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Report

Five siRNAs Targeting Three SNPs May Provide Therapy for Three-Quarters of Huntington's Disease Patients

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Summary

Among dominant neurodegenerative disorders, Huntington's disease (HD) is perhaps the best candidate for treatment with small interfering RNAs (siRNAs) [1–9]. Invariably fatal, HD is caused by expansion of a CAG repeat in the *Huntingtin* gene, creating an extended polyglutamine tract that makes the Huntingtin protein toxic [10]. Silencing mutant *Huntingtin* messenger RNA (mRNA) should provide therapeutic benefit, but normal Huntingtin likely contributes to neuronal function [11–13]. No siRNA strategy can yet distinguish among the normal and disease *Huntingtin* alleles and other mRNAs containing CAG repeats [14]. siRNAs targeting the disease isoform of a heterozygous single-nucleotide polymorphism (SNP) in *Huntingtin* provide an alternative [15–19]. We sequenced 22 predicted SNP sites in 225 human samples corresponding to HD and control subjects. We find that 48% of our patient population is heterozygous at a single SNP site; one isoform of this SNP is associated with HD. Several other SNP sites are frequently heterozygous. Consequently, five allele-specific siRNAs, corresponding to just three SNP sites, could be used to treat three-quarters of the United States and European HD patient populations. We have designed and validated selective siRNAs for the three SNP sites, laying the foundation for allele-specific RNA interference (RNAi) therapy for HD.

Results

Current strategies for designing single-nucleotide-selective small interfering RNAs (siRNAs) rely on SNPs that produce a purine:purine mismatch between the siRNA guide strand and the counter-selected messenger RNA (mRNA) target [15, 20]. Only 4 of the 12 nucleotide mismatches satisfy this criterion. Even when purine:purine mismatches are available, single-mismatch siRNAs vary in their selectivity, ranging in one study, for example, from 4.3- to 133-fold discrimination between the fully complementary targeted RNA and the mismatched, counter-selected RNA [15].

Sequencing and Analysis of *Huntingtin* SNP Sites in HD and Control Patients

We sequenced 12 PCR amplicons spanning 22 known single-nucleotide polymorphism (SNP) sites in *Huntingtin* by using genomic DNA from 109 Huntington's disease (HD) patients and 116 non-HD controls (Figure 1A). The sequenced DNA encompassed six complete coding exons and the portion of exon 67 that contains the stop codon and part of the 3′ untranslated region (UTR). Twenty-two of the SNP sites were reported in the SNPper database [21, 22]. Four of these reported SNP sites were present only as a single isoform in our population. We identified an additional two sites by resequencing exons 2–67 in the *Huntingtin* locus from six HD patient samples. Table 1 reports the frequency of heterozygosity for each SNP site for patient and control DNA.

Of the 24 SNPs, rs362307 at nucleotide (nt) 9633 (exon 67) of the mRNA was significantly associated with HD ($p = 0.0000523$). After Bonferroni correction for multiple testing, the association remained significant ($p = 0.000890$). More than 48% of the HD patients that we examined—which are believed to be representative of the US and European patient pool—were heterozygous at this site (Table 1). The U isoform of the rs362307 SNP comprised 26% of *Huntingtin* alleles among the patients that we tested, but only 6% of alleles among the controls. This finding suggests that a single, allele-specific siRNA selectively targeting the U mRNA isoform of this SNP could be used to treat nearly half of this patient population. To confirm our statistical analysis, we used a previously reported method to determine the rs362307 SNP isoform linked to the CAG repeat expansion allele [23] for 16 patient blood samples. Of the 16 patients, 8 were heterozygous at this site; of the 8, the U isoform was linked to the expanded CAG repeat for 7 patients (Table S1 available online). We conclude that the U isoform of this SNP is associated with the disease allele of *Huntingtin* mRNA.

An Additional Two SNPs Achieve Patient Coverage > 75%

Eight other SNP sites were each heterozygous in > 33% of our patient population but did not show a statistically significant association with HD. Because no particular isoform of these SNPs is associated with HD in our patient population, each SNP site requires two distinct, isoform-selective siRNAs. We calculated the maximum coverage (i.e., the number of patients with at least one heterozygous SNP site) for all possible combinations of one to seven SNPs. Adding two additional SNP sites covered ~75% of our patient population. Using four or more SNP sites as potential targets for siRNA therapy is not predicted to provide much additional benefit; using even seven SNP sites achieves < 80% coverage but would require 13 isoform-selective siRNAs (Figure 1B).

