付表 19-4　受検者における受検の満足度④【2013 年 10 月~2014 年 9 月】

	安心できる雰囲気について								プライバシー保護について							
	MSM以外の男性		女性		MSM		合計		MSM以外の男性		女性		MSM		合計	
大阪府吹田保健所																
とても満足	69	63.9%	39	75.0%	5	62.5%	**113**	**67.3%**	67	62.0%	32	61.5%	5	62.5%	**104**	**61.9%**
やや満足	19	17.6%	6	11.5%	2	25.0%	**27**	**16.1%**	16	14.8%	8	15.4%	2	25.0%	**26**	**15.5%**
やや不満	2	1.9%	1	1.9%	0	0.0%	**3**	**1.8%**	7	6.5%	4	7.7%	0	0.0%	**11**	**6.5%**
とても不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	0	0.0%	2	3.8%	0	0.0%	**2**	**1.2%**
無回答	18	16.7%	6	11.5%	1	12.5%	**25**	**14.9%**	18	16.7%	6	11.5%	1	12.5%	**25**	**14.9%**
合計	108	100.0%	52	100.0%	8	100.0%	**168**	**100.0%**	108	100.0%	52	100.0%	8	100.0%	**168**	**100.0%**
大阪府四条畷保健所																
とても満足	44	84.6%	25	75.8%	7	87.5%	**76**	**81.7%**	44	84.6%	22	66.7%	7	87.5%	**73**	**78.5%**
やや満足	5	9.6%	6	18.2%	1	12.5%	**12**	**12.9%**	4	7.7%	7	21.2%	1	12.5%	**12**	**12.9%**
やや不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	1	1.9%	2	6.1%	0	0.0%	**3**	**3.2%**
とても不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**
無回答	3	5.8%	2	6.1%	0	0.0%	**5**	**5.4%**	3	5.8%	2	6.1%	0	0.0%	**5**	**5.4%**
合計	52	100.0%	33	100.0%	8	100.0%	**93**	**100.0%**	52	100.0%	33	100.0%	8	100.0%	**93**	**100.0%**
大阪府枚方保健所																
とても満足	26	68.4%	24	82.8%	4	80.0%	**54**	**75.0%**	27	71.1%	20	69.0%	5	100.0%	**52**	**72.2%**
やや満足	6	15.8%	4	13.8%	1	20.0%	**11**	**15.3%**	4	10.5%	5	17.2%	0	0.0%	**9**	**12.5%**
やや不満	0	0.0%	1	3.4%	0	0.0%	**1**	**1.4%**	1	2.6%	4	13.8%	0	0.0%	**5**	**6.9%**
とても不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**
無回答	6	15.8%	0	0.0%	0	0.0%	**6**	**8.3%**	6	15.8%	0	0.0%	0	0.0%	**6**	**8.3%**
合計	38	100.0%	29	100.0%	5	100.0%	**72**	**100.0%**	38	100.0%	29	100.0%	5	100.0%	**72**	**100.0%**
大阪府藤井寺保健所																
とても満足	132	69.8%	60	60.6%	26	70.3%	**218**	**67.1%**	127	67.2%	56	56.6%	23	62.2%	**206**	**63.4%**
やや満足	35	18.5%	17	17.2%	9	24.3%	**61**	**18.8%**	35	18.5%	19	19.2%	12	32.4%	**66**	**20.3%**
やや不満	2	1.1%	5	5.1%	0	0.0%	**7**	**2.2%**	3	1.6%	6	6.1%	0	0.0%	**9**	**2.8%**
とても不満	0	0.0%	1	1.0%	0	0.0%	**1**	**0.3%**	2	1.1%	2	2.0%	0	0.0%	**4**	**1.2%**
無回答	20	10.6%	16	16.2%	2	5.4%	**38**	**11.7%**	22	11.6%	16	16.2%	2	5.4%	**40**	**12.3%**
合計	189	100.0%	99	100.0%	37	100.0%	**325**	**100.0%**	189	100.0%	99	100.0%	37	100.0%	**325**	**100.0%**
大阪府茨木保健所																
とても満足	280	72.5%	119	70.8%	62	80.5%	**461**	**73.1%**	262	67.9%	107	63.7%	60	77.9%	**429**	**68.0%**
やや満足	46	11.9%	23	13.7%	9	11.7%	**78**	**12.4%**	55	14.2%	37	22.0%	10	13.0%	**102**	**16.2%**
やや不満	8	2.1%	5	3.0%	1	1.3%	**14**	**2.2%**	13	3.4%	2	1.2%	2	2.6%	**17**	**2.7%**
とても不満	1	0.3%	3	1.8%	0	0.0%	**4**	**0.6%**	2	0.5%	4	2.4%	0	0.0%	**6**	**1.0%**
無回答	51	13.2%	18	10.7%	5	6.5%	**74**	**11.7%**	54	14.0%	18	10.7%	5	6.5%	**77**	**12.2%**
合計	386	100.0%	168	100.0%	77	100.0%	**631**	**100.0%**	386	100.0%	168	100.0%	77	100.0%	**631**	**100.0%**
大阪府富田林保健所																
とても満足	29	74.4%	18	69.2%	6	75.0%	**53**	**72.6%**	25	64.1%	17	65.4%	6	75.0%	**48**	**65.8%**
やや満足	3	7.7%	5	19.2%	1	12.5%	**9**	**12.3%**	5	12.8%	4	15.4%	1	12.5%	**10**	**13.7%**
やや不満	1	2.6%	1	3.8%	0	0.0%	**2**	**2.7%**	3	7.7%	2	7.7%	0	0.0%	**5**	**6.8%**
とても不満	0	0.0%	0	0.0%	1	12.5%	**1**	**1.4%**	0	0.0%	1	3.8%	1	12.5%	**2**	**2.7%**
無回答	6	15.4%	2	7.7%	0	0.0%	**8**	**11.0%**	6	15.4%	2	7.7%	0	0.0%	**8**	**11.0%**
合計	39	100.0%	26	100.0%	8	100.0%	**73**	**100.0%**	39	100.0%	26	100.0%	8	100.0%	**73**	**100.0%**
大阪府和泉保健所																
とても満足	16	25.4%	19	39.6%	3	42.9%	**38**	**32.2%**	16	25.4%	16	33.3%	3	42.9%	**35**	**29.7%**
やや満足	7	11.1%	3	6.3%	1	14.3%	**11**	**9.3%**	7	11.1%	4	8.3%	0	0.0%	**11**	**9.3%**
やや不満	1	1.6%	0	0.0%	0	0.0%	**1**	**0.8%**	1	1.6%	0	0.0%	0	0.0%	**1**	**0.8%**
とても不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	0	0.0%	0	0.0%	1	14.3%	**1**	**0.8%**
無回答	39	61.9%	26	54.2%	3	42.9%	**68**	**57.6%**	39	61.9%	28	58.3%	3	42.9%	**70**	**59.3%**
合計	63	100.0%	48	100.0%	7	100.0%	**118**	**100.0%**	63	100.0%	48	100.0%	7	100.0%	**118**	**100.0%**
大阪府池田保健所																
とても満足	56	75.7%	37	69.8%	3	37.5%	**96**	**71.1%**	50	67.6%	36	67.9%	3	37.5%	**89**	**65.9%**
やや満足	11	14.9%	10	18.9%	4	50.0%	**25**	**18.5%**	16	21.6%	7	13.2%	3	37.5%	**26**	**19.3%**
やや不満	1	1.4%	2	3.8%	0	0.0%	**3**	**2.2%**	1	1.4%	3	5.7%	1	12.5%	**5**	**3.7%**
とても不満	0	0.0%	0	0.0%	0	0.0%	**0**	**0.0%**	0	0.0%	3	5.7%	0	0.0%	**3**	**2.2%**
無回答	6	8.1%	4	7.5%	1	12.5%	**11**	**8.1%**	7	9.5%	4	7.5%	1	12.5%	**12**	**8.9%**
合計	74	100.0%	53	100.0%	8	100.0%	**135**	**100.0%**	74	100.0%	53	100.0%	8	100.0%	**135**	**100.0%**
chotCASTなんば																
とても満足	2489	65.5%	1362	66.8%	866	66.6%	**4717**	**66.1%**	2456	64.6%	1324	64.9%	830	63.8%	**4610**	**64.6%**
やや満足	942	24.8%	491	24.1%	328	25.2%	**1761**	**24.7%**	881	23.2%	474	23.2%	325	25.0%	**1680**	**23.5%**
やや不満	128	3.4%	101	5.0%	53	4.1%	**282**	**4.0%**	196	5.2%	139	6.8%	83	6.4%	**418**	**5.9%**
とても不満	13	0.3%	16	0.8%	9	0.7%	**38**	**0.5%**	35	0.9%	30	1.5%	17	1.3%	**82**	**1.1%**
無回答	227	6.0%	69	3.4%	45	3.5%	**341**	**4.8%**	231	6.1%	72	3.5%	46	3.5%	**349**	**4.9%**
合計	3799	100.0%	2039	100.0%	1301	100.0%	**7139**	**100.0%**	3799	100.0%	2039	100.0%	1301	100.0%	**7139**	**100.0%**

