A genetics-first approach to identify novel variants of the calcium sensing receptor associated with autosomal dominant hypocalcemia type 1

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Summary

Background

- An estimated 1 in 10 people suffer from a rare disease, and a central challenge in rare disease is accurate diagnosis. Autosomal dominant hypocalcemia type 1 (ADH1) is a rare genetic disease caused by gain-of-function (GOF) variants in the calcium sensing receptor (CaSR), encoded by the gene *CASR*.
- Like many rare diseases, **ADH1 has primarily been studied in small cohorts** selected based on their clinical characteristics, which can lead to **biased ascertainment**. This bias can lead to inaccuracies in our understanding of both which genotypes cause GOF of CaSR and which phenotypes result from GOF of CaSR.
- To better define these genotypes and phenotypes, we are conducting an **integrated analysis of data from population-based biobanks, a sponsored testing program, and in vitro experiments**.

Results and discussion

- To identify putative GOF CaSR variants in the UK Biobank (UKB), we **scored CaSR variants based on their association with ADH1 phenotypes**. We replicated this analysis in All of Us (AOU) and found that 4 of these variants (Asn1074Asp, Asn345Asp, Gln1040Glu, and Gly1019Arg) have suggestive associations in both biobanks and a minor allele frequency consistent with previously established ADH1 variants.
- To further validate our findings, we evaluated data from patients participating in a **sponsored testing program** for suspected genetic hypoparthyroidism. Four of identified variants (Asn1074Asp, Ala364Glu, Thr972Met, and Phe815del) also show suggestive signals in the UKB or All of Us.
- Next, to better define the phenotypes associated with GOF CaSR variants, we conducted a **phenome-wide association study (pheWAS)** using a set of high confidence GOF CaSR variants and identified 83 phenotypes below a phenome-wide significance level of $3x10^{-5}$. These phenotypes may be novel additions to the ADH1 phenotypic spectrum.
- Finally, we determined that the frequency of previously established GOF variants was 2.8–4.0 in 100,000 individuals across four biobanks (gnomAD, TOPMed, the UKB, and AOU), similar to a previous report on the Geisinger cohort.
- We are **replicating the pheWAS in AOU** and **testing variants experimentally** for increased calcium sensitivity.
- Our work **clarifies the genotypes and phenotypes associated with ADH1 and may improve ADH1 diagnosis.**

ADH1 is a rare genetic disorder characterized by hypoparathyroidism, hypocalcemia, hypercalciuria, and can be accompanied by muscle problems, seizures, and kidney complications

ADH1 is a rare genetic form of hypoparathyroidism caused by GOF variants in the calcium sensing receptor (CaSR), which is encoded by the gene *CASR*. The clinical manifestations of the disease were discovered in ~1981 (**Figure 1**). The CaSR regulates calcium homeostasis by controlling parathyroid hormone secretion. Like many rare diseases, ADH1 has mostly been studied in relatively small cohorts selected based on their clinical characteristics, which can lead to biased ascertainment. **This bias can lead to inaccuracies in our understanding of both which genotypes cause GOF of CaSR and which phenotypes result from GOF of CaSR.**

To better define these genotypes and phenotypes, we are conducting an integrated analysis of data from population-based biobanks, a sponsored testing progratm, and *in vitro* experiments. The goal of this study is to clarify which genotypes lead to GOF of CaSR and which phenotypes result (**Figure 2**).

Figure 1. Milestones in ADH1 research and clinical manifestations.