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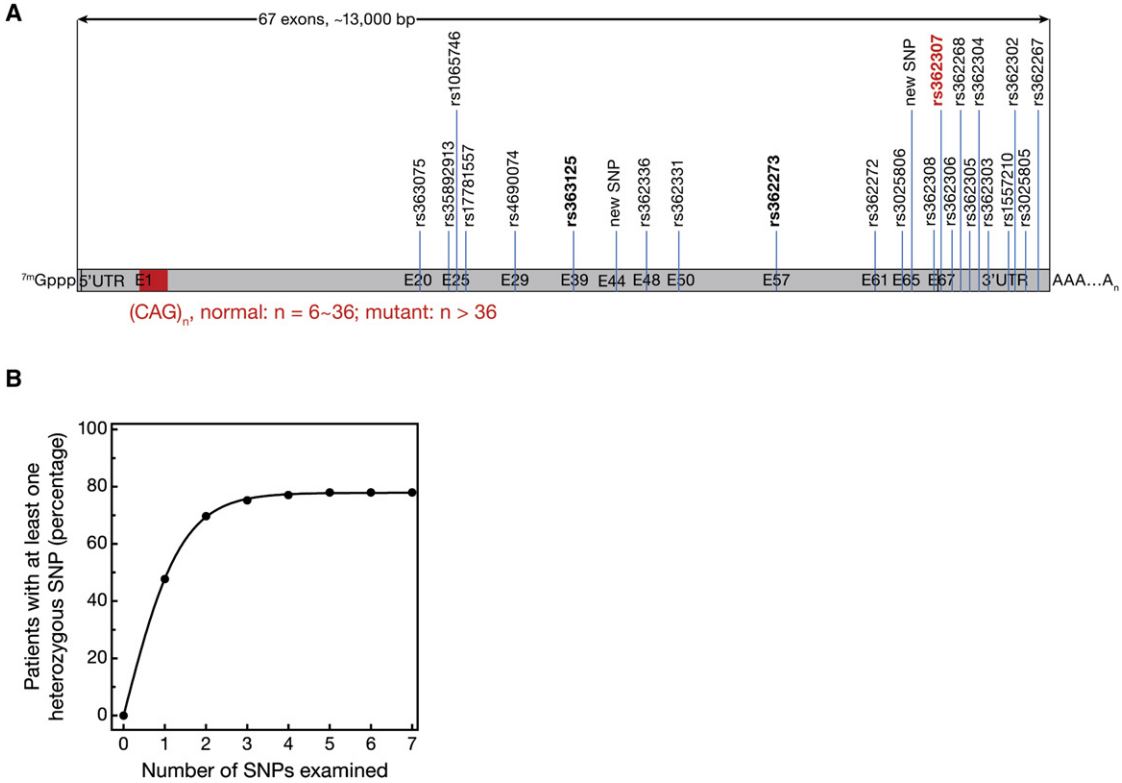

Figure 1. Analysis of SNPs in the Human *Huntingtin* mRNA

(A) We sequenced PCR amplicons from genomic DNA from 109 HD patients and 116 controls spanning 22 SNP sites within the *Huntingtin* mRNA. The SNP at nucleotide 9633 (rs362307, shown in red) is associated with HD, and sites for which we have designed siRNAs are in bold.

(B) The maximum percentage of patients to have at least one heterozygous SNP with any combination of one to seven SNPs was calculated with the experimentally determined frequency of heterozygosity for the SNP sites in our study. Three SNPs cover ~75% of the patient population analyzed here.

