Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 25/11/2019;
Date Revised: 21/02/2020;
Date Published Online: 25/02/2020;

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**How to Cite:**
Ali Q, Jamal A, Ullah S, Waqar AB (2020). Epidemiology, incidence and mortality of Nasopharynx Cancer in Southeast Asia: an update report. Adv. Life Sci. 7(2): 91-97.

**Keywords:**
HIV; AIDS; APOBEC3G; Polymorphism

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# Male predominant association with Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G variants (rs6001417, rs35228531, rs8177832) predict protection against HIV-1 infection

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## Abstract

Background: Human immunodeficiency virus (HIV) infection, it is a global health concern mainly lead to acquired immune deficiency syndrome (AIDS). There are numerous limitations of this infection particularly in the form of host factors which may limit and interfere HIV-1 replication. The most notable host factors which hinder HIV-1 DNA propagation is the apolipoprotein B mRNA-editing enzyme, catalytic polypeptide- like 3G (APOBEC3G). Any genetic polymorphism of this substantial host factor may impact the host susceptibility pattern to HIV viral infection in different part of the world. The aim of this study to examine genetic variants (rs6001417, rs35228531, rs8177832) effecting HIV-1 infection.

Method: Three variants of APOBEC3G gene polymorphism were genotyped while using RT-PCR method. Frequency distribution of these genotypes was evaluated in both the HIV-1 and healthy group.

Results: The rs6001417 CG (p = 0.03) and rs35228531 CT (p = 0.01) genotypes were found as protective elements, while rs35228531 TT (p = 0.02) and rs8177832 AA (p = 0.03) genotypes had shown susceptibility against the HIV-1 infection. Our data suggest, rs35228531 CT (p = 0.003) and rs8177832 AA (P = <0.001) genotypes have predominant incidences in HIV-1 male population than healthy control.

Conclusion: We predict rs6001417 CG, rs35228531 CT as protective and rs35228531 TT, rs8177832 AA genotypes as a predisposing tool, against the HIV-1 infection in a section of Pakistani population. In addition, male gender was found predominantly high in both protective genotype rs35228531 CT ( p = 0.003) and predisposing genotype rs8177832 AA ( p = <0.001). The predominant contribution may help the patient to be predict about the status of HIV infection, however, extra efforts are required to study larger cohort of patients to better elucidate the association.

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## Introduction

HIV-1 infection is a complex and global health dilemma. Due to its fatal nature, approximately 37 million individuals worldwide are infected with HIV, with each passing year 3.1 million new cases emerge [1-3]. Recent reports suggest that HIV infection is highly prevalent in Sub-Saharan countries [4], however currently in Pakistan it is the leading cause of morbidity and mortality as it hits approximately 83,468 individuals. Major causes of HIV transmission are contaminated blood and sexual contacts [5,6].

The human immune system provides resistance against foreign objects through numerous immune strategies, leading to hindrance and elimination of foreign objects. In the process, advance and novel immune mechanisms are evolved [7-11]. Advancements in human genetic studies elucidate that both viral and host genetic factors are critically involved in genetic susceptibility or protection toward the infectious agents [12-14]. Moreover, the individuals with homozygousΔ32 allelic variant of the CCR5 protein have significant and influential outcome, however it does not exhibit complete resistance to HIV infection. Interestingly, truncated CCR5 is not a known co-receptor for HIV virus, yet it provides protection against HIV infection [15,16]. Also, the ethnic differences have crucial role in host predisposition and/or protection against the disease [17-19]. Subsequently, relevance of these genetic and ethnic differences to a particular disease has started to fascinate the researchers worldwide and thus manifesting the better understanding of their potential role in HIV infection or disease progression.

Mounting body of evidence suggests that multiple host factors affect pathogenesis of HIV-1/AIDS. Likely factors include; Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G), Chemokine Receptor 5 (CCR-5), Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), Tripartite motif 5a (TRIM5a), Tetherin, and SAM-domain HD-domain containing protein (SAMHD1) [5,20-23]. The possible defense mechanisms exhibited by these elements are antagonized by accessory viral proteins [24,25].

