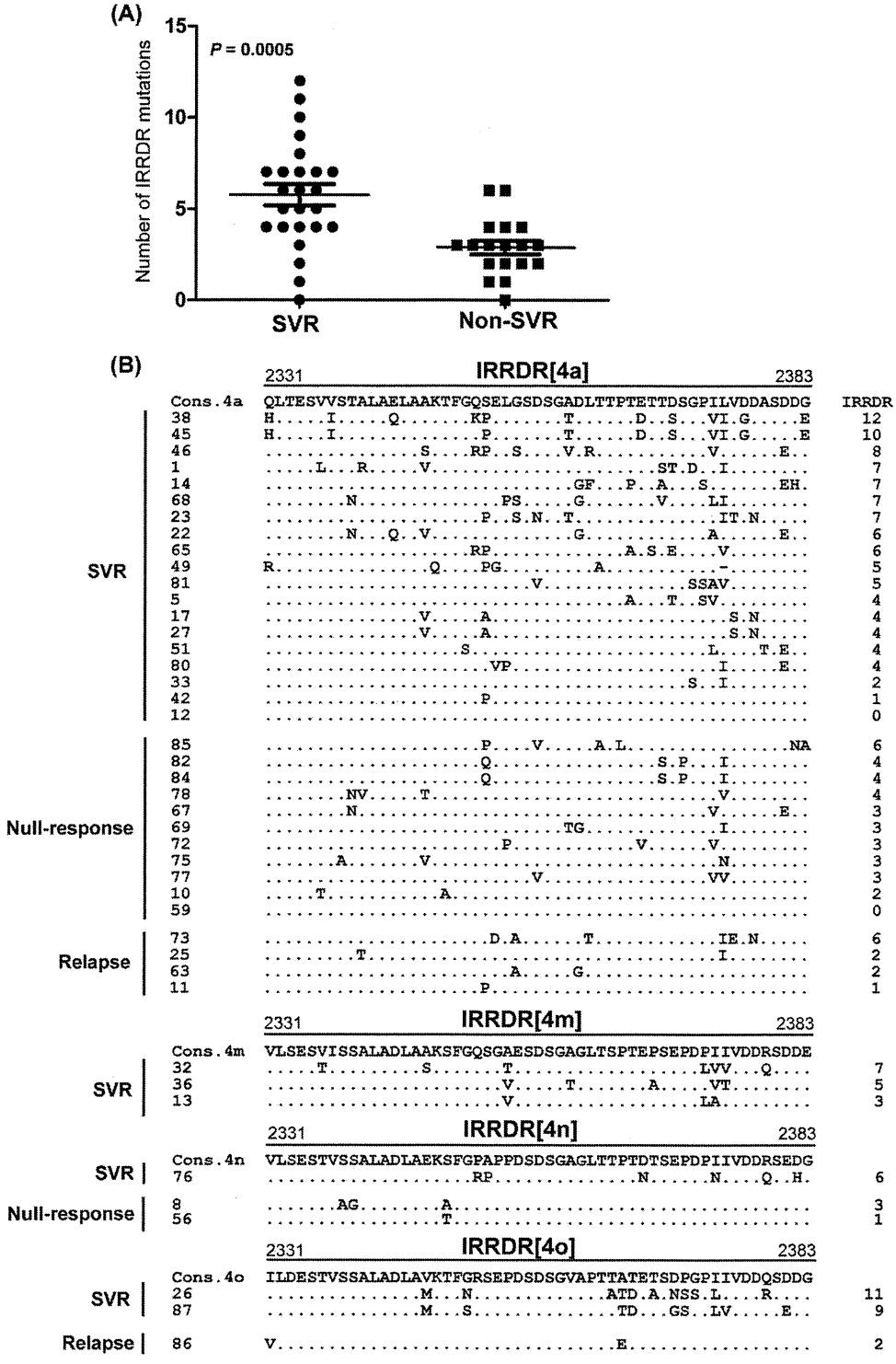

FIG 2 Correlation between IRRDR[HCV-4] sequence variations and treatment outcome. (A) Average number of amino acid mutations in IRRDR[HCV-4] obtained from SVR and non-SVR patients. (B) Alignment of IRRDR[HCV-4] sequences obtained from SVR and non-SVR patients with HCV-4a, -4m, -4n, and -4o. The consensus sequence (Cons) of each subtype is shown on the top. The numbers along the sequence indicate the amino acid positions. Dots indicate residues identical to those of the Cons sequence. The numbers of the mutations in each IRRDR (4a, 4m, 4n, or 4o) are shown on the right.

