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NDT Perspectives

Genetics of calcium homeostasis in humans: continuum between monogenic diseases and continuous phenotypes

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ABSTRACT

Extracellular calcium participates in several key physiological functions, such as control of blood coagulation, bone calcification or muscle contraction. Calcium homeostasis in humans is regulated in part by genetic factors, as illustrated by rare monogenic diseases characterized by hypo or hypercalcaemia. Both serum calcium and urinary calcium excretion are heritable continuous traits in humans. Serum calcium levels are tightly regulated by two main hormonal systems, i.e. parathyroid hormone and vitamin D, which are themselves also influenced by genetic factors. Recent technological advances in molecular biology allow for the screening of the human genome at an unprecedented level of detail and using hypothesis-free approaches, such as genome-wide association studies (GWAS). GWAS identified novel loci for calcium-related phenotypes (i.e. serum calcium and 25-OH vitamin D) that shed new light on the biology of calcium in humans. The substantial overlap (i.e. *CYP24A1, CASR, GATA3; CYP2R1*) between genes involved in rare monogenic diseases and genes located within loci identified in GWAS suggests a genetic and phenotypic continuum between monogenic diseases of calcium homeostasis and slight disturbances of calcium homeostasis in the general population. Future studies using whole-exome and whole-genome sequencing will further advance our understanding of the genetic architecture of calcium homeostasis in humans. These findings will likely provide new insight into the complex mechanisms involved in calcium homeostasis and hopefully lead to novel preventive and therapeutic approaches.

Keyword: calcium, monogenic, genome-wide association studies, genetics

INTRODUCTION: CONTROL OF CALCIUM HOMEOSTASIS

Extracellular calcium participates in several key physiological functions, such as control of blood coagulation, bone calcification or muscle contraction. Even if important calcium fluxes are exchanged between organs implicated in the import, storage and export in and out of the organism (the intestine, bone and kidneys), serum calcium concentration is kept stable, with variations that do not exceed 2–3% and that are mainly due to circadian rhythmicity [1]. However, even with a thorough control of its concentration, calcium reaches saturation levels in the plasma and only strong inhibitors, e.g. magnesium, fetuin A or albumin, can prevent precipitation and stabilize calciprotein particles [2]. Although calcium has crucial physiological roles, it can do harm if deposited inappropriately.

Regulation of extracellular calcium concentration and biomineralization depends upon a complex network of hormones and intricate feedback loops. Like sodium homeostasis, which is regulated by both, a short-term-acting and G-protein-coupled receptor (GPCR)-dependent system (angiotensin 2) and a long-term-acting and nuclear receptor-dependent hormone (aldosterone), calcium homeostasis is maintained mainly by two systems, the parathyroid hormone (PTH) and vitamin D.

PTH

Biologically active PTH is a peptide that interacts, with high affinity, with PTH receptor 1 (PTHR1), triggering downstream intracellular signalling. PTH is synthetized as a pre-prohormone

in the parathyroid glands and is secreted in a pulsatile manner, with infradian and circadian rhythms, in the blood stream as a 1–84 fragment (intact PTH). The half-life of biologically active PTH in the circulation is only a few minutes before it is degraded in a variety of smaller processed fragments with largely unknown functions [3, 4]. Recent data indicate that oxidation of PTH in certain clinical circumstances such as renal insufficiency could decrease the affinity of PTH for its receptor [5]. Parathyroid glands exert two major functions regarding calcium: sensing plasma calcium concentration—free-ionized calcium only—and adapting secretion of PTH. Once released in the bloodstream, PTH augments serum calcium levels directly by increasing calcium reabsorption by the kidney, calcium release by the bone and indirectly by increasing intestinal calcium uptake.

