HIV-1 Subtype Diversity and Prevalence of Primary Drug Resistance in a Single-Center Pediatric Cohort in Germany

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Keywords

HIV-1-infected children · Subtype · Drug-resistant mutations · Mother-to-child transmission

Abstract

Objectives: Data on drug-resistant mutations (DRMs) in HIV-1-infected therapy-naïve children are scarce. The aim of this study was to determine the HIV-1 subtype distribution and the prevalence of DRMs in therapy-naïve HIV-1-infected children who received routine care at the University Hospital Düsseldorf, Düsseldorf, Germany. **Methods:** Records of all HIV-1-infected children who received routine care between January 2005 and December 2015 were analyzed retrospectively. The collected data included demographics, clinical characteristics, CD4 cell count, viral load, HIV-1 subtype, and resistance genotype at baseline. **Results:** 83 HIV-1-infected children received routine care during the observation period. HIV-1 subtypes were available in 61/83 patients (73.5%) and baseline HIV-1 resistance in 24 (29%). The prevalence of major DRMs was 29% (21% nucleoside reverse-transcriptase inhibitors [NRTIs], 12.5% non-NRTIs, and 4% protease inhibitors). Minor mutations in the protease gene were common (58%). Non-B subtypes were predominant (77%). **Conclu-**

sions: We report a predominance of non-subtype-B infections and a higher prevalence of DRMs compared to other pediatric cohorts from resource-rich settings. The difference in HIV-1 subtype distribution is due to the fact that a relevant proportion of pediatric patients in Germany are immigrants from high-prevalence settings in sub-Saharan Africa where non-B subtypes predominate.

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Introduction

The introduction of combined antiretroviral therapy (ART) has dramatically changed the course of infections with human immunodeficiency virus type 1 (HIV-1) in adults and children [1, 2]. The use of ART in pregnancy and as prophylaxis in infants for the prevention of mother-to-child transmission (MTCT) has led to a pronounced reduction of vertical HIV-1 transmission, but a selection of drug-resistant mutations (DRMs) in infected infants is increasingly being reported [3, 4]. Some HIV-1 subtypes and recombinants seem to accelerate the emergence of DRMs [4, 5]. However, data on the clinical benefit of baseline resistance testing in children are currently lack-

ing. German guidelines have recommended screening for baseline DRMs since 2006 and European guidelines (PENTA) have recommended screening in all therapy-naïve children since 2009 [6, 7]. The prevalence of DRMs in therapy-naïve and treated patients has been well-documented in adults but data on DRMs in therapy-naïve children are limited [8]. In US- and European-based cohort studies, the prevalence of drug resistance in therapy-naïve children ranged between 9–30% [8–12].

The prevalence of transmitted DRMs in HIV-infected therapy-naïve adults in Germany was documented in the RESINA Study, and found to be 9.2% between 2001 and 2009 (nucleoside reverse-transcriptase inhibitors [NRTIs]) 5.8%, non-NRTIs [NNRTI] 2.7%, and protease inhibitors [PIs] 2.7%) [13]. There are no data on DRMs and HIV-1 subtypes of HIV-1-infected children living in Germany.

The aim of this study was to determine the HIV-1 subtype diversity and the prevalence of DRMs in therapy-naïve HIV-1-infected children who received routine care between January 2005 and December 2015 at the University Hospital Düsseldorf in Germany.

Material and Methods

Records of all HIV-1-infected children who received routine care between January 2005 and December 2015 at the Department of Pediatrics at the University Hospital Düsseldorf, were reviewed and analyzed retrospectively. The study was approved by the local ethics committee (internal study No. 4858).

The data included demographic profile (ethnicity, country of birth, sex, mode of transmission, perinatal antiretroviral exposure, age at diagnosis, year of HIV diagnosis), CDC stage, CD4 cell count, HIV-1 viral load, HIV-1 subtype and baseline genotypic resistance profile prior to the initiation of ART.

For genotypic resistance analysis, the HIV-1 RNA was isolated from EDTA plasma and analyzed for resistance mutations in the protease (PR) and reverse-transcriptase (RT) genes as previously described [14]. Sequencing was performed by classical Sanger sequencing analysis. Classification of HIV-1 subtypes was performed by sequence analysis of the PR/RT sequences with the NCBI genotyping tool (<https://www.ncbi.nlm.nih.gov/projects/genotyping>) or the COMET HIV-1 tool (<https://comet.liv.lu>). The detected drug resistance mutations were defined according to the Stanford Drug Resistance Database (<http://hivdb.stanford.edu>).