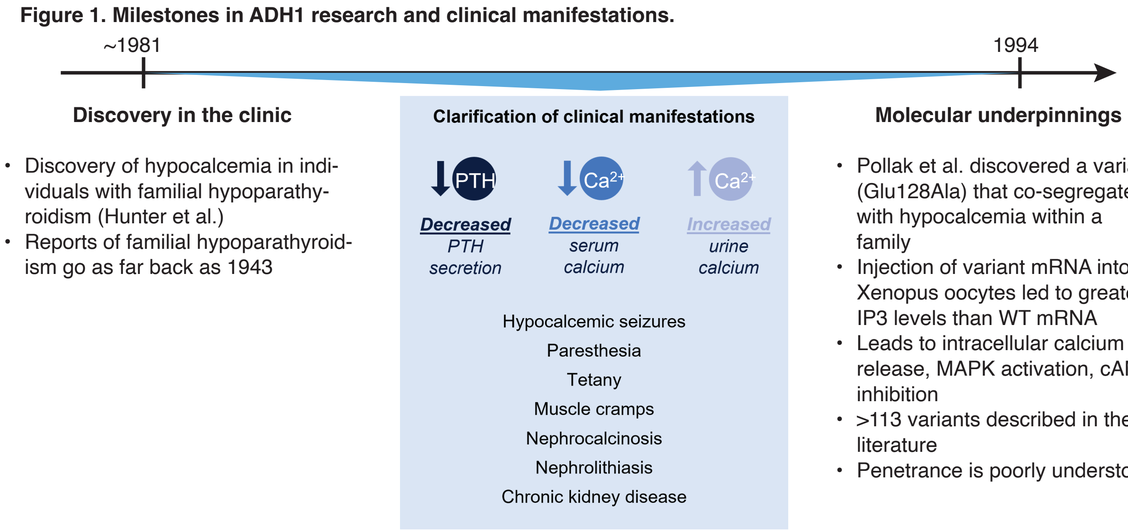
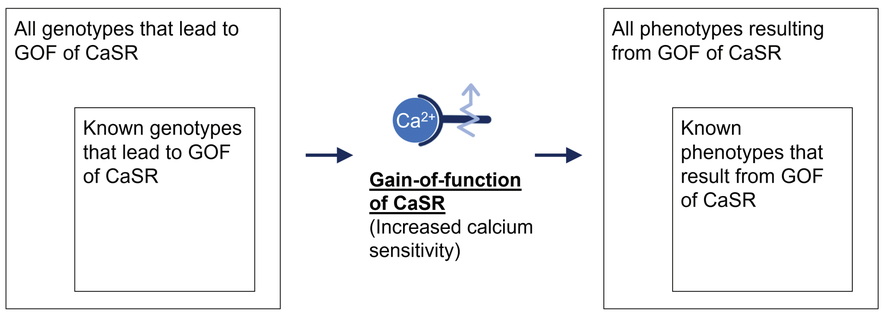
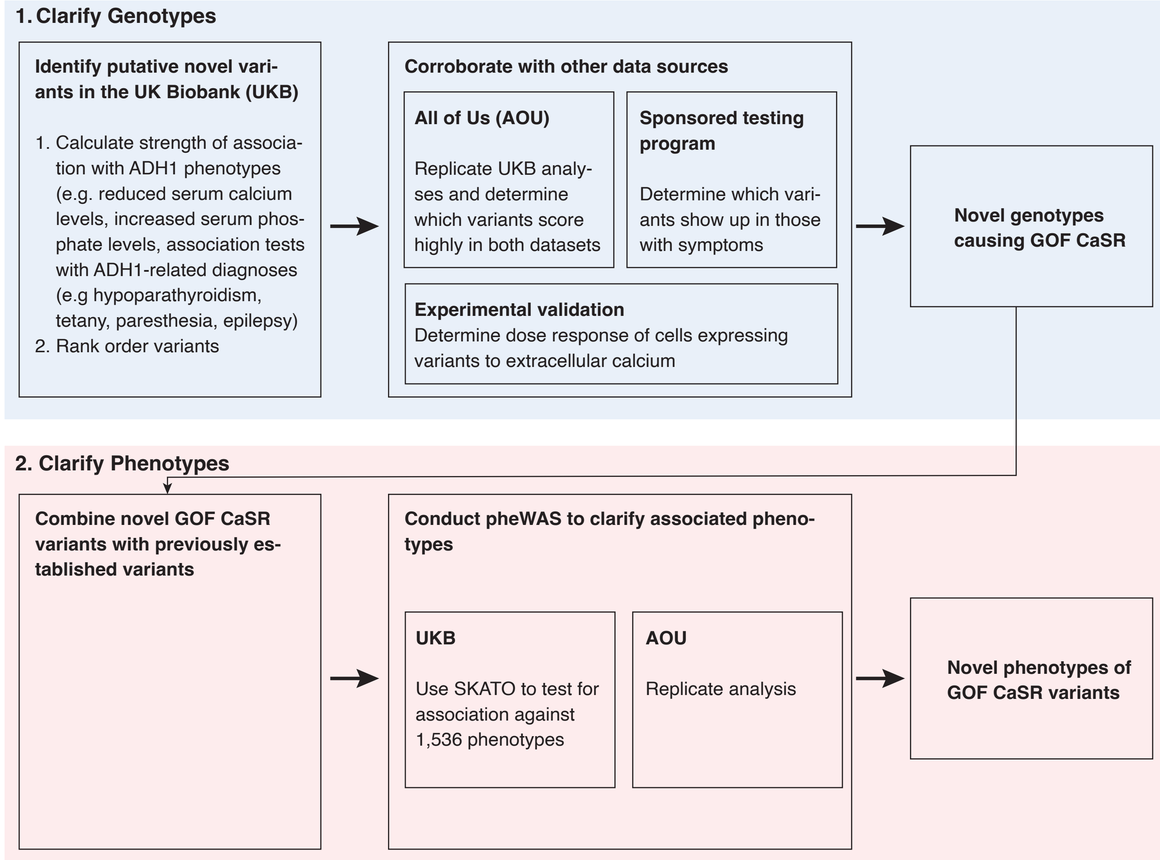