付表 20　MSM 受検者における年齢層別分析【2013 年 10 月~2014 年 9 月】

	年齢階級10歳区分						合計	Pearson
	19歳以下	20-29歳	30-39歳	40-49歳	50-59歳	60歳以上		X2 による
	n=72	n=795	n=554	n=352	n=106	n=63	n=1942	P値
行政区分								
大阪市	11 15.3%	130 16.4%	154 27.8%	117 33.2%	38 35.8%	33 52.4%	483 24.9%	**<0.01**
大阪府	8 11.1%	85 10.7%	46 8.3%	25 7.1%	6 5.7%	8 12.7%	178 9.2%	
chotCASTなんば	53 73.6%	580 73.0%	354 63.9%	210 59.7%	62 58.5%	22 34.9%	1281 66.0%	
今回を除いて、これまでにHIV検査（エイズ検査）を受けたことがありますか？								
ある	23 31.9%	498 62.6%	437 78.9%	276 78.4%	88 83.0%	47 74.6%	1369 70.5%	**<0.01**
ない(今回初めて)	48 66.7%	294 37.0%	116 20.9%	75 21.3%	18 17.0%	16 25.4%	567 29.2%	
無回答	1 1.4%	3 0.4%	1 0.2%	1 0.3%	0 0.0%	0 0.0%	6 0.3%	
今回はどなたと来られましたか？								
1人で来た	33 45.8%	512 64.4%	413 74.5%	270 76.7%	91 85.8%	52 82.5%	1371 70.6%	**<0.01**
家族・恋人	6 8.3%	55 6.9%	23 4.2%	16 4.5%	0 0.0%	1 1.6%	101 5.2%	
友達・その他	18 25.0%	94 11.8%	26 4.7%	11 3.1%	3 2.8%	3 4.8%	155 8.0%	
無回答	15 20.8%	134 16.9%	92 16.6%	55 15.6%	12 11.3%	7 11.1%	315 16.2%	
今回、あなたは自分で検査を受けようと思いましたか？								
自分で受けようと決めた	47 65.3%	677 85.2%	509 91.9%	331 94.0%	99 93.4%	59 93.7%	1722 88.7%	**<0.01**
人から勧められた、または誘われた	25 34.7%	117 14.7%	43 7.8%	20 5.7%	7 6.6%	4 6.3%	216 11.1%	
無回答	0 0.0%	1 0.1%	2 0.4%	1 0.3%	0 0.0%	0 0.0%	4 0.2%	
対象地域								
大阪府	57 79.2%	664 83.5%	463 83.6%	291 82.7%	94 88.7%	54 85.7%	1623 83.6%	0.63
大阪府以外の地域	15 20.8%	131 16.5%	91 16.4%	61 17.3%	12 11.3%	9 14.3%	319 16.4%	
問25　検査満足度　2) 話し方・言葉づかい								
とても満足	51 70.8%	591 74.3%	394 71.1%	251 71.3%	70 66.0%	41 65.1%	1398 72.0%	0.18
やや満足	14 19.4%	158 19.9%	115 20.8%	65 18.5%	24 22.6%	13 20.6%	389 20.0%	
やや不満	3 4.2%	9 1.1%	15 2.7%	7 2.0%	2 1.9%	2 3.2%	38 2.0%	
とても不満	1 1.4%	4 0.5%	0 0.0%	2 0.6%	1 0.9%	0 0.0%	8 0.4%	
無回答	3 4.2%	33 4.2%	30 5.4%	27 7.7%	9 8.5%	7 11.1%	109 5.6%	
問25　検査満足度　3) 質問しやすい雰囲気								
とても満足	51 70.8%	570 71.7%	371 67.0%	241 68.5%	70 66.0%	36 57.1%	1339 68.9%	0.20
やや満足	15 20.8%	171 21.5%	131 23.6%	75 21.3%	23 21.7%	18 28.6%	433 22.3%	
やや不満	3 4.2%	16 2.0%	19 3.4%	6 1.7%	5 4.7%	1 1.6%	50 2.6%	
とても不満	0 0.0%	4 0.5%	2 0.4%	3 0.9%	0 0.0%	0 0.0%	9 0.5%	
無回答	3 4.2%	34 4.3%	31 5.6%	27 7.7%	8 7.5%	8 12.7%	111 5.7%	
問25　検査満足度　5) 安心できる雰囲気								
とても満足	49 68.1%	564 70.9%	365 65.9%	231 65.6%	70 66.0%	38 60.3%	1317 67.8%	0.20
やや満足	18 25.0%	168 21.1%	135 24.4%	74 21.0%	22 20.8%	17 27.0%	434 22.3%	
やや不満	2 2.8%	23 2.9%	20 3.6%	16 4.5%	5 4.7%	0 0.0%	66 3.4%	
とても不満	0 0.0%	7 0.9%	3 0.5%	2 0.6%	1 0.9%	0 0.0%	13 0.7%	
無回答	3 4.2%	33 4.2%	31 5.6%	29 8.2%	8 7.5%	8 12.7%	112 5.8%	
問25　検査満足度　4) プライバシー保護								
とても満足	52 72.2%	541 68.1%	339 61.2%	208 59.1%	65 61.3%	38 60.3%	1243 64.0%	**<0.01**
やや満足	14 19.4%	181 22.8%	133 24.0%	79 22.4%	20 18.9%	15 23.8%	442 22.8%	
やや不満	0 0.0%	30 3.8%	41 7.4%	29 8.2%	10 9.4%	2 3.2%	112 5.8%	
とても不満	2 2.8%	10 1.3%	10 1.8%	7 2.0%	3 2.8%	0 0.0%	32 1.6%	
無回答	4 5.6%	33 4.2%	31 5.6%	29 8.2%	8 7.5%	8 12.7%	113 5.8%	
季刊誌 南界堂通信(ロゴ画像)								
見ていない	72 100%	788 99.1%	545 98.4%	342 97.2%	104 98.1%	58 92.1%	1909 98.3%	**<0.01**
見た	0 0.0%	7 0.9%	9 1.6%	10 2.8%	2 1.9%	5 7.9%	33 1.7%	
コミュニティセンターdista(ロゴ画像)								
見ていない	63 87.5%	681 85.7%	468 84.5%	310 88.1%	94 88.7%	62 98.4%	1678 86.4%	0.05
見た	9 12.5%	114 14.3%	86 15.5%	42 11.9%	12 11.3%	1 1.6%	264 13.6%	

Ⅲ. 研究成果刊行物一覧

研究論文別刷

Ⅳ. 研究成果の刊行に関する一覧表・刊行物

著者	タイトル	雑誌名	巻号	ページ	出版年
Mayumi Imahashi, Taisuke Izumi, Dai Watanabe, Junji Imamura, Kazuhiro Matsuoka, Hirotaka Ode, Takashi Masaoka, Kei Sato, Noriyo Kaneko, Seiichi Ichikawa, Yoshio Koyanagi, Akifumi Takaori-Kondo, Makoto Utsumi, Yoshiyuki Yokomaku, Takuma Shirasaka, Wataru Sugiura, Yasumasa Iwatani, Tomoki Naoe	Lack of Association between Intact/Deletion Polymorphisms of the APOBEC3B Gene and HIV-1 Risk	PLOS ONE	DOI: 10.1371/journal.pone.	0092861	2014
Yasuharu Hidaka, Don Operario, Hiroyuki Tsuji, Mie Takenaka, Hirokazu Kimura, Mitsuhiro Kamakura, Seiichi Ichikawa	Prevalence of Sexual Victimization and Correlates of Forced Sex in Japanese Men Who Have Sex with Men	PLOS ONE	Vol. 9 Issue 5	E95675	2014
纐纈ゆき、金子典代、市川誠一	若年女性における過去と現在の性感染症予防行動と情報入手状況の比較	日本ウーマンズヘルス学会誌（別冊）	Vol.13	53-62	2014
松下修三、市川誠一、生島嗣、木村哲、荒木順子	座談会「治療が予防になる時代のコミュニティセンター事業」	HIV 感染症と AIDS の治療(別冊)	5巻2号	4-19	2014

PLOS ONE

Lack of Association between Intact/Deletion Polymorphisms of the *APOBEC3B* Gene and HIV-1 Risk

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Abstract

Objective: The human APOBEC3 family of proteins potently restricts HIV-1 replication *APOBEC3B*, one of the family genes, is frequently deleted in human populations. Two previous studies reached inconsistent conclusions regarding the effects of *APOBEC3B* loss on HIV-1 acquisition and pathogenesis. Therefore, it was necessary to verify the effects of APOBEC3B on HIV-1 infection *in vivo*.

Methods: Intact (I) and deletion (D) polymorphisms of *APOBEC3B* were analyzed using PCR. The syphilis, HBV and HCV infection rates, as well as $CD4^+$ T cell counts and viral loads were compared among three *APOBEC3B* genotype groups (I/I, D/I, and D/D). HIV-1 replication kinetics was assayed *in vitro* using primary cells derived from PBMCs.

Results: A total of 248 HIV-1-infected Japanese men who have sex with men (MSM) patients and 207 uninfected Japanese MSM were enrolled in this study. The genotype analysis revealed no significant differences between the *APOBEC3B* genotype ratios of the infected and the uninfected cohorts ($p = 0.66$). In addition, HIV-1 disease progression parameters were not associated with the *APOBEC3B* genotype. Furthermore, the PBMCs from D/D and I/I subjects exhibited comparable HIV-1 susceptibility.

Conclusion: Our analysis of a population-based matched cohort suggests that the antiviral mechanism of APOBEC3B plays only a negligible role in eliminating HIV-1 *in vivo*.

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Introduction

Human APOBEC3 proteins are cellular cytidine deaminases that play crucial roles in the inhibition of retroviral replication, including that of HIV-1 [1–3]. The molecular mechanisms underlying APOBEC3-mediated HIV-1 restriction are primarily dependent on the editing [1,2] and/or non-editing activities [4,5] of these enzymes. The family of genes encoding the seven APOBEC3 proteins (APOBEC3A, B, C, DE, F, G, and H) is positioned in a tandem array on human chromosome 22 [6]. HIV-1 produces an accessory protein, Vif, that invalidates the antiviral functions of the APOBEC3 proteins by mediating the ubiquitination-proteasomal degradation of APOBEC3 in virus-producing cells [7]. APOBEC3C, DE, F, G, and H (haplotype II) are vulnerable to HIV-1 Vif-mediated degradation, whereas APOBEC3A and B are resistant [8–12].

Among the members of the APOBEC3 family, APOBEC3G has been consistently shown to possess powerful anti-HIV-1 activity in cell-based systems [1,2], and this protein may affect the pathogenesis of HIV-1 infection *in vivo* [13–19]. However, there is little consensus regarding the degree to which the other APOBEC3 family members, especially APOBEC3B, are able to restrict HIV-1 replication *in vitro* and *in vivo*. The anti-HIV-1 activity of APOBEC3B is undetectable when this gene is stably expressed in a human T cell line [20] and is detected only weakly after the transient transfection of HEK 293T or HeLa cells [20–22]. Because these findings have varied according to the experimental conditions employed, there is a fundamental

question whether the expression of human APOBEC3B, DE, and F plays a critical role in HIV-1 restriction *in vivo*. The potential role of APOBEC3B in modulating HIV-1 replication *in vivo* is of particular interest because this protein is resistant to HIV-1 Vif-mediated degradation [20,22–24].

