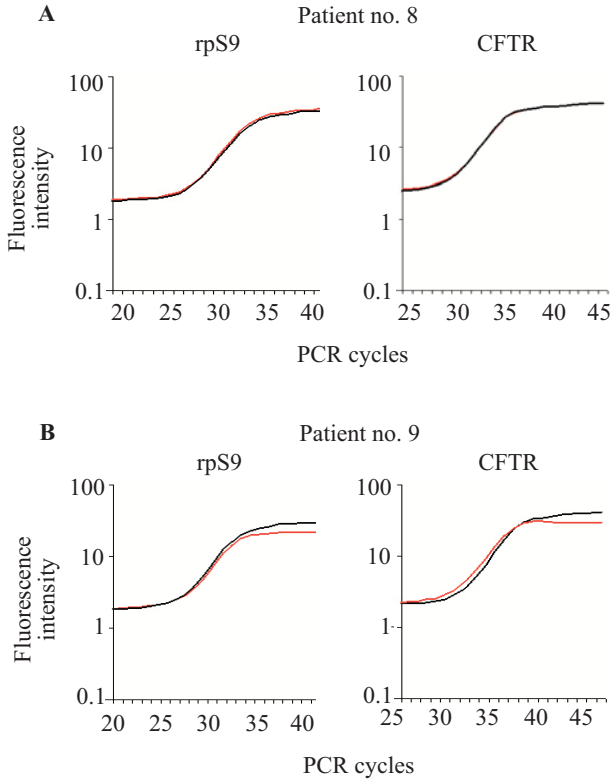
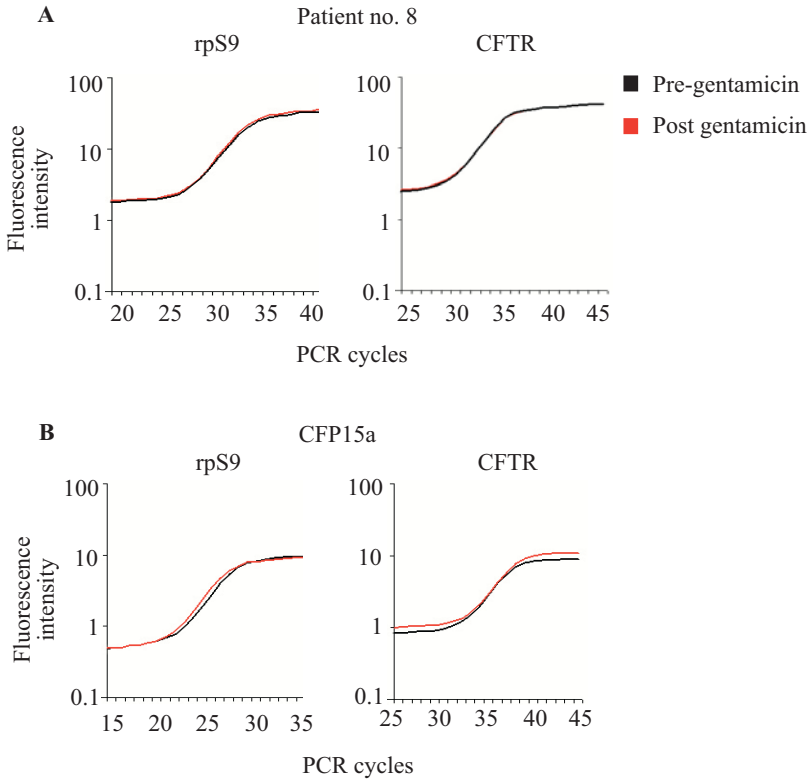
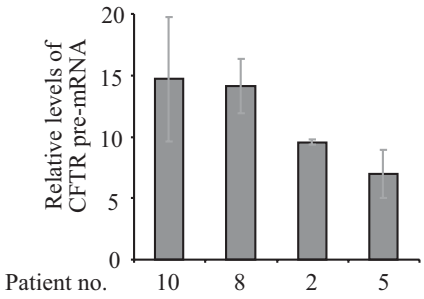
Supplemental Figure 1



