Prime Editing advancements enable *in vivo* therapeutic correction of *ATP7B* p.H1069Q and p.R778L mutations in Wilson's disease

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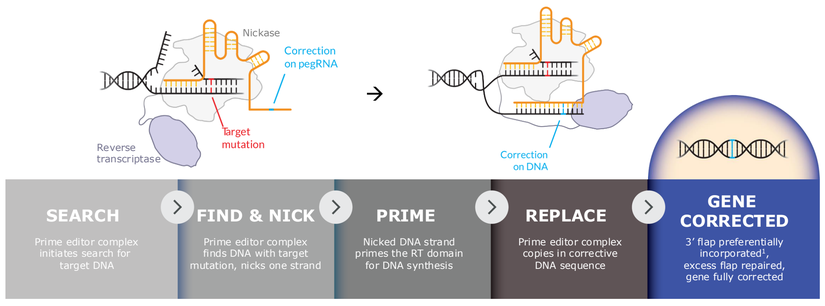
Background

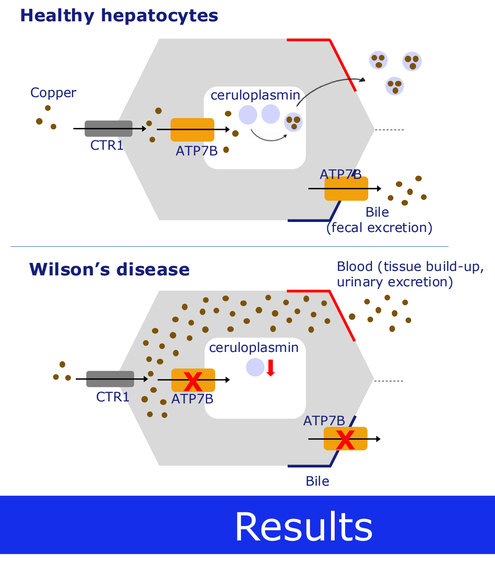
How Prime Editing works



Prime Editing for safe and effective treatment of >90% of genetic diseases

- Targeted and precise search and replace genome editing enables highly effective disease-causing mutation correction while minimizing unintended edits

Wilson's Disease Overview



Disease severity

- Common liver and systemic disease presenting in teens to 20's (prevalence approx. 1:30,000)
- Leads to liver failure, neurocognitive decline and premature death

Human Biology

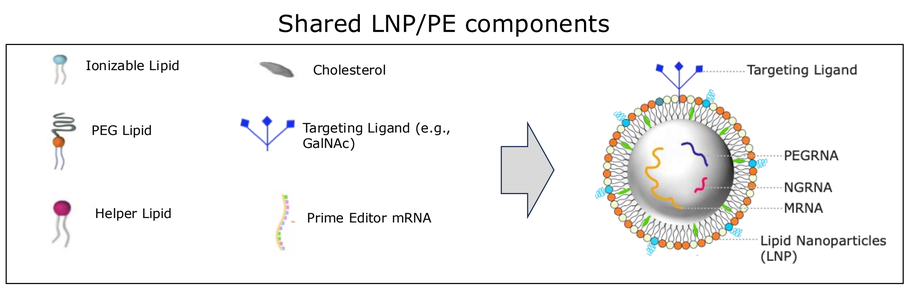
- Autosomal recessive due to loss of function mutations in *ATP7B*
- Affects copper homeostasis, leading to toxic accumulation of copper in liver and brain
- H1069Q and R778L are two prevalent mutations found in up to 50% of patients
- Correction of 20-30% of hepatocytes may be curative

Prime Medicine therapeutic approach

- Prime's universal liver targeted LNP, to deliver RNA Prime Editors to patient liver to correct mutations in ATP7B

