REVIEW

Cardiovascular Therapeutics

Thiazide-Induced Hyponatraemia: Epidemiology and Clues to Pathogenesis

Mark Glover[1] & Jenny Clayton[2]

1 Division of Therapeutics and Molecular Medicine, University of Nottingham, Nottingham, United Kingdom
2 Department of Diabetes and Endocrinology, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Keywords
Hypertension; Molecular cardio-biology; Renal dysfunction; Vascular biology.

Correspondence
Dr. M. Glover, Division of Therapeutics and Molecular Medicine
Queen's Medical Centre
Nottingham, NG7 2UH, UK.
Tel.: +44 115 8231063;
Fax: +44 115 8231059;
E-mail: mark.glover@nottingham.ac.uk

doi: 10.1111/j.1755-5922.2011.00286.x

Both the authors are joint first authors.

SUMMARY

Thiazide diuretics are one of the most widely used and cost-effective classes of antihypertensive agents worldwide. Thiazides however have a significant side effect profile and are frequently insufficient to normalize blood pressure alone. Thiazide-induced hyponatraemia (TIH) is a major adverse effect, affecting up to one in seven patients receiving these drugs. TIH is more common in females, the elderly and those of low body weight and may cause symptoms such as confusion, falls and seizures. It is a common cause of hospital admission in the elderly.

Although TIH occurs at least as frequently as hypokalaemia, much less is understood about the mechanism by which this occurs. Thiazides lower blood pressure by reducing the reabsorption of sodium from the distal nephron by inhibition of the NaCl cotransporter. The molecular mechanism by which this occurs together with the little known role of thiazides in regulating water reabsorbtion from the collecting ducts is discussed and the relevance to TIH evaluated.

TIH is highly reproducible by thiazide rechallenge suggesting there may be a genetic predisposition. Both targeted resequencing of candidate genes and genome wide association techniques offer promising strategies by which such genetic contributions may be investigated. The rewards for uncovering the molecular mechanisms underlying TIH and the regulation of distal nephron sodium and water absorption are significant; not only could it inform the design of better tolerated, more efficacious thiazide-like antihypertensive agents but it may also facilitate the pharmacogenomic profiling of hypertensive patients to avoid thiazides in those likely to suffer adverse effects.

Introduction

Thiazide diuretics are one of the oldest, most widely prescribed and cost-effective classes of antihypertensive medication worldwide [1,2] with all cause mortality benefits equal to angiotensin-converting enzyme inhibitors and calcium channel antagonists [3]. Thiazides however are no panacea; they have a significant side effect profile. Some adverse effects are directly related to inhibition of the thiazide-sensitive NaCl cotransporter (NCC) in the distal convoluted tubule (DCT) such as hypokalemia, hypercalcaemia and possibly hypomagnesemia, but most appear to be idiosyncratic off-target effects including gout, photosensitization, impotence, and impaired glucose tolerance [4]. Hence, a better understanding of the molecular mechanisms that are responsible for the adverse effects of thiazide diuretics should aid the design of novel antihypertensive diuretics with fewer side effects and could even lead, through pharmacogenomic profiling, to the personalized prescribing of antihypertensive medications.

In this review, we address the epidemiology, clinical features, and pathophysiology of thiazide-induced hyponatraemia (TIH).

Epidemiology

TIH is less well characterized than hypokalaemia yet occurs at least as frequently, affecting up to 14% of patients prescribed thiazides in primary care [5]. Hyponatraemia is important in hospitalized patients too; it is the most frequent electrolyte abnormality observed amongst medical in-patients and affects up to a third of elderly patients taking thiazides on admission [6,7]. For patients hospitalized with severe hyponatraemia (sodium <125 mM) the role of thiazide diuretics is even more stark: thiazides are responsible for over a quarter of such presentations and are by far the most common cause of drug-induced severe hyponatraemia [8].

Large scale prospective trials of thiazides have not demonstrated a uniform reduction in serum sodium concentration [9,10] and yet some individuals are clearly affected, implying that there may be subgroups of the treated population who are susceptible [5,11]. Risk factors predisposing to TIH include:

- Increasing age [5,11–15] with those over 70 years carrying a threefold higher relative risk, possibly due to age related reduction in free water clearance [16].

- Low body weight [11].
- Hypokalaemia [11].
- Female gender [12,13,17], although this may be confounded by the over representation of female patients in thiazide-treated cohorts [11].

Clinical Presentation

The onset of TIH is typically within 2 weeks [18] however in some individuals plasma sodium can fall within hours of thiazide ingestion and severe hyponatraemia can develop in less than 2 days [12,13]. TIH may less commonly develop after months or years [11] when additional factors such as polypharmacy, comorbidities and decline in renal function may contribute. Although hyponatraemia is a continuous variable it may be usefully classified into mild (Na^+ 134–131 mmol/L), moderate (Na^+ 125–130 mmol/L), or severe ($Na^+ < 125$ mmol/L).

Symptoms depend on the speed of onset and degree of hyponatraemia. Patients with a sodium concentration above 125 mmol/L are often asymptomatic although may experience gait and attention deficits with increased risk of falls [19] and fractures [20]. Once sodium drops below 120 mmol/L most patients have significant symptoms [21] including nausea and vomiting, muscular weakness, headache causes, confusion and ataxia. Severe and rapidly evolving hyponatraemia causes cerebral oedema and can lead to seizures, coma, brain damage, respiratory arrest, brain stem herniation, and death [22,23].

The majority of patients with TIH are clinically euvolaemic with low or normal plasma urea, creatinine, urate, and potassium [24]. Urinary osmolality is high relative to plasma and often exceeds it [12,25,26]. Urinary sodium concentration is usually greater than 20 mmol/L [25,26].

Differential Diagnosis of TIH

The etiology of hyponatraemia is often multifactorial [8] and other causes or contributing factors need to be considered (Table 1). A careful assessment of volume status is important both for diagnosis and management.

