, A. D. Monforte et al., “When to start highly active antiretroviral therapy in chronically HIV-infected patients: evidence from the ICONA study,” AIDS, vol. 15, no. 8, pp. 983–990, 2001.

P. Kelly, T. Shawa, S. Mwanamakondo et al., “Gastric and intestinal barrier impairment in tropical enteropathy and HIV: limited impact of micronutrient supplementation during a randomised controlled trial,” BMC Gastroenterology, vol. 10, article 72, 2010.

M. Duong, L. Piroth, G. Peytavin et al., “Value of patient self-report and plasma human immunodeficiency virus protease inhibitor level as markers of adherence to antiretroviral therapy: relationship to virologic response,” Clinical Infectious Diseases, vol. 33, no. 3, pp. 386–392, 2001.

J.-C. Schmit and B. Weber, “Recent advances in antiretroviral therapy and HIV infection monitoring,” Intervirology, vol. 40, no. 5-6, pp. 304–321, 1998.

L. W. Barrett, S. Fletcher, and S. D. Wilton, “Regulation of euakaryotic gene expression by the untranslated gene regions and other non-coding elements,” Cellular and Molecular Life Sciences, vol. 69, no. 21, pp. 3613–3634, 2012.

X. Pichon, L. A. Wilson, M. Stoneley et al., “RNA binding protein/RNA element interactions and the control of translation,” Current Protein and Peptide Science, vol. 13, no. 4, pp. 294–304, 2012.

E. Askar, G. Ramadori, and S. Mihm, “Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection,” Journal of Medical Virology, vol. 82, no. 11, pp. 1859–1868, 2010.

B. P. dos Santos, J. V. Valverde, P. Rohr et al., “TLR7/8/9 polymorphisms and their associations in systemic lupus erythematosus patients from southern Brazil,” Lupus, vol. 21, no. 3, pp. 302–309, 2012.

K. M. Beima-Sofie, A. W. Bigham, J. R. Lingappa et al., “Toll-like receptor variants are associated with infant HIV-1 acquisition and peak plasma HIV-1 RNA level,” AIDS, vol. 27, no. 15, pp. 2431–2439, 2013.

V. Goldschmidt, G. Bleiber, M. May, R. Martinez, M. Ortiz, and A. Telenti, “Role of common human TRIM5α variants in HIV-1 disease progression,” Retrovirology, vol. 3, article no. 54, 2006.

J. H. Jovanbakh, P. An, B. Gold et al., “Effects of human TRIM5α polymorphisms on antiretroviral function and susceptibility to human immunodeficiency virus infection,” Virology, vol. 354, no. 1, pp. 15–27, 2006.

H. Price, P. Lacap, J. Tuff et al., “A TRIM5α exon 2 polymorphism is associated with protection from HIV-1 infection in the Pumwani sex worker cohort,” AIDS, vol. 24, no. 12, pp. 1813–1821, 2010.