Development of Allele-Specific siRNAs

The HD-associated SNP site at position 9633 of the *Huntingtin* mRNA does not fall into the category of SNPs that are predicted to be readily amenable to selective targeting because it does not create a purine:purine mismatch between siRNA and mRNA [15, 20]. However, our analysis of *Huntingtin* SNPs in HD patients and controls (Figure 1B and Table 1) suggests that a practicable RNA-silencing therapy for HD requires an siRNA that targets the disease isoform at this site but spares the normal *Huntingtin* mRNA. To this end, we designed siRNAs targeting the U isoform of the position 9633 SNP. We tested both the efficacy and selectivity of the siRNAs in cultured human HeLa cells cotransfected with the siRNA and luciferase reporters containing in their 3′ UTRs either the U or C isoform of the sequence containing the SNP. In our hands, such luciferase reporter assays are good predictors of the efficacy and selectivity of siRNAs for endogenous mRNA targets. Figures S1 and S2 present an example with a pair of siRNAs—one fully matched and one bearing a position 10 (P10) mismatch—that targets an SNP site (rs363125) in endogenous *Huntingtin* mRNA in HeLa cells. Previous work has shown that such SNP-selective siRNAs can reduce mutant *Huntingtin* levels while leaving normal *Huntingtin* intact [24].

siRNAs whose guide strand was fully matched to the U isoform, which is associated with HD but mismatched at position 10 or position 16 to the C isoform, were functional but failed to discriminate between U and C reporter mRNAs (Figure S3A). (siRNAs that bear purine:pyrimidine mismatches to their counterselected targets generally show poor discrimination [15].) We also tested single mismatches at positions 2 through 9 (Table S2) but found that all of these were less specific than the most selective position 10 + seed mismatch. Double-mismatch strategies based on a position 16 mismatch with the counterselected isoform had very low activity (Table S2).

Previous work has shown that adding a second mismatch can improve the ability of siRNA to discriminate between alleles [25]. We reasoned that adding a mismatch in the seed sequence of the siRNA might sufficiently destabilize our siRNA so that the doubly mismatched siRNA would lose its ability to silence the wild-type *Huntingtin* mRNA, and pairing at the SNP site would allow the singly mismatched siRNA to retain silencing activity for the disease allele. Therefore, we tested doubly mismatched siRNAs combining a seed mismatch with a position 10 mismatch. We prepared siRNAs that are predicted to mismatch at position 10 with the normal *Huntingtin* mRNA and that also bear an additional mismatch to both normal and disease alleles at one of the six seed positions (2–7). Mismatches at positions 5 or 6, combined with a position 10 mismatch with the counterselected isoform, resulted in a reduction or loss of silencing of the SNP-mismatched target while retaining good activity against the SNP-matched target (Figure S3B).

Table 2 reports "discrimination ratios"—the ratio of the IC_{50} of the siRNA for the counterselected target to the IC_{50} of the targeted mRNA. The P10 (SNP) + P5 siRNA ($IC_{50P10\ mismatch}$ > 20; $IC_{50P10\ match}$ = 0.62 ± 0.43 nM) had a discrimination ratio > 32 and, at 20 nM, the highest concentration tested, reduced expression of the counterselected reporter by only 33%. The P10 + P6 siRNA achieved no appreciable reduction in