The association of gender with spontaneous clearance of HCV has already been reported showing link between female gender and rs12979860 CT genotype and facilitation in the spontaneous clearance of hepatitis C virus potentially [26]. It has been also reported that TNFAIP3 rs2230926G may be preliminary predictor of systemic lupus erythematous onset in males [27].

APOBEC3G (antiviral Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G) located on chromosome #22q13 [28] is a host intrinsic element, which combats HIV-1 infection [29]. Virion infectivity factor (vif), a structural part of HIV-1 is able to counteract APOBEC3G antiviral activity by targeting it via proteasomes [30]. The vif protein derived from different subtypes, A, B, CRF01_AE, and CRF02_AG, portray comparable anti APOBEC3G activity. On contrary subtype C vif protein depicts maximal anti APOBEC3G activity [31]. The APOBEC3G protein demonstrates its antiviral action by incorporating itself into newly synthesized viral particles, in the absence of the vif, triggering deamination of cytosine (C) to uracil (U) in newly synthesized mutant viral particles. APOBEC3G polymorphisms, such as (H186R) rs8177832 are associated with pathogenesis in different ethnic groups and various HIV-1 subtypes [32,33], however this association is not reported in other populations [33]. The previous studies did not take concern with circulating recombinant forms of HIV-1, nor examine the effect of APOBEC3G polymorphisms in Pakistani ethnic groups.

The emphasis of present study is to understand and evaluate the role of rs6001417, rs35228531, rs8177832 variants of APOBEC3G polymorphism in HIV-1 infection with circulating recombinant in Pakistani population.

## Methods

This study included a total of 206 individuals involving 100 HIV-1 seropositive and 106 HIV-1 sero-negative individuals. Both groups were obtained in the Imperial Diagnostic & Research Center-Imperial College of Business Studies Lahore, Pakistan. This study was approved by the Institutional Ethical Review Board (IERB), Faculty of Health and Allied Sciences (FHAS), Imperial College of Business Studies (ICBS), Lahore. After obtaining the written consent, whole blood was collected from each participant in EDTA container.

### HIV-1 Detection

Plasma samples obtained by centrifugation at 3500 rpm for 5 minutes and were tested for HIV–1 infection, using Alere determine HIV-1 or 2 tests (Product code: 7D2346 CE Chicago, Illinois, United States) as per previously reported protocol [34].

### Genotypic determination

The extraction of genomic DNA was performed from whole blood leukocytes, using a non-enzymatic salting-out method [35]. APO-BEC-3G (rs6001417, rs35228531, rs8177832) loci were determined using Fast Real-Time PCR (RT-PCR) Systems (Applied Bio-system Step One TM).

The 184bp DNA fragment of three variants APO-BEC-3G (rs6001417, rs35228531, rs8177832) was amplified by using (SYBR GreenER qPCR super MIX Universal kit Invitrogen USA). The primer sequences used are given in Table 1. The total volume of PCR mixture was 25ul, containing DNA template (1 ul), each primer (1 ul) and 10 ul commercial super Mix containing Taq-polymerase, MgCl2, dNTPs, Syber green fluorescent dye and PCR buffer. Thermocycling was performed as; initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 55°C for 30s and extension at 68°C for 30s.The final extension was performed at 72°C for 07 minutes.

The amplified products were run on 2% agarose gel along with 50 bp ladder (Fermentas USA) and visualized under Wealtec Gel doc system (Fermentas USA).

