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Identification and antiviral activity of common polymorphisms in the APOBEC3 locus in human populations

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ABSTRACT

There are seven members of the APOBEC3 family in humans (APOBEC3A through APOBEC3H) that have antiviral activity against retroviruses and/or retroelements. To determine whether variants in APOBEC3 genes in human populations have altered antiviral activity, we identified and functionally tested novel single nucleotide variants (SNVs) in APOBEC3 genes present in the 1000 Genome Project dataset. We found that common variants minor allele frequency (> 1%) of APOBEC3A, C, F, and G do not affect protein function. However, we found that two common novel polymorphisms in APOBEC3D decrease antiviral activity against HIV-1, and one polymorphism decreases activity against Alu retrotransposons. We characterized the diversity of APOBEC3 genes in three human populations and find significant evidence that APOBEC3D has evolved under purifying selection in recent human history. These data suggest that the activity of APOBEC3D has been maintained in human populations for a cellular function in host defense.

Introduction

The APOBEC3 family of retroviral restriction factors contains seven paralogs in humans (APOBEC3A through APOBEC3H) (Jarmuz et al., 2002; OhAinle et al., 2006). APOBEC3s are cytidine deaminases that inhibit the replication of a diverse array of viruses and retrotransposons (Chen et al., 2006; Esnault et al., 2005; Mangeat et al., 2003; Okeoma et al., 2007; Sheehy et al., 2002; Turelli et al., 2004). Most APOBEC3 proteins are constitutively expressed in viral target cells, though some APOBEC3 proteins are induced by interferon (Koning et al., 2009; Refsland et al., 2010). APOBECSs inhibit viral replication during reverse transcription by inducing hypermutation of viral genomes and by deamination-independent methods (Harris et al., 2003; Macmillan et al., 2013; Newman et al., 2005; Zhang et al., 2003).

Most lentiviral genomes contain an antagonist of APOBEC3s, which is encoded by the *vif* gene in HIV-1, to overcome the restriction imposed by APOBEC3s (Mariani et al., 2003; Sheehy et al., 2003). This has led to an evolutionary 'arms race' between hosts and viruses that has left a signature of positive selection, or rapid evolution, in host and viral genes (Compton and Emerman, 2013). Many APOBEC3 genes, including APOBEC3G, APOBEC3H, and APOBEC3D, which was called "APOBEC3DE" in earlier papers, have evolved under positive selection for millions of years in primates (Duggal et al., 2011; OhAinle et al., 2008; Sawyer et al., 2004). At least one APOBEC3 gene within an Old World monkey species has acquired population-specific polymorphisms in recent history that allow host evasion from lentiviral infection (Compton et al., 2012), suggesting that APOBEC3s may have rapidly evolved in recent primate history as well.

Within humans, several APOBEC3s are known to have common polymorphisms that render them defective. For example, two variants of APOBEC3H have decreased protein expression (OhAinle et al., 2008), and deletions of APOBEC3B are common in some human populations (Kidd et al., 2007). Polymorphisms in APOBEC3F and APOBEC3G have also been described (An et al., 2004; Mulder et al., 2010; Reddy et al., 2010). However, whether variants that have functional consequences are present in other APOBEC3 genes is unknown. Moreover, the only allele of the human APOBEC3D gene that has been functionally tested for antiviral activity is less active against lentiviruses than the orthologous chimpanzee APOBEC3D gene (Duggal et al., 2011). Because of widespread interest in understanding the contributions of human genetics to viral susceptibility and disease, we sought to comprehensively identify and functionally characterize variants of APOBEC3 genes in human populations.

Using the 1000 Genome Project dataset, we identify 21 amino acid-altering mutations in the APOBEC3 locus, of which nine are reported

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for the first time. We find that six common (minor allele frequency, MAF > 1%) single nucleotide variants (SNVs) in APOBEC3A, C, F, and G have no effect on antiviral activity. However, two SNVs in APOBEC3D decrease antiviral activity against HIV-1, and one of these two has decreased activity against Alu retrotransposons. To better understand the evolutionary pressures acting on the APOBEC3 locus, we perform neutrality tests and find that APOBEC3D is subject to purifying selection in humans. These results highlight the conserved role of APOBEC3D in host defense and suggest that APOBEC3D variants may be only slightly deleterious.

Results

Common polymorphisms in the APOBEC3 locus

To identify single nucleotide variants in the APOBEC3 locus, we accessed the 1000 Genome Project Phase I genotypes for the coding regions of each APOBEC3 gene, which consists of genotypes from 913 geographically diverse individuals of African, Asian, and European ancestries. We also obtained insertions and deletions for 911 of the same individuals from the 1000 Genome Project Integrated Phase 1 release. A summary of the most common (MAF > 1%) codon-altering variants found in APOBEC3A through H is found in Table 1 and the SNVs functionally tested in this study in Fig. 1.

The 1000 Genome Project dataset contains 12 previously reported common variants in the APOBEC3 locus, including the deletion of APOBEC3B (Kidd et al., 2007), the deletion of residue 15 in APOBEC3H (OhAinle et al., 2008), and SNVs in APOBEC3F (A108S, V231I, Y307C) (Mulder et al., 2010), APOBEC3G (H186R, Q275E) (An et al., 2004; Reddy et al., 2010), and APOBEC3H (R18L, R105G, E121K/D, D178E) (OhAinle et al., 2008). These polymorphisms were previously identified using smaller datasets because of their very high frequency. In addition to these previously reported variants, nine additional common variants can also be found in this dataset, including SNVs in APOBEC3A (T19A), APOBEC3B (K62E, P98L, T146K, L189P, A254V), APOBEC3C (S188I), and APOBEC3D (R97C, R248K) (Fig. 1 and Table 1). Using the 1000 Genome Project data, we have now identified all common polymorphisms in the APOBEC3 locus by eliminating an ascertainment bias.