CALCIUM-SENSING RECEPTOR

In contrast to other ions for which it has been postulated that sensors exist but were never discovered, the identification of the calcium-sensing receptor (CASR) by Brown and Hebert was instrumental in deciphering a main passive regulator of serum calcium concentration [6]. First, by its expression in the parathyroid gland, CASR directly connects calcium sensing and control of PTH secretion and its downstream consequences. However, the effects of the CASR on calcium homeostasis extend beyond the parathyroid glands. Expressed in the intestine and the bone, CASR may regulate calcium fluxes in these organs even though its precise role is not fully established [7, 8]. In contrast, in the kidney, CASR has been shown to control serum calcium concentration independently of its effect on PTH secretion. Indeed, using double CASR–PTH knockout mice, Kantham *et al.* [9] showed that CASR defends against hypercalcaemia independently from PTH. Toka *et al.* [10] did provide additional evidence consistent with these results by studying CASR kidney-specific knockout mice. Finally, an elegant study by Loupy *et al.* [11] showed that CASR in the thick ascending limb regulates serum calcium concentration independently of PTH. Altogether, these studies displayed strong evidence that regulation of renal calcium excretion by CASR directly influences calcium homeostasis and identified CASR as a primary regulator of serum calcium concentration, independently of its effect on PTH secretion.

VITAMIN D

To fit with the high affinity in the ligand pocket of the nuclear receptor vitamin D receptor (VDR) and produce its effects, vitamin D needs to be activated by two hydroxylation steps, at position 25 and 1. It is not yet fully elucidated, which enzyme hydroxylates vitamin D at position 25 in the liver. One candidate is the mitochondrial cytochrome P450, subfamily 27, polypeptide 1 (CYP27A1), which is, however, mainly involved in cholesterol and bile acid metabolism and has high-capacity but only low-affinity 25-hydroxylase activity [12]. Since mice and humans with a *CYP27A1* mutation exhibit normal 25-OH vitamin D concentrations, this enzyme probably plays a minor role in the transformation of vitamin D [13]. Another proposed enzyme involved in the 25-hydroxylation of vitamin D in the liver is the low-capacity, high-affinity microsomal cytochrome P450, subfamily 2R, polypeptide 1 (CYP2R1) [14]. CYP2R1 plays a crucial role in the metabolism of vitamin D, since patients and mice with homozygous mutations in this gene display low-circulating 25-OHD levels and rickets [15]. Surprisingly, double knockout mice for CYP2R1 and CYP27A1 exhibit normal levels of serum 25(OH) vitamin D, and no rickets, suggesting that another 25-hydroxylase exists and needs to be identified, at least in the mouse [16]. The second and highly regulated hydroxylation is much better characterized. CYP27B1 (1-alpha hydroxylase) is a highly regulated enzyme from the CYP450 family. Calcium, phosphate, PTH, calcitonin and the fibroblast growth factor 23 (FGF23)-Klotho axis are the main regulatory factors of CYP27B1, whereas $1,25(OH)_2$ vitamin D inhibits, in a negative feedback loop on CYP27B1, its own production. Vitamin D homeostasis depends, however, not only on production, but also on degradation, which is also highly regulated. Cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1), hydroxylates $1,25(OH)_2$ vitamin D and targets it for further catabolism into calcitroic acid and excretion. CYP24A1 is regulated, at the transcriptional level, in an antagonistic manner compared with CYP27B1, by calcium, phosphate, PTH, FGF23, Klotho and is activated by high levels of circulating $1,25(OH)_2$ vitamin D, thus inducing its own degradation. Active $1,25(OH)_2$ vitamin D stimulates intestinal calcium absorption, increases bone mineralization, but also bone resorption and favours calcium reabsorption by the kidney.

The main mechanisms of calcium homeostasis described here have been further explored at the molecular level by geneticists. This review now emphasizes how studies on rare monogenic diseases in patients bearing disturbances of calcium homeostasis allowed precise dissection of the molecular pathways involved in these pathologies. Moreover, we will show that hypothesis-free genome-wide association studies (GWAS) conducted in large population-based samples are powerful in detecting new genetic determinants of serum calcium and in validating known actors involved in the control of serum calcium levels. This review does not cover non-genetic causes of hyper or hypocalcaemia.