Table 1 Causes of hyponatraemia. Syndrome of Inappropriate Anti-Diuretic Hormone (SIADH)

Impaired capacity of renal water excretion or sodium reabsorption		
Hypovolaemic	Euvolaemic	Hypervolaemic
Renal sodium loss	Thiazide diuretics	Congestive heart failure
Diuretics	Hypothyroidism	Cirrhosis
Hyperglycaemia	Glucocorticoid deficiency	Nephrotic syndrome
Adrenal insufficiency	SIADH	Renal failure
Salt-wasting nephropathies	Malignancy	Pregnancy
Cerebral salt wasting		
Extrarenal sodium loss		
Diarrhoea		
Vomiting		
Haemorrhage		
Excessive sweating		
Third spacing of fluids		
Burns		
Pancreatitis		
Bowel obstruction		
Excessive water intake		
Primary polydipsia		
Sodium-free irrigant solutions		
Inappropriate intravenous fluid replacement (e.g., 5% Dextrose solution)		

Management of TIH

There is little consensus on electrolyte monitoring in patients prescribed a thiazide or on the management of TIH. As the onset of TIH is usually within 1–2 weeks [18] it would seem prudent to advise measurement of sodium concentration after 2 weeks of treatment and annually thereafter. If hyponatraemia occurs management will depend on the sodium concentration, the presence of symptoms and indication for the thiazide.

Mild and Moderate Hyponatraemia (Sodium >125 mmol/L)

Thiazide cessation and replacement with an alternative antihypertensive agent is desirable. TIH usually corrects within 1–5 days of drug cessation but can take up to 2 weeks [27]. However in asymptomatic patients this needs to be balanced against the potential risk of poorly treated hypertension if other agents fail to control blood pressure adequately. In moderate hyponatraemia (Na < 130) fluid restriction is sometimes advocated [28].

Severe Hyponatraemia (Sodium <125 mmol/L)

These patients are likely to be symptomatic and are often managed in hospital. A delicate balance must be struck between the of risks of cerebral oedema and the risks of osmotic demyelination syndrome related to the rate of correction of the hyponatraemia [29]. In the absence of severe manifestations such as seizures or coma conservative treatment (thiazide cessation) is advised. The majority of patients will be euvolaemic but if hypovolaemic, normal saline should be infused. In the presence of severe manifestations (seizures or coma) management in a high dependency or critical care setting is required. Hypertonic (3%) saline may be given cautiously with frequent electrolyte monitoring (every 2–3 h); formulae can be used to guide the infusion rate [30] although these do not account for ongoing urinary or other losses and should not be regarded as a substitute to frequent reassessment of clinical and biochemical parameters. Relatively small increases in serum sodium concentration (3–7 mmol/L) substantially reduce cerebral oedema [31] and often terminate seizures [32,33]. An initial correction rate of 1–2 mmol/L/h is advised for the first few hours but thereafter, to reduce the risk of osmotic demyelination syndrome, the rate of correction should be slowed aiming for a daily correction rate of no more than 12 mmol/L [22].

Pathophysiology

Despite careful clinical and biochemical characterization and the identification of risk factors predisposing to TIH, little is understood of the molecular mechanisms by which hyponatraemia occurs. Unlike hypokalaemia, hyponatraemia is dose-independent [5,8] suggesting that this may be an idiosyncratic effect. Although some individuals develop marked hyponatraemia, the average plasma sodium concentration within the thiazide treated population is unchanged [5] implying that there may be defined subgroups of patients who are susceptible.

Moreover, recurrence of hyponatraemia is highly reproducible in patients with previous TIH by single dose thiazide rechallenge [34]. This suggests that there may be genetic predisposition to the development of TIH, given the controlled dietary conditions of the study population.

A priori, the molecular mechanisms underlying a possible genetic predisposition to TIH must either result in diminished sodium reabsorption, inappropriate water retention or a combination of the two and suggests that defective gene products may reside in the distal nephron.

In the following sections, we review the mechanisms of sodium and water reabsorption along the nephron and possible mechanisms underlying TIH.

Mechanisms of Sodium Reabsorption

The kidneys filter some 170 L of plasma daily containing around 23 moles of sodium, of which over 99.5% is reabsorbed by a series of sodium transporters along the nephron (Figure 1). The greatest fraction of filtered sodium (60–70%) is reabsorbed in the proximal convoluted tubule (PCT) by the Na^+/H^+ exchanger NHE3 [35].

A further 20–30% is reabsorbed in the Thick Ascending Limb of the Loop of Henle (TAL) by the Na-K-2Cl transporter NKCC2, the target of Loop diuretics such as furosemide [36]. Potassium is recycled across the apical membrane, in a rate limiting manner, by the renal outer medullary potassium channel, ROMK, whereas Cl^- exits the cell via the basolateral membrane through chloride channels and transporters including CLCNKB. Mutations within any of these genes (NKCC2, ROMK, and CLCNKB) leads to Bartter syndrome: type I, II, and III, respectively [37–40]. Bartter syndrome is a salt wasting condition, mimicking treatment with loop diuretics and is characterized by hypokalemia, metabolic alkalosis, hypercalciuria and a tendency to hypotension. More recently, type IV and V Bartter syndromes have been described caused by mutation of Barttin (an essential cofactor for CLCNKB) and the basolateral calcium sensing receptor (CaSR) (by leading to ROMK inhibition), respectively [41,42].