Table 1. Frequency of Heterozygosity for 24 SNP Sites in the *Huntingtin* mRNA

Location in mRNA (Position, nt)	Reference Number	Percent Heterozygosity	
		Controls	HD Patients
ORF, exon 20 (2822)	rs363075	G/A, 10.3% (G/G, 89.7%)	G/A, 12.8% (G/G, 86.2%; A/A, 0.9%)
ORF, exon 25 (3335)	rs35892913	G/A, 10.3% (G/G, 89.7%)	G/A, 13.0% (G/G, 86.1%; A/A, 0.9%)
ORF, exon 25 (3389)	rs1065746	G/C, 0% (G/G, 100%)	G/C, 0.9% (G/G, 99.1%)
ORF, exon 25 (3418)	rs17781557	T/G, 12.9% (T/T, 87.1%)	T/G, 1.9% (T/T, 98.1%)
ORF, exon 29 (3946)	rs4690074	C/T, 37.9% (C/C, 50.9%; T/T, 11.2)	C/T, 35.8% (C/C, 59.6%; T/T, 4.6%)
ORF, exon 39 (5304)	*rs363125*	*C/A, 17.5% (C/C, 79.0%; A/A, 3.5%)*	*C/A, 11.0% (C/C, 87.2%; A/A, 1.8%)*
ORF, exon 44 (6150)	exon 44 (new)	G/A, 0% (G/G, 100%)	G/A, 2.8% (G/G, 97.2%)
ORF, exon 48 (6736)	rs362336	G/A, 38.7% (G/G, 49.6%; A/A, 11.7%)	G/A, 37.4% (G/G, 57.9%; A/A, 4.7%)
ORF, exon 50 (7070)	rs362331	T/C, 45.7% (T/T, 31.0%; C/C, 23.3%)	T/C, 39.4% (T/T, 49.5%; C/C, 11.0%)
ORF, exon 57 (7942)	*rs362273*	*A/G, 40.3% (A/A, 48.2%; G/G, 11.4%)*	*A/G, 35.2% (A/A, 60.2%; G/G, 4.6%)*
ORF, exon 61 (8501)	rs362272	G/A, 37.1% (G/G, 51.7%; A/A, 11.2%)	G/A, 36.1% (G/G, 59.3%; A/A, 4.6%)
ORF, exon 65 (9053)	rs3025806	A/T, 0% (C/C, 100%)	A/T, 0% (C/C, 100%)
ORF, exon 65 (9175)	exon 65 (new)	G/A, 2.3% (G/G, 97.7%)	G/A, 0% (G/G, 100%)
ORF, exon 67 (9523)	rs362308	T/C, 0% (T/T, 100%)	T/C, 0% (T/T, 100%)
3′UTR, exon 67 (9633)[a]	*rs362307*	*C/T, 13.0% (C/C, 87.0%)*	*C/T, 48.6% (C/C, 49.5%; T/T, 1.9%)*
3′UTR, exon 67 (9888)	rs362306	G/A, 36.0% (G/G, 52.6%; A/A, 11.4%)	G/A, 35.8% (G/G, 59.6%; A/A, 4.6%)
3′UTR, exon 67 (9936)	rs362268	C/G, 36.8% (C/C, 50.0%; G/G 13.2%)	C/G, 35.8% (C/C, 59.6%; G/G, 4.6%)
3′UTR, exon 67 (9948)	rs362305	C/G, 20.2% (C/C, 78.1%; G/G 1.8%)	C/G, 11.9% (C/C, 85.3%; G/G, 2.8%)
3′UTR, exon 67 (10060)	rs362304	C/A, 22.8% (C/C, 73.7%; A/A, 3.5%)	C/A, 11.9% (C/C, 85.3%; AA, 2.8%)
3′UTR, exon 67 (10095)	rs362303	C/T, 18.4% (C/C, 79.8%; T/T, 1.8%)	C/A, 11.9% (C/C, 85.3%; T/T, 2.8%)
3′UTR, exon 67 (10704)	rs1557210	C/T, 0% (C/C, 100%)	C/T, 0% (C/C, 100%)
3′UTR, exon 67 (10708)	rs362302	C/T, 4.3% (C/C, 95.7%)	C/T, 0% (C/C, 100%)
3′UTR, exon 67 (10796)	rs3025805	G/T, 0% (G/G, 100%)	G/T, 0% (G/G, 100%)
3′UTR, exon 67 (11006)	rs362267	C/T, 36.2% (C/C, 52.6%; T/T, 11.2%)	C/T, 35.5% (C/C, 59.8%; T/T, 4.7%)

The SNP sites for which we tested siRNAs are in italics.
[a] These siRNAs provide good discrimination at the HD-associated SNP site.

expression of the mismatched reporter, even at 20 nM ($IC_{50P10\ mismatch}$ > 20 nM), but was less effective against the matched reporter ($IC_{50P10\ match}$ = 1.5 ± 0.31 nM), yielding a lower discrimination ratio. We often observed such a trade-off between the efficacy and the selectivity of SNP-specific siRNAs. We also designed and tested an siRNA targeting the C isoform;

Table 2. Validation of siRNAs Designed to Distinguish between Matched and Mismatched SNP Isoforms