GENETICS OF CALCIUM HOMEOSTASIS

Familial aggregation and heritability

Both serum calcium and urinary calcium excretion have been shown to be heritable continuous traits in humans [17]. In 1747 pairs from the UK Adult Twin Registry, heritability was estimated to be 33% (95% CI: 21–45%) for serum calcium and 52% (95% CI: 41–61%) for 24-h urinary calcium excretion [17]. The unimodal distributions of serum calcium (symmetric and close to normal) and 24-h urinary calcium excretion (positively skewed, close to log-normal) in the general

population suggest that numerous genetic factors (i.e. polygenic traits) play a role in their control. The existence of rare monogenic diseases with disturbances in calcium homeostasis further underscores the importance of genetic factors. Similar to other phenotypes, it is likely that a genetic and phenotypic continuum exists between rare monogenic disorders of calcium homeostasis and genetic factors responsible for the control of calcium homeostasis in the general population (Figure 1). This continuum can be described as follows: (i) several mutations in a single gene can lead to the same monogenic disease (e.g. *VDR, CASR* [18]); (ii) different mutations in a single gene may lead to several monogenic disorders (e.g. *CASR*); (iii) although the genetic effect size within a monogenic context is usually large, other genes may modify the penetrance of the main mutation (e.g. *CASR–PTH* [19]); (iv) genes involved in rare monogenic diseases may play a role in common complex diseases and phenotypes (e.g. *CASR, CYP24A1*); (iv) a polygenic disorder/phenotype is considered to result from the addition of many small genetic effects, although one cannot exclude a mixture of big and small effects.

Genome-wide association studies

GWAS have made an important contribution to our understanding of the genetic determinants of human traits [20]. GWAS allow a hypothesis-free exploration of the entire human genome and identify genomic regions robustly associated with phenotypes of interest. Current DNA chips usually include 500 000 to 1 million single nucleotide polymorphisms (SNPs) to be genotyped at an affordable cost. During the past decade >2000, robust associations have been published in GWAS [20].

What are the principles underlying GWAS?

Once participants have been genotyped, several steps are conducted until a clean data set is obtained. In a second stage, additional genetic variants are being inferred using the linkage disequilibrium structure (so-called 'haplotype blocks') of the human genome and publicly available HapMap and/or 1000 Genomes data (so-called 'imputed SNPs'). First generation GWAS focused on common alleles (i.e. with a minor allele frequency usually >5%). More recent DNA chips now also allow the measuring of rarer genetic variants. The fact that GWAS focused on common variants does not imply that some of the association signals from GWAS do not arise from multiple rare variants. This results from the fact that a GWAS signal is not restricted to the so-called top SNP (i.e. SNPs with the lowest association P-value in a given region), but also includes the entire region. The length of the captured region strongly depends on the local linkage disequilibrium structure (i.e. correlation structure).

Once a specific region of the genome has been identified as being robustly associated with a phenotype of interest, a much additional work is needed to unravel the causal variant(s). Whenever a single gene is included in the associated region, this task is much easier than when dozens of genes are present. Also, the SNPs most significantly associated with the phenotype of interest are unlikely to be the true underlying causal variant.

When compared with candidate gene studies published before the GWAS era, GWAS findings are more robust in that they have to be replicated before the results can be published. As most GWAS associations correspond to small effect sizes, very large sample sizes are usually needed to replicate findings. GWAS are, therefore, less likely to generate false-positive findings than earlier candidate gene studies that were often underpowered and hard to replicate. Of note, the size of the effect from a GWAS signal provides limited information on the importance of the corresponding gene in the phenotype under study. SNPs available in genome-wide DNA arrays only represent a small subset of all SNPs throughout the human