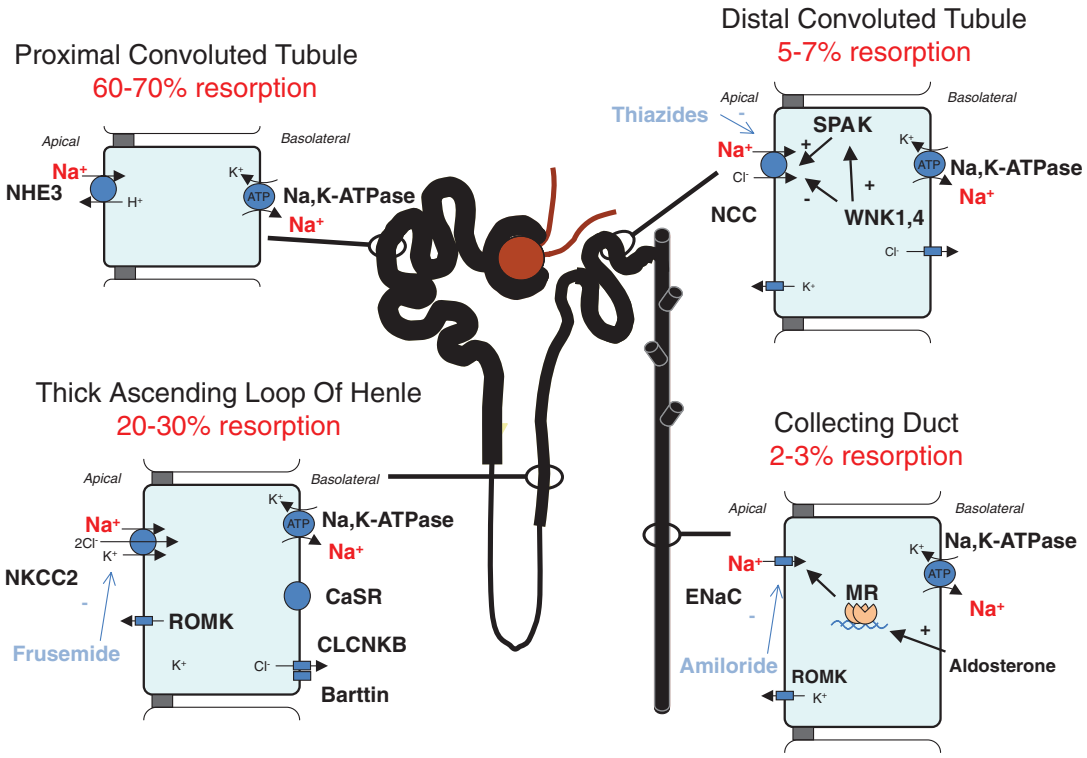

Figure 1 Schematic representation of the nephron showing principal sites of sodium reabsorption. In the proximal convoluted tubule via NHE3, Na^+/H^+ exchanger isoform 3; in the thick ascending Loop of Henle via the Frusemide-sensitive NKCC2, $Na^+/K^+/2Cl^-$ cotransporter; in the distal convoluted tubule by the thiazide-sensitive NCC, Na^+/Cl^- cotransporter and in the collecting duct via the amiloride-sensitive epithelial sodium channel (ENaC). ROMK (renal outer medulla potassium channel), CLCNKB (chloride channel kidney B), CaSR (Calcium Sensing Receptor), SPAK (STE-20/SPS1 proline/alanine-rich kinase), WNKs 1/4 (with no lysine kinases 1/4) and MR (mineralocorticoid receptor) are also shown.

Of the remaining 10% of sodium reaching the distal nephron, 5–7% is reabsorbed by the thiazide-sensitive Na-Cl cotransporter, NCC in the DCT. The DCT is the site of two other monogenic diseases. First, loss of-function mutations within NCC lead to Gitelman syndrome [43], which resembles therapy with thiazide diuretics. Second, Gordon syndrome [44] (pseudohypoaldosteronism type 2 or PHAII), the clinical mirror of Gitelman syndrome, which results from increased NCC membrane expression; it is characterized by hypertension and hyperkalemic acidosis and caused by mutations within one of two members of a novel family of regulatory kinases called the "With No Lysine (K)" kinases (WNK1 and WNK4) [45,46].

A final 2–3% of filtered sodium is reabsorbed in the connecting tubule (CNT) and the collecting duct (CD) by the amiloride-sensitive epithelial sodium channel (ENaC). Gain of function mutations in ENaC cause Liddle syndrome, a condition of hypokalaemic alkalosis and low renin hypertension [47]. Conversely loss of function ENaC mutation leads to an autosomal recessive syndrome of salt wasting termed pseudohypoaldosteronism type 1 [48].

ENaC regulation by aldosterone is reflected by syndromes of hyper- and hypo-tension caused by gain- and loss-of function mutations in the mineralocorticoid receptor: the syndrome of activating mineralocorticoid receptor mutation [49] and a mild form of autosomal dominant pseudohypoaldosteronism type 1, respectively [50].

Mechanisms of water reabsorption

Osmotically driven water reabsorption through renal tubules and the vasa recta is orchestrated by a family of water-permeable transporters, the aquaporins (AQPs). First characterized by Agre in 1992 [51] there are now 11 identified isoforms of this transporter, each with subtly different properties and expression patterns. Some are only permeable to water (AQPs 0, 1, 2, 4, 5, and 6) whilst others are also permeable to ammonia (AQPs 3, 7, 8, 9, and possibly 10) or urea (AQP 7, 9, and possibly 3) [52].

Around 90% of glomerular filtrate is reabsorbed in the PCT and descending Loop of Henle via AQP1, which is expressed in apical and basolateral membranes of the proximal nephron and vasa recta [53]. Whilst the proximal tubule has constitutively high water permeability, the ascending LoH is relatively impermeable to water, which leaves the distal nephron, in particular the principal cells of the CD, to regulate solute-free water excretion.

Water reabsorption in the CD is under the control of antidiuretic hormone (ADH/vasopressin). AQP2 is the principal ADH-regulated aquaporin and is expressed in the apical membrane, subapical vesicles and basolateral membrane of principal cells [54].

ADH binds to and activates V2 receptors on the basolateral membrance of principal cells, causing a rise in intracellular cAMP by activation of the GTP-binding subunit Gαs (Figure 2). This activates protein kinase A (PKA) which is then recruited to subapical membrane vesicles containing AQP2 by PKA-anchoring proteins [55]. PKA then phosphorylates AQP2 at Serine 256 causing vesicular fusion and thus AQP2 expression in the apical membrane. ADH also affects long term AQP2 regulation by changes in protein expression [56].