Figure 2. The goal of this study is to clarify both genotypes that cause increased CaSR activity and the phenotypes that result from increased CaSR activity.



Methods



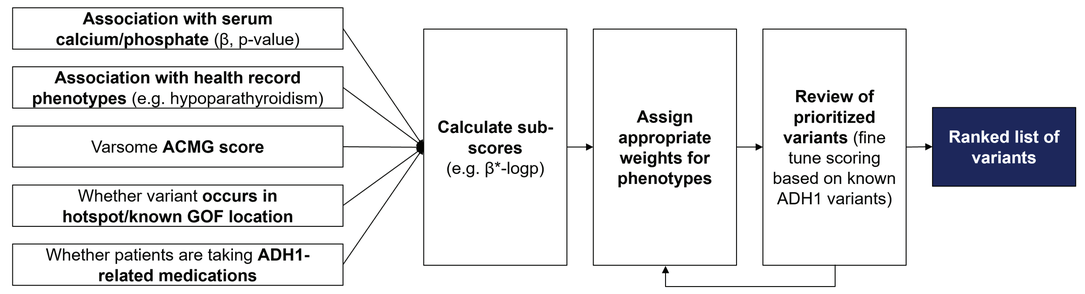
Confirmation of phenotypes of previously established ADH1 variants

- First, in the UK Biobank (UKB) we confirmed the association of 3 out of 4 previously reported variants found in non-Hispanic White probands with reduced serum calcium level, a hallmark phenotype of ADH1.
  - Val104Ile was also associated with reduced serum calcium level in 4 individuals of Indian ancestry ($p = 2x10^{-6}$).

Protein alteration	# hets	# homs	Calcium (mean, mmol/L)	Calcium (std, mmol/L)	Calcium (beta)	Calcium (p-value)	Phosphate (mean, mmol/L)	Phosphate (std, mmol/L)	Phosphate (beta)	Phosphate (p-value)	ICD codes
Thr151Met	1	0	1.96	—	-0.43	3.8E-06	1.57	—	0.37	1.6E-02	
Asn124Lys	2	0	2.20	0.01	-0.20	2.4E-03	1.25	0.02	0.04	6.9E-01	Hypoparathyroidism
Val104Ile	1	0	2.13	—	-0.24	8.8E-03	1.46	—	0.35	2.5E-02	Tetany
Thr888Met	2	0	2.29	0.07	-0.10	1.3E-01	1.33	0.04	0.14	2.1E-01	

Variants in the UK Biobank and All of Us were scored according to strength of association with ADH1 phenotypes

Figure 3. Variant scoring methodology.



- We scored 576 missense or stop-gain/frameshift CASR variants in the UKB within each ancestry based on regression modeling of ADH1 phenotypes, such as diagnosis code-based phenotypes (**Figure 3**).
- Following variant scoring, we selected the 46 variants with a score above a suggestive threshold of 1.5 for further examination. **Figure 4** shows the top 14 variants and their sub-scores.
- We replicated our score in AOU, in which there were 527 missense or stop-gain/frameshift variants. 237 of these were common across both biobanks. We found that, of the 28 shared variants with a score greater than 1.5 in All of Us, 10 variants had a score greater than 1.5 in both biobanks, 4 of these variants (Asn1074Asp, Asn345Asp, Gln1040Glu, and Gly1019Arg) have suggestive associations in both biobanks and a minor allele frequency consistent with previously established ADH1 variants.