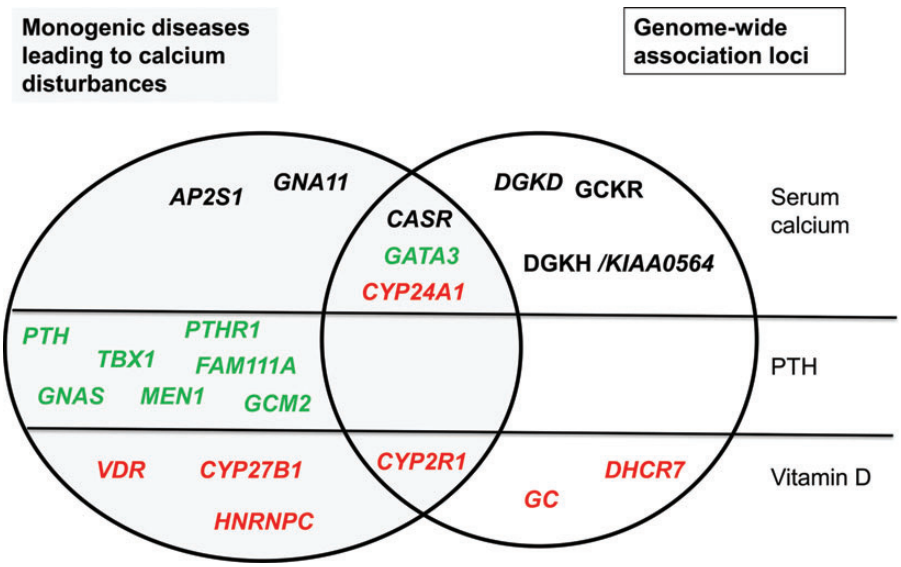

FIGURE 1: Genes involved in regulation of serum calcium concentration. Genes have been identified either by studies of monogenic diseases or by GWAS performed on serum calcium and on vitamin D. Genes represented in green are mainly involved in the PTH axis and those in red are mainly involved in the vitamin D axis. No GWAS has been conducted on PTH levels so far.

genome and genomic regions (and genes) are not equally well covered on these chips. A logical consequence of the need to replicate findings across studies (and hence across settings) is that GWAS results are typically robust to environmental conditions. The presence of a strong gene-by-environment interaction may therefore not be captured in GWAS, unless this specific interaction is adequately explored.

GWAS for calcium-related phenotypes

So far, only a few GWAS have focused on phenotypes related to calcium [21–23]. For serum total calcium, the largest study published so far comes from the CalciGen consortium and identified six genomic regions as being robustly associated with serum calcium in 39 400 individuals of European descent from 17 population-based cohorts. This study explored the genetic regions associated with total serum calcium in the general population under free living conditions. The six replicated SNPs for serum calcium were (i) rs1570669 near *CYP24A1*, (ii) rs10491003 upstream of *GATA3* (GATA-binding protein 3), (iii) rs7481584 in *CARS* (cysteinyl-tRNA synthetase), (iv) rs1550532 in *DGKD* (diacylglycerol kinases delta), (v) rs7336933 near *DGKH* (diacylglycerol kinases eta)/*KIAA0564* and (vi) rs780094 in *GCKR* [glucokinase (hexokinase 4) regulator] [23]. This study also confirmed the strong association of rs1801725 in the *CASR* locus with serum calcium that had been previously identified in two separate GWAS [21, 22]. The first three signals implicate regions involved in Mendelian disorders of calcium homeostasis. Two loci located on different chromosomes are near genes that encode two different diacylglycerol kinases, namely DGKH and DKGD. Although further studies are needed to confirm that these two genes are indeed responsible in these signals, this result suggests a role of diacylglycerol kinases in calcium homeostasis in humans.

Altogether, loci identified by the CalciGen consortium only explain a tiny fraction of the variance of serum calcium levels [23]. The top hit for serum calcium (rs1801725, a missense *CASR* variant), by far the largest effect of all genome-wide significant signals for serum calcium, only explains 1.26% of serum calcium variance [22], despite the fact the *CASR* plays a major role in calcium homeostasis. This sharply contrasts with the high heritability of serum calcium levels. The reasons for this 'missing heritability', which is shared by most complex phenotypes, are a hotly debated issue in the current literature [24]. It is likely that a larger GWAS meta-analysis would identify additional serum calcium loci. It may appear as a surprise that genes such as *TRPV5* (transient receptor potential cation channel, subfamily V, member 5), *TRPV6* (transient receptor potential cation channel, subfamily V, member 6), *ATP2B1* (ATPase, Ca^{++} transporting, plasma membrane 1), *PTH, PTHR*, *VDR, Klotho* and *SLC8A1* [solute carrier family 8 (sodium/calcium exchanger), member 1], known to play a key role in calcium transport in the intestine, bone and/or kidney or in calcium homeostasis, did not come up in the CalciGen GWAS meta-analysis. Such an absence of association does not at all imply that these genes are not important for calcium homeostasis in humans. We are not aware that any GWAS for urinary calcium excretion or fractional excretion of calcium have been published so far.