Results

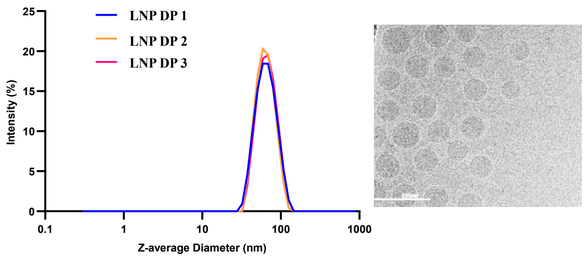
1 Prime Medicine's Universal LNP contains a novel GalNAc liver targeting ligand



- ✓ Validated as a delivery mechanism
- ✓ Increases potency
- ✓ Improves safety profile
- ✓ Improves biodistribution profile

2 Prime Medicine's Universal LNP can be used to generate multiple drug products

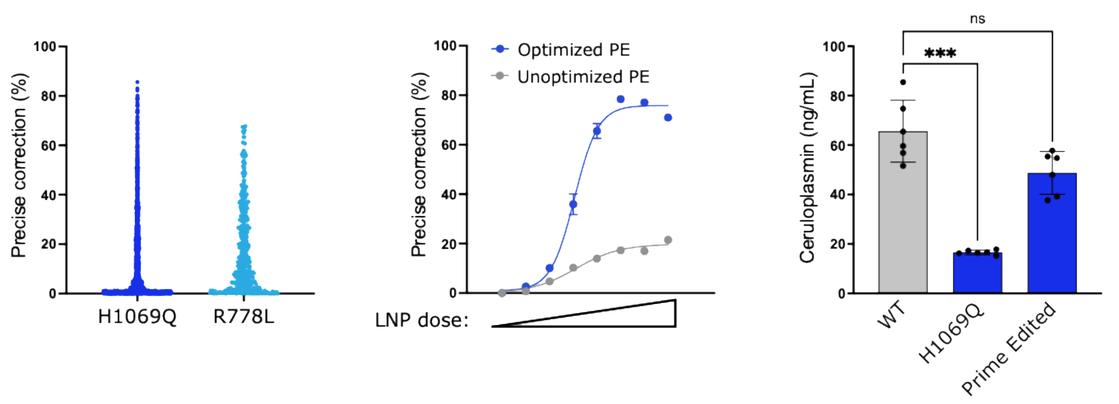
	Avg. Size (nm)	PDI	%EE
LNP DP 1	60	0.056	99.3
LNP DP 2	59	0.045	97.9
LNP DP 3	60	0.052	98.8



- Prime Medicine's Universal LNP keeps constant 6 out of the 8 components of the LNP and PE cargo for all liver programs
- By swapping out only the guide RNAs new drug products are made

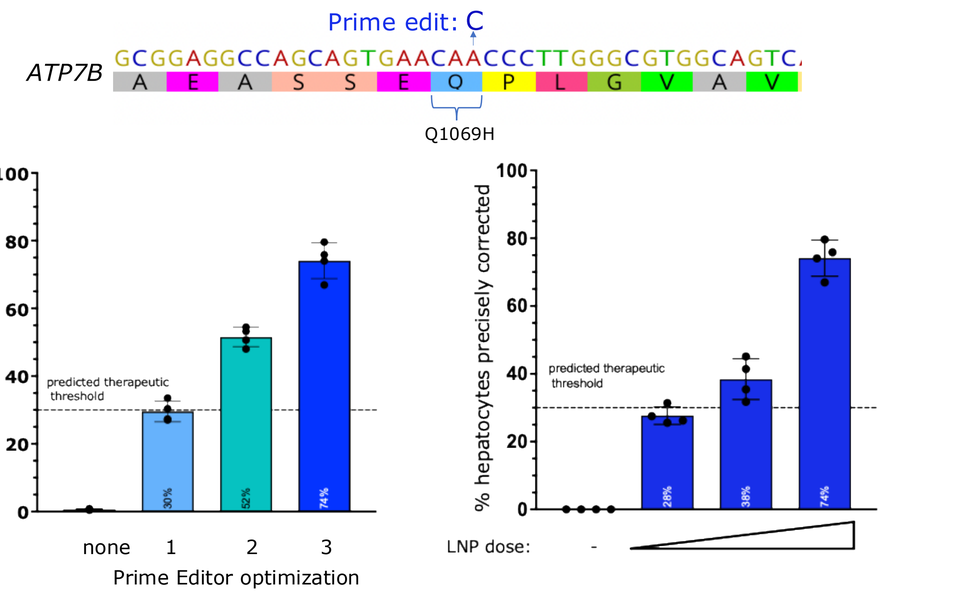
3 Screening and validation identifies highly active Prime Editors for H1069Q and R778L correction