A polymorphic deletion of a 29.5-kb segment between *APOBEC3A* exon 5 and *APOBEC3B* exon 8 has been identified in human populations; this polymorphism causes the loss of the entire APOBEC3B coding region [25]. A particularly high frequency of *APOBEC3B* deletion has been found among Asians [25]. According to Kidd et al., the deletion allele is rare in Africans (1%) and Europeans (6%), more common in East Asians (36%) and Amerindians (58%), and almost fixed in Oceanians (93%) [25].

Two independent groups have reported contrasting findings concerning the effects of the *APOBEC3B* gene deletion on HIV-1 acquisition and disease progression [26,27]. An et al. determined that the deletion allele genotype correlated with a higher risk of HIV-1 infection, whereas a study conducted by Itaya et al. concluded that the deletion polymorphism had no effect on HIV-1 acquisition and the rate of disease progression to AIDS. An et al. included 4 patients with homozygous deletions of *APOBEC3B* in their HIV-1-seropositive cohorts of 656 European and 296 African-American individuals but no homozygotes for the deletion in their seronegative groups, which prevented a proper evaluation of the impact of the deletion polymorphism on HIV-1 acquisition and pathogenesis [26]. In contrast, the study conducted by Itaya et al. in Japan utilized inappropriate enrollment [27], because the enrolled patients were all hemophiliacs who had survived HIV-1 infection for at least 10 years prior to the study and the information for individuals who had progressed to AIDS and death before the enrollment date was excluded.

To examine the impact of the *APOBEC3B* deletion polymorphism on HIV-1 infection risk *in vivo*, this study enrolled a matched cohort in Japan and investigated the impact of *APOBEC3B* gene intact/deletion polymorphisms on HIV-1 susceptibility and pathogenesis. In addition, we analyzed the effects of different *APOBEC3B* genotypes on HIV-1 replication kinetics *in vitro*.

Materials and Methods

Sample Collection

A total of 248 Japanese HIV-1-positive men who have sex with men (MSM) who were patients at Nagoya Medical Center (n = 203) and Osaka Medical Center (n = 45) were enrolled in this study from November 2011 to February 2013. The control group comprised 207 Japanese HIV-1-negative MSM who were recruited at the Nagoya Lesbian & Gay Revolution Plus (NLGR+) festival in June 2012. The study protocol was approved by the ethics committees of Nagoya Medical Center (registration number 2011-430) and Osaka Medical Center. Written informed consent was obtained from all the participants. The control subjects recruited at the NLGR+ festival provided anonymous consent. To collect information regarding their sex, nationality, age, and sexuality, anonymous questionnaires collated with linked numbers were obtained.

Genotyping

The *APOBEC3B* intact (I) and deletion (D) alleles were genotyped using a previously reported polymerase chain reaction (PCR) method [26] with slight modifications. Of note, the "intact (I)" in this study is used for the "insertion" that originally reported by Kidd et al. [25]. Briefly, the primer sets for amplifying the Deletion and Insertion 2 fragments were the same as those previously described [26], although one additional set of primers for the Insertion 1 fragement was replaced by the two following oligonucleotide primers: Insertion3_F: 5′-GAGTG-GAAGCGCCTCCTC-3′ and Insertion3_R: 5′-CTCCTGGCCAGCCTAGC-3′. The QIAamp DNA Blood Mini Kit (Qiagen, Valencia, USA) was used according to the manufacturer's protocol to extract genomic DNA from whole blood (patients) or from buccal mucosa (controls).

Analysis of Viral Replication Capacity and Infectivity

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood samples from different HIV-1-negative donors with the I/I and D/D *APOBEC3B* genotypes (n = 5 for each) using Ficoll-Hypaque density gradient centrifugation (Pharmacia, Uppsala, Sweden). The PBMCs were then subjected to negative selection with the MACS CD4 T Cell Isolation Kit (Miltenyi Biotec, Cologne, Germany) to purify primary $CD4^+$ T cells. The cells were activated with 1 μg/ml of phytohemagglutinin (PHA) (Pharmacia) for 72 hours, infected with HIV-1 NL4-3 for 24 hours with a multiplicity of infection (MOI) of 0.01, washed twice, and maintained in RPMI-1640 medium with 20% fetal bovine serum (FBS), penicillin (50 U/ml)/streptomycin (50 μg/ml) (Invitrogen, Carlsbad, USA), and 20 U/ml interleukin-2 (IL-2) (Roche Applied Science, Mannheim, Germany). The culture supernatants were assayed for the p24 antigen using the HIV-1 p24 Antigen Assay Kit (Coulter Corporation, Fullerton, USA) on the day of infection and on days 2, 4, 6, 8, 10, and 13 after infection. To analyze the viral infectivity of the infected PBMCs, culture supernatants were harvested six days post-infection and inoculated into TZM-bl cells [29] in black 96-well plates. The viral infectivity was assessed 48 hours post-infection by detecting β-galactosidase activity using the Galacto-Star System (Applied Biosystems, Foster City, USA).

Quantification of APOBEC3 mRNA

To analyze the mRNA expression levels of members of the APOBEC3 family, unstimulated $CD4^+$ cells from three different genotyped subjects were prepared for RNA isolation. The induction rates of mRNA transcription for APOBEC3A or APOBEC3G were analyzed using monocyte-derived macrophages (MDMs). Briefly, monocytes were isolated from PBMCs from each genotyped healthy donor using CD14 MicroBeads (Miltenyi Biotec). The enriched $CD14^+$ cells were plated at a cell density of 1×10^6/ml in 12-well plates in RPMI-1640 medium (Sigma, St. Louis, USA) with penicillin (50 U/ml)/streptomycin (50 μg/ml) for three hours, followed by the addition of 10% FBS and 10 ng/ml macrophage colony stimulating factor (M-CSF) (Peprotech, Rocky Hill, USA). Adherent cells were cultured for eight days to facilitate their differentiation into MDMs. Differentiated MDMs either received no stimulation or were stimulated with 100 U/ml of recombinant human interferon (IFN)-α (Sigma) for six hours and were then lysed for RNA isolation. As previously described [14,30], total RNA isolated using the QIAamp RNA Blood Mini Kit (Qiagen) was used to synthesize cDNA with the SuperScript III First-Strand Synthesis System (Invitrogen) using random hexamers. The cDNA levels were quantified using real-time PCR in a Thermal Cycler Dice Real Time System (TP800) (Takara Bio, Shiga, Japan). The real-time PCR was employed to analyze the levels of APOBEC3, β-actin, and GAPDH mRNA, and the assays were performed according to the manufacturer's protocol using SYBR Premix DimerEraser (Takara Bio). The primer sets for the real-time PCR were purchased from FASMAC Co., Ltd. (Atsugi, Japan) and the oligonucleotide sequences are

shown in Table S1. The gene expression levels were calculated using the ΔΔCt (Ct; cycle threshold) and are presented as the ratio of APOBEC3 mRNA to β-actin or GAPDH mRNA.

Statistical Analysis

The relationships between *APOBEC3B* genotype and baseline characteristics were assessed using the Fisher exact test for categorical variables. The Mann-Whitney *U*-test was used for continuous variables. All the statistical analyses were performed with the statistical software EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More specifically, this software is a version of R commander (version 1.6–3) modified to add statistical functions that are frequently used in biostatistics [31]. All the p values were two-tailed. The effects of *APOBEC3B* gene deletion on the disease progression of HIV-1 were evaluated based on the CD4+ T cell counts and log10 HIV-1 viral load (RNA copy number/ml) at more than two time points before the start of antiretroviral therapy (ART). Patients whose $CD4^+$ T cell counts and HIV-1 viral loads were measured at fewer than two time points were excluded from the statistical analyses of these factors. Other related infectious diseases were identified in the patients using the following definitions. If the rapid plasma reagin test and/or the *Treponema pallidum* latex agglutination (TPHA) test were positive, the patient was considered positive for syphilis. Patients were considered hepatitis B virus (HBV)-positive if either hepatitis B surface antigen (HBsAg) or hepatitis B core antibody (HBcAb) was present. In addition, patients were considered hepatitis C virus (HCV) carriers if they tested positive for HCV antibodies.

Results

APOBEC3B Genotype Frequencies in the Cohorts

The demographics of the HIV-1-positive and HIV-1-negative cohorts are shown in Table 1. A total of 248 HIV-1-infected Japanese MSM patients and 207 uninfected Japanese MSM were enrolled and analyzed in this study. To conduct a matched cohort study, all the participants were recruited from Nagoya and Osaka in the central area of Japan. First, a comparative analysis of the *APOBEC3B* genotype among the participants indicated that there were no significant differences in *APOBEC3B* genotype frequency between the HIV-1-positive (D/D 7.7%, I/D 44.0%, and I/I 48.4%) and HIV-1-negative (D/D 8.7%, I/D 39.6%, and I/I 51.7%) cohorts ($p = 0.66$) (Table 1). A comparison of the distributions of the *APOBEC3B* deletion allele in the HIV-1-positive and HIV-1-negative cohorts revealed that the D allele occurred in the HIV-1-positive (29.6%) and HIV-1-negative subjects (28.5%) at comparable rates ($p = 0.71$). We also analyzed the cDNA sequences of *APOBEC3B* I allele isolated from the Japanese healthy donors with the I/I or I/D genotypes (Table S1). There was one variant (rs#2076109): K62 (allele frequency, or $AF = 0.4$) (E62 as the reference) although we could not detect any other variants changing the amino acid sequences within the 15 alleles. According to the 1000 Genome database, the variant ($AF = 0.373$) appears globally distributed but not limited in Japan or Asia. In addition, we tested the antiviral effect of APOBEC3B E62 and the variant with an overexpression system using 293T cells (Figure S1). The results demonstrated that the E62 variant had equivalent antiviral activity to APOBEC3B K62 *in vitro*. These data suggest that the I alleles in our Japanese cohorts are not strongly biased in terms of genetic and functional features.

Next, we analyzed the HIV-1-positive individuals for the prevalence of HBV, HCV, and syphilis, as well as for HIV-1 disease progression at a minimum of two time points before the commencement of ART. The prevalence of each infectious disease is presented in Table 2. The frequencies of the three *APOBEC3B* genotypes (D/D 7.6%, I/D 45.4%, and I/I 47.0%) among the 132 HBV-positive patients were not significantly different from those of the 94 HBV-negative individuals (D/D 9.6%, I/D 41.5%, and I/I 48.9%) ($p = 0.69$). In addition, the *APOBEC3B* genotype distributions did not differ significantly between the HCV-positive and HCV-negative patients ($p = 1.00$) or between the syphilis-positive and syphilis-negative patients ($p = 0.62$) (Table 2).