Reference Number	siRNA Guide Strand	SNP Position	Primary Mismatch	Secondary Mismatch Position	Secondary Mismatch	IC50 (nM)		Discrimination Ratio
						Match	Mismatch	
rs363125	5′-agcguugaaguacugucccca-3′	10	G:A	none	none	0.17 ± 0.11	0.27 ± 0.25	1.6
rs363125	5′-agcguugaauuacugucccca-3′	10	U:C	none	none	0.18 ± 0.09	0.22 ± 0.07	1.2
rs363125	5′-ucuucuagcguugaaguacug-3′	16	G:A	none	none	0.36 ± 0.24	>20	>55
rs363125	5′-ucuucuagcguugaauuacug-3′	16	U:C	none	none	0.74 ± 0.40	>20	>27
rs362307	5′-cacaagggcgcagacuuccaa-3′	10	G:U	none	none	0.36 ± 0.04	0.77 ± 0.16	2.1
rs362307	5′-uacaagggcacagacuuccaa-3′	10	A:C	none	none	0.16 ± 0.09	0.14 ± 0.10	0.87
rs362307	5′-gcagggcacaagggcgcagac-3′	16	G:U	none	none	0.73 ± 0.12	0.72 ± 0.12	0.99
rs362307	5′-ucagggcacaagggcacagac-3′	16	A:C	none	none	0.19 ± 0.02	0.20 ± 0.06	1.1
rs362307	5′-cgcaagggcacagacuuccaa-3′	10	A:C	2	G:U	1.0 ± 0.35	1.9 ± 0.27	1.9
rs362307	5′-cauaagggcacagacuuccaa-3′	10	A:C	3	U:G	3.0 ± 1.8	3.5 ± 1.6	1.2
rs362307	5′-caccagggcacagacuuccaa-3′	10	A:C	4	C:U	1.0 ± 0.22	1.6 ± 1.2	1.6
rs362307	5′-cacacgggcacagacuuccaa-3′	10	A:C	5	C:U	0.62 ± 0.43	>20	>32
rs362307	5′-cacaauggcacagacuuccaa-3′	10	A:C	6	U:C	1.5 ± 0.31	>20	>13
rs362307	5′-cacaagugcacagacuuccaa-3′	10	A:C	7	U:C	1.3 ± 0.51	5.9 ± 1.9	4.5
rs362307	5′-cacaauggcgcagacuuccaa-3′	10	G:U	6	U:C	3.2 ± 2.2	>20	>6
rs362273	5′-guugaucuguagcagcagcuu-3′	10	U:G	none	none	0.09 ± 0.14	0.01 ± 0.006	0.11
rs362273	5′-guugaucugcagcagcagcuu-3′	10	C:A	none	none	0.12 ± 0.06	0.44 ± 0.11	3.7
rs362273	5′-cucgggguugaucuguagcag-3′	16	U:G	none	none	0.01 ± 0.002	0.007 ± 0.002	0.70
rs362273	5′-cucgggguugaucugcagcag-3′	16	C:A	none	none	0.01 ± 0.003	0.004 ± 0.001	0.41
rs362273	5′-ucugaucuguagcagcagcuu-3′	10	U:G	2	C:A	0.01 ± 0.002	0.06 ± 0.008	5.9
rs362273	5′-uucgaucuguagcagcagcuu-3′	10	U:G	3	C:A	0.02 ± 0.003	0.29 ± 0.04	15
rs362273	5′-uuuuaucuguagcagcagcuu-3′	10	U:G	4	U:C	0.03 ± 0.006	0.37 ± 0.11	11
rs362273	5′-uuugcucuguagcagcagcuu-3′	10	U:G	5	C:U	0.02 ± 0.003	0.59 ± 0.08	31
rs362273	5′-uuugaccuguagcagcagcuu-3′	10	U:G	6	C:A	0.02 ± 0.002	0.06 ± 0.015	2.7
rs362273	5′-uuugauuuguagcagcagcuu-3′	10	U:G	7	U:G	0.006 ± 0.001	0.10 ± 0.02	17
rs362273	5′-uuugcucugcagcagcagcuu-3′	10	C:A	5	C:U	0.15 ± 0.04	0.74 ± 0.11	4.9

IC_{50} values are given as the average ± SD for at least three independent experiments. The IC_{50} is reported as > 20 nM for siRNAs that failed to achieve half-maximal inhibition at the highest concentration tested. Underline indicates the site of the primary mismatch; italics indicate the site of the secondary mismatch.