predictive factors of SVR for PEG-IFN/RBV treatment outcome in HCV-4 infection, first, all available data of baseline patients' parameters and IRRDR[HCV-4] polymorphism were entered in a univariate logistic analysis. This analysis yielded 3 factors that were correlated or nearly correlated with SVR: IRRDR[HCV-4]≥4 ($P = 0.0004$), patient's age (<42 years; $P = 0.03$), and HCV RNA titer (<5,200 IU/ml; $P = 0.08$). Subsequently, these 3 factors were entered in multivariate logistic regression analysis. This anal-

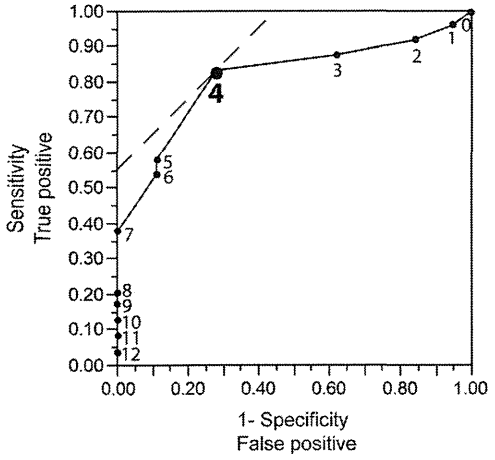

FIG 3 ROC curve analysis of IRRDR[HCV-4] sequence heterogeneity for SVR prediction. The solid line curve shows the AUC. Solid circles with numerals plotted on the curve represent different numbers of IRRDR mutations analyzed. The dashed line in the upper left corner indicates the optimal number of IRRDR[HCV-4] mutations for SVR prediction, which yields the highest sensitivity (84%) and the highest specificity (72%).

ysis revealed that the IRRDR[HCV-4]≥4 was the only independent predictive factor for SVR in HCV-4 infection (Table 4). We then assessed SVR predictability by means of IRRDR[HCV-4]≥4. As shown in Table 5, IRRDR[HCV-4]≥4 would predict SVR with a positive predictive value (PPV) of 81% ($P = 0.002$) and sensitivity of 84%. On the other hand, IRRDR[HCV-4]≤3 would predict non-SVR with a negative predictive value (NPV) of 76% ($P = 0.02$) and specificity of 72%. Thus, the degree of sequence variation in IRRDR[HCV-4] would yield useful positive and negative predictive markers for PEG-IFN/RBV therapy outcome in HCV-4-infected patients.

DISCUSSION

Both host and viral genetic factors have been implicated in influencing the clinical response to PEG-IFN/RBV therapy for HCV infection (22). It has recently been reported that host genetic polymorphisms near or within the IL28B gene on chromosome 19 show a critical impact on the treatment outcome of patients infected with HCV-1 (20, 37, 39). As for the viral factor(s), polymorphisms of NS5A and core regions of a given HCV genotype have been linked to a difference in SVR rates (3, 4, 13, 18, 30). This hypothesis was mostly inferred from studies carried out with Asian populations, in particular Japanese, with HCV-1b infection. However, whether it can be applied to non-Asian populations infected with non-HCV-1 is still unknown. To the best of our knowledge, this is the first study that specifically examines the relationship between HCV genome heterogeneity, in particular in NS5A and core regions, and PEG-IFN/RBV treatment outcome in Egyptian patients infected with HCV-4. In analogy with our previous studies that identified IRRDR as a significant determinant for PEG-IFN/RBV treatment outcome in Japanese patients infected with HCV-1b, -2a, and -2b (12–16), we have demonstrated in the present study that sequence heterogeneity within IRRDR is closely associated with the ultimate treatment outcome in Egyptian patients infected with HCV-4. A high degree of sequence variation in IRRDR[HCV-4], i.e., more than 4 (IRRDR ≥ 4), significantly correlated with SVR, while a low degree of sequence variation in this region (IRRDR ≤ 3) correlated with non-SVR, null response, and relapse. The majority of patients with SVR (84%) had HCV with IRRDR of ≥4. In contrast, nearly two-thirds (72%) of the patients with non-SVR had HCV with IRRDR ≤ 3 ($P = 0.0004$) (Table 3). Notably, 21 of the 26 patients infected with HCV with IRRDR[HCV-4]≥4 achieved SVR. Accordingly, the PPV and NPV of IRRDR[HCV-4]≥4 for SVR and non-SVR patients were 81% ($P = 0.002$) and 76% ($P = 0.02$), respectively (Table 5). Our present results thus strongly suggest that the degree of sequence heterogeneity within IRRDR[HCV-4] would be a useful marker for prediction of treatment outcome in HCV-4 infection.