We also assessed the rates of both $CD4^+$ T cell decline and plasma viral load increase at different time points after the first patient visit to the hospital prior to ART treatment. As shown in Figure 1, the changes in the $CD4^+$ T cell counts (cells/μl/day) and viral loads (log_{10} copies/ml/day) did not differ significantly according to *APOBEC3B* genotype (CD4: $p = 0.054$; viral load: $p = 0.96$). The data from the 46 patients (D/D 6.5%, I/D 41.3%, and I/I 52.2%) who began ART before the second measurement of the $CD4^+$ T cells and viral loads were excluded from the analysis. Of these 46 patients, 32 (D/D 3.1%, I/D 50.0%, and I/I 46.9%) began ART shortly after their first hospital visit due to AIDS onset; this decision was based on the domestic clinical guidelines of the Ministry of Health, Labor, and Welfare of Japan. There were no significant differences in the proportions of the *APOBEC3B* genotypes between the patients with $CD4^+$ T cell count and viral load data from at least two time points and the 46 patients without complete data ($p = 0.91$). Detailed demographic information on the HIV-1 (+) patients is shown in Table 3. Moreover, we analyzed the non-ART periods from the first diagnosis through the ART introduction and set two groups: longer and shorter than median days from diagnosis to ART. As the genotype frequencies were compared (Table 4), the results showed no significant difference in the *APOBEC3B* genotype between the two groups ($p = 0.96$).

Moreover, we performed deep sequencing of the HIV-1 proviral DNAs that were isolated from the I/I, I/D or D/D patients' PBMCs, and then analyzed the hypermutation rates on APOBEC3-prefered dinucleotide sequences: GG>AG and GA>AA mutations. The results showed that the hypermutation frequencies vary among different individuals although the levels of GA>AA hypermutation relative to the GG>AG are comparable among the three *APOBEC3B* genotypes (Figure S2). The data suggest that the APOBEC3B is not likely a major contributor to introduce hypermutations on the proviral DNAs in HIV-1(+) patients' PBMCs.

The Effects of *APOBEC3B* Genotype on Other APOBEC3 Expression Profiles

To assess whether the *APOBEC3B* gene deletion altered the expression of the other proximal *APOBEC3* genes, we compared mRNA expression profiles in fresh, unstimulated primary $CD4^+$ cells of each *APOBEC3B* genotype: D/D, I/D, and I/I. As shown in Figure 2A, the mRNA expression levels of APOBEC3A, which is the *APOBEC3* family member located closest to the *APOBEC3B* gene, were not significantly different between the I/I and D/D genotype groups ($p = 0.63$), although the levels would likely vary considerably among individuals. As expected, APOBEC3B mRNA expression levels were not detected in the D/D subjects (Figure 2A). The APOBEC3B mRNA levels in the I/D subjects were somewhat lower than in the I/I subjects, although this difference was not statistically significant (Figure 2A, $p = 0.12$). Moreover, the relative levels of APOBEC3C, DE, F, G, and H mRNA were comparable among the I/I, I/D, and D/D subjects (Figure 2A). We also analyzed APOBEC3B mRNA levels in

Table 1. *APOBEC3B* genotype frequency in HIV-1-positive patients and HIV-1-negative controls.

	HIV-1		
	Negative (%)	**Positive (%)**	**p[a]**
Genotype			
D/D	18/207 (8.7)	19/248 (7.7)	0.66
I/D	82/207 (39.6)	109/248 (44.0)	
I/I	107/207 (51.7)	120/248 (48.4)	
Allele			
D	118/414 (28.5)	147/496 (29.6)	0.71
I	296/414 (71.5)	349/496 (70.4)	

[a]Determined using the Fischer exact test.
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PBMCs isolated from healthy donors and HIV-1 seropositive patients with or without the ART. Similar to the pattern of APOBEC3B mRNA levels in the CD4+ T cells of three genotyped subjects (Figure 2A), the mRNA expression is slightly lower in the I/D genotyped PBMCs than in the I/I whereas no detectable level of APOBEC3B mRNA in the D/D PBMCs (Figure S3). The different expression levels between the I/D and I/I PBMCs were not statistically significant (Figure S3). Moreover, comparative analysis showed that the APOBEC3B mRNA level of each I/I or I/D genotype appears relatively higher in the HIV-1 (+) patients, regardless the ART-treatment, than in the uninfected donors. However, the difference was not statistically significant (Figure S3).

In the *APOBEC3B* D allele, the APOBEC3A mRNA contains a 3′-untranslated region of APOBEC3B's and is subject to the upstream regulatory elements of the *APOBEC3A*. Thus, we further assessed whether the degrees to which APOBEC3A and APOBEC3G mRNA expression was stimulated by IFN-α in MDMs differed between the *APOBEC3B* I/I and D/D genotypes. The APOBEC3G mRNA expression was used as a control because the gene is distal to the *APOBEC3B* loci on the genome. As shown in Figure 2B, IFN-α stimulation resulted in APOBEC3A mRNA increases in the I/I and D/D MDMs of 1,999±1,190-fold and 1,251±264-fold, respectively. The APOBEC3G mRNA levels increased upon IFN-α stimulation by 28.6±41.8-fold (I/I) and 38.9±18.0-fold (D/D). A comparison of the mRNA expression magnitudes between the two homozygous *APOBEC3B* genotypes revealed no significant differences ($p = 0.4$ and $p = 0.4$ for APOBEC3A and APOBEC3G, respectively).

The Effects of *APOBEC3B* Genotype on HIV-1 Susceptibility *in Vitro*

We further analyzed the viral replication kinetics in primary PBMCs isolated from D/D or I/I donors. At an MOI of 0.01, the efficiency of HIV-1 replication was comparable between the D/D and I/I genotypes (Figure 3A). The p24 antigen levels in the culture supernatant from the I/I and D/D PBMCs were $8.7 \pm 3.0 \times 10^5$ pg/ml and $1.3 \pm 0.2 \times 10^6$ pg/ml, respectively, on day 8 ($p = 0.31$) and $8.4 \pm 0.2 \times 10^5$ pg/ml and $1.3 \pm 0.3 \times 10^6$ pg/ml, respectively, on day 6 ($p = 0.13$). At the peak of infection (day 6), the virus-containing supernatants derived from D/D and I/I PBMCs exhibited comparable levels of infectivity ($p = 0.86$) (Figure 3B). These data suggest that the different *APOBEC3B* deletion genotypes are not associated with significantly different levels of HIV-1 susceptibility *in vitro*.

Table 2. *APOBEC3B* genotype frequency and clinical parameters in HIV-1-positive patients.

	APOBEC3B genotype			
	D/D (%)	**I/D (%)**	**I/I (%)**	**p[a]**
HBV				
Positive	10/132 (7.6)	60/132 (45.4)	62/132 (47.0)	0.69
Negative	9/94 (9.6)	39/94 (41.5)	46/94 (48.9)	
Unknown	0/22 (0)	10/22 (45.5)	12/22 (54.5)	
HCV				
Positive	0/7 (0)	3/7 (42.9)	4/7 (57.1)	1.00
Negative	19/241(7.9)	106/241 (44.0)	116/241 (48.1)	
Syphilis				
Positive	9/116 (7.8)	47/116 (40.5)	60/116 (51.7)	0.62
Negative	10/131 (7.6)	61/131 (46.6)	60/131 (45.8)	
Unknown	0/1 (0)	1/1 (100)	0/1(0)	

[a]Determined using the Fischer exact test.
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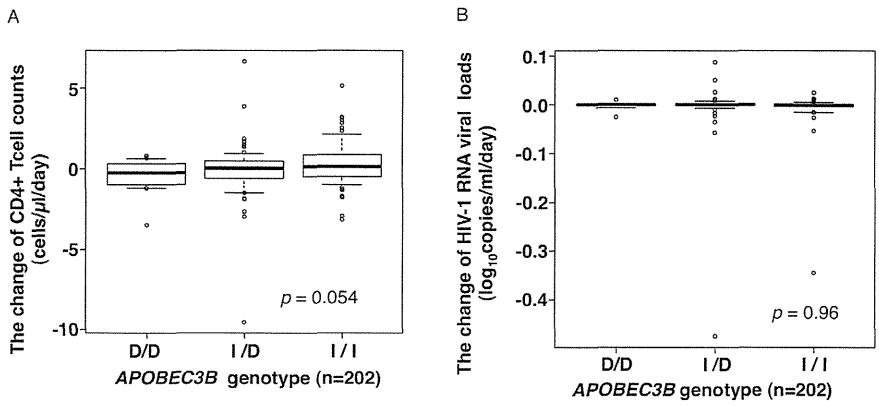

Figure 1. Analysis of effects of genotype on parameters of HIV disease progression in the HIV-1-infected cohort. (A) Changes in $CD4^+$ T cell counts (cells/μl/day) (n = 202). (B) Changes in HIV-1 RNA levels (log_{10} copies/ml/day) in plasma (n = 202). The box plots show data between the 25th and 75th percentiles with central horizontal lines representing the median, and with whiskers showing the 10th and 90th percentiles. The open circles represent outliers with data >1.5-fold of the interquartile range. All the p values were determined using the Kruskal-Wallis test.
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Discussion

There is only limited information about the roles played by APOBEC3 family members *in vivo*, with the exception of APOBEC3G. Previously, two independent groups reported conflicting conclusions regarding the impact of the *APOBEC3B* gene deletion on human HIV-1 infection *in vivo*, and this issue remains unclear [26,27]. Therefore, to determine the effects of different *APOBEC3B* genotypes on HIV-1 infection *in vivo* and *in vitro*, we investigated the frequencies of intact and deletion polymorphisms of the *APOBEC3B* gene in a matched cohort in Japan.