### Statistical Analysis:

The data was shown as Mean ± SD. The frequencies of different genotypes were calculated by direct counting. The Variables in the category were compared in different groups by using the Chi - square test. All statistical analyses were done by using SPSS 22. The independent t –test was applied for the demonstration of quantitative variation in both the patient and control groups. P-value < 0.05 was considered statistically significant.

| Variant's names | Forward primers | Reverse primers |
|---|---|---|
| rs6001417 | CGCGTGCCACCATGAAGATC | GTTACAGTCAGCGCCAGACCT |
| | GTCCGCGTGCCACCATGAAGATG | |
| rs35228531 | GCGACAATTTGAATCGGT | GAGGGATGTCGTGTGACCTT |
| | GCGACAATTTGAATCGGC | |
| rs8177832 | GAGCCTTGGAATAATCTGCCTA | AGGGAGACCCTCACCTGAGAA |
| | TTGAGCCTTGGAATAATCTGCG | |

**Table 1:** Primers of APO-BEC-3G polymorphism genotyping

Note: This table shows two forward primers and one reverse primer for each variant of APO-BEC-3G gene polymorphism

## Results

Generally, in 2018, a total of 129,079 new A total of 206 individuals were recruited in current study. The population investigated consisted of 100 HIV-1 infected patients and 106 healthy controls. Age and gender wise distribution of the positive cases and healthy groups are shown in Table 2. The mean ages were 39.98±11.38 and 35.94±9.84 in the control and HIV-1 groups respectively. Genotype frequencies of all the three APOBEC3G loci (rs6001417, rs35228531, rs8177832) for both groups are shown (Table 2).

| APO-BEC-3G variants | Control (n = 106) | Cases (n = 100) | *P*-value |
|---|---|---|---|
| **rs6001417 Genotypes** | | | |
| CC | 50 (47.2%) | 66 (66.0%) | 0.13 |
| CG | 45 (42.5%) | 27 (27.0%) | 0.03 (protective) |
| GG | 11 (10.4%) | 07 (7.0%) | 0.34 |
| **rs35228531** | | | |
| CC | 56 (52.8%) | 58 (58.0%) | 0.85 |
| CT | 44 (41.5%) | 25 (25.0%) | 0.01 (protective) |
| TT | 06 (5.7%) | 17 (17.0%) | 0.02 (risk toward the disease) |
| **rs8177832** | | | |
| AA | 43 (40.6%) | 66 (66.0%) | 0.03 (risk toward the disease) |
| AG | 41 (38.7%) | 26 (26.0%) | 0.08 |
| GG | 22 (20.8%) | 08 (08.0%) | 0.06 |
| **Gender** | | | |
| Male | 67 (63.2%) | 54 (76.0%) | |
| Female | 39 (36.8%) | 46 (24.0%) | 1.4 |
| Age | 39.98±11.38 | 35.94±9.84 | 0.1 |

**Table 2:** Association of HIV-1 cases with APO-BEC-3G variants genotypes in 100 patients

Note: This table shows the associations of HV-1 and APO-BEC-3G genotypes. Use HIV-1 individuals as the dependent variable, APO-BEC-3G variant's genotypes as independent variables. So, rs6001417 CG genotype is significantly associated with a higher number than GG and CC genotype in both the groups, the rs6001417 CG and rs35228531 CT genotypes may help and predict the protective role in Healthy individuals. Moreover, it is also predicted a predisposing role of and rs35228531 TT and rs8177832 AA genotypes against the HIV-1 infection.

### Association between APOBEC3G rs6001417 variant and HIV-1 status

We found that the frequencies of APOBEC3G (rs6001417) CC, CG, and GG genotypes were 66.0%, 27.0%, 7.0% in HIV-1 and 47.2%, 42.5% and 10.4% in the control group (Table 2). Individuals reported positive for HIV-1 infection had lower frequencies of the APOBEC3G (rs6001417) CG, GG genotypes, whereas, CC genotype was increased in HIV-1 patients compared to controls. Thus, higher prevalence of CG genotype in the control group might indicate its possible protective role against HIV-1 infection (45 (42.5%) vs 27 (27.0%), p = 0.03) (Figure 1).