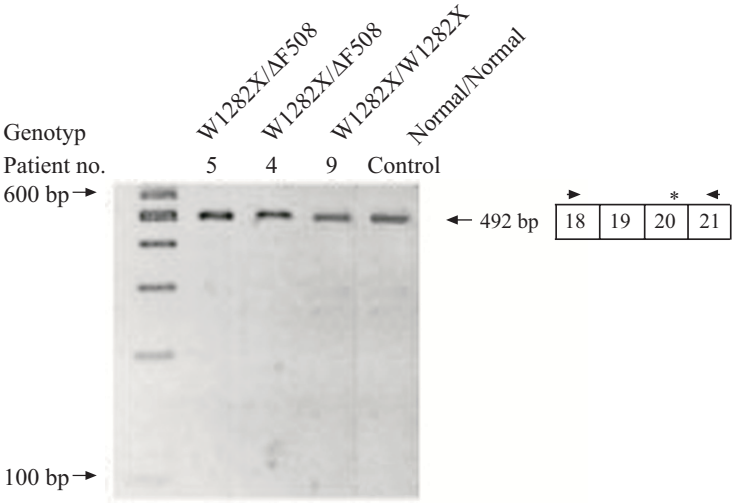
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental figure legends

Supplemental Figure 1. Levels of CFTR nonsense transcripts in repeated nasal epithelium samples of CF patients. Examples of real-time PCR of the CFTR and the rpS9 genes of patients 8 and 9, in two different time points (marked in black and red).

Supplemental Figure 2. Levels of CFTR nonsense transcripts in nasal epithelium of CF patients and in cell lines, before and after gentamicin treatment. Examples of real-time PCR of the CFTR and the rpS9 genes, of RNA from patient 8 (**A**) and CFP15a cell line (**B**), before and after gentamicin treatment.

Supplemental Figure 3. Relative levels of CFTR pre-mRNA in samples derived from CF patients. In the analysis we included only RNA samples in which no DNA (or negligible amounts) could be detected by PCR using primers for the Albumin gene, which is not expressed in nasal epithelium. The level of CFTR pre-mRNA was measured by real-time PCR and normalized to the level of rpS9. The relative levels of CFTR pre-mRNA are shown as average ± s.e.m.

Supplemental Figure 4. Splicing pattern of the W1282X region. Examples of RT-PCR performed on RNA samples. Left lane - 100 bp DNA ladder. The observed 492 bp band is expected from normally spliced CFTR mRNA. The scheme on the right shows the amplified region: the location of the W1282X mutation is marked by an asterisk, the PCR primers are marked by arrow heads.

Supplemental results

Correct splicing of CFTR exon 20 in transcripts carrying the W1282X mutation

We further analyzed the possibility that the level of transcripts available for the readthrough treatment is modulated by skipping over the PTC containing exon (69). Analysis of the splicing pattern of CFTR exon 20 in the pre-treatment RNA samples, where the W1282X mutation is located revealed one PCR product in the expected size from normal splicing of this exon, at all time points, both in patients who responded to the treatment and in those who did not respond (Supplemental Figure 4). Sequence

analysis of these PCR products revealed the expected sequence from normal splicing of exon 20. Similar to the finding in the patients, splicing analysis of exon 20 in CFP15a cells revealed no skipping over the W1282X mutation (data not shown) indicating that alternative splicing of exon 20 has no role in modulating the endogenous CFTR nonsense transcripts. These results indicate that skipping over the W1282X mutation does not play a role in modulating the response to gentamicin treatment.

69. Maquat, L.E. 2002. NASty effects on fibrillin pre-mRNA splicing: another case of ESE does it, but proposals for translation-dependent splice site choice live on. *Genes Dev*. **16**:1743–1753.