The comparison of *APOBEC3B* genotypes in HIV-1-infected patients and HIV-1-negative controls revealed similar *APOBEC3B* genotype distributions in the two groups: D/D 7.7%, I/D 44.0%, and I/I 48.4% in the infected cohort versus D/D 8.7%, I/D 39.6%, and I/I 51.7% in the uninfected cohort (p = 0.66). In addition, no significant associations between the *APOBEC3B* genotype and the subclinical parameters of disease progression were observed among the HIV-1-positive patients. We also found no differences between the mRNA expression profiles of other APOBEC3 family members in PBMCs. Furthermore, the IFN-α-stimulated mRNA induction rates for APOBEC3A and APOBEC3G in MDMs did not differ between the D/D and I/I genotypes. Moreover, the HIV-1 susceptibility levels in PBMCs were comparable between the two genotypes. Considered together, our findings suggest that the loss of APOBEC3B is not significantly associated with HIV-1 acquisition and pathogenesis *in vivo* and with HIV-1 susceptibility *in vitro*, which fully supports the results of the cohort study conducted by Itaya et al [27].

There are two possible explanations for the lack of APOBEC3B involvement in HIV-1 restriction. First, the APOBEC3B protein cannot be incorporated into viral cores. Efficient HIV-1 restriction requires that APOBEC3 family proteins are packaged into virions through associations with viral and/or nonviral RNA [1,2,28–30] and that the proteins are localized to the plasma membrane in virus-producing cells [31]. APOBEC3G colocalizes with HIV-1 RNA and cellular RNA in P bodies [32] and are dispersed throughout the cytoplasm that facilitate interactions with HIV-1 Gag proteins and their incorporation into nascent virions [1,2]. In contrast, APOBEC3B predominantly localizes to the nucleus [20,21,33], which may prevent its incorporation into virions.

The second possible explanation is that the low expression level of APOBEC3B in PBMCs [22,34,35] is insufficient to block HIV-

Table 3. Demographics of the cohorts.

	HIV-1 Negative (n = 207)	HIV-1 Positive (n = 248)
Age (years), median [IQR][a]	33 [26–39]	40 [36–51]
Year of diagnosis, median [IQR] [a]	NA[b]	2008 [2005–2010]
ART naïve at entry, n (%)	NA[b]	20 (8%)
$CD4^+$ cell count at entry (cells/mm^3), median [IQR] [a]	NA[b]	451 [294–534]
HIV-1 viral load at entry (copies/mL), median [IQR] [a]	NA[b]	61 [<40–410]
History of AIDS, n (%)	NA[b]	32 (13%)
Days from diagnosis to entry, median [IQR] [a]	NA[b]	1470 [539–2256]
Observation periods for disease progression (days), median [IQR] [a]	NA[b]	56 [28–88]
Days from diagnosis to ART, median [IQR] [a]	NA[b]	88 [38–599]

[a]IQR denotes interquartile range.
[b]NA, Not applicable.
doi:10.1371/journal.pone.0092861.t003

Table 4. *APOBEC3B* genotype frequency on the days from diagnosis to ART (n = 246).

	days from diagnosis to ART		
	88 days> (%)	88 days< (%)	p[a]
Genotype			
D/D	9 (3.7)	10 (4.1)	0.961
I/D	49 (19.9)	59 (24.0)	
I/I	57 (23.2)	62 (25.2)	

(Median days from diagnosis to ART = 88 days).
[a]Determined using the Fischer exact test.
The diagnosis date of 2 patients(each patient's genotype is I/I and I/D, respectively.) are unknown.
doi:10.1371/journal.pone.0092861.t004

1 replication, as shown in Figure 3. Similar to the HIV-1 results, overexpressed APOBEC3B potently suppresses HBV replication *in vitro* [36]. However, a study by Abe et al. on the frequency of the D/D genotype in HBV carriers demonstrated that the *APOBEC3B* gene deletion was not responsible for chronic HBV infection [37]. These data suggest that the high expression of APOBEC3B *in vitro* may produce exaggerated effects on both HIV-1 and HBV infection *in vitro*.

All the participants enrolled in this study were Japanese MSM, according to the information provided on anonymous questionnaires. Because approximately 80% of the HIV-1-positive patients in Japan are MSM [38], we investigated the effects of *APOBEC3B* deletion polymorphisms on this major mode of HIV-1 transmission rather than on the two other major modes (injection drug use and heterosexual intercourse). However, the effect of *APOBEC3B* genotype is less likely to be dependent on the mode of HIV-1 transmission because APOBEC3B mRNA expression in hematopoietic cells is lower and less tissue-specific than that of most of the other APOBEC3 family members [22,34,35].

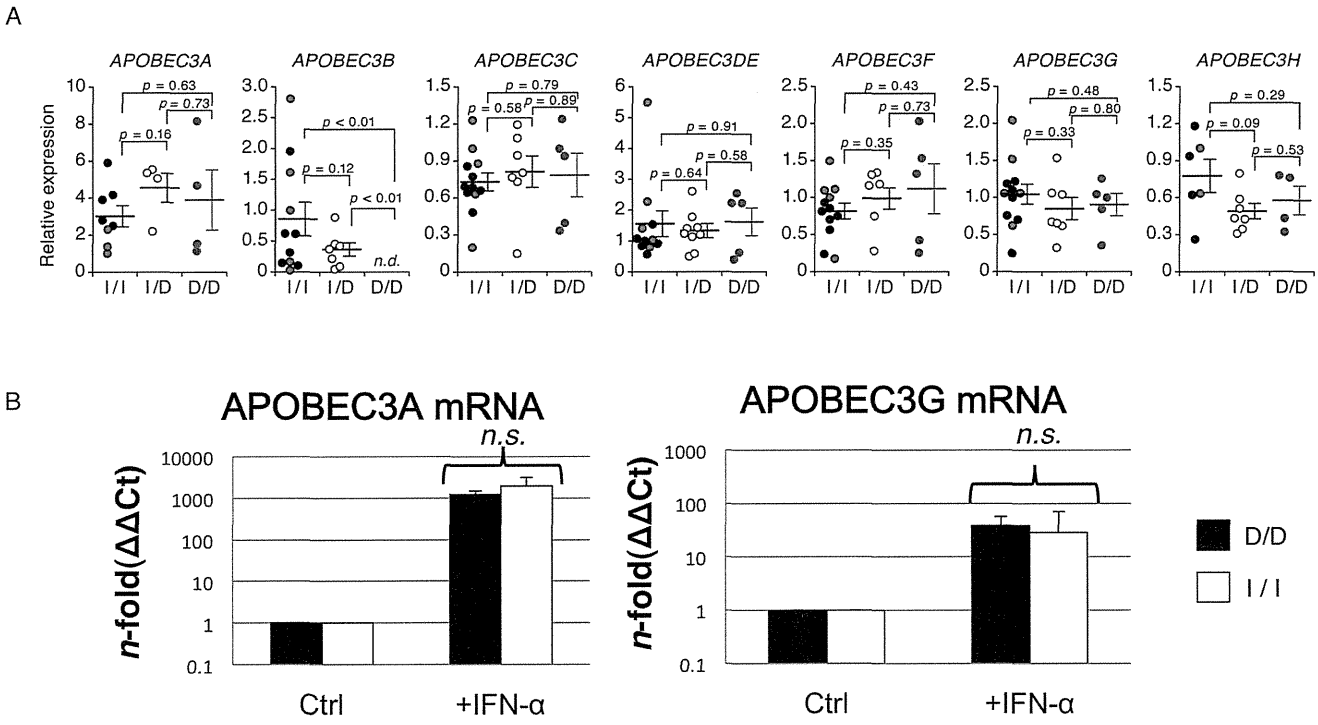

Figure 2. APOBEC3 mRNA expression levels depending on *APOBEC3B* genotype. (A) Comparison of mRNA expression levels of APOBEC3 in $CD4^+$ cells isolated from intact (I/I), hemizygous (I/D) and deletion (D/D) individuals of healthy donors. The relative mRNA expression levels of APOBEC3A (I/I, n = 8; I/D, n = 4; D/D, n = 4), APOBEC3B (I/I, n = 11; I/D, n = 7; D/D, n = 5), APOBEC3C (I/I, n = 12; I/D, n = 7; D/D, n = 5), APOBEC3DE (I/I, n = 11; I/D, n = 9; D/D, n = 5), APOBEC3F (I/I, n = 12; I/D, n = 7; D/D, n = 5), APOBEC3G (I/I, n = 12; I/D, n = 7; D/D, n = 5), and APOBEC3H (I/I, n = 6; I/D, n = 7; D/D, n = 4) were determined using quantitative RT-PCR and were normalized to GAPDH. The red (I/I) or gray (D/D) dots represent the expression levels of donors whose PBMCs were used for the *in vitro* kinetics of HIV-1 replication and infectivity in Figure. 3. The p values were calculated using Welch's *t*-test. The error bar represents the standard error of the mean (SEM). (B) APOBEC3A (A3A) and APOBEC3G (A3G) mRNA expression levels under basal conditions (Ctrl) and after stimulation with 100 U/ml (+IFN-α) of interferon (IFN)-α in $CD14^+$ MDMs isolated from healthy control subjects. The black and white bars indicate D/D (n = 3) and I/I (n = 4) individuals, respectively. The *p* values were calculated with the Mann-Whitney *U*-test. The error bars represent the standard deviation. *n.d.*, not detected. Ct, cycle threshold. *n.s.*, not significant ($p = 0.4$ for both cases).
doi:10.1371/journal.pone.0092861.g002

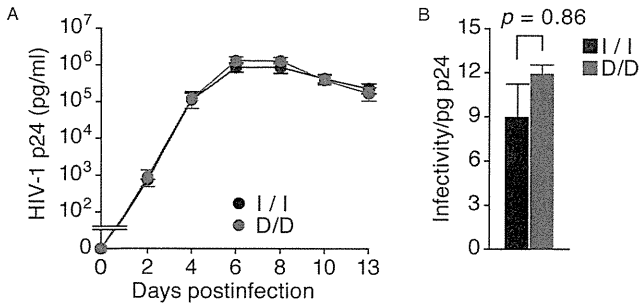

Figure 3. The kinetics and infectivity of HIV-1 depending on *APOBEC3B* genotype. (A) The kinetics of HIV-1 replication in PBMCs isolated from I/I (black dot) or D/D (gray dot) subjects (n = 5 each). (B) The infectivity values of virus-containing supernatants derived from I/I (black bar) and D/D (gray bar) PBMCs six days post-infection are provided relative to the values normalized with equal amounts of p24. The assay was performed using samples from three donors, and a representative result is shown. The p values were calculated using Welch's *t*-test. The error bars represent the SEM.
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In the D/D genotype, APOBEC3A mRNA expressed from the genome has a 3′-untranslated region corresponding to that of APOBEC3B. In addition, the genomic location of the APOBEC3G coding region is closer to the highly IFN-responsive transcriptional element of *APOBEC3A* in the D/D genome than to in the I/I. Therefore, we evaluated whether *APOBEC3B* gene deletion altered the IFN-stimulated gene induction of the other APOBEC3 family members. Our results suggest that the 29.5-kb genomic deletion of *APOBEC3B* does not significantly affect the expression profiles of the proximal *APOBEC3* family genes. Therefore, it is unlikely that the loss of the *APOBEC3B* gene in the D/D population leads to functional compensation via the mRNA expression modulation of the other APOBEC3 family members. Interestingly, Biasin et al. have demonstrated that increased levels of APOBEC3G mRNA in PBMCs, (primarily $CD14^+$ MDMs) following exposure to IFN-α correlated with HIV-1 susceptibility both *in vivo* and *in vitro* [13]. Our results showed that the induction magnitude of APOBEC3G mRNA upon the IFN-α stimulation was similar between the I/I and D/D genotypes (Figure 2B). This suggests that different HIV-1 susceptibility observed by Basin et al. is unlikely linked to the *APOBEC3B* intact/deletion genotypes.