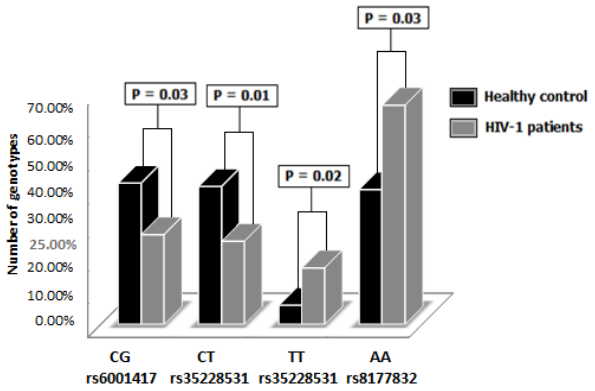


**Figure 1:** Association of protective rs6001417 CG and rs35228531 CT genotypes and predisposing rs35228531 TT Allele toward the HIV-1 status analysis.

Note: Genotypes frequency were noted by using Pearson chi square test analysis, which also predict the significance level (<0.05) of genotypes among the healthy and infected individuals and data is shown in percentages.

### Association between APOBEC3G rs35228531 variant and HIV-1 status

APOBEG3G (rs35228531) CC, CT and TT genotypes were found to be 52.8%, 41.5%, 5.7% and 58.0%, 25.0%, 17.0% in control and HIV-1 positive individuals, respectively (Table 2). The levels of CC genotype were comparable in both control and infected group (p = 0.85), however, CT genotype was significantly higher in control compared to the infected group (41.5% vs 25.0%, p = 0.01).

Furthermore, our data suggest that TT genotype frequency was significantly increased in HIV-1 positive cases compared to control group (17.0% vs 5.7%, p = 0.02). (Figure 1).

### Association between APOBEC3G rs8177832 variant and HIV-1 status

APOBEG3G (rs8177832) AA, AG and GG genotypes were found as 40.6%, 38.7%. 20.8% and 66.0%, 26.0%, 08.0% in control and HIV-1 positive individuals, respectively (Table 2). The levels of AG and GG genotypes were comparable in both control and infected groups (p = 0.08 and p = 0.06, respectively), however, AA genotype was significantly lower in control compared to the infected group (40.6% vs 66.0%, p = 0.03) (Figure 1).

Furthermore, we performed correlation analysis in between all the genotypes and the three studied variants of APOBEC3G in both groups but did not find any significant correlation (Table 2).

### Analysis of gender with Protective (rs6001417) CG and (rs35228531) CT genotype

The frequency of protective genotypes of the above-mentioned variants are shown in Table 3. The frequency of rs6001417 CG genotype was slightly higher in the males but did not show statically significance (27.5% vs 45.3%), p = 0.34) (Figure 2). However, rs35228531 CT genotype prevalence was significantly higher in male group, comparatively 42.1%) vs 21.2%; p = 0.003 (Figure 2).

| APO-BEC-3G varaiants | | Male | Female | *P*-value |
|---|---|---|---|---|
| rs6001417 | Genotypes | | | |
| | CC | 75 (62.5%) | 41 (47.7%) | 0.001 |
| | CG | 34 (27.5%) | 38 (45.3%) | 0.34 |
| | GG | 12 (10.0%) | 06 (07.0%) | 0.15 |
| rs35228531 | | | | |
| | CC | 60 (49.6%) | 54 (63.5%) | 0.57 |
| | CT | 51 (42.1%) | 18 (21.2%) | 0.003 |
| | TT | 10 (08.3%) | 13 (15.3%) | 0.53 |
| rs8177832 | | | | |
| | AA | 74 (61.2%) | 35 (41.2%) | < 0.001 |
| | AG | 35 (28.9%) | 32 (37.6%) | 0.71 |
| | GG | 12 (09.9%) | 18 (21.2%) | 0.27 |

**Table 3:** Association between APO-BEC-3G polymorphism variants and gender

Note: This table shows the association of dependent variable (gender, i.e. male and female) with independent variable APO-BEC-3G polymorphism genotypes. APO-BEC-3G rs35228531 CT may link with gender Male in the protective mechanism, resp. The rs8177832 AA genotype also account for raised level of Male gender and pertaining to its predisposing role.