The importance of the ADH-AQP2 system to human water regulation is underlined by nephrogenic diabetes insipidus resulting from mutation in either the V2 receptor (X-linked) or AQP2 (in which separate mutations result in either autosomal dominant or recessive forms of the disease) [57].

It may at first seem paradoxical that thiazides are a more frequent cause of TIH than loop diuretics given that NCC reabsorbs much less filtered sodium than NKCC1/2. However whilst loop diuretics cause a reduction in medullary concentrating gradient (and thus diminished water reabsorption), thiazides do not, leaving water reabsorption relatively unaffected and thus inducting hyponatraemia more readily.

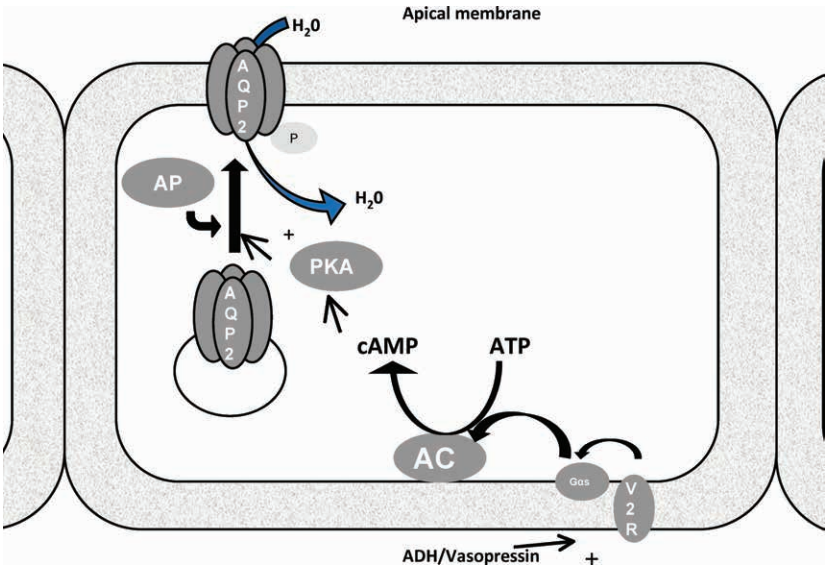

Figure 2 Schematic representation of a principal cell in the collecting duct showing water reabsorption by the vasopressin-aquaporin system. ADH (antidiuretic hormone also known as vasopressin), V2R (vasopressin 2 receptor), Gαs (G-protein alpha subunit), AC (adenylate cyclase), ATP (adenosine triphosphate), cAMP (cyclic adenosine monophosphate), PKA (protein kinase A), AP (PKA anchoring proteins), and AQP2/AQP2P (aquaporin subunit 2 in nonphosphorylated and phosphorylated forms, respectively) are shown.

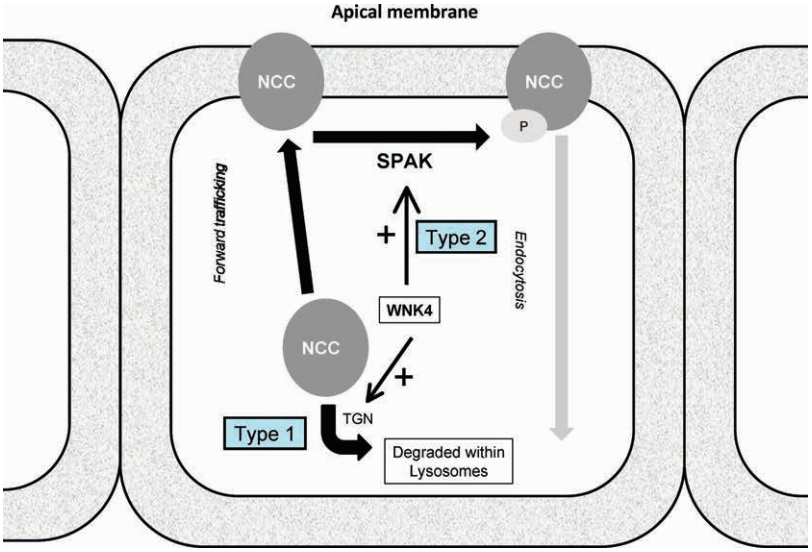

Figure 3 Schematic representation of the two principal mechanisms of regulation of the thiazide-sensitive Na^+/Cl- cotransporter, NCC. *Type 1* regulation affects forward trafficking of NCC to the apical membrane. At least for wild type WNK4 this appears to be by diversion of NCC through the trans-golgi network (TGN) for degradation in lysosomes. *Type 2* regulation of NCC determines intrinsic transporter kinetics by phosphorylation of key N-terminal serine/threonine residues such as T58. This phosphorylation is mediated by STE-20/SPS1 proline/alanine-rich kinase (SPAK) which in turn is activated by WNK4.

Possible Molecular Mechanisms Underlying TIH

Disordered NCC Regulation?

Much has been learnt regarding the regulation of the thiazide-sensitive Na-Cl cotransporter, NCC, from the study of monogenic syndromes of loss of function (Gitelman) or gain of function (Gordon) mutations and these reflect the molecular mechanisms of NCC regulation detailed *in vitro* (Figure 3).

Type 1 Gitelman mutations encode transporters which fail to be trafficked to the apical membrane and are retarded in the endoplasmic reticulum (ER) or Golgi apparatus [58], resulting in a reduced maximum transporting capacity (Bmax). Conversely, *Type 2* NCC mutations encode transporters which are trafficked to the membrane but which display reduced transporter activity (Vmax) [58].

Type 1 NCC regulation (trafficking regulation) is mediated by WNK kinases (specifically WNKs 1, 3, and 4). In the case of (wild type) WNK4 this involves diversion of NCC for degradation in lysosomes by the AP3 signalling pathway [59].