The first published GWAS for 25(OH)D was conducted in 4501 individuals of European descent and identified the rs2282679 near *GC* [group-specific component (vitamin D-binding protein], rs3829251 near *NADSYN1* (NAD synthetase 1)/*DHCR7* (7-dehydrocholesterol reductase) and rs2060793 near *CYP2R1* variants as being significantly associated with 25(OH)D levels [25]. In a later GWAS meta-analysis, three loci achieved genome-wide significance and replication for 25 (OH) vitamin D levels in 30 000 participants of European descent: (i) rs2282679 in *GC*; (ii) rs12785878 near *NADSYN1*/*DHCR7* and (ii) rs10741657 near *CYP2R1*, whereas the *CYP24A1* rs6013897 variant had genome-wide significance in the pooled sample [26]. These loci collectively explained between 1 and 4% of 25(OH) variance. *GC* is involved in vitamin D transport; *DHCR7* is involved in the synthesis, and *CYP2R1* in the hydroxylation, of cholesterol. Identification of *CYP2R1* by GWAS for 25(OH)D strengthens its implication as the main 25-hydroxylase for vitamin D. A GWAS conducted in 572 Caucasian children with asthma did not identify additional variants associated with 25(OH)D [27]. So far variants identified in GWAS only explain a small proportion (~5%) of the heritability of 25(OH)D estimated by twin- and family studies [28]. Additional studies are needed to further decipher genetic determinants of 25(OH) vitamin D levels in humans.

A single GWAS, in 229 Hispanic Americans, was conducted for 1,25(OH)2D and no genome-wide significant signal was identified, yet 8 SNPs from a set of 50 selected SNPs were replicated for 1,25(OH(2)D in a larger cohort: rs6680429, rs1348864, rs4559029, rs12667374, rs7781309, rs10505337, rs2486443 and rs2154175 [29]. Larger sample sizes are clearly needed to achieve sufficient power to detect and replicate signals across studies.

Rare monogenic diseases involved in serum calcium control

Genetic determinants of serum calcium levels are numerous and can be grouped into those affecting the CASR, PTH, vitamin D and other calcium regulatory axis. Since the pioneer work of Fuller Albright in the 1930s, large collections of rare conditions in which plasma calcium is disturbed either towards higher or lower levels have been identified, described, explored and categorized. With the emergence of molecular medicine, old unique entities have been subcategorized in a variety of conditions that can now be better explained and treated, e.g. familial hypocalciuric hypercalcaemia (FHH), previously thought to be a single condition which is now genetically heterogeneous, with at least three causative genes identified [*CASR, GNA11* (guanine nucleotide-binding protein (G protein), alpha 11), *AP2S1* (adaptor-related protein complex 2, sigma 1 subunit)]. If precise and complete clinical descriptions of patients are still mandatory, new powerful molecular tools have facilitated and accelerated the diagnosis and the discovery of genes causing orphan diseases. Some of the main rare diseases affecting calcium plasma levels are discussed hereafter (Table 1). Owing to space constraints, only a selection of conditions and publications is highlighted here and this review is neither systematic nor exhaustive.