### Analysis of gender with Predisposing (rs35228531) TT and (rs8177832) AA genotypes

We found that the distribution of TT genotype was similar in male and female groups [male (08.3%) vs female (15.3%); p = 0.53] however, rs8177832 AA genotype levels were higher in male than female [male (61.2%) vs female (41.2%); p = < 0.001] (Figure 2).

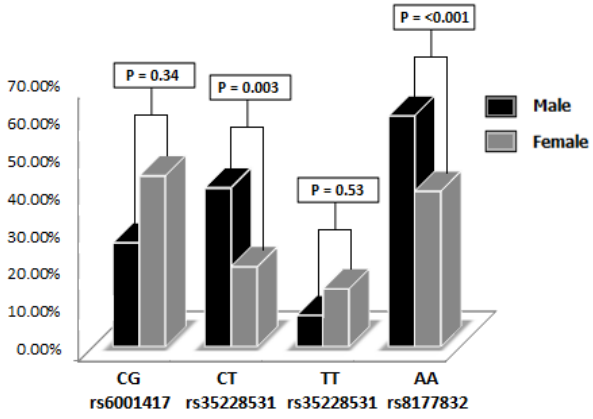


**Figure 2:** Distribution of male and female in protective and predisposing APO-BEC-3G variants genotypes.

Note: Data of genotypes is shown in percentages and chi square analysis was done for the prediction of *p*-value among the male and female gender

### Predictive value of protecting and predispose features associated with APOBEG3G variants genotypes

As we have mentioned in our earlier results sections that the protective features are linked with rs6001417 CG, rs35228531 CT, and predisposing with rs35228531 TT, rs8177832 AA genotype against HIV-1 infection. In addition, we examined the predictive value of these four genotypes as well by doing statistical analysis, using SPSS version 22.0. Both the rs6001417 CG and rs35228531 CT genotypes were appeared as protective genotypes, we also explored positive predictive value (PPV) which was 42.5% and 41.5%, respectively (Table 4), which predicts the chance in percentage to reduce the risk of being infected with HIV-1 virus. Moreover, the rs35228531 TT and rs8177832 AA genotype had shown susceptibility towards the onset of HIV-1 infection. The PPV calculated was 17.0%, 66.0%, which predicts the role of these genotypes regarding the increased risk of being infected with HIV-1 virus (Table 4).

| APO-BEC-3G variants | PPV | NPV | Specificity | Sensitivity |
|---|---|---|---|---|
| rs6001417 CG | 42.5% | 27.0% | 30.6% | 38.1% |
| rs35228531 CT | 41.5% | 25.0% | 28.7% | 36.9% |
| rs35228531 TT | 17.0% | 5.7% | 6.7% | 14.5% |
| rs8177832 AA | 66.0% | 37.0 % | 55.8% | 47.4% |
| PPV (positive predictive value), NPV (negative predictive value). | | | | |

**Table 4:** Prediction of APO-BEC-3G polymorphism genotypes in HIV-1 infection

Note: According to this table, rs6001417 CG and rs35228531 CT genotype predicting a protective role against HIV-1 disease in healthy individuals. Whereas rs35228531 TT and rs8177832 AA genotype predicting a predisposing role towards the HIV-1 individuals. We examined positive and negative predictive value of these four genotypes.