Type 2 NCC regulation (activity regulation) is determined by the phosphorylation of N-terminal Ser/The residues [60,61]. Human NCC T60 (homologous to mouse T58) appears most important, reflected by T60M mutation causing Gitelman syndrome in some pedigrees [62]. STE20-related Proline Alanine rich Kinase (SPAK), phylogenetically related to the WNK kinases, has recently been recognized as the effector of *type 2* regulation and a potential antihypertensive drug target [58]. Intronic, gain of function SPAK mutations, are associated with human hypertension [63], whereas kinase-inactivating, loss of function SPAK mutation causes a salt wasting hypotensive phenotype akin to Gitelman syndrome in knock-in mice [64].

It is possible that NCC or WNK mutation could predispose to TIH for example by producing thiazide hypersensitivity and augmenting thiazide-induced distal nephron sodium loss. Unpublished preliminary work within our group used Sanger sequencing using genomic DNA from a small number of affected patients ($Na^+ < 125$ mM) and matched controls. Specifically sequencing of all 26 NCC exons and WNK4 exon 7 (encoding the acidic motif in which most PHA2 mutations are clustered) was undertaken [65]. Although rare as well as common polymorphisms within NCC and WNK4 were identified this failed to reach statistical significance. The most promising SNP (T465C) was located within NCC exon 11 with an odds ratio of 2.1, $P = 0.03$.

However, NCC regulation has emerged as significantly more complex than first appreciated and is orchestrated by a scaffold of interacting proteins [4] including several new NCC regulators such as apoptosis-associated tyrosine kinase (AATYK) [66], protein phosphatase 4 (PP4) [67], ATP-sensitive inward rectifier potassium channel 10 (KCNJ10) [68], γ-adducin [69] and the angiotensin 1 receptor (AT1R) [70] whose genes would now also be candidates for investigation.

Disordered aquaporin regulation?

It is possible that TIH patients may tend towards excessive water consumption or reabsorption from the distal nephron; although biochemical parameters closely resemble SIADH there are no reproducible differences in ADH secretion [34]. Patients who develop TIH display reduced free water clearance, impaired maximum urine dilution [16] and acute weight gain on single dose thiazide rechallenge [34].

Microperfusion studies suggest that thiazides induce acute upregulation of AQP2 when applied to the apical but not basolateral membrane of CD cells, increasing their water permeability [71,72]. Thiazide-induced water permeability is inhibited by the functional ADH antagonist PGE2 or inhibitors of PKA (presumably by countering the ADH mediated rise in intracellular cAMP) and augmented by indomethacin [71,72]. This correlates well with clinical observations of an indomethacin-induced reduction

in free water clearance of healthy controls as well as the potentiation of thiazide-induced serum hypo-osmolarity by indomethacin polypharmacy [16].

The possibility that non-NCC effects of thiazides account for TIH may be argued from the absence of clinically significant hyponatraemia in Gitelman syndrome [73]. Indeed the literature on severe hyponatraemia in Gitelman syndrome consists of a solitary patient after prolonged polydypsia [74].

The Future for TIH Research

That particular individuals within the thiazide-treated population develop hyponatraemia which is highly reproducible on single-dose thiazide rechallenge [34] makes genetic analysis of such individuals a promising strategy to uncover the molecular mechanisms underlying this disorder.

There are two principal strategies, which could be used to investigate the genetic basis of TIH; targeted resequencing of candidate genes and Genome Wide Association Study (GWAS).

Targeted resequencing using high throughput next generation techniques with deep coverage would permit investigation of hitherto prohibitive numbers of patients and candidate genes given the rapidly reducing cost of this technology. A limited number of candidate genes can be identified; for example, NCC and SPAK, which mediate thiazide-sensitive sodium reabsorption from the DCT and AQP2 and the V2 (vasopressin/ADH) receptor, which regulate water absorption in the CD.

This strategy has the potential to identify rare variants in these genes. Rare genetic variants are predicted to be enriched for functional alleles and to exhibit stronger effect sizes than common variants as a consequence of the purifying selection pressure they are subject to. That rare polymorphisms contribute to human phenotypes is well established as illustrated by the effect that such polymorphisms in renal sodium transporters, including NCC, have on human blood pressure [75]. Next generation resequencing, by identifying rare polymorphisms, is already starting to show results in the field of lipid regulation [76].

There are however several disadvantages to a candidate gene approach, albeit with the aid of higher throughput next generation sequencing techniques. First, for all the recent advances in understanding NCC and aquaporin regulation there is much that remains unknown. Thus even an expanded and potentially lengthy list of candidate genes containing all known regulators of NCC and aquaporins may not contain the causative variant. Moreover, the critical polymorphism may be in non-NCC/AQP mediated sodium and water pathways such as reduced downstream sodium absorptive capacity in the CDs.

Secondly a list of candidate genes should also include those which mediate thiazide pharmacokinetic pathways, many of which are poorly understood. Thiazide pharmacokinetics vary significantly between agent; bioavailability varies between 60% (hydrochlorothiazide) and 90% (bendroflumethiazide), most are at least 95% bound to plasma proteins and, being sulphonamide derivatives, are excreted into the urine via the organic acid transporter, SLC22A6 (OAT1) in the PCT [77]. The degree to which thiazides are excreted unchanged in the urine also varies widely from 100% for hydrochlorothiazide to as little as 30% for bendroflumethiazide. The remaining 70% of bendroflumethiazide undergoes clearance by a poorly characterized metabolic pathway [78].

Thirdly, many of the candidate genes are large, e.g., WNK1 and polymorphisms producing phenotypic variance such as blood pressure are intronic (e.g., as seen with SPAK [63] and WNK1 [79]) such that sequencing of all candidate genes, both introns and exons, may be inefficient.

It may be that whole genome techniques such as whole exome sequencing or Genome Wide Association Study (GWAS) may be more successful. Whilst whole exome sequencing would address coding regions only GWAS could potentially uncover intronic variants. GWAS has enjoyed considerable success in identifying pharmacogenomic polymorphisms responsible for a variety of adverse drug phenomenon; examples include simvastatin induced myopathy due to polymorphism of the hepatic anion-transporter SLO1B1(OATP1B1) [80] and HLA haplotype in association with lumaricoxib induced hepatitis [81], amoxicillin-clavulanate induced cholestasis [82,83], carbamazepine induced Stevens-Johnson syndrome [84], abacavir hypersensitivity [85] and ximelagatran induced hepatitis [86].

