The Influence of host genetic factors on HIV-1 infection

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SUMMARY

The clinical course of HIV-1 infection can be highly variable between individuals, with extremes of disease progression within 2 years, or a continuous asymptomatic infection for more than 15 years. Moreover, certain people are relatively resistant to HIV-1 infection, reflected in their uninfected status despite high levels of sexual risk behavior. Next to viral factors, the genetic make-up of an individual has been implicated to play a role in the susceptibility to HIV-1 infection and/or the rate of progression to disease. Already a lot of research has focused on host genetic factors and several candidate genes were identified to be associated with HIV-1 susceptibility and pathogenesis. However, these host genetic markers can only explain a small fraction of the diversity observed in HIV-1 infection.

In this thesis we performed genetic association studies to reveal additional host genetic factors that possibly play a role in the susceptibility to HIV-1 infection, the clinical course of infection, and the immune response post-seroconversion.

Human TRIM5α can inhibit HIV-1 replication, albeit it only modestly, by targeting the capsid of incoming HIV-1 and thereby interfering with viral replication. Single nucleotide polymorphisms (SNPs) H43Y and R136Q in TRIM5α are associated with altered antiviral activity of TRIM5α on HIV-1 in vitro. In Chapter 2, we screened HIV-1 infected individuals from the Amsterdam Cohort Studies (ACS) for these polymorphisms and subsequently analyzed how these genotypes were associated with the clinical course of infection. Individuals who were homozygous for the 43Y genotype had an accelerated disease progression when compared to carriers of the 43H genotype. For participants who carried the minor allele of the 136Q genotype we observed a delayed progression to a viral load above the median (10^4.5 copies per ml plasma), but only after the emergence of CXCR4-using HIV-1 variants. Naïve CD4+ T-cells, which are selectively targeted by CXCR4-using HIV-1 variants, expressed a higher level of Trim5α when compared to memory CD4+ T-cells. Finally, we observed that the 136Q allele, in combination with the 5'UTR -2GG genotype, was associated with an accelerated progression to AIDS. These results indicate that variants in Trim5α may influence the clinical course of HIV-1 infection.

The first genome-wide association study (GWAS) on HIV-1 infection revealed SNPs in the human leukocyte antigen (HLA)-C and the HLA-complex P5 (HCP5) gene region to be associated with viral load at set-point and several SNPs in high LD in the ring finger protein 39 (RNF39) and zinc ribbon domain-containing 1 (ZNRD1) gene region to be associated with CD4+ T-cell count drop. We were able to confirm the associations between the minor allele variants of SNP rs9264942 -35kb HLA-C and rs2395029 HCP5 and a lower viral load at set-point in the ACS (Chapter 3). Furthermore, these two SNPs were also significantly associated with disease progression, although not independent from viral load and CD4+ T-cell count at set-point. We did not identify an association of the SNPs in the RNF39/ZNRD1 gene region with the clinical course of HIV-1 infection.
In **Chapter 4**, the association of SNP genotypes with the clinical course of HIV-1 infection was analyzed in a genome wide association study using progression to AIDS or AIDS-related death as a phenotype. Several interesting signals were identified to be associated with survival time after HIV-1 infection to AIDS-diagnosis and/or AIDS-related death, albeit that none of the signals passed the threshold for genome-wide significance. Four SNPs from the top ten associations with AIDS or AIDS-related death were also found to be associated with disease progression in the French Genomics of Resistance to Immunodeficiency Virus (GRIV) cohort (P < 0.05). The results from this GWAS emphasizes that besides the already identified association of the HLA gene region with viral load, other host genetic factors may be associated with the clinical course of HIV-1 infection.

In **Chapter 5** we looked for alleles that are more prevalent in long-term non-progressors (LTNPs) with a detectable viral load from the French GRIV cohort, with seronegative French controls, to reveal SNP genotypes that are associated with disease progression, without necessarily exerting their effect through controlling viral load. SNP rs2234358 in the C-X-C chemokine receptor 6 (CXCR6) had the strongest signal in this association analysis and could be confirmed in a candidate gene approach comparing LTNPs from the ACS and two American cohorts with uninfected individuals. The combined P-value of these analyses passed the genome-wide significance threshold. This study emphasizes the power to detect host genetic markers influencing disease progression making use of extreme phenotypes, and highlights the possible role of CXCR6 in the clinical course of HIV-1 infection.