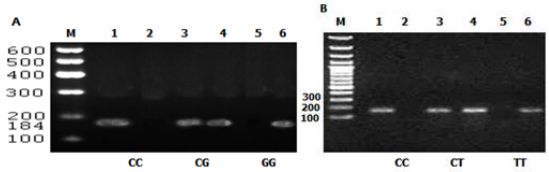


**Figure 3:** Agarose gel electrophoresis patterns for genetic variations of APO-BEC-3G gene polymorphism.

Note: The RT- PCR analysis shows an electrophoresis pattern of three genotypes. (A) Lane M, 184 bp marker; Lane 1, homozygous C genotype; Lane 2, heterozygous C/G genotype; Lane 3, homozygous G genotype of APO-BEC-3G rs6001417. (B) M lane indicates 184 bp marker; Lane 1 shows homozygous C genotype; Lane 2, heterozygous C/T genotype and Lane 3 express homozygous T genotype of APO-BEC-3G rs35228531.

### Multivariate logistic regression analysis of Male gender with protective and predisposing genotypes

We employed multi-nominal logistic regression to interpret the association between APOBEG3G variants (protective and predisposing) genotypes and gender in the healthy control and HIV-1 group and analyzed the following factors: APOBEG3G rs35228531 CT (CC and TT were taken as reference genotypes) and gender (male vs female). These factors independently and significantly contributed to protection against HIV-1 infection, rs35228531 CT vs CC (reference genotype)

(OR, 2.47; CI, 1.28 - 04.77; p = 0.007) and CT vs TT (reference genotype) (OR, 3.41; CI, 1.24 – 9.32; P = 0.017). The CT (protective genotype) (51 (42.1%) vs 18 (21.2%); (p = < 0.001) was more prevalent in male than female group (Table 5) Factor involving in the predisposition of the HIV-1 infection in rs35228531 TT (predisposed genotype) had a comparable association with male and female gender in the disease progression (effecting by CC and CT genotypes; OR, 0.37; CI, 0.1 – 1.01; P = 0.06 and OR, 0.29; CI, 0.10 – 0.80; p = 0.017, respectively) (Table 5).

| Characteristics | | OR | 95% CI | P-value |
|---|---|---|---|---|
| APO-BEC-3G variants | | - | - | - |
| Dependent variable | **Reference variable** | - | - | - |
| rs6001417 | | | | |
| **Male** | Female | 1.79 | 1.00 – 3.23 | 0.04 |
| **CC** | GG | 1.01 | 0.35 – 2.95 | 0.97 |
| **CC** | CG | 2.46 | 1.32 – 4.59 | 0.005 |
| CG | **GG** | **0.41** | **0.13 – 1.23** | **0.11** |
| CG | **CC** | **0.40** | **0.21 – 0.75** | **0.005** |
| **GG** | CC | 0.98 | 0.33 – 2.85 | 0.97 |
| **GG** | CG | 2.42 | 0.81 – 7.25 | 0.11 |
| rs35228531 | | | | |
| **CC** | TT | 1.37 | 0.55 – 3.43 | 0.49 |
| **CC** | CT | 0.40 | 0.20 – 0.77 | 0.007 |
| CT | **TT** | **3.41** | **1.24 – 9.32** | **0.017** |
| CT | **CC** | **2.47** | **1.28 – 4.77** | **0.007** |
| TT | **CC** | **0.37** | **0.1 – 1.01** | **0.06** |
| TT | **CT** | **0.29** | **0.10 – 0.80** | **0.017** |
| rs8177832 | | | | |
| AA | **GG** | **4.17** | **1.72 – 10.11** | **0.002** |
| AA | **AG** | **2.30** | **1.19 – 4.44** | **0.013** |
| **AG** | GG | 1.81 | 0.74 – 4.41 | 0.19 |
| **AG** | AA | 0.43 | 0.22 – 0.83 | 0.013 |
| **GG** | AG | 0.55 | 0.22 – 1.34 | 0.19 |
| **GG** | AA | 0.24 | 0.09 – 0.58 | 0.002 |
| **CI, Confidence interval, OR, Odd ratio, APO-BEC-3G** | | | | |