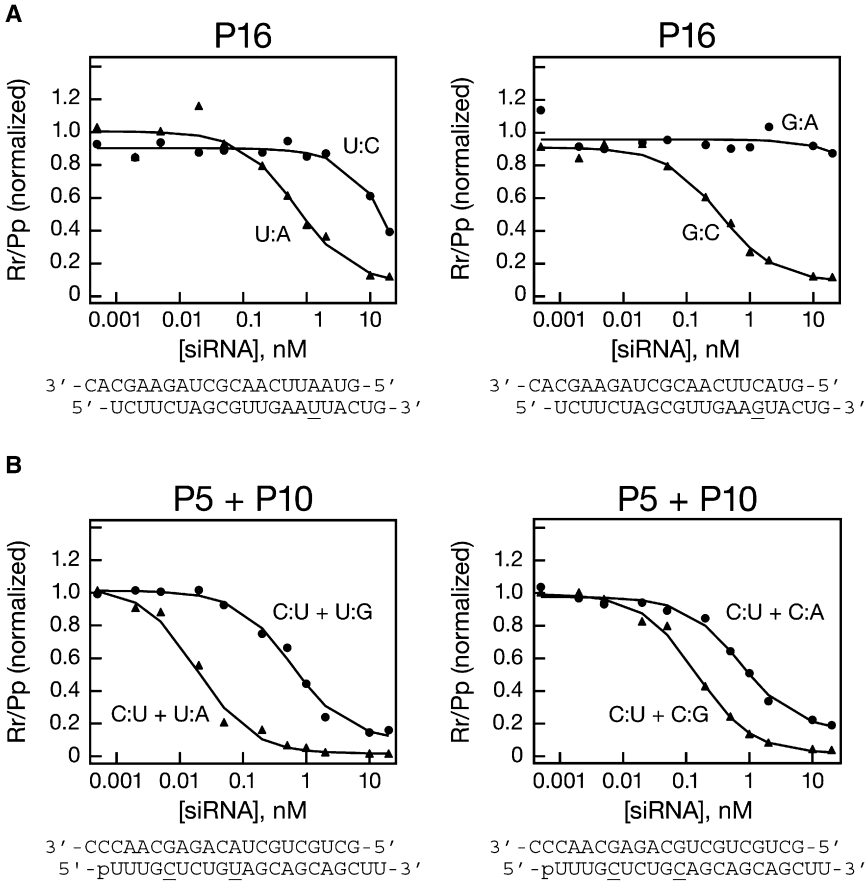

Figure 2. Representative Data for the Development of Isoform-Specific siRNAs Targeting Two Additional SNP Sites

(A) siRNAs mismatched at position 16 discriminated between luciferase reporter mRNAs bearing either the C or the A isoform of the rs363125 SNP site.

(B) siRNAs bearing a mismatch to the SNP site at position 10 and an additional position 5 mismatch discriminated between the G and A isoforms of the rs362273 SNP site.

though it was less active than the siRNA targeting the U isoform, it selectively targeted the P10-matched allele ($IC_{50P10\ mismatch}$ > 20; $IC_{50P10\ match}$ = 3.2 ± 2.2 nM) (Figure S3C and Table 2).

To cover 75% of HD patients requires siRNAs targeting additional SNPs. Because no specific nucleotide isoform of these SNP sites is associated with HD, selective siRNAs are needed for both isoforms. Our long-term strategy would be to screen patients to determine the SNP isoform associated with the expanded CAG repeat *Huntingtin* allele [23] and select the corresponding siRNA for therapy. As a first step toward this goal, we tested each siRNA's ability to target one isoform of the SNP while minimizing silencing of the other isoform. For the SNP site rs363125, which lies at nt 5304 (exon 39) and occurs as either an A or a C, a single mismatch was sufficient to provide a high degree of selectivity for the fully matched target for both the A (>27-fold discrimination; $IC_{50mismatch}$ > 20 nM; $IC_{50match}$ = 0.74 ± 0.40 nM) and C ($IC_{50mismatch}$ > 20 nM; >55-fold discrimination; $IC_{50match}$ = 0.36 ± 0.24 nM) isoforms (Figures 2A and S4 and Table 2). For a second SNP, rs362273, which lies at nt 7942 (exon 57) in the *Huntingtin* mRNA and occurs as either an A or a G, the P10 (SNP) + P5 siRNA design targeting the A isoform of the SNP provided ~30-fold selectivity ($IC_{50P10\ mismatch}$ = 0.59 ± 0.08 nM; $IC_{50P10\ match}$ = 0.02 ± 0.003 nM), whereas the siRNA targeting the G isoform ($IC_{50P10\ mismatch}$ = 0.74 ± 0.11 nM; $IC_{50P10\ match}$ = 0.15 ± 0.04 nM) gave ~4.9-fold selectivity (Figures 2B and S5 and Table 2).