Prime Editor screening in cell lines | Hit validation in primary humanized mouse hepatocytes | Ceruloplasmin in H1069Q patient iHEPs



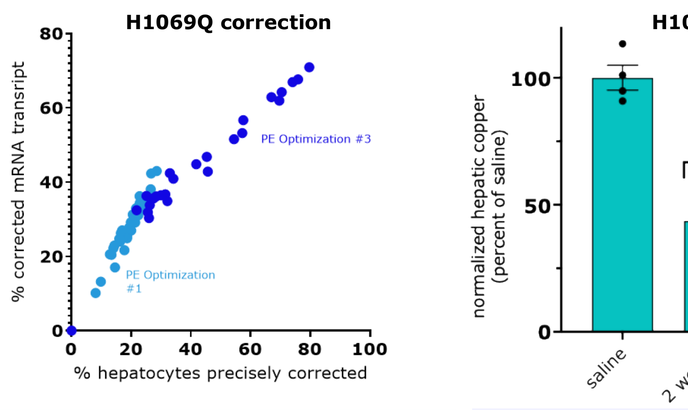
- High throughput screening in hepatocyte cell lines identifies several candidate Prime Editor components capable of >60% editing for H1069Q and R778L
- Further optimization of PE in *ATP7B* H1069Q humanized mouse hepatocytes greatly improves Prime Editor potency and efficacy
- Prime Editor normalizes ceruloplasmin abundance in H1069Q patient induced human hepatocytes (iHeps)

4 Improvements to Prime Editor cargo in Universal LNP enables therapeutic correction in fully humanized *ATP7B* H1069Q WD mouse models

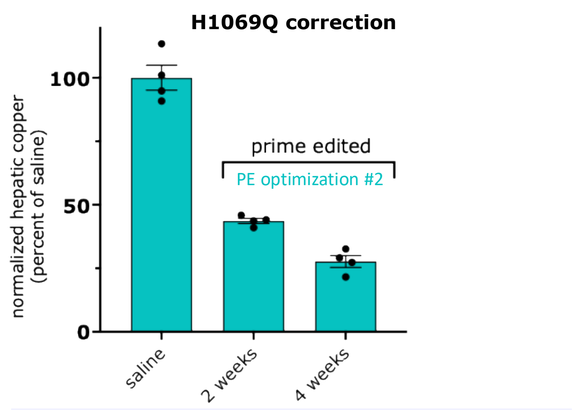


- Optimization of LNP components improve Prime Editing to up to 80% of hepatocytes in humanized *ATP7B* H1069Q mouse models
- Dose-dependent editing was observed for H1069Q correction, exceeding 30% therapeutic threshold at clinically relevant dose