Consistent with previous reports of APOBEC3 variants in HapMap populations, the deletion of APOBEC3B, the polymorphisms in APOBEC3F at codons 108 and 231, and the polymorphisms in APOBEC3H at codons 15, 105, 121, and 178 are found at their highest frequencies in populations with Asian or European ancestries (Fig. 1). In contrast, SNVs in APOBEC3A, APOBEC3C, APOBEC3D, and APOBEC3G are found at their highest frequencies in populations of African ancestry. Thus, all APOBEC3 paralogs contain at least one common non-synonymous polymorphism in a human population. In addition, most variants in the APOBEC3 locus have population-specific distributions, indicative of demographic events or directional selection acting in a population-specific manner.

Common polymorphisms in APOBEC3A, C, F, and G do not affect antiviral activity

All common variants in APOBEC3H have been well described functionally in previous studies: the deletion of residue 15 decreases protein stability; the R105G mutation decreases protein stability, alters cellular localization, and reduces RNA binding; the D121K mutation decreases sensitivity to HIV-1 Vif; and the R18L and D178E mutations have no effect on protein function (Harari et al., 2009; Li et al., 2010; OhAinle et al., 2008; Tan et al., 2009; Wang et al., 2011; Zhen et al., 2012, 2010). The loss of APOBEC3B in many individuals suggests that APOBEC3B is not essential for human health, and the insensitivity of APOBEC3B to HIV-1 Vif (Doehle et al., 2005) suggests it is not relevant to HIV-1 infection. Therefore, for the rest of our functional analyses of SNVs in APOBEC3 genes, we focused on those found in APOBEC3A, C, D, F, and G.

Human APOBEC3A and APOBEC3C inhibit the replication of LINE-1 (Bogerd et al., 2006; Muckenfuss et al., 2006), with the activity of APOBEC3A more potent than APOBEC3C. APOBEC3A T19A and APOBEC3C S1881 mutations are present at frequencies near 10% in African populations and < 1% in other populations (Table 1). As no SNVs in either paralog have been previously described, we tested whether these APOBEC3A or APOBEC3C variants affect their activity against a retroelement, LINE-1. Each of these mutations was introduced into a plasmid containing epitope-tagged APOBEC3A or APOBEC3C. We co-transfected increasing amounts of APOBEC3 plasmids with a plasmid containing the non-LTR human retrotransposon LINE-1, which expresses neomycin resistance after retrotransposition. Because APOBEC3A is a stronger inhibitor of LINE-1 (Niewiadomska et al., 2007), we used 10 times less APOBEC3A plasmid DNA compared to APOBEC3C in these assays. After selection in G418, neomycin-resistant colonies were counted as a measure of LINE-1 activity. Although APOBEC3A T19A and APOBEC3C S188I had slightly decreased protein expression compared to wild-type APOBEC3A or APOBEC3C (Fig. 2A and B, upper panels), these polymorphisms did not affect the ability of APOBEC3A or APOBEC3C to restrict the activity of LINE-1 in a dose-dependent manner (Fig. 2A and B, lower panels). These data suggest that the SNVs in APOBEC3A and APOBEC3C do not alter their capacity to restrict LINE-1.

Human APOBEC3F has potent antiviral activity against HIV-1Δ*vif* and is sensitive to HIV-1 Vif (Bishop et al., 2004; Liddament et al., 2004; Wiegand et al., 2004; Zheng et al., 2004), but reports vary in its ability to inhibit LINE-1 (Bogerd et al., 2006; Hulme et al., 2007; Stenglein and Harris, 2006). In this dataset, APOBEC3F Y307C is present at a low frequency in African and European populations (MAF < 5%) and is absent in Asian populations.

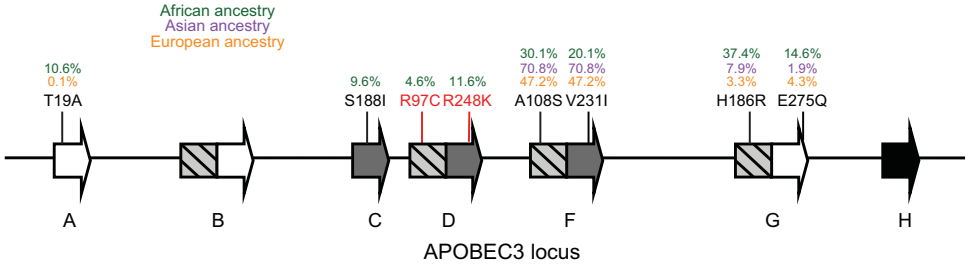

Fig. 1. Variants in the APOBEC3 locus tested in this study. The human APOBEC3 locus with polymorphisms tested in this study. Polymorphisms in red have functional effects. Shading of APOBEC3 genes represents regions of homology. Percentages in green represent the derived allele frequency in individuals of African ancestry, purple represents individuals of Asian ancestry, and orange represents individuals of European ancestry. Diagram is not to scale. The entire dataset of SNVs is in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).