Table 1. Monogenic diseases leading to serum calcium disturbances

Target	Gene	Mechanism	Disease	OMIM	Effect on plasma calcium
CASR	*CASR*	Loss of function, Heterozygous	FHH type 1	145980	Hypercalcaemia
		Loss of function, Homozygous	NSHPT	239200	Hypercalcaemia
		Activating mutation	ADH type 1, including Bartter-like syndrome	601198	Hypocalcaemia
	GNA11	Loss of function	FHH type 2	145981	Hypercalcaemia
		Activating mutation	ADH type 2	615361	Hypocalcaemia
	AP2S1	Loss of function	FHH type 3	600740	Hypercalcaemia
Parathyroid gland – PTH axis	*MEN1*	Loss of Function	MEN type 1 with hyperparathyroidism	131100	Hypercalcaemia
	PTH	Loss of function	Familial isolated hypoparathyroidism	146200	Hypocalcaemia
	GCM2	Loss of function	Familial isolated hypoparathyroidism	146200	Hypocalcaemia
	TBX1 and others (?)	Deletion chromosome 22	Di George, velocardiofacial, Catch22 syndrome	188400	Hypocalcaemia
	FAM111A	Loss of function	Hypoparathyroidism and bone dysplasia	615292	Hypocalcaemia
	GATA3	Loss of function	Hypoparathyroidism, renal dysplasia, sensorineural deafness	146255	Hypocalcaemia
	GNAS	Loss of function	Pseudo-hypoparathyroidism	103580	Hypocalcaemia
	PTHR1		Chondrodysplasia		Hypercalcaemia
			- Murk Jansen	156400	
			- Blomstrand	215045	
			- Eiken	600002	
Vitamin D	*CYP27B1*	Loss of function	Vitamin D-dependent rickets 1A	264700	Hypocalcaemia
	CYP2R1	Loss of function	Vitamin D-dependent rickets 1B	600081	Hypocalcaemia
	VDR	Loss of function	Vitamin D-dependent rickets 2A	277440	Hypocalcaemia
	HNRNPC	Loss of function	Vitamin D-dependent rickets 2B	164020	Hypocalcaemia
	CYP24A1	Loss of function	Infantile hypercalcaemia	143880	Hypercalcaemia

Secondary causes were omitted (e.g. hypomagnesaemia with secondary hypocalcaemia, hypophosphataemia…).

CONDITIONS AFFECTING THE PTH AXIS

PTH being produced mainly by the parathyroid glands in humans, every inherited condition affecting the presence of the glands will be translated into major calcium dysregulation. Indeed, hypoplasia or aplasia of the parathyroid glands, such as seen in Di George syndrome (due to hemizygous deletion of ∼1.5–3 Mb on the 22q11.2 locus and affecting in particular TBX1 [30]) or in familial isolated hypoparathyroidism (due to mutations in the genes coding for *GCM2* (glial cells missing, Drosophila, homologue 2) [31]) results in severe hypoparathyroidism and hypocalcaemia. A similar phenotype, with yet preserved glands, can be seen in patients carrying loss of function mutations in the PTH-coding gene. The reader is encouraged to consult the excellent review of Gregorieva and Thakker for further information on these conditions [32]. New entities for hypoparathyroidism have been elucidated. *FAM111A* (family with sequence similarity 111, member A) is a gene of unknown function recently identified as causative for Kenny–Caffey syndrome and for osteocraniostenosis, including hypocalcaemia, primary hypoparathyroidism and bone anomalies [33]. Its role in PTH and bone metabolism is still unclear. *GATA3* is a transcription factor involved in the development of the kidney (ureteric bud), and parathyroid gland. Mutations in *GATA3* lead to hypoparathyroidism, deafness and renal dysplasia, an autosomal-dominant trait in which hypocalcaemia and very low levels of PTH are associated with renal dysplasia and sensorineural deafness [34].

In contrast, MENIN is a tumour suppressor protein involved in chromatin remodelling and expressed in several glandular tissues. When mutated, the coding gene *MEN1* (multiple endocrine neoplasia syndrome type 1) leads to hypomorphic MENIN production that results in a complex MEN1. Primary hyperparathyroidism is one of the most frequently encountered complications of the syndrome, leading to classic hypercalcaemia/hypophosphataemia with inappropriately high PTH levels.

PTH mediates its effect via PTHR1 which is a GPCR. Mutations in the genes coding for PTHR1 and for G protein alpha S (encoded by *GNAS*) that transduces the signal intracellularly, leads to peripheral resistance to PTH effects. This entity is called pseudo-hypoparathyroidism and leads to severe bone anomalies (chondrodysplasia of several types, including Eiken syndrome [35]) and renal resistance to PTH action. As *GNAS* is highly methylated, the phenotype depends on the parental inheritance of the mutated gene. A detailed description of the different subtypes of pseudo-hypoparathyroidism is beyond the scope of this review but can be found in Levine *et al.* [36].