Discussion

Targeted reduction of mutant *Huntingtin* mRNA is considered an ideal strategy for treating HD. The primary obstacles to the development of such a therapy have been concerns about the number of siRNAs that would require testing in clinical trials. It is not clear whether drug regulatory agencies will permit patient-specific siRNAs to be used in humans without large-scale clinical trials. Such trials are, of course, not possible if only small numbers of patients share a common SNP isoform. Our results suggest that there is sufficient heterozygosity at a small number of SNP sites among American and European HD patients to support SNP-specific siRNA therapy. Targeting just three SNPs with five siRNAs should cover the majority of HD patients in the population studied here. This is possible because of the presence of several highly heterozygous SNPs and because a single SNP isoform for SNP rs362307 is associated with HD. In a parallel study, Hayden and colleagues likewise report that SNP rs362307 is associated with HD [26]. One siRNA targeting this HD-associated isoform should target the mutant *Huntingtin* allele in nearly 50% of our patient population. We have developed an siRNA that selectively targets the disease-associated isoform of this SNP in cultured human cells. In the near future, preclinical testing of this siRNA for efficacy, selectivity, and safety is clearly of the highest importance.

What of the ~25% of patients predicted to be beyond the reach of the five siRNAs developed here? Unfortunately, our analysis predicts that a very large number of siRNAs will be required to provide siRNA therapy for this subpopulation. Adding an additional four siRNAs (for a total of nine siRNAs corresponding to five SNP sites) only increases the treatable patient population by 3%. A further increase in the number of siRNAs provides no real additional benefit.

Finally, we find that, by using potentiating mismatches in the seed sequence, isoform-selective siRNAs can be designed for SNP sites predicted to be poor candidates for the development of allele-selective siRNAs. Our data suggest that a single siRNA directed against a SNP isoform associated with HD could be used to treat nearly half of the US and European HD population. Clearly, an siRNA directed against this SNP isoform, such as the siRNA presented here, merits thorough preclinical validation to test its promise as a candidate therapy for HD.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, five figures, and three tables and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)00878-1.