**Table 5:** Multi-nominal logistic regression analysis of Male gender to the protective and predisposing genotypes of APO-BEC-3G variants

Note: This table shows the MLM (multi logistic model) analysis, which predicted the male gender contribution, the rs35228531 CT a protective genotype strongly favors by Male than Female gender after comparing with CC and TT genotypes, whereas rs6001417 CG a protective genotype show comparable association against GG and CC genotypes with both male and female genders. The rs35228531 TT a predisposing genotype also equally contributed by both the gender than CT and TT whereas, rs8177832 AA genotype favoring by Male as well after compared with GG and AA genotypes.

Moreover, our analysis also revealed the significant effect of male gender on rs8177832 AA a predisposed genotype towards HIV-1 infection (effecting by AG and GG genotypes; OR, 4.17; CI, 1.72 – 10.11; p = 0.002 and OR, 2.30; CI, 1.19 – 4.44; p = 0.013, respectively) (Table 5). HIV-1 studied individuals with rs6001417 CG (protective genotype) almost didn't show any distinct contribution for both male and female ((effecting by GG and CC genotypes; OR, 0.41; CI, 0.13 – 1.23; P = 0.11 and OR, 0.40; CI, 0.21 – 0.75; P = 0.005, respectively) (Table 5).

## Discussion

Continual exposure to HIV infection may not certainly result in AIDS occurrence [36]. Multiple genetic and immune factors help in HIV acquirement, pathogenesis and AIDS progression at various stages of HIV life-cycle. So, HIV infection activates multiple intrinsic host factors that confer resistance to HIV pathogenesis. One of the important factors that substantiate intrinsic inhibition to HIV infection is APOBEC3G genetic host factor [24,37]. APOBEC3G single nucleotide polymorphisms (SNPs) are of particular importance. Its numerous SNPs have been studied in American [38] and European [39] populations in relation to its influence on AIDS development and progression. To our knowledge, the role of APOBEC3G in relation to HIV-1 infection has not been reported before in Pakistani population. Therefore, in current study we have investigated the frequency distribution of the three variants of APOBEC3G gene polymorphism in residents of Pakistan [40] and association of these variants with HIV-1 pathogenesis.

Our results suggest that the most common genotype prevalence was CC in APOBEC3G variants (rs6001417, rs35228531),and AA in rs8177832 followed by CG, GG and CC, CT and AG,GG respectively, in both HIV-1 infected and uninfected populations. Briefly, in rs6001417 homozygous genotypes CC (p = 0.13) and GG (p = 0.34) distribution was quite comparable in both groups. However, heterozygous genotype CG incidence is significantly elevated in control than HIV-1 positive group (p = 0.03). We assume that higher incidence in control group exhibits the possible protective role against HIV-1 infection [41].

Similarly, in rs35228531 variant CC genotype occurrence was comparable in both groups (p = 0.85). On other hand, TT genotype distribution is more prevalent in infected group (p = 0.02). Thus, indicates a potential role in susceptibility related to HIV infection. Yet, CT a heterozygous genotype is distributed more frequently in control group (p = 0.01). This demonstrates the possible protective feature of CT genotype against HIV-1 pathogenesis. Moreover, AA genotype of rs8177832 variant has been shown a significant contribution towards the HIV infected individuals in the region (P = <0.001), while AG and GG attained a comparable participation in both the infected and healthy individuals. In brief, AA genotype has assumed the highest in HIV-1 group may depict the possible predisposed role towards HIV-1 infection.