5 Correction of H1069Q in humanized mouse models produces corrected mRNA transcripts and reduces liver copper

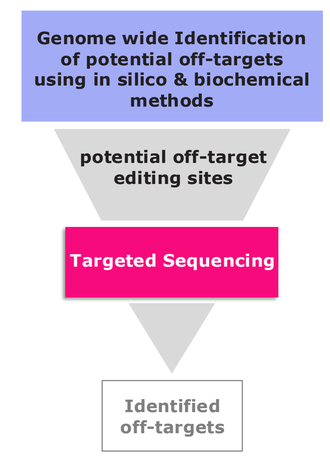


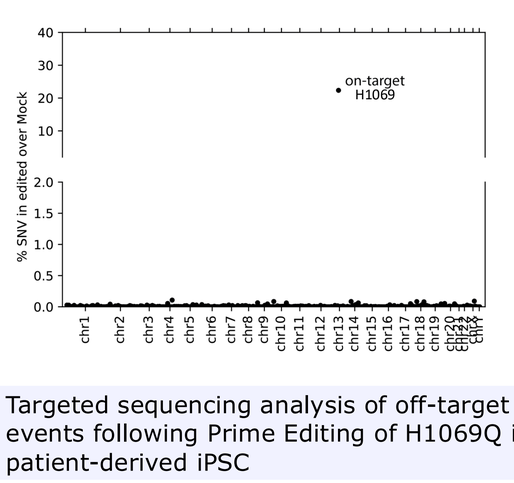
- DNA correction of H1069Q by Prime Editing correlates with corrected mRNA



- Time-dependent 75% reduction in liver copper using optimization 2 Prime Editor
- Further copper studies ongoing with Prime Editor Optimization #3

6 Preliminary off-target analysis for H1069Q Prime Editor does not identify off-target (SNVs or indels) events genome-wide



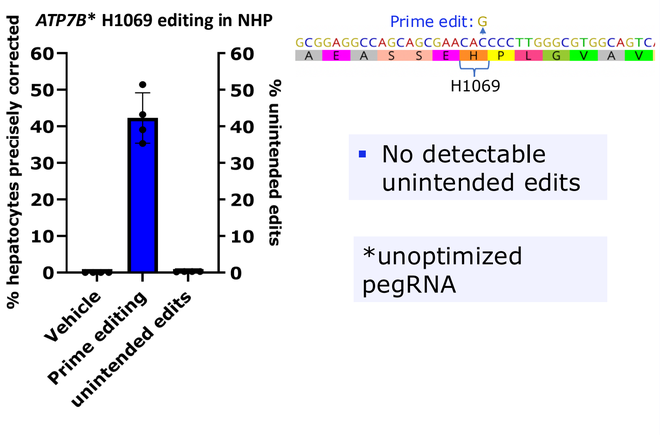


- Targeted sequencing analysis of off-target events following Prime Editing of H1069Q in patient-derived iPSC

7 Universal LNP-formulated surrogate H1069 Prime Editor *in vivo* in NHP achieves up to 51% precise editing in liver hepatocytes

Prime's Universal LNP has an excellent safety profile in NHP

- Well-tolerated with no acute reactions, clinical observations, or body weight changes
- Minimal LFT abnormalities
- No change in platelet, coagulation, or blood count
- No change in blood biochemistry
- Minimal change in IL6 production
- No other cytokine changes
- No change in liver histopathology (H&E)



- No detectable unintended edits

*unoptimized pegRNA

Conclusions

- Successfully identified several candidate Prime Editor components capable of >60% editing for *ATP7B* p.H1069Q and p.R778L
- LNP-formulated Prime Editors using Prime Medicine's Universal liver targeted LNP efficiently correct (up to 80%) the H1069Q mutation in a fully humanized *ATP7B* Wilson's disease mouse model without detectable unintended edits
- Preliminary off-target analysis demonstrated H1069Q Prime Editors do not result in detectable off-target events in patient-derived iPSC
- LNP-formulated Prime Editors resolve copper accumulation in humanized mouse livers
- Using Prime Medicine's Universal LNP with a surrogate Prime Editor RNA cargo, up to 51% editing at the H1069 locus was observed in NHP *in vivo*
- Prime Medicine's Universal LNP is well-tolerated in NHP with a favorable safety profile
- These data support the advancement of a potential one-time, curative approach for Wilson's disease patients H1069Q or R778L mutations