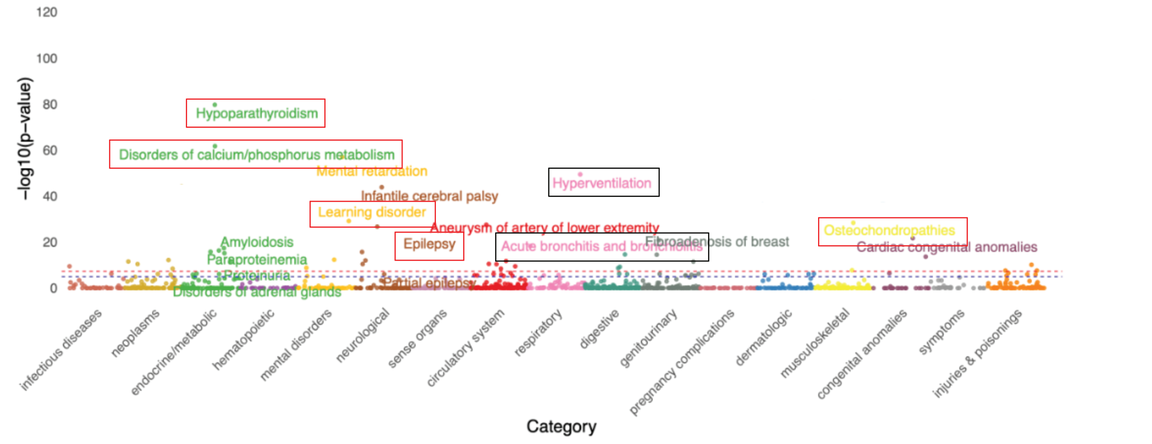
	Protein alteration	Allele count	Total score	Calcium	Phosphate	Phenotype	Medications	Location	Varsome
Known GOF	Leu159Val	1	14.1	0.0	-0.1	13.9	0.0	0.0	0.3
	Glu556Asp	3	8.6	1.5	0.0	6.4	0.0	0.5	0.2
	Thr151Met	1	8.4	7.0	0.7	0.0	0.0	0.5	0.3
	Asn124Lys	2	6.6	1.6	0.0	3.3	1.0	0.5	0.3
	His766Gln	3	4.1	1.5	0.0	1.5	1.0	0.0	0.1
	Glu481Ala	1	3.3	1.0	-0.1	1.8	0.0	0.5	0.1
	Gln944Ter	1	3.2	2.9	0.0	0.0	0.0	0.0	0.3
	Ile78LysfsTer4	1	3.2	0.0	0.0	2.9	0.0	0.0	0.3
	Met74Leu	4	3.1	-1.4	-0.1	3.7	0.7	0.0	0.2
	Val104Ile	1	2.8	1.5	0.6	0.0	0.0	0.5	0.2
	Gly778Cys	2	2.5	2.3	-0.1	0.0	0.0	0.0	0.3
	Asn541Ser	4	2.2	0.6	0.1	1.3	0.0	0.0	0.1
	Ser247Phe	12	2.1	0.0	0.0	1.9	0.1	0.0	0.1
	Cys6Ser	1	2.1	0.0	-0.1	2.0	0.0	0.0	0.1

A sponsored testing program revealed further novel putative ADH1 variants

- 169 patients participated in a sponsored testing program for suspected genetic hypoparthyroidism.
- We identified a pathogenic/likely pathogenic variant or variant of uncertain significance (VUS) of CASR in 21% (36/169) of participants.
- Of the 26 variants identified, 13 were previously not associated with ADH1, and 4 of identified variants (Asn1074Asp, Ala364Glu, Thr972Met, and Phe815del) also show suggestive signals in the UKB or All of Us.

We used both known and novel GOF variants to conduct a pheWAS to reveal novel phenotypes associated with GOF of CaSR

- 1,518 phecodes were tested for association with the 20 top scoring rare variants in the UKB using SKATO.
- Established associations were confirmed (e.g. hypoparathyroidism $p=10^{-79}$, disorders of calcium/phosphorus metabolism $p=10^{-61}$, epilepsy $p=10^{-26}$, and learning disorder $p= 10^{-29}$, osteochondropathies $p = 10^{-28}$).
- Further associations suggest pleitropy of CASR variants beyond previously established ADH1 phenotypes (e.g. hyperventilation $p=10^{-49}$ and bronchitis $p=10^{-18}$ may be related with CASR's role in the lung). These associations require further study.



Conclusion

- Our work demonstrates how large, publicly available genomic datasets can be used to perhaps improve diagnosis by better defining genotypes that cause ADH1 and the phenotypes that result.
- Next steps:
  - We have selected a subset of variants for *in vitro* testing of whether they show increased sensitivity to calcium.
  - Replication of pheWAS in AOU.
  - Replication of our analyses in the Mass General Brigham biobank.

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