Methods

Patients' characteristics

Patients of the evaluation cohort were recruited by the Department of Hepatology of the University Leipzig, by the German competence network "Kompetenznetz Hepatitis" and by 19 and 20 centers in Germany (INDIV-1), respectively. Chronically infected patients of the evaluation cohort were treated with interferon-based therapy consisting of pegylated (peg) interferon (IFN) and ribavirin. They received the recommended doses. Treatment duration ranged from 48 to 72 weeks depending on the individual treatment response.

The samples of the patients from Anti-D cohorts were collected by the treating hepatologists in the original referral centers from 2011 to 2012. At least once a year all regional centres followed the women if they were accessible. Data collection during regular follow-up visits in the referral centers comprised the assessment of the women's clinical status and included the documentation of relevant biochemical parameters.The present study represents a subgroup (n=305) of the 35-year interim analysis performed by Wiese et al.[24] of the original German cohort of 1978/79. Characteristics of the Irish cohort are described in Kenny-Walsh et al.[25] Patients with chronic hepatitis had been followed more frequently than women with self-limited hepatitis C. Women with chronic HCV infection got the opportunity for treatment with dual combination therapy of pegIFN and ribavirin and recently with directly acting antivirals (DAAs).

Genotyping

Samples of chronic and cleared subjects were randomly assigned to plates and blinded to lab personnel. Sequencing was used for confirmation of results from the melt curve analysis. Furthermore, samples which failed clear genotyping by melting curve analysis were sequenced. Sequencing was performed with the same primers used for RT-PCR as mentioned

in the Supplementary Table 1, with BigDye Terminator and a capillary sequencer from Applied Biosystems (Darmstadt, Germany

Table S1: Sequences of primer and probes for melting curve analysis, sequencing and site directed mutagenesis. FL=Fluorescein, LC=Light Cycler, mut=mutagenesis, PH=phosphate, SNP=single nucleotide polymorphism

TLR9 SNP	Primer/probes	Sequence (5'-3')
rs187084	sense	TCCCAGCAGCAACAATTCATTA
	antisense	CTGCTTGCAGTTGACTGTGT
	sensor	ATCACTGCCCTCAAGAAGCT-FL
	anchor	LC640-ACATTCCAGCAGGGGAATAAGACGATA-PH
	mut sense	gataaaagatcactgccctcaagaagctgacattccagc
	mut antisense	gctggaatgtcagcttcttgagggcagtgatcttttatc
rs5743836	sense	ATGGGAGCAGAGACATAATGGA
	antisense	CTGCTTGCAGTTGACTGTGT
	sensor	GGAGTTTCCAGGCAGAGG-FL
	anchor	LC610-ACAGCACATCCCAAGGCCCT-PH
	mut sense	gagacttgggggagtttccaggcagagggaa
	mut antisense	ttccctctgcctggaaactcccccaagtctc