Recent studies of tumors such as breast cancers [39–41] and lymphomas [42] have shown that increased expression of APOBEC3B *in vivo* was linked to the chronic induction of mutations and/or instability in genomic DNA. We did not observe any significant diagnosable HIV-associated cancers in our short-term cohort study. It may be necessary to continue our prospective studies for a longer period. In addition, because other studies have suggested that *APOBEC3B* gene deficiency is associated with higher susceptibility to two other ancient pathogens, human T-cell leukemia virus type 1 [43,44] and *Plasmodium falciparum* (the causative agent of malaria) [45], it would be beneficial to further investigate the correlations between *APOBEC3B* genotype and susceptibility to unknown pathogens.

Conclusions

Our analysis of a population-based matched cohort provided important evidence that the loss of the *APOBEC3B* gene is not associated with risk of HIV-1 infection and disease progression. In addition, the *in vitro* kinetics of HIV-1 replication and the infectivity of the virus in PBMCs were comparable between the D/D and I/I subjects. These results suggest that the APOBEC3B antiviral mechanism plays only a negligible role in eliminating HIV-1 *in vivo*. This finding may explain why HIV-1 has not evolved a Vif-based strategy to counteract APOBEC3B restriction. Further analyses to explore the role(s) of APOBEC3B in human are also required in other cohorts with diverse genetic backgrounds in Asia.

Supporting Information

Figure S1 Overexpression of two APOBEC3B variants and the antiviral effect of the variants *in vitro*. **(A)** A DNA fragment of the complete *APOBEC3B* open reading frame was amplified by RT-PCR from each RNA sample of healthy donors with APOBEC3B K62 (A3B K62) and E62 (A3B E62). Each of the fragment was replaced into the *APOBEC3G* gene position of the pcDNA A3G (Myc-His) WT (A3G WT) plasmid as previously described [8]. The primer sets for amplification of APOBEC3B cDNA were used as follows: the 1st PCR, 5′- gagcgggacagggacaagcg and 5′- aacccaggtctctgccttcc; the 2nd PCR, 5′-tcgagcggccgcatgaatccacagatcagaaatccg and 5′- cgataacaagcttgtttccctgattctggagaatggc. The resultant APOBEC3B expression plasmids, pcDNA APOBEC3B K62 and pcDNA APOBEC3B E62, contain a C-terminal MycHIS tag (consisting of Myc and hexa-histidine epitopes). The sequences of both the insert and the boundary regions for the APOBEC3B expression plasmids were verified by DNA sequencing. The expression or control (Vector) plasmids were transfected into human embryonic kidney cells (HEK 293T) by using FuGENE HD (Promega, Madison, USA). At 48 hr after transfection, cell lysates were prepared with Laemmli buffer containig 2.5% 2-Mercaptoethanol and analyzed by western blot. Protein bands were probed with anti-β-tubulin rabbit polyclonal antibody (1/2,500) (ab6046, Abcam, Cambridge, USA) or anti-His mAb (1/3,000) (D291-3, Medical & Biological Laboratories Co., Nagoya, Japan) as previously reported [8]. **(B)** The effect of two APOBEC3B variants on HIV-1 infectivity *in vitro* was analyzed. For virus production, 293T cells were cotransfected with 1 μg of pNL4-3 WT (HIV-1 WT) or pNL4-3*vif*(–) (HIV-1 *vif*(–)) plus 1 (black) or 0.1 (gray) μg of pcDNA APOBEC3B K62, pcDNA APOBEC3B E62, pcDNA 3.1 (–) (Vector), or pcDNA A3G (Myc-His) WT. Because it has been reported that the antiviral effect of APOBEC3B on HIV-1 *in vitro* can be observed when overexpressed in 293T cells but not T cell lines [20], we used 293T cells for the virus production. Virus infectivity was determined using TZM-bl cells [29]. Relative infectivity as relative light units (RLU) was calculated by normalizing for the amount of input CA, determined by p24 antigen ELISA (ZeptoMetrix, Buffalo, USA). Three independent experiments were performed. Results from one representative experiments are shown. A3G, APOBEC3G.
(TIF)

Figure S2 Quantitative hypermutation analysis of APOBEC3-prefered dinucleotide motifs in the proviral DNA isolated from PBMCs of HIV-1 (+) patients. **(A)** Genomic DNAs from patients' PBMC (n = 4, for each *APOBEC3B* genotype I/I, I/D, and D/D) were extracted using the QIAamp DNA Blood Mini Kit. The proviral DNA fragments were prepared by nested PCR using the PrimeSTAR GXL DNA Polymerase (Takara Bio). For the first PCR, a 2,877-bp DNA fragment of *pol* (RT-IN) region (nt 2,388–5,264 according to the numbering positions of HXB2 strain, K03455) and a 1,095-bp fragment of *vif* region (nt 4,899–5,993) were independently amplified with 300 nM of each primer set: *pol*, DRRT1L (5′-atgataggggaattg-

gaggttt) and DRIN1R (5′-cctgtatgcagaccccaatatg); *vif*, DRVIF1F (5′-cgggtttattacagggacagcag) and DRVIF1R (5′-gctgtctccgcttcttcctgccat). For the nested PCR, a 2,735-bp (*pol*, nt 2,485–5,219) and an 859-bp (*vif*, nt 4,953–5,812) fragment were generated using primer sets, DRRT7L (5′-gacctacacctgtcaacataattgg)/DRRT7R (5′-cctagtgggatgtgtacttctgaactta) and DRVIF2F (5′-ctctggaaaggtgaaggggcagta)/DRVIF2R (5′-gaataatgcctattctgctatg), respectively. The resulting PCR products were purified with the QIAquick PCR Purification kit (Qiagen) and quantified with the Quant-iT dsDNA BR kit (Life Technologies). Paired-end DNA libraries were prepared using the Nextera DNA sample prep kit (Illumina, San Diego, USA) according to the manufacture's protocol. The DNA libraries were sequenced on a MiSeq (Illumina) using the MiSeq reagent kit v2 to produce 250 bp ×2 paired-end reads. The reads generated by deep sequencing were mapped onto the reference sequence of HXB2 strain by BWA 0.7.3a program (http://bio-bwa.sourceforge.net). Then, sequences of a 150-base pairs-long region were extracted and the sequences containing bases with quality scores under 30 were omitted by our *in house* program. **(B)** Among the extracted sequences, the hypermutation types and the numbers of the dinucletitide sequences, GG>AG (red) and GA>AA (blue), were analyzed. In order to detect hypermutations, the unique sequences with >5-fold coverage depth were used. The frequency (%) of hypermutaion is shown as mutation rates per dinucleotide (GG or GA) sequence with two color-coded scales below. The positions of the hypermutations in each patient sample are represented based on the nucleotide position of HXB2 strain. Since no sequences at the 3′-end part of *vif* (5470–5619) in sample ID #15, 47 and 73 were mapped onto HXB2 (panel A), the hypermutation frequency in the portion (5,485–5,619) is not shown. **(C)** The cumulative histograms represent number of hypermuated positions (y-axis) for GG>AG (red) or GA>AA (blue) at the degree of hypermutation (%) (x-axis). Bars were denoted for every 10% of the frequency degree.
(PDF)

Figure S3 Relative expression levels of APOBEC3B mRNA in three different *APOBEC3B* genotyped subjects of healthy donors or HIV-1-infected patients. Total RNA samples were isolated from PBMCs of three different genotyped subjects, intact (I/I); hemizygous (I/D); and deletion (D/D) individuals, of healthy donors (Uninfected), or HIV-1 (+) patients before (Naïve) or after (Treated) cART. Each genotype includes 3 samples for each status. Relative APOBEC3B mRNA expression levels were determined by using RT-qPCR using the Thermal Cycler Dice Real Time System (TP800) (Takara Bio, Shiga, Japan). The qPCR cycle at which amplification was detectable above a background threshold (threshold cycle, or Ct) was calculated and normalized to β-Actin. The relative expression levels are presented as the ΔΔCt (*n*-fold) of APOBEC3B mRNA to β-actin mRNA. cDNA sytheisis and qPCR was performed in duplicate for each sample, and the mean values and standard deviations for each genotype group (n = 3) are shown. The p values were calculated using Kruskal-Wallis test. The error bar represents the standard deviation. *n.d.*, not detected.
(TIF)

Table S1 Oligonucleotide primers used for real-time PCR of *APOBEC3* and control. A real-time PCR assay was performed for *APOBEC3* and control genes (Gene symbol) using each of forward primer (S) and reverse primer (AS) sets.
(DOC)

Table S2 APOBEC3B variations in the I/D and I/I genotyped healthy donors. APOBEC3B cDNAs from I/D and I/I-genotyped healthy donors (n = 5 each) were amplified by the nested RT-PCR and cloned into pUC118 plasmids. The *APOBEC3B* cDNA sequences were determined by DNA sequencing. The individual APOBEC3B variants analyzed are shown. A3B, APOBEC3B.
(DOC)

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Author Contributions

Conceived and designed the experiments: MI TI NK SI YK ATK MU YY WS YI TN. Performed the experiments: MI TI KM TM KS YI. Analyzed the data: MI TI DW JI KS HO YK ATK YY WS YI. Contributed reagents/materials/analysis tools: MI DW JI SI MU YY TS. Wrote the paper: MI TI YK ATK WS YI TN.