The molecular mechanism underlying the possible involvement of this region in IFN responsiveness of the virus is still unknown. The significant difference among IRRDR sequence patterns may suggest genetic flexibility of this region. Indeed, the C-terminal portion of NS5A was shown to tolerate sequence insertions and deletions (29). This flexibility might play an important role in modulating the interaction with various host systems, including IFN-induced antiviral machineries. It is also possible that the genetic flexibility of IRRDR is accompanied by compensatory changes elsewhere in the viral genome and that these compensatory changes affect overall viral fitness and responses to IFN-based therapy (8, 29, 41). Also, it is worth noting that IRRDR is among the most variable sequences across the different genotypes and subtypes of HCV (25) whereas its upstream and downstream sequences show a higher degree of sequence conservation (15). This may suggest that whereas the upstream and downstream sequences have a conserved function(s) across all the HCV genotypes, IRRDR sequences have a genotype-dependent or even a strain-dependent function(s).

A mutation at position 70 of the core protein of HCV-1b has been reported to be correlated with PEG-IFN/RBV treatment out-

TABLE 3 Correlation between NS5A sequence heterogeneity and virological responses in HCV-4 infection

Factor	No. of isolates/total no. (%)				*P* value for SVR versus:		
	SVR	Non-SVR	Null response	Relapse	Non-SVR	Null response	Relapse
IRRDR ≥ 4	21/25 (84)[a]	5/18 (28)	4/13 (31)	1/5 (20)	0.0004	0.003	0.01
IRRDR ≤ 3	4/25 (16)	13/18 (72)[b]	9/13 (69)	4/5 (80)			
IRRDR ≥ 5	16/25 (64)[a]	2/18 (11)	1/13 (8)	1/5 (20)	0.0006	0.002	0.14
IRRDR ≤ 4	9/25 (36)	16/18 (89)[b]	12/13 (92)	4/5 (80)			
IRRDR ≥ 3	22/25 (88)[a]	11/18 (61)	10/13 (77)	1/5 (20)	0.066	0.39	0.006
IRRDR ≤ 2	3/25 (12)	7/18 (39)[b]	3/13 (23)	4/5 (80)			

[a] Sensitivity (proportion of SVR patients with the favorable factor).
[b] Specificity (proportion of non-SVR patients with the unfavorable factor).