Results

Study Cohort Characteristics

A total of 83 patients were seen in the study period. Overall, 46% children were male and 54% female. More than half (66%) had one or both parents of African origin

Table 1. Demographic characteristics and HIV-1 subtype of the HIV-1-infected children

Total number of children	83
Sex	
Female	45
Male	38
Ethnicity	
Caucasian	18
African	55
Asian	6
Other	4
Country of birth	
Germany	46
Other	30
Unknown	7
Mode of transmission	
Vertical	77
Horizontal	1
Unknown	5
MTCT prophylaxis of any kind	
Yes	13
No	70
Year of HIV diagnosis	
Before 2005	46
2006–2010	22
2011–2015	15
Age at diagnosis	
<1 year	31
1–4 years	27
≥5 years	20
Unknown	5
HIV-1 group M subtype	
B	14
A1	3
CRF01_AE	6
CRF02_AG	13
CRF06_CPX	5
CRF11_CPX	1
CRF13_CPX	2
C	8
G	4
D	2
F	3
Unknown	22

and 22% were Caucasians. In 46 patients (55%), HIV-1 infection was diagnosed before 2005. The most prevalent mode of transmission was vertical infection (93%) (Table 1). The majority of infected children (60%) were born in Germany and did not receive prophylaxis to prevent MTCT, mainly due to the unknown HIV status of the mother during pregnancy. Thirteen patients (15%) received some kind of prophylaxis to prevent MTCT (ante-partum, intrapartum, or neonatal) but none of them had a viral load of <40 copies/mL at birth.

Table 2. Prevalence of drug-resistant mutations in 24 therapy-naïve HIV-1-infected children

Patient No.	Sex	Age at baseline resistance testing	CD4/ μ L	VL cp/mL	Subtype	NRTI	NNRTI	PI (major)	PI (minor)	Perinatal ART exposure or a maternal history of ART
1	F	3 months	637	999,000	CRF13_CPX	none	none	none	L10I, K20I	no
2	F	16 years	363	9,934	G	none	none	none	L10I, K20I	no
3	M	8 months	1,946	4,089,367	CRF06_CPX	none	none	none	L10I	no
4	M	8 months	1,032	3,600,000	C	none	none	none	T74S	no
5	F	3 months	3,058	906,490	CRF02_AG	none	none	none	K20I	yes
6	M	6 weeks	1,150	2,500,000	CRF01_AE	M41L D67N K70R V75M M184V L210W L215Y	K101E V106M E138A G190A	none	L10I K20I	yes
7	F	3 months	2,889	3,197,440	B	D67DG	none	none	none	yes
8	F	12 months	363	>10,000,000	CRF02_AG	none	none	none	K20I	no
9	M	1 month	2,545	1,069,635	A1	A62V	none	none	none	yes
10	M	2 weeks	564	>17,000,000	CRF02_AG	none	none	M46LM	K20I	yes
11	F	10 years			CRF11_CPX	none	E138A	none	none	no
12	F	8 years	852	158,447	C	none	none	none	none	no
13	F	1 month	3,732	>500,000	CRF02_AG	none	none	none	L10I, K20I	yes
14	M	13 years	378	10,000	A1	none	none	none	none	no
15	M	6 years	6	409,445	CRF01_AE	none	none	none	L10I	no
16	M	9 months	1,235	15,305	CRF06_CPX	none	none	none	L10I, K20I	no
17	M	13 years	354	8,513	C	K70EG M184V	K103N	none	L24IV	no
18	M	8 years	510	91,920	CRF-02AG	none	none	unknown	unknown	no
19	F	9 years	unknown	unknown	C	none	none	none	none	no
20	M	12 years	828	17,261	F1	none	none	none	L10LV	no
21	M	8 years	258	41,446	C	none	none	none	none	no
22	F	2 years	1,101	123,385	CRF06_CPX	none	none	none	L10I, K20I	no
23	M	12 years	351	264,792	B	K219KR	none	none	none	no
24	F	2 years	1,492	23,506	CRF02_AG	none	none	none	none	no

VL, viral load (HIV-1 RNA); NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor; ART, antiretroviral therapy.

Forty-one patients (49%) started ART before 2005. Twenty-seven patients were therapy-naïve at their first visit at our center during the observation period. Fifteen patients started therapy at other centers during the observation period and were then referred to us.

HIV Diversity

The HIV-1 subtype was available in 73% (61/83) of the children (Table 1). Only 23% (14/61) were infected with subtype B. Thus, non-B subtypes were most prevalent in this pediatric cohort, at 77% (47/61). The most frequently detected non-B subtypes were the circulating recombinant form CRF02_AG at 28% (13/47), followed by subtype C at 17% (8/47). Regarding the children with available genotypic resistance testing before the initiation of

ART ($n = 24$), 92% (22/24) harbored non-B subtype virus strains (Table 2).