CASR-ASSOCIATED DISEASES

Human loss-of-function mutation in the *CASR* gene causes FHH when one allele is mutated and neonatal severe hyperparathyroidism (NSHPT) when both alleles are affected. FHH is characterized by decreased urinary calcium excretion and

PTH-dependent hypercalcaemia, while NSHPT leads to severe early hyperparathyroidism and hypercalcaemia [37]. In mice in which *CASR* has been deleted, the severe phenotype mimicking human NSHPT could be rescued by suppressing PTH, as elegantly showed in experiments during which *PTH* and *CASR* were simultaneously inactivated [38]. The fact that these mice harbour only slightly elevated, but highly variable plasma calcium suggests that the regulation of serum calcium concentration and PTH by the CASR occurs independently. The hypocalciuria observed in *CASR* KO mice was not rescued in the double *CASR–PTH* knockout animals, suggesting that CASR in the kidney is necessary for normal calcium excretion by preventing excessive calcium reabsorption independently of PTH [39]. In contrast, gain-of-function mutations in the *CASR* gene observed in humans lead to autosomal-dominant hypocalcaemia type 1 (ADH1) [40]. Similarly, mice carrying an activating mutation in *CASR* exhibit hypocalcaemia, hypoparathyroidism, and hyperphosphataemia and decreased renal calcium reabsorption [41].

Further sequencing of *CASR*-negative FHH patients provided new insights into our understanding of the regulatory pathways of the CASR and identified genetic heterogeneity in this syndrome. FHH type 1 is the classical form due to *CASR* mutations. FHH type 2 is phenotypically similar, but results from loss-of-function mutations in a gene coding for the protein G alpha 11 (*GNA11*) [42]. This indicates that this protein G is implicated in the downstream intracellular signalling of CASR. Interestingly, activating mutations in the same gene could lead to the mirror image, i.e. ADH type 2, as seen with CASR. Finally, FHH type 3 patients, who have a different and more severe clinical course, including increased serum PTH concentrations, hypophosphataemia and osteomalacia were found to carry mutations in the gene coding for adaptor protein 2 sigma subunit (*AP2S1*), a protein involved in clathrin-mediated endocytosis [43]. This suggests that regulation of CASR endocytosis is critical for its normal function. This finding perfectly illustrates the fact that studying rare monogenic diseases may uncover new pathogenic mechanisms and in this case, identify new regulatory pathways for CASR and calcium homeostasis.

CONDITIONS AFFECTING THE VITAMIN D AXIS

In family-based studies, heritability estimates ranged from 20 to 80% for circulating levels of vitamin 25-OH-D(3) and from 30 to 48% for 1,25-OH(2)-D(3) levels [44, 45], which justifies the search for genetic determinants. In a twin study in Vietnam, heritability of 25(OH) vitamin D was high and significant in winter (70%), but not in summer, which suggests a predominant role of environmental conditions in summer [46].

Active $1,25(OH)_2$ vitamin D level is the result of the fine regulation of production and degradation by cytochrome P450 enzymes. Genetic syndromes affecting production and degradation of 1,25 $(OH)_2$ vitamin D have been described and further subcategorized. Inactivating mutations in the *CYP27B1* gene, coding for 1-alpha hydroxylase, lead to vitamin D-resistant rickets type 1a [47]. More debated is the role of CYP2R1 in vitamin D-resistant rickets type 1b, as discussed previously. Active $1,25(OH)_2$ vitamin D produces its effect by its interaction with the VDR. Mutations in *VDR* provide severe peripheral resistance to the effect of $1,25(OH)_2$ vitamin D and an autosomal