Whichever technique is followed, uncovering the mechanisms underlying TIH remains a pressing issue. Not only would an understanding of the molecular details of the mechanisms involved aid the design of new antihypertensive thiazide-like drugs but it would also bring Medicine one step closer to an era of individualized prescribing based on pharmacogenomic profiling [87].

Acknowledgments

We are grateful to Professor I.P. Hall and Dr A.M. Freyer for their help and guidance in the preparation of this manuscript.

Conflict of Interests

The authors have no conflict of interest.

References

1. Williams B, Poulter NR, Brown MJ, et al. Guidelines for management of hypertension: Report of the fourth working party of the British Hypertension Society, 2004 – BHS IV. *J Hum Hypertens* 2004;**18**:139–185.
2. National Institute of Clinical Excellence (NICE). CG34 Hypertension: Full guideline (new recommendations and the evidence they are based on). Available at: http://guidance.nice.org.uk/CG34/Guidance/pdf/English. 2006 [Accessed 5 May 2011].
3. The ALLHAT officers and coordinators for the ALLHAT collaborative research group. Major outcomes in high-risk hypertensive patients randomized to angiotensin converting enzyme inhibitor or calcium channel blocker vs diuretic. *JAMA* 2002;**288**:2981–2997.
4. Glover M, Zuber Am, O'Shaughnessy KM. Hypertension, dietary salt intake, and the role of the thiazide-sensitive sodium chloride transporter NCCT. *Cardiovasc Ther* 2010;**1**:68–76.
5. Clayton JA, Rodgers S, Blackey J, Avery A, Hall IP. Thiazide diuretic prescription and electrolyte abnormalities in primary care. *Br J Clin Pharmacol* 2005;**61**:87–95.
6. Roe PF. Hyponatraemia and diuretics. *Lancet* 1975;**1**:1146–1147.
7. Byatt CM, Millard PH, Levin GE. Diuretics and electrolyte disturbances in 1000 consecutive