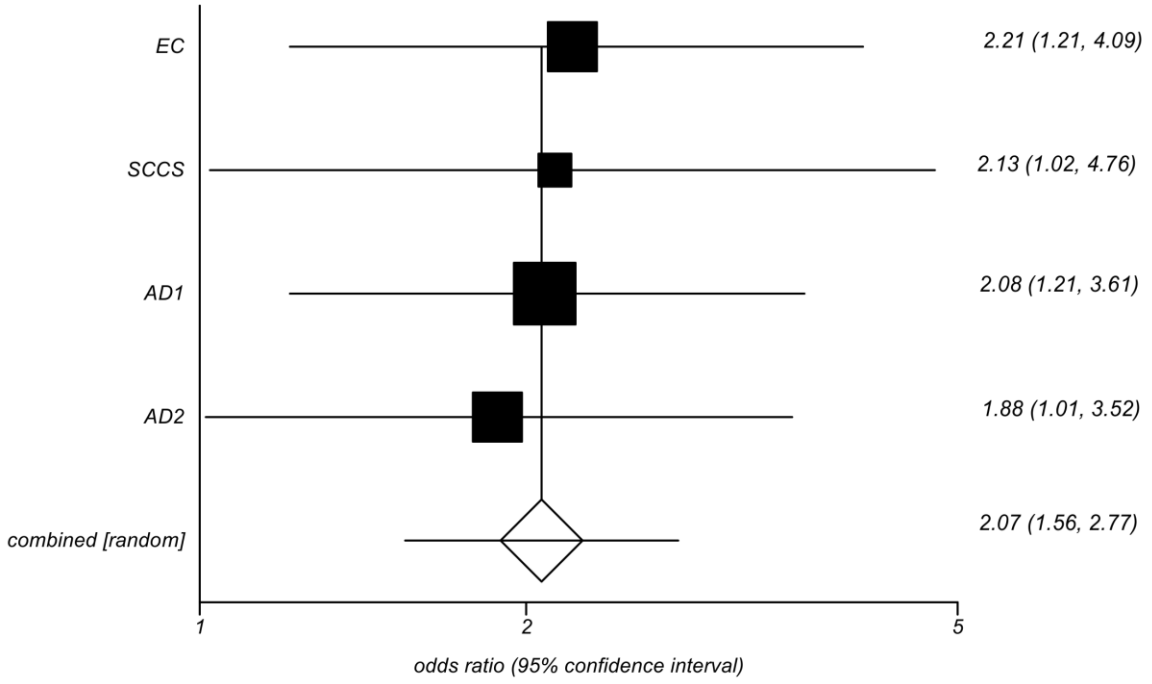
TLR9 reporter gene assay

1.5×10^5 Nawalma R 20 B cells, in 500µl medium were seeded and immediately transfected in triplicate 24-well with 300ng of a pGL3-based *TLR9* promoter *Firefly* luciferase reporter plasmid with or without (WT) point mutations corresponding to *TLR9* SNPs (*TLR9*-rs187084C, *TLR9*-rs5743836C) or lacking the *TLR9* promoter region (pGL3_basic), 10ng pRL-TK *Renilla* luciferase reporter (Promega) and 100ng of a GFP-expressing plasmid (to

monitor transfection efficacy) using HP-DNA Extreme Gene Transfection Reagent (Roche). 48 h after transfection, luciferase activities were determined using the Dual-Luciferase reporter assay system (Promega) on a Fluostar Optima Instrument (BMG Labtech).

Results

Figure S1: Meta-analysis forest plot showing a strong association between spontaneous viral clearance of HCV infection in women and the C-allele of TLR9 rs187084. Analyis was performed with StatsDirect 2.8.0 software (Cheshire, UK). EC=evaluation cohort, SCCS=Swiss HCV cohort, AD1=German Anti-D cohort, AD2=Irish Anti-D cohort.



Random effects model
Pooled odds ratio = 2.07 (95% CI: 1.56 to 2.77), p<0.0001
Cochran Q = 0.15923; df = 3; p = 0.9839
Inconsistency I^2 = 0%

Combination of *TLR9* rs187084 and *IFNL4* rs12979860

Table S2: Association of combined genotypes of *TLR9* rs187084/*IFLN4* rs12979860 with spontaneous viral clearance in women of the overall cohort. N=number, SC=spontaneous viral clearance, OR=odds ratio, CI=confidence interval, p=p-value, REF=reference

TLR9 **rs187084**	***IFNL4*** **rs12979860**	**N**	**SC**	**OR [95% CI]**	**p-value**
TT	**CC**	109	46%	REF	
CT		146	62%	1.95 [1.18-3.23]	p=0.009
CC		45	58%	1.62 [0.80-3.26]	p=0.181
CT/CC		191	61%	1.87 [1.16-3.00]	p=0.01
TT	**CT**	162	15%	REF	
CT		141	29%	2.36 [1.34-4.15]	p=0.003
CC		61	30%	2.41 [1.20-4.85]	p=0.014
CT/CC		202	29%	2.37 [1.40-4.03]	p=0.001
TT	**TT**	37	19%	REF	
CT		37	22%	1.18 [0.38-3.68]	p=0.773
CC		15	20%	1.07 [0.24-4.85]	p=0.929
CT/CC		52	21%	1.15 [0.40-3.31]	p=0.796

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