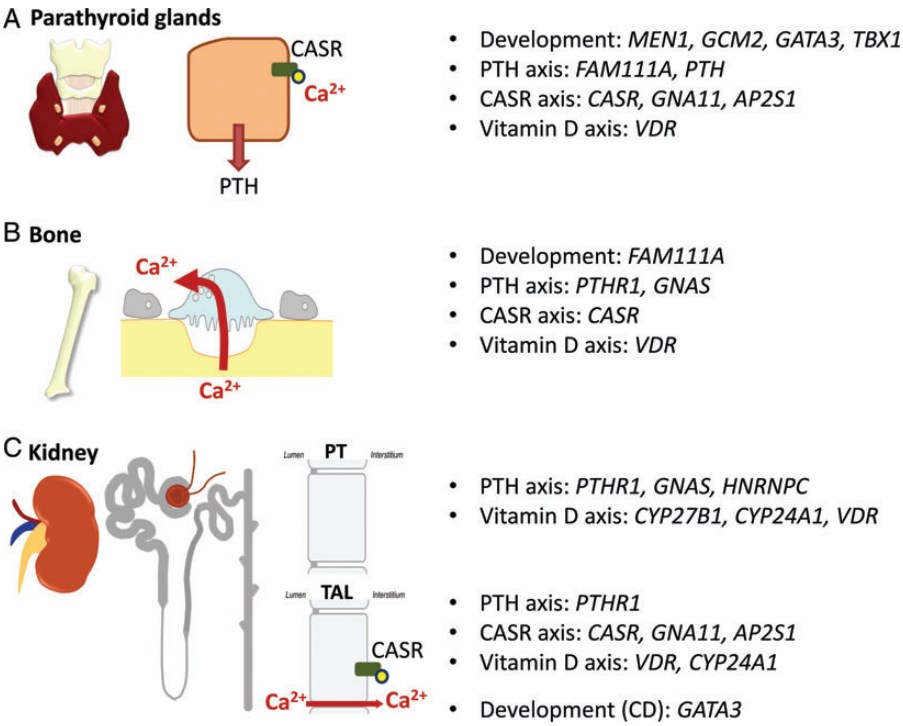

FIGURE 2: Tissue expression of genes involved in the regulation of serum calcium concentration. (**A**) Genes present in parathyroid glands. (**B**) Genes expressed in the bone. (**C**) Genes in the kidneys. PT, proximal tubule; TAL, thick ascending limb; CD, collecting duct.

recessive disorder called vitamin D-resistant rickets type 2a characterized by hypocalcaemia, hyperparathyroidism and rickets [48]. The numerous *VDR* mutations described in this latter disorder have been reviewed recently by Malloy and Feldmann [49]. Vitamin D-resistant rickets type 2b results from mutations in a protein associated with the transduction complex.

Catabolism of $1,25(OH)_2$ vitamin D to biologically inactive $1,24,25(OH)_3$ vitamin D and calcitroic acid is achieved by the P450 24-hydroxylase (CYP24A1), expressed in the proximal and distal tubule of the kidney, as well as in the collecting duct [50]. Human *CYP24A1* mutations have been reported in idiopathic infantile hypercalcaemia [51]. If this rare condition is due to clear loss of function mutations in the *CYP24A1* gene, more subtle genetic variant affecting only slightly the enzymatic activity may lead to an increased sensitivity to vitamin D.

CONCLUSION

Calcium homeostasis in humans is regulated in part by genetic factors, as illustrated by rare monogenic diseases. Serum calcium levels are tightly regulated by two main hormonal systems, i.e. PTH and vitamin D, which are themselves also influenced by genetic factors. Recent technological advances in molecular biology allow screening the human genome at an unprecedented level of detail and using hypothesis-free approaches, such as GWAS. GWAS identified novel loci for calcium-related phenotypes that shed new light on the biology of calcium in humans (Figure 2). There is likely a genetic and phenotypic continuum between rare monogenic diseases of calcium homeostasis and slight disturbances of calcium homeostasis in the general population. Future studies using whole-exome and whole-genome sequencing will further advance our understanding of the genetic architecture of calcium homeostasis in humans. These findings will likely provide new insight into the complex mechanisms involved in calcium homeostasis and hopefully lead to novel preventive and therapeutic approaches.

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CONFLICT OF INTEREST STATEMENT

This review has not been submitted nor published elsewhere.

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