geriatric admissions. *R J Soc Med* 1990;**83**:704–708.
8. Clayton JA, Le Jeune IR, Hall IP. Severe hyponatraemia in medical in-patients: Aetiology, assessment and outcome. *Q J Med* 2006;**99**:505–511.
9. Ashfar N, Locksley R, Arieff AI. Neurological manifestations and morbidity of hyponatraemia: Correlation with brain water and electrolytes. *Medicine (Baltimore)* 1976;**55**:121–129.
10. Leonetti G, Rapelli A, Salvetti A, Scapellato L. Long-term effects of indapamide: Final results of a two year Italian multicentre study in systemic hypertension. *Am J Cardiol* 1990;**65**:67H–71H.
11. Elliott WJ, Weber RR, Murphy MB. A double-blind, randomized, placebo-controlled comparison of the metabolic effects of hydrochlorothiazide and indapamide. *J Clin Pharmacol* 1991;**31**:751–757.
12. Chow KM, Szeto CC, Wong TY, Leung CB, Li PK. Risk factors for thiazide-induced hyponatraemia. *Q J Med* 2003;**96**:911–917.
13. Ashraf N, Locksley R, Arieff AI. Thiazide-induced hyponatraemia associated with death or neurologic damage in outpatients. *Am J Med* 1981;**70**:1163–1168.
14. Sharabi Y, Illan R, Kamari T, Cohen H, Nadler M, Messerli FH, Grossman E. Diuretic induced hyponatraemia in elderly hypertensive women. *J Hum Hypertens* 2002;**16**:631–635.
15. Abramow M, Cogan E. Clinical aspects and pathophysiology of diuretic-induced hyponatraemia. *Adv Nephrol Necker Hosp* 1984;**13**:1–28.
16. Fidler HM, Goldman J, Bielawska CA, Rai GS, Hoffrband BI. A study of plasma sodium levels in elderly people taking amiloride or triamterine in combination with hydrochlorothiazide. *Postgrad Med J* 1993;**69**:797–799.
17. Clark BA, Shannon RP, Rosa RM, Epstein FH. Increased susceptibility to thiazide induced hyponatraemia in the elderly. *J Am Soc Nephrol* 1994;**5**:1106–1111.
18. Ashouri OS. Severe diuretic-induced hyponatraemia in the elderly. *Arch Int Med* 1986;**146**:1355–1357.
19. Sonnenblick M, Friedlander Y, Rosin AJ. Diuretic-induced severe hyponatraemia. Review and analysis of 129 reported patients. *Chest* 1993;**103**:601–606.
20. Renneboog B, Musch W, Vandemergel X, Manto MU, Decaux G. Mild chronic hyponatraemia is associated with falls, unsteadiness and attention deficits. *Am J Med* 2006;**119**:71.e1–8.
21. Kengne FG, Andres C, Sattar L, Melot C, Decaux G. Mild hyponatraemia and risk of fracture in the ambulatory elderly. *Q J Med* 2008;**101**:583–588.
22. Adrogue HJ, Madias NE. Hyponatraemia. *N Eng J Med* 2000;**342**:1581–1589.
23. Kumar S, Berl T. Sodium. *Lancet* 1998;**352**:220–228.
24. Spital A. Diuretic-induced hyponatraemia. *Am J Neprol* 1999;**19**:447–452.
25. Fuisz RE, Lauler DP, Cohen P. Diuretic-induced hyponatraemia and sustained antidiuresis. *Am J Med* 1962;**33**:783–791.
26. Fichman MP, Vorherr H, Kleeman CR, Telfer N. Diuretic-induced hyponatraemia. *Ann Int Med* 1971;**75**:853–863.
27. Hamburger S, Koprivica B, Ellerbeck E, Covinsky JO. Thiazide-induced syndrome of inappropriate secretion of antidiuretic hormone. *JAMA* 1981;**246**:1235–1236.
28. Mann SJ. The silent epidemic of thiazide-induced hyponatraemia. *J Clin Hypertens* 2008;**10**:477–484.
29. Berl T. Treating hyponatraemia. damed if we do and damned if we don't. *Kidney Int* 1990;**37**:1006–1018.
30. Adrogue HJ, Madias NE. Aiding fluid prescription for the dysnatremias. *Internsive Care Med* 1997;**23**:309–316.
31. Sterns RH. The treatment of hyponatraemia: First, do no harm. *Am J Med* 1990;**88**:557–560.
32. Worthley LI, Thomas PD. Treatment of hyponatraemia seizures with intravenous 29.2% saline. *BMJ (Clin Res Ed)* 1986;**292**:168–170.
33. Sarnaik AP, Meert K, Hackbarth R, Fleischmann L. Management of hyponatraemia seizures in children with hypertonic saline: A safe and effective strategy. *Crit Care Med* 1991 ;**19**:758–762.
34. Friedman E, Shadel M, Halkin H, and Farfel Z.Thiazide-induced hyponatremia. Reproducibility by single dose rechallenge and an analysis of pathogenesis. *Ann Int Med* 1989;**110**:24–30.
35. Bobulescu IA, Moe W. Luminal Na(+)/H(+) exchange in the proximal tubule. *Pflugers arch* 2009;**458**:5–21.
36. Bachmann S, Bostanjoglo M, Schmitt R, Ellison DH. Sodium-transport-related proteins in the mamalian distal nephron— Distribution, ontogeny and functional aspects. *Anat Embryol (Berl)* 1999;**200**:447–468.
37. Simon BD, Karet FE, Rodriguez-Soriano J, et al. Genetic heterogeneity of Bartter's syndrome revealed by mutation in the K^+ channel, ROMK. *Nat Genet* 1996;**14**:152–156.
38. Simon BD, Karet FE, Hamdan JM, DiPietro A, Sanjad SA, Lifton RP. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-Cl cotransporter NKCC2. *Nat Genet* 1996;**13**:183–188.
39. Simon DB, Nelson-Williams C, Bia MJ, et al. Gitelman's varient of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl transporter. *Nat Genet* 1996;**12**:24–30.
40. Simon BD, Bindra RS, Mansfield TA, et al. Mutation in the basolateral Chloride channel CLCNKB, cause Bartter syndrome type III. *Nat Genet* 1997;**17**:171–178.
41. Estevez R, Boettger T, Stein V, Birkenhäger R, Otto E, Hildebrandt F, and Jentsch TJ. Barttin is a Cl channel beta-subunit crucial for renal Cl reabsorption and inner K secretion. *Nature* 2001;**414**:558–561.
42. Vargas-Poussou R, Huang C, Hulin P, et al. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol* 2002;**13**:2259–2266.
43. Gitelman HJ, Graham JB, Welt LG. A new familial disorder characterised by hypokalaemia and hypomagnesaemia. *Trans Assoc Am Physicians* 1966;**79**:221–235.
44. Gordon R, Geddes R, Pawsey C, O'Halloran W. Hypertension and severe hyperkalaemia associated with suppression of renin and aldosterone and completely reversed by dietary sodium restriction. *Aust Ann Med* 1970;**4**:287–294.
45. Disse-Nicodeme S, Achard J, Desitter I, Hout A, Fournier A, Corvol P, Jeunemaitre X. A new locus on chromosome 12p 13.3 for Pseudohypoaldosteronism Type II, an autosomal dominant form of hypertension. *Am J Hum Genet* 2000;**67**:302–310.
46. Mansfield TA, Simon DB, Farfel Z, et al. Multilocus linkage of famelial hyperkalaemia and hypertension, famelial yperaldosteronism type II to chromosomes 1q31–42 and 17p11–21. *Nat Genets* 1997;**16**:202–205.
47. Liddle GW, Bledsoe T, Coppage WS Jr. A familial renal disorder simulating primary aldosteronism but with negligible aldosterone secretion. *Trans Assoc Am Physicians* 1963;**76**:199–213.
48. Chang S, Grunder S, Hanukoglu A, et al. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis,Pseudohypoaldosteronism type I. *Nat Genet* 1996;**12**:248–253.
49. Geller D, Farahi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. *Science* 2000;**289**:119–123.
50. Sartorato P, Lapeyraque A-L, Armanini D, et al. Different inactivating mutations of the minerolocorticoid receptor in fourteen families affected by Type I Pseudohypoaldosteronism. *J Clin Endocrinol Metab* 2003;**88**:2508–2517.
51. Preston GM, Carrol TP, Guggino WB, Agre P. Appearance of water channels in *Xenopus* Oocytes expressing red cell CHIP28 protein. *Science* 1992;**256**:385–387.
52. Litman T, Sogaard R, Zeuthen T. Ammonia and urea permeability of mammalian aquaporins. *Hanb Exp Pharmacol* 2009;**190**:327–358.
53. Nejsum LN. The renal plumbing system: Aquarorin water channels. *Cell Mol Life Sci* 2005;**62**:1692–1706.
54. Nielsen S, Smith BL, Christensen EI, Agre P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capiliary endothelia. *Proc Natl Acad Sci USA* 1993;**90**:7275–7279.
55. Robben JH, Knoers NV, Deen PM. Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol* 2006;**291**:F257-F270.
56. Bichet DG. Lithium, cyclic AMP signaling, A-kinase anchoring proteins, and aquaporin 2. *J Am Soc Nephrol* 2006;**17**:920–922.
57. Sands JM, Bichet DG. Nephrogenic diabetes incipidus. *Ann Int Med* 2006;**144**: 186–194.