To identify additional variations that are associated with HIV-1 pathogenesis, one may need to look into low-frequency SNPs (MAF < 5%) in large study samples. GWAS data from the American MultiCenter AIDS Cohort Study (MACS), the French GRIV cohort and the ACS were reanalyzed; comparing in total 365 LTNPs and 147 rapid progressors (RPs) with 1394 uninfected individuals (**Chapter 6**). More than 8,000 SNPs with a MAF < 5% were tested. Three signals on chromosome 6, which were in moderate LD with each other, including HCP5 rs2395029, and a fourth polymorphism in an exon of Rho-type GTPase-activating protein (RICH2), were identified to be associated with non-progression. Thus, by targeting low-frequency SNPs in a multi-cohort GWAS analysis, a potentially novel host genetic factor influencing HIV-1 disease progression was revealed.

We performed in **Chapter 7** a GWAS in High Risk Sero-Negatives (HRSN), who have remained HIV-1 negative despite multiple high-risk exposures to the virus, to identify novel genetic markers associated with HIV-1 susceptibility. The prevalence of the CCR5Δ32 homozygous phenotype was higher in HRSN when compared to HIV-positive individuals, emphasizing the exposed uninfected phenotype. Several SNPs were potentially associated with HIV-1 acquisition when HRSN were compared with HIV-1 positive individuals, although none of the signals was genome-wide significant. Furthermore, the minor allele frequency (MAF) of two out of the top 10 SNPs associated with HIV-1 susceptibility were also significantly reduced in HIV-1 positive
individuals when compared with healthy controls, both in the ACS and the French GRIV cohort. Interestingly, two additional gene regions potentially being associated with HIV-1 acquisition, have previously been identified as HIV-1 dependency factors for infection and replication in *in vitro* siRNA screens.

Not much is known about how human genetic variation might influence cross-neutralizing antibody responses after HIV-1 infection. Therefore, a GWAS was performed to identify associations between host genetic polymorphisms and the presence of HIV-1 specific cross-reactive neutralizing activity (CrNA) in sera of participants of the ACS, as described in Chapter 8. Three SNPs in high LD in the N-acetylglucosaminyltransferase IV (GNTIVH) gene region, and some signals close to major histocompatibility complex I chain-related protein A (MICA) and in the HCP5 gene were identified to have strong P-values for the association with CrNA, although none of them reached genome-wide significance. Moreover, the HLA-B*57 allele was found to be strongly correlated with CrNA in HIV-1 infected individuals. These results emphasize that host genetic factors seem to play a role in the development of cross-neutralizing activity, although confirmation of these signals in other cohorts is necessary.

In the AIDS epidemic in the Netherlands, an increase of viral load at set-point over calendar time has been reported. Indeed, viral load at set-point in post-2003 seroconverters was significantly higher when compared to pre-2003 seroconverters, as reported in Chapter 9. In the pre-2003 seroconverters the CCR5wt/Δ32 genotype and the minor alleles of HCP5 rs2395029 and -35 HLA-C rs9264942 were all individually associated with a lower viral load at set-point. However, this association was no longer observed for HCP5 rs2395029 and CCR5wt/Δ32 in the post-2003 seroconverters. We concluded from these results that increased viral load at set-point at a population level coincides with a lost effect of these host genetic markers on viral load control over calendar time at a population level.

In this thesis several host genetic markers were identified that possibly play a role in HIV-1 acquisition, disease progression and the presence of CrNA, both in the ACS and in meta-analysis studies including other cohorts. Better understanding of the genetic variations and how they influence HIV-1 infection and pathogenesis may ultimately lead to novel therapy strategies and vaccine development to combat the AIDS epidemic.