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Prevalence of Sexual Victimization and Correlates of Forced Sex in Japanese Men Who Have Sex with Men

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Abstract

Studies of men who have sex with men (MSM) in diverse geographic and cultural contexts have identified health challenges affecting this population. MSM might be particularly vulnerable to sexual victimization and forced sex. The aim of this research study was to examine prevalence of sexual victimization and correlates of forced sex among Japanese MSM. We recruited a sample of 5,731 Japanese MSM who completed an internet-administered survey. Participants reported on history of different types of sexual victimization, unprotected anal sex, other health risk behaviors, exposure to gay-related teasing and bullying, depression, and suicidality. Over one-fifth of the sample (21.4%) reported experiencing at least one form of sexual victimization, and 8.7% reported a history of forced sex. MSM who had ever experienced forced sex were significantly more likely to report experiencing psychological risks (depression OR = 1.55, 95% CI = 1.28–1.89; attempted suicide OR = 2.25, 95% CI = 1.81–2.81; other forms of bullying OR = 1.38, 95% CI = 1.13–1.68) and other behavioral risks (unprotected anal sex OR = 1.56, 95% CI = 1.29–1.90; sex venue attendance OR = 1.27, 95% CI = 1.04–1.54; methamphetamine use OR = 1.57, 95% CI = 1.05–1.36), compared to MSM who had not experienced forced sex. Efforts to develop holistic and integrated health services for Japanese MSM are warranted, particularly related to psychosocial determinants of HIV prevention. However, due to cultural factors that emphasize familial and social relations and that stigmatize same-sex behavior, Japanese MSM might experience challenges to seeking social support and health services. Interventions must be provided in safe and non-judgmental settings where Japanese MSM feel comfortable disclosing their health and social support needs.

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Introduction

Globally, there have been an increasing number of studies examining health and psychosocial risk factors affecting men who have sex with men (MSM) [1]. Much of this attention has focused on disproportionate HIV prevalence among MSM across international settings [2–4]. However, additional research has also shown that MSM in diverse geographic regions may also experience psychological and social vulnerabilities – such as discrimination and interpersonal violence – which can contribute to further health challenges in this population [5–9]. A nascent literature has examined health outcomes in Japanese MSM. Some of the documented health challenges among Japanese MSM include HIV risk [10], drug use [11], and suicidal ideation and attempted suicide [12]. Studies to date suggest a need for enhanced understanding into the risk factors for health problems in Japanese MSM.

Previous research has suggested that adverse behavioral and psychosocial health indicators in Japanese MSM might be due, in part, to exposure to social stressors [13]. For example, stigma, homophobic abuse, and victimization are forms of social stress reported in MSM samples in Japan [12,13]. Experience of homophobic stigma has been shown to be related to psychological problems and sexual risk behaviors in MSM populations in other parts of Asia [14–17]. This finding is consistent with minority stress theory [18], which postulates that exposure to negative social or interpersonal events can compromise the psychological well-being of sexual minority individuals and thereby contribute to higher prevalence of mental and physical health problems in MSM.

Sexual victimization is an extreme form of social and interpersonal stress that MSM may experience, and can contribute to further psychological and behavioral health risks among those who have been victimized. Sexual victimization can be defined as any form of involuntary sexual interaction or contact with another person, which can occur in childhood as well as in adulthood [19]. Studies have shown that MSM with a history of childhood sexual victimization show greater sexual risk behavior in adulthood and have higher prevalence of HIV infection compared with their MSM peers who have not experienced sexual victimization [20]. There are few studies that have examined adult sexual victimi-

zation experiences among men. This may be due, in part, to underreporting of adult sexual victimization, stigma about discussion of sexual victimization in adult men, and myths about men's vulnerability to sexual victimization [21]. However, a review of research on sexual victimization in adult men found that MSM were more likely to report experiences of adult sexual victimization compared with heterosexual men [21]. Forced sex is a specific type of sexual victimization that has extremely adverse physical and mental health consequences among male victims. Individuals with a history of forced sex can have long-term risk for HIV, trauma, and maladaptive health risk behaviors [22].

To date, there are no known studies exploring sexual victimization, including forced sex, among Japanese MSM and its potential role in affecting the health and psychological well-being in this population. To enhance understandings of the health of Japanese MSM, the aims of this paper are to explore (i) the prevalence of different types of sexual victimization in a large population sample, and (ii) associations between history of forced sex and other psychosocial and behavioral risk factors. Because this is an understudied topic, findings from this analysis can provide insight for future research and potentially guide interventions to address the health and well-being of MSM in Japan who have a history of sexual victimization and forced sex.

Method

Recruitment

The internet was used to recruit a diverse sample of Japanese MSM for a study of health behaviors and well-being. The internet has been argued to be an acceptable method for collecting large, heterogeneous samples of hard-to-reach populations [23,24]. Internet technology can be helpful in reaching gay, bisexual, and questioning men who are less comfortable attending homosexual-themed venues, such as bars and nightclubs. Data collection through the internet can also increase the opportunity for participants to respond anonymously by avoiding face-to-face contact [11,13], which might be a barrier to participation due to MSM stigma in Japanese culture. Informational announcements about the study were placed on internet websites catering to Japanese MSM audiences. In addition to posting banners on gay-related websites, recruitment strategies included: flyers distributed in gay venues, announcements posted in gay organizational newsletters as well as in gay magazines, and announcements posted at social network websites catering to gay men. We designed the internet banners and informational flyers in a manner that would draw the attention of MSM, e.g., using physically attractive male models. However, we designed a range of recruitment media (e.g., information-only announcements without pictures or using gay-relevant slogans and symbols) to minimize bias associated with recruiting men solely based on their response to sexually suggestive images. Announcements provided information about the research project and eligibility for participation. Potential participants were directed to an internet site to learn more about the study. Participant inclusion criteria included: 1) being a Japanese male who has ever had sex with men; 2) having internet access; 3) having Japanese written language fluency.

Procedure

Participants who met inclusion criteria entered a secured internet website to complete the anonymous online survey. The website first presented informed consent information. If participants understood the purposes of the study and agreed to the terms of participation, they clicked an "Agree" button, and they then accessed the questionnaire. All items and response options were presented in Japanese language, and participants' responses were immediately saved in a firewall-protected database. To minimize the chances that participants would complete the survey multiple times, we examined internet protocol addresses and internet providers encoded within the data and, if encoded information appeared similar, checked the demographic data for redundant information. Internet protocol addresses were deleted before conducting analysis. Using a procedure validated previously in an internet study of MSM in Japan, we asked participants to define two terms that were identified through earlier formative research as well-known colloquial expressions in the Japanese MSM/gay community (which translated into English would mean "gay men/gay society" and "heterosexual") [11,13]. Data from men who were unable to define the terms were excluded from analysis. Data were collected between August 11 and November 30, 2005. The study protocol was approved by the Ethics Committee of Nagoya City University School of Nursing.

In total, 6,260 participants attempted to complete the questionnaire, 196 people were excluded due to missing data, 140 were excluded because of data duplication or because they could not define the slang terms, 73 were excluded because they were not males, and 120 were excluded because they did not live in Japan.

Measures

Participants reported demographic characteristics, including age, highest educational level, and sexual orientation (gay, bisexual, heterosexual, undecided, unsure and other). Participants were asked whether they had ever experienced a range of sexual interactions *against their will*, including the following: being undressed by another person, target of verbal sexual abuse, forced to kiss another person, sexually touched by another person, forced to touch the genitals of another person, forced to engage in vaginal sex with a female, forced to engage in oral sex (with a male or female), forced to engage in anal sex with a male, and any other form of unwanted sexual interaction. Participants also described whether they had ever been harassed or bullied in school by others due to their sexuality, and whether they had close gay/bisexual friends to whom they could confide in and close heterosexual friends to whom they could confide in. They completed the Japanese version of the Center for Epidemiologic Studies Depression Scale (CES-D); participants were categorized as being moderately depressed based on a score greater than 16 [25]. Participants also reported whether they had ever attempted suicide. Finally, participants reported their HIV status, frequency of condom use when engaging in anal sex, and recent methamphetamine use.

Data analysis

Statistical analysis was conducted using SPSS v.21. First, we described the prevalence of different types of sexual victimization, sociodemographic variables, and self-reported psychosocial and behavioral health variables. Second, we examined correlates of forced sex, using chi-square tests to assess bivariate associations and multivariable logistic regression to identify independent associations controlling for other co-variates. Multivariable regression analysis was based on procedures described in Hosmer & Lemeshow [26], in which we entered into the regression model any variable that had a moderate bivariate association with forced sex at $p<.25$. Adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) were reported.

Table 1. Participant characteristics and associations with history of forced sex in a sample of Japanese MSM (n = 5,731).