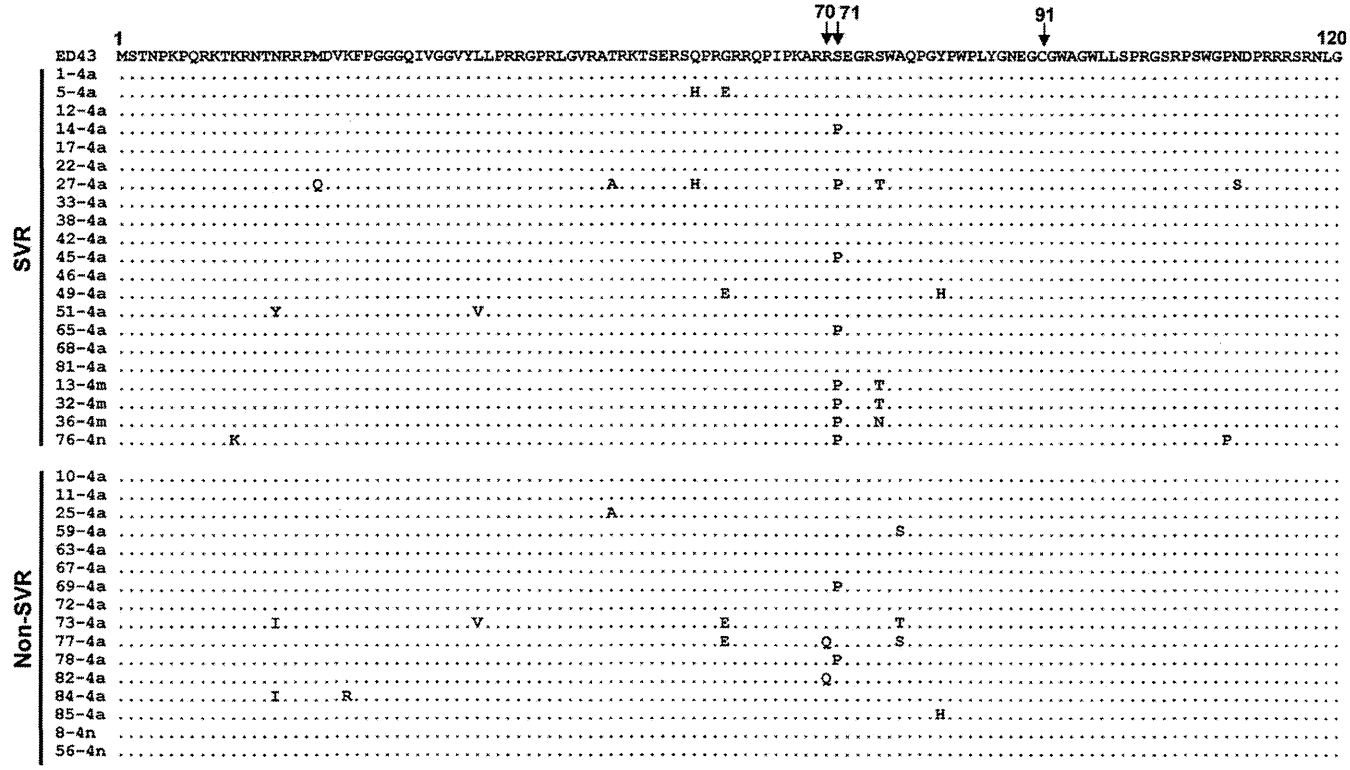

FIG 4 Sequence alignment of the core protein of HCV-4 isolates. Core protein sequences (aa 1 to 120) of HCV-4 obtained from SVR and non-SVR patients are aligned. The prototype sequence of ED43 (10) is shown on the top. The numbers along the sequence indicate the amino acid positions. Dots indicate residues identical to those of the prototype sequence.

come (4, 12). In the present study, however, we found no significant correlation between core protein polymorphism and treatment outcome in HCV-4 infection. The residue at position 70 of the core protein of all but two HCV-4 isolates analyzed in this study was Arg (Fig. 4), which is known to be associated with SVR in HCV-1b infection (4, 12). This high degree of sequence conservation at position 70 might be the reason for the lack of significant correlation between core protein polymorphism and treatment outcome in HCV-4 infection.

Single nucleotide polymorphisms (SNPs) near the IL28B region have been identified as the strongest baseline predictors of SVR to PEG-IFN/RBV in patients with HCV-1 infection. More recently, in two major studies that were carried out exclusively with HCV-4-infected patients (9, 11), the CC genotype of rs12979860 IL28B SNP was also strongly associated with SVR. It is worth noting that although the SVR rate was more than 80% among the patients with the CC genotype, these patients represented only around 40% of total SVR cases in both studies. Furthermore, the CC genotype was found in only 34% of all Egyptian patients analyzed (9). Taken together, those observations support the idea that in addition to IL28B polymorphism, there should be an additional factor(s) that influences SVR. In this context, an interplay between IRRDR and IL28B polymorphisms might explain why some patients with undesirable IL28B genotype achieve SVR and why some patients infected with HCV isolates with IRRDR[HCV-4]≥4 do not achieve SVR. Further comprehensive study is needed to validate the importance of IRRDR and IL28B polymorphisms in predicting the treatment outcome of HCV-4-infected patients.

In conclusion, the present study emphasizes the importance of IRRDR sequence heterogeneity in the prediction of PEG-IFN/RBV treatment outcome for different HCV genotype infections in

TABLE 4 Univariate and multivariate analyses for identification of independent predictive factors for SVR in HCV-4-infected patients treated with PEG-IFN/RBV therapy

Univariate analysis		Multivariate analysis	
Variable	*P* value	Odds ratio (95% CI)	*P* value
IRRDR mutations (IRRDR ≥ 4 versus IRRDR ≤ 3)	0.0004	10.5 (1.12–98.91)	0.04
Age (<42 years)	0.03		
HCV-RNA (<5,200 IU/ml)	0.08		

TABLE 5 PPV, NPV, sensitivity, and specificity of IRRDR sequence heterogeneity on the likelihood of achieving SVR and non-SVR in HCV-4 infection

Factor	PPV	NPV	Sensitivity[c]	Specificity[d]
IRRDR ≥ 4	81% (21/26)[a]		84% (21/25)	
IRRDR ≤ 3		76% (13/17)[b]		72% (13/18)

[a] $P = 0.002$.
[b] $P = 0.02$.
[c] Proportion of SVR patients who were infected with HCV isolates with IRRDR of ≥4.
[d] Proportion of non-SVR patients who were infected with HCV isolates with IRRDR of ≤3.

different ethnic groups, including Egyptian patients infected with HCV-4.

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No conflicts of interest exist.

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