Drug-Resistant Mutations

Results of baseline genotypic resistance testing were available in 24/83 patients (29%). The main reason for missing genotypic results at baseline was the initiation of ART before the implementation of routine resistance testing ($n = 41$). Further reasons for missing genotypes were therapy initiation at another center ($n = 13$) and unsuccessful amplification of the plasma samples ($n = 2$). The reason was unknown in 3 patients.

Baseline resistance testing revealed major HIV-1 DRMs in the virus strains of 7/24 (29%) patients (21% NRTIs, 12.5% NNRTIs, and 4% PIs) (Table 2). Two pa-

tients (#6 and #17) presented viruses with a dual class resistance, i.e., for NRTIs and NNRTIs. No patient was infected with a 3-drug-class-resistant variant. Besides major DRMs, several polymorphisms were observed in the RT gene of all therapy-naïve-infected children. DRMs were predominantly detected in 4/6 patients with neonatal or maternal ART exposure (#6, #7, #9, and #10) (Table 2). In 3 patients (#11, #23, and #17), DRM-presenting viruses were found in children without a history of maternal ART (antepartum and intrapartum) or neonatal ART. One patient (#17), a vertically infected boy diagnosed at the age of 13 years, harbored a virus with NRTI (K70EG, M184V) and NNRTI (K103N) mutations. His country of birth was Angola at a time when maternal ART and prophylaxis to prevent MTCT were not available. He had also previously been denied access to ART. A phylogenetic analysis of the HIV quasiespecies of the child and the mother confirmed the vertical HIV-1 transmission; however, analysis of the mother's HIV-1 quasiespecies did not present the DRMs detected in the child. Another patient (#10) harbored a variant with a major PI mutation (M46L) at baseline. The medical history of this patient revealed maternal ART prior to pregnancy but no maternal ART during pregnancy and no neonatal ART to prevent MTCT. Overall, minor PI mutations were identified in the HIV-1 protease gene of 14 (58%) patients, including the polymorphisms L10I and K20I, and the atypical mutation T74S.

Discussion

In this retrospective pediatric cohort study in Düsseldorf, Germany, the prevalence of HIV-1 infections with non-subtype-B variants was 77%, thus showing a considerably higher frequency than in other pediatric cohorts in resource-rich settings, e.g., 11% non-B variants in a Spanish cohort (1993–2010) and 3–35% in pediatric US cohorts (2.7% in The Bronx [2011] and 16.7% in New York [2001–2002], NY, and 35% on Rhode Island [1991–2012]) [10–12, 15]. The higher frequency of non-subtype-B variants observed in our study was mainly due to the fact that more than half (66%) of the patients had one or both parents of African origin where HIV-1 non-subtype-B variants are predominant [16].

With an overall frequency of 29%, we observed a higher prevalence of drug resistance in therapy-naïve children than in other pediatric cohorts [8–12, 17, 18]. The prevalence rate of major DRMs in therapy-naïve children was 6.8% in 1998–2010 in the UK and 13.6% in

1993–2010 in a Spanish cohort ($n = 44$) [9, 10]. Masquelier et al. [18] reported zidovudine resistance in 20% of the children born in France and enrolled in the ARNS-EPF French national cohort in 1994–1999. In the US pediatric cohorts, the rate of DRMs was in the range of 12–24% [4, 11]. Overall, the usage of different mutation lists (e.g., from the International AIDS Society [IAS], Agence Nationale de Recherche sur le SIDA [ANRS], or Stanford HIV databases) for the identification of DRMs in the different studies presents a bias in the comparability of the prevalence of DRMs across different cohorts [19].

To date, there are no comparable data available from other pediatric cohorts in Germany. RT gene DRMs were the most prevalent mutations in our cohort (21% NRTIs and 12.5% NNRTIs). In addition to the typically described DRMs in the RT gene, as detected in patients #6, #9, and #17, atypical amino acid substitutions at resistance-associated positions like D67G or K219R were also found (#7 and #23). Although their influence on drug susceptibility is not clearly defined, they were scored as DRMs according to the Stanford Drug Resistance Database (<http://hivdb.stanford.edu>). The RT mutation E138A detected in patient #11 infected with the recombinant form CRF11_cpx is a polymorphic mutation in up to 5% of viruses, depending on the HIV-1 subtype [20]. Nevertheless, the presence of E138A prior to therapy may reduce the antiviral activity of rilpivirine, which has to be considered for the selection of an active drug regimen [21]. One patient (#10) harbored the most likely transmitted PI mutation, M46L. This patient's mother did not receive any ART during pregnancy, did not disclose her HIV-1 status, and denied being HIV-1-infected even after vertical HIV-1 infection was diagnosed in her child. Later, findings confirmed maternal ART prior to pregnancy and very poor adherence. Detailed information about the type of maternal ART prior to pregnancy was missing. M46L is described as a non-polymorphic mutation selected for primarily by Indinavir, nelfinavir, fosamprenavir, atazanavir and lopinavir, leading to a reduced susceptibility to PI treatment [22–24]. Two of the screened patients (#6 and #17) carried viruses with dual class resistance, to NRTIs and NNRTIs. Interesting was the finding of virus variants presenting the M184V mutation, which are characterized by impaired viral replication. The mother of patient #6 received ART before and during pregnancy, but she had high viremia throughout pregnancy and at birth. Thus, the acquisition of the drug-resistant viruses in patient #6 could be explained by MTCT. In contrast, the acquisi-