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References

1. Xia, H., Mao, Q., Eliason, S.L., Harper, S.Q., Martins, I.H., Orr, H.T., Paulson, H.L., Yang, L., Kotin, R.M., and Davidson, B.L. (2004). RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. Nat. Med. *10*, 816–820.
2. Machida, Y., Okada, T., Kurosawa, M., Oyama, F., Ozawa, K., and Nukina, N. (2006). rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. Biochem. Biophys. Res. Commun. *343*, 190–197.
3. Wang, Y.L., Liu, W., Wada, E., Murata, M., Wada, K., and Kanazawa, I. (2005). Clinico-pathological rescue of a model mouse of Huntington's disease by siRNA. Neurosci. Res. *53*, 241–249.
4. Xia, X., Zhou, H., Huang, Y., and Xu, Z. (2006). Allele-specific RNAi selectively silences mutant SOD1 and achieves significant therapeutic benefit in vivo. Neurobiol. Dis. *23*, 578–586.
5. Xia, H., Mao, Q., Paulson, H.L., and Davidson, B.L. (2002). siRNA-mediated gene silencing in vitro and in vivo. Nat. Biotechnol. *20*, 1006–1010.
6. Harper, S.Q., Staber, P.D., He, X., Eliason, S.L., Martins, I.H., Mao, Q., Yang, L., Kotin, R.M., Paulson, H.L., and Davidson, B.L. (2005). RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc. Natl. Acad. Sci. USA *102*, 5820–5825.
7. DiFiglia, M., Sena-Esteves, M., Chase, K., Sapp, E., Pfister, E., Sass, M., Yoder, J., Reeves, P., Pandey, R.K., Rajeev, K.G., et al. (2007). Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc. Natl. Acad. Sci. USA *104*, 17204–17209.
8. Ralph, G.S., Radcliffe, P.A., Day, D.M., Carthy, J.M., Leroux, M.A., Lee, D.C., Wong, L.F., Bilsland, L.G., Greensmith, L., Kingsman, S.M., et al. (2005). Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. Nat. Med. *11*, 429–433.
9. Raoul, C., Abbas-Terki, T., Bensadoun, J.C., Guillot, S., Haase, G., Szulc, J., Henderson, C.E., and Aebischer, P. (2005). Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. Nat. Med. *11*, 423–428.
10. The Huntington's Disease Collaborative Research Group. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell *72*, 971–983.
11. Auerbach, W., Hurlbert, M.S., Hilditch-Maguire, P., Wadghiri, Y.Z., Wheeler, V.C., Cohen, S.I., Joyner, A.L., MacDonald, M.E., and Turnbull, D.H. (2001). The HD mutation causes progressive lethal neurological disease in mice expressing reduced levels of huntingtin. Hum. Mol. Genet. *10*, 2515–2523.
12. Cattaneo, E., Zuccato, C., and Tartari, M. (2005). Normal huntingtin function: An alternative approach to Huntington's disease. Nat. Rev. Neurosci. *6*, 919–930.
13. Dragatsis, I., Levine, M.S., and Zeitlin, S. (2000). Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nat. Genet. *26*, 300–306.
14. Caplen, N.J., Taylor, J.P., Statham, V.S., Tanaka, F., Fire, A., and Morgan, R.A. (2002). Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. Hum. Mol. Genet. *11*, 175–184.
15. Schwarz, D.S., Ding, H., Kennington, L., Moore, J.T., Schelter, J., Burchard, J., Linsley, P.S., Aronin, N., Xu, Z., and Zamore, P.D. (2006). Designing siRNA that distinguish between genes that differ by a single nucleotide. PLoS Genet. *2*, e140.
16. Ding, H., Schwarz, D.S., Keene, A., Affar, E.-B., Fenton, L., Xia, X., Shi, Y., Zamore, P.D., and Xu, Z. (2003). Selective silencing by RNAi of a dominant allele that causes amyotrophic lateral sclerosis. Aging Cell *2*, 209–217.
17. Dahlgren, C., Zhang, H.Y., Du, Q., Grahn, M., Norstedt, G., Wahlestedt, C., and Liang, Z. (2008). Analysis of siRNA specificity on targets with double-nucleotide mismatches. Nucleic Acids Res. *36*, e53.
18. Du, Q., Thonberg, H., Wang, J., Wahlestedt, C., and Liang, Z. (2005). A systematic analysis of the silencing effects of an active siRNA at all single-nucleotide mismatched target sites. Nucleic Acids Res. *33*, 1671–1677.
19. Miller, V.M., Gouvion, C.M., Davidson, B.L., and Paulson, H.L. (2004). Targeting Alzheimer's disease genes with RNA interference: An efficient strategy for silencing mutant alleles. Nucleic Acids Res. *32*, 661–668.
20. Dykxhoorn, D.M., Schlehuber, L.D., London, I.M., and Lieberman, J. (2006). Determinants of specific RNA interference-mediated silencing of human beta-globin alleles differing by a single nucleotide polymorphism. Proc. Natl. Acad. Sci. USA *103*, 5953–5958.
21. Riva, A., and Kohane, I.S. (2004). A SNP-centric database for the investigation of the human genome. BMC Bioinformatics *5*, 33.
22. Riva, A., and Kohane, I.S. (2002). SNPper: Retrieval and analysis of human SNPs. Bioinformatics *18*, 1681–1685.
23. Liu, W., Kennington, L.A., Rosas, H.D., Hersch, S., Cha, J.H., Zamore, P.D., and Aronin, N. (2008). Linking SNPs to CAG repeat length in Huntington's disease patients. Nat. Methods *5*, 951–953.
24. van Bilsen, P.H., Jaspers, L., Lombardi, M.S., Odekerken, J.C., Burright, E.N., and Kaemmerer, W.F. (2008). Identification and allele-specific silencing of the mutant huntingtin allele in Huntington's disease patient-derived fibroblasts. Hum. Gene Ther. *19*, 710–719.
25. Ohnishi, Y., Tamura, Y., Yoshida, M., Tokunaga, K., and Hohjoh, H. (2008). Enhancement of allele discrimination by introduction of nucleotide mismatches into siRNA in allele-specific gene silencing by RNAi. PLoS ONE *3*, e2248.
26. Warby, S.C., Montpetit, A., Hayden, A.R., Carroll, J.B., Butland, S.L., Visscher, H., Collins, J.A., Semaka, A., Hudson, T.J., and Hayden, M.R. (2009). CAG expansion in the Huntington disease gene is associated with a specific and targetable predisposing haplogroup. Am. J. Hum. Genet. *84*, 351–366.