		Total		Lifetime experience of forced sex		
		n	%	n	%	*p-value*
Overall		5,731		500	8.7	
Age group	12–19	371	6.5	36	9.7	0.002
	20–29	2,432	42.4	253	10.4	
	30–39	2,037	35.5	149	7.3	
	40–49	652	11.4	48	7.4	
	50+	205	3.5	11	5.4	
	Missing	34	0.6	3	8.8	
Sexual orientation	Gay	3,868	67.5	337	8.7	0.25
	Bisexual	1,484	25.9	138	9.3	
	Other	379	6.6	25	6.6	
Educational level	No university degree	2,496	43.6	236	9.5	0.089
	University degree	3,235	56.4	264	8.2	
Ever been teased verbally with words such as "homosexual, faggot, fag"	No	2,605	45.5	193	7.4	0.001
	Yes	3,126	54.5	307	9.8	
Ever been bullied other than verbal teasing	No	3,146	54.9	220	7	<0.001
	Yes	2,585	45.1	280	10.8	
Depression in past week	Not depressed	3,510	61.2	235	6.7	<0.001
	Depressed	2,221	38.8	265	11.9	
Ever attempted suicide	No	4,926	86	358	7.3	<0.001
	Yes	805	14	142	17.6	
Went to any sex venues in 6 months	No	2,714	47.4	205	7.6	0.003
	Yes	3,017	52.6	295	9.8	
Have close gay/bisexual friends	No	2,000	34.9	156	7.8	0.069
	Yes	3,731	65.1	344	9.2	
Have close heterosexual friends	No	2,370	4.4	175	7.4	0.003
	Yes	3,361	58.6	325	9.7	
HIV status	Negative	5,425	94.7	457	8.4	0.002
	Positive	306	5.3	43	14.1	
Ever used methamphetamine	No	5,520	96.3	467	8.5	0.001
	Yes	211	3.7	33	15.6	
Unprotected anal intercourse in 6 months	No	2,941	51.3	198	6.7	<0.001
	Yes	2,790	48.7	302	10.8	

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Results

Data from a total of 5,731 respondents are included in this analysis (Table 1). The mean age was 30.8 years (SD = 8.9, range = 12–82), with 6.5% between 12 and 19 years old, 42.4% between 20 and 29 years old, 35.5% between 30 and 39 years old, 11.4% between 40 and 49 years old, and 3.6% over the age of 50 (n = 34 did not report their age). Over half (56.4%) of the sample had completed a University degree. Over two-thirds (67.5%) identified themselves as gay and 25.9% identified as bisexual. Over half (54.5%) had been verbally teased with words such as "homosexual, faggot, fag" and 45.1% had experienced other forms of bullying. The majority of participants reported having close gay/bisexual male friends (65.1%) as well as close heterosexual friends (58.6%). Over one-third (38.8%) reported moderate levels of depression (CES-D >16), and 14.0% had ever attempted suicide. Overall, 5.3% identified as HIV-positive, 48.7% reported having unprotected anal sex in the past six months, 52.6% had visited a sex venue in the past six months, and 3.7% had ever used methamphetamines.

Prevalence of sexual victimization experiences are reported in Table 2. Overall, 21.4% of the sample reported experiencing any of the types of sexual victimization assessed in this survey. The most common forms of sexual victimization included unwanted sexual touching (16.7%), being undressed (10.5%), being forced to kiss someone (9.6%) and being forced to touch someone's genitals (8.8%). A total of 500 participants (8.7% overall; 40.8% of those who reported any sexual victimization) reported ever experiencing any forced sex. Overall, 6.5% experienced forced anal sex, 5.9% experienced forced oral sex, and 2.0% experienced forced vaginal sex.

Bivariate correlates of a history of forced sex are listed in Table 1. Forced sex was associated with younger age, experience

Table 2. Prevalence of different forms of lifetime sexual victimization in a sample of Japanese MSM (n = 5,731).

Forms of sexual victimization	n	%
Undressed	600	10.5
Abused with obscene words	392	6.8
Forced kiss	553	9.6
Touched	957	16.7
Forced to touch genital part	507	8.8
Forced vaginal sex	113	2
Forced oral sex	338	5.9
Forced anal sex	372	6.5
Other	215	38
Any forced sex (vaginal, oral, anal)	500	8.7
Any forms of sexual victimization (any of above)	1,224	21.4

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of verbal teasing due to being gay, experience of other forms of bullying due to being gay, depression, history of attempted suicide, having close heterosexual friends, HIV-positive status, unprotected anal sex, vising a sex venue, and history of methamphetamine use ($p<.01$).

Variables that were independently associated with a history of forced sex are shown in Table 3. Based on multivariable regression analysis, forced sex was shown to be significantly associated with depression (OR = 1.55, 95% CI = 1.28–1.89), history of attempted suicide (OR = 2.25, 95% CI = 1.81–2.81), experience of bullying (OR = 1.38, 95% CI = 1.13–1.68), having close heterosexual friends (OR = 1.26, 95% CI = 1.03–1.55), visiting a sex venue (OR = 1.27, 95% CI = 1.04–1.54), having unprotected anal sex (OR = 1.56, 95% CI = 1.29–1.90), HIV positive status (OR = 1.57, 95% CI = 1.10–2.24), and ever using methamphetamines (OR = 1.57, 95% CI = 1.05–2.36).

Discussion

Over one-fifth (21.4%) of this large sample of MSM in Japan reported experiencing at least one form of sexual victimization as assessed in this study, and 8.7% reported a history of forced sex. MSM who had ever experienced forced sex were significantly more likely to report experiencing psychological risks (i.e., depression, attempted suicide, other forms of bullying) and other behavioral risks (unprotected anal sex, sex venue attendance, methamphetamine use) compared with their peers who did not experience forced sex. These cross-sectional findings suggest that assessing for sexual victimization and addressing the consequences of forced sex might be an important component of clinical screenings or public health interventions related to HIV prevention and mental health services for MSM in Japan.

Findings here are consistent with studies from other settings which indicate that HIV and other health disparities affecting MSM must be understood in the context of psychosocial stressors and contextual factors that determine health risk behaviors among members of this population [18]. Consequently, integrated and holistic approaches to health care for MSM may be warranted – particularly approaches that consider history of adverse psychological and behavioral co-factors that need intervention [1].

Capacity to provide holistic health services to MSM in Japan, however, is currently limited. Among Japanese MSM, 80% have not disclosed their sexual orientation to parents, thus these men may experience difficulty seeking help from their family members. Although poor mental health status such as depression was apparent in this population, experience of accessing mental health services was low [27]. These findings suggest that MSM may experience difficulty seeking support from parents as well as in medical care settings, potentially due to the fear of prejudice and discrimination. Professionals such as mental care providers, nurses, public health professionals providing HIV counseling and testing, and clinical psychologists would benefit from improved training to understand about the needs of this population, in order to provide adequate professional services and support to MSM. Japanese MSM would benefit from resources that identify health service providers or health settings that are friendly and competent in working with sexual minority patients and populations. Currently, there are no known publically available resources to help MSM in Japan identify health services in general, especially mental health care. Development of referral networks, brochures, and websites with information about appropriate and confidential services for MSM is warranted.

There are notable strengths to this study. This is the first known study of the prevalence of sexual victimization and correlates of forced sex in Japanese MSM. Use of the internet allowed us to recruit a large sample of MSM, and suggests the utility of internet and social media for outreach and recruitment to MSM in Japan, a population that might otherwise be hard to reach. Findings expose a need for appropriate and confidential health services for MSM, and suggest the role of sexual victimization as a determinant of behavioral health and psychosocial problems in this population.

Limitations to this study must be considered. First, the study used a cross-sectional design which prevents interpretation of causality or temporal order among variables. Second, although this is a large MSM sample, participants were recruited using non-representative sampling methods. Because we did not use targeted recruitment efforts to achieve a sociodemographically representative sample, findings might not be generalizable to MSM who do not access gay-themed internet or periodical content or men who felt uncomfortable completing an online survey. Third, self-report measures might have been affected by social desirability or recall biases. Fourth, because this was an exploratory study, and the first of its kind in Japan, we did not have access to culturally validated measures of sexual victimization and other risk behaviors in Japanese MSM. Although measures of sexual risk and other health behaviors in this survey have also been reported in previous studies of Japanese MSM [11,13], future research must better assess the

Table 3. Multivariable regression to identify independent correlates of forced sex in a sample of Japanese MSM (n = 5,731).

		Lifetime experience of forced sex		
		AOR	**95% CI**	*p-value*
Age group	12–19	ref.		
	20–29	1.03	(0.70–1.51)	0.877
	30–39	0.71	(0.48–1.07)	0.102
	40–49	0.8	(0.50–1.28)	0.354
	50+	0.68	(0.33–1.38)	0.282
	Missing	0.99	(0.28–3.50)	0.983
Sexual orientation	Gay	ref.		
	Bisexual	1.24	(1.00–1.55)	0.055
	Other	0.81	(0.53–1.25)	0.343
Educational level	No university degree	ref.		
	University degree	1.02	(0.84–1.23)	0.865
Ever been teased verbally with words such as "homosexual, faggot, fag"	No	ref.		
	Yes	1.13	(0.93–1.39)	0.229
Ever been bullied other than verbal teasing	No	ref.		
	Yes	1.38	(1.13–1.68)	0.002
Depression in past week	Not depressed	ref.		
	Depressed	1.55	(1.28–1.89)	<.001
Ever attempted suicide	No	ref.		
	Yes	2.25	(1.81–2.81)	<.001
Went to any sex venues in 6 months	No	ref.		
	Yes	1.27	(1.04–1.54)	0.017
Have close gay/bisexual friends	No	ref.		
	Yes	1.12	(0.90–1.38)	0.307
Have close heterosexual friends	No	ref.		
	Yes	1.26	(1.03–1.55)	0.027
HIV status	Negative	ref.		
	Positive	1.57	(1.10–2.24)	0.014
Ever used methamphetamine	No	ref.		
	Yes	1.57	(1.05–2.36)	0.029
Unprotected anal intercourse in 6 months	No	ref.		
	Yes	1.56	(1.29–1.90)	<.001

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psychometric properties and cultural sensitivity of sexual behavior and victimization measures for use with this population. Fifth, most measures of sexual behavior, victimization, and other risk behaviors in this survey assessed lifetime experience, resulting in limited inferences about temporal windows which might affect health risk.

Conclusion

In summary, this study highlights the role of prior sexual victimization in contributing to the psychological and behavioral risks of MSM in Japan. Findings reported here correspond with a substantial literature (mostly conducted in the West) on the associations of sexual victimization – including childhood sexual victimization as well as adult victimization – on psychological adjustment and future sexual risk outcomes. Efforts to address these issues among Japanese MSM are warranted. Such efforts must be mindful of cultural and social factors that might challenge provision of holistic services to Japanese MSM, and which might also present barriers to access of health service and disclosure of problems among Japanese MSM.

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Author Contributions

Conceived and designed the experiments: YH. Performed the experiments: YH HK MK SI. Analyzed the data: YH DO HT MT. Contributed reagents/materials/analysis tools: YH. Wrote the paper: YH DO HT MT HK MK SI.

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研　究

若年女性における過去と現在の性感染症予防行動と情報入手状況の比較

Comparison of STI-related sex behavior and access to STI-related information of women between when they were 19 years old and present age

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