tion of DRMs in patient #17 remains unclear. He was diagnosed at the age of 13 years and harbored NRTI and NNRTI mutations without a history of ART exposure. His country of birth was Angola, at a time when maternal ART and prophylaxis to prevent MTCT were not available, and previous ART had been denied. A phylogenetic analysis of the HIV quasiespecies of the child and the mother confirmed the vertical HIV-1 transmission but the mother's virus did not harbor these DRMs. We speculate that the patient intermittently or transiently received ART with or without the knowledge of the mother. The mother consistently denied drug administration. This case emphasizes the importance of baseline resistance testing in every child regardless of their ART history.

Besides the major DRMs, we also identified a relevant proportion of patients carrying minor mutations, particularly L10I and K20I in the protease gene. L10I is a polymorphic mutation in the protease gene associated with reduced susceptibility to the protease inhibitors reported for subtype B variants [25, 26]. Regarding non-subtype-B variants, L10I is a frequent polymorphism [27]. K20I is the consensus amino acid in subtype G and CRF02_AG viruses and also other recombinants coding the subtype G in the protease, like CRF06_cpx or CRF13_cpx. The PI-selected accessory mutation T74S detected in patient #4 is also described as a polymorphism in most non-B subtypes [28].

The frequency of DRMs in therapy-naïve HIV-infected adults in Germany was well-documented in the RESINA Study. The study reported an average DRM prevalence of 9.2% in 2001–2009 (NRTIs 5.8%, NNRTIs 2.7%, and PIs 2.7%) in North Rhine-Westphalia, Germany [13]. Moreover, subtype B was predominant (72%) and recombinant forms were found in only 6% of the therapy-naïve patients. This difference in HIV-1 diversity compared to our pediatric cohort is mainly due to differences in ethnic origin and mode of transmission. In the RESINA adult cohort, 81% patients were of Caucasian origin, mainly German, 80% were male, and the mode of transmission was mainly sexual (51% MSM). In our pediatric cohort, most patients or their parents were immigrants from Africa where HIV-1 non-subtype-B variants are predominant [16]. Due to the increasing accessibility to ART and strategies to prevent MTCT in sub-Saharan Africa, MTCT of HIV-1 is decreasing. However, among those who do become infected, the prevalence of DRMs, especially NNRTI resistance, is high. A recently published prospective observational study from South Africa demonstrated a high prevalence of NNRTI resistance

(52%) among infants who fail HIV-prophylaxis [29]. In another study from South Africa conducted in 2011, 56.8% of HIV-1-infected children harbored NNRTI, 14.8% harbored NRTI, and 1.3% harbored PI mutations [3]. Since a relevant percentage of new HIV-1-infected children seen at our center are immigrants from sub-Saharan Africa, this selection of DRMs being reported might have an impact on the prevalence of DRMs in the future.

Despite the limitations of this pediatric study on HIV-1 subtype distribution and baseline drug resistance (its retrospective nature, small sample size, and sparse information on maternal drug history and maternal resistance profiles), it provides important information on the frequency of transmitted drug resistance in HIV-1-infected children. To date, it is the first study on HIV-1 subtype diversity and prevalence of DRMs in therapy-naïve HIV-1-infected children receiving routine care at a center in Germany. This cohort study reveals a different HIV-1 subtype distribution with predominant non-B subtype infections versus the therapy-naïve HIV-1-infected adults in the RESINA cohort in Germany [13]. This difference is mainly attributed to the different ethnic backgrounds with a higher proportion of immigrants from sub-Saharan Africa in the pediatric cohort. Our results emphasize the importance of baseline screening for the presence of resistance-associated mutations in all HIV-1-infected children before the initiation of ART.

Disclosure Statement

There are no conflicts of interest.

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