58. Glover M, O'Shaughnessy KM. STK39 and WNK Kinases—A new target for blood pressure treatment? *Curr Opin Nephrol Hypertens* 2011;**20**:16–22.
59. Subramanya AR, Liu J, Ellison DH, Wade JB, Welling PA. WNK4 diverts the thiazide-sensitive NaCl cotransporter to the lysosome and stimulates AP-3 interaction. *J Biol Chem* 2009;**287**:18471–18480.
60. Richardson C, Rafiqi FH, Karlsson HKR, et al. Activation of the thaizide-sensitive Na-Cl cotransporter by the WNK-regulated kinases SPAK and OSR1. *J Cell Sci* 2008;**121**:675–684.
61. Glover M, Mercier Zuber A, O'Shaughnessy KM. Renal and Brain Isoforms of WNK3 have opposite effects on NCCT. *J Am Soc Nephrol* 2009;**20**:1314–1322.
62. Lin S-H, Shiang J-C, Huang C-C, Yang S-S, Hsu Y-J, Cheng C-J. Phenotype and Genotype Analysis in Chinese Patients with Gitelman's Syndrome. *J Clin Endocrinol Metab.* 2005;**90**:2500–2507.
63. Wang Y, O'Connell JR, McArdle PF, et al. Whole-genome association study identifies STK39 as a hypertension susceptibility gene. *Proc Natl Acad Sci USA* 2009;**106**:226–231.
64. Rafiqi FH, Mercier Zuber A, Glover M, et al. Role of the WNK-activated SPAK kinase in regulating blood pressure. *EMBO Mol Med* 2010;**2**:1–13.
65. Clayton JA. The pharmacoepidimiology of hyponatraemia. Thesis/dissertation, University of Nottingham, 2006.
66. Gagnon KBE, England R, Diehl L, Delpire E. Apoptosis-associated tyrosine kinase scaffolding of protein phosphatase 1 and SPAK reveals a novel pathway for Na-K-Cl cotransporter regulation. *Am J Physiol Cell Physiol* 2007;**292**:C1809–C1815.
67. Glover M, Zuber AM, Figg N, O'Shaughnessy KM. The activity of the thiazide-sensitive Na+Cl—Cotransporter is regulated by protein phosphatase, PP4. *Can J Physiol Pharmacol* 2010;**88**:986–995.
68. Scholl UI, Choi M, Liu T, et al. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by muttaions in *KCNJ10*. *Proc Natl Acad Sci USA* 2009;**106**:5842–5847.
69. Dimke H, San Cristobal P, de Graaf M, Lenders JW, Deinum J, Hoenderop JG, Bindels R. Gamma adducin stimulates the thiazide-sensitive NaCl cotransporter. *J Am Soc Nephrol* 2011; **22**:508–517.
70. San Cristobal P, Pacheco-Alvarez D, Richardson C, et al. Angiotensin II signaling increases activity of the renal NaCl cotransporter through a WNK4-SPAK dependent pathway. *Proc Natl Acad Sci USA* 2009;**106**:4384–4389.
71. Magaldi AJ. New insights into the paradoxical effect of thiazides in diabetes insipidus therapy. *Nephrol Dial Transplant.* 2000;**15**:1903–1905.
72. Cesar KR, Magaldi AJ.Thiazide induces water absorption in the inner medullary collecting duct of normal and Brattleboro rats. *Am J Physiol Renal Physiol* 1999;**277**:756–760.
73. Simon DB, Nelson-Williams C, Bia MJ, et al. Gitelman's varient of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl transporter. *Nat Genet.* 1996;**12**:24–30.
74. Schepkens H, Stubbe I, Hoeben H, Vanholder R, Lameire N. Severe hyponatraemia and hypouricaemia in Gitelman's syndrome. *Nephrol Dial Transplant.* 2001;**16**:2250–2252.
75. Weizhen J, Foo JN, O'Roak BJ, et al. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. *Nat Genet.* 2008;**5**:592–599.
76. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, Cohen JC. Rare loss-of-function mutations in *ANGPTL* family members contribute to plasma triglyceride levels in humans. *J Clin Invest.* 2009;**119**:70–79.
77. Uwai Y, Saito H, Hashimoto Y, Inui K-I. Interaction and transport of thiazide diuretics, loop diuretics and acetazolamide via rat renal organic anion transporter rOAT1. *J Pharmacol Expt Ther* 2000;**295**:1261–265.
78. Beermann B, Groschinsky-Grind M, Lindstrom B. Pharmacokinetics of Bendroflumethiazide. *Clin Pharmacol Ther* 1977;**22**:385–388.
79. Delaloy C, Lu J, Hounot AM, Disse-Nicodeme S, Gasc JM, Corvol P, Jeunemaitre X. Multiple promotors in the WNK1 gene: One controls the expression of a kidney-specific kinase-defective isoform. *Mol Cell Biol* 2003;**23**:9208–9221.
80. The SEARCH collaborative group. SLCO1B1 varients and statin-induced-myopathy—A genomewide study. *N Eng J Med* 2008;**359**:789–799.
81. Singer JB, Lewitzky S, Leroy E, et al. A genome-wide study identifies HLA alleles associated with lumaricoxib-related liver injury. *Nat Genet* 2010;**42**:711–716.
82. Hautekeete ML, Horsmans Y, Van Waeyenberge C, et al. HLA association of amoxicillin-clavulanate-induced hepatitis. *Gastroenterology* 1999;**117**:1181–1186.
83. J O'Donohue, K Oien, P Donaldson, J Underhill, M Clare, R MacSween, P Mills. Co-amoxiclav jaundice: Clinical and histological features and HLA class II association. *Gut* 2000;**47**:717–720.
84. Chung WH, Hung SI, Hong HS, et al. A marker for Stevens-Johnson syndrome. *Nature* 2004;**428**:486.
85. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;**359**:727–732.
86. Kindmark A, Jawaid A, Harbron CG, et al. Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. *Pharmacogenomics J* 2010;**8**:186–195.
87. O'Shaughnessy KM. HapMap, pharmacogenomics, and the goal of personalized prescribing. *Br J Clin Pharmacol* 2006;**61**:783–786.