In a previous study, carried out in Burkina Faso-West Africa, the role of the aforementioned variants has been substantiated in regard with providing protection against HIV-1 infection and rapid disease progression (up to 2 to 5 folds) [42]. Moreover, rs8177832 (H186R) mutation association with high HIV-1 loads and decreased CD4+ count in south African females was also shown[38]. However, another group could not establish the association between APOBEC3G variants (including rs6001417 and rs8177832 but not rs35228531) and a disease progression phenotype in white French HIV-1 seropositive individuals [43,44]. Contradictory paradigm of APOBEC3G role against HIV-1 infection may be attributed to either prevalence of non-identical virus strains in different regions or genetic variations in different populations and ethnic group. A study of 3,073

subjects from six different cohorts demonstrated that the distribution of this genetic variation in African Americans was 37% but rare in European American (<3%) and in Europeans (5%) [7]. Moreover, the SNPs of APOBEC3G interacting proteins such as Vif and CUL5 may also influence the disease progression [45].

We have shown that, the effect of APOBEC3G variants is gender-depended i.e, rs35228531 CT genotype frequency is higher in male than female ($p < 0.001$) (Table 3). Apparently, the profound incidence of rs35228531 CT genotype supports the males in term of protection against the HIV-1 infection. However, our results further indicate that in variant rs8177832 genotype AA incidence is also higher in male then female ($p < 0.001$) (Table 3). The elevated incidence of variant rs8177832 genotype AA makes male group more vulnerable to the pathogenesis of HIV-1 infection. To the best of our knowledge, there is no prior study or report showing the association between the gender and APOBEC3G gene polymorphism in HIV-1 infection.Role of SNP genotypes and their association with male gender has been reported previously in systemic lupus erythematous (SLE) patients [27]. Moreover, we have recently reported that both genders favor IL28B rs12979860 CT genotype (OR: 4.80; CI: 2.22–10.35; $p = 0.0005$) and (OR: 3.47; CI: 1.63–7.43; $p = 0.001$) for male and female, respectively, in spontaneous clearance of HCV infection [26,27].

Our results showed that, APOBEC3G rs6001417 CG and rs35228531 CT genotypes provide protection with 42.5% and 41.5% positive predictive value (PPV) respectively, for HIV-1 infection while rs35228531 TT genotype with predictive value of 17.0% had shown a predispose role against the HIV-1 studied population. In a previous study, using HCV patients, showed the predictive role of SNP rs12979860 genotypes of IL28B in HCV infection [46,47]. Themulti logistic model (MLM) analysis, which predicted the predominant male group contribution in elevating protection against HIV-1 infection and aggravating the onset of the infection. The rs35228531 genotype CT promotes the protective probability 3 and 2-fold by male than female group. In a previous study carried out on Burkina Faso population none of the rs35228531 genotypes (CT, CC, TT) demonstrated the role in protecting against HIV-1. Our findings showed CC genotype had 3-times the risk of being infected. Concurrently, our multinomial logistic regression analysis substantiates that the rs8177832 genotype AA carriers pose 4 and 2-fold predisposing toward the infection OR=4.17 [95% CI: 1.72-10.11, p=0.002] and OR=2.30 [95%CI: 1.19-4.44, p=0.013] (vs genotypes GG and AG, respectively) by male than female gender.

In current study, we have reported the possible biological role of APOBEC3G gene variants (rs35228531, rs35228531 and rs6001417) in promoting and/or repressing the HIV-1 infection between male and female in Pakistani population. As far as we know, this is the first study examining the role of APOBEC3G gene variants on HIV-1 infection in a Pakistani population in both gender, where we found male a possible predominant . In short, male gender was found predominatly high in both protective gentotype rs35228531 CT ($p = 0.003$) and predisposing genotype rs8177832 AA ($p = <0.001$). This predomiant contribution may help the patient to be predict about the status of HIV infection. However, the major limitation, is that the current study was carried out on a limited sample size. The findings, based on such a sample size / population fraction are not enough to infer to the whole population. In order to have a better understanding of the above-mentioned association further investigations are needed for a substantial part of the population.

## Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' Contribution

The conduction and designing of this study was done by Qaisar Ali and Arshad Jamal and lab support provided by Sajjad Ullah whereas, Ahmed Bilal Waqar supervised this research.

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