NEPHROGENIC SYNDROME OF INAPPROPRIATE ANTIDIURESIS SECONDARY TO AN ACTIVATING MUTATION IN THE ARGININE VASOPRESSIN RECEPTOR AVPR2

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Abstract

Context

Nephrogenic syndrome of inappropriate antidiuresis (NSIAD), resulting from activating mutations in the arginine vasopressin receptor type 2 (AVPR2) is a rare cause of hyponatraemia. However, its true prevalence may be under-estimated and it should be considered in the investigation of unexplained hyponatraemia, with implications for management and targeted gene testing.

Objective

We describe a structured approach to the investigation of hyponatraemia in a young patient, which allowed a diagnosis of NSIAD to be made. We review current knowledge of NSIAD, and use a structural modelling approach to further our understanding of the potential mechanisms by which the causative mutation leads to a constitutively active AVPR2.

Design

Clinical and biochemical investigation of hyponatraemia; a formal water load test with measurement of arginine vasopressin levels (AVP); sequencing of *AVPR2;* and computed structural modelling of the wild-type and constitutively activated mutant receptors.

Results

A 38-year-old man presented with intermittent confusion and nausea associated with hyponatraemia and a biochemical picture consistent with syndrome of inappropriate antidiuretic hormone (SIADH). Adrenocortical and thyroid function, and an acute intermittent porphyria screen were normal. Cross-sectional imaging of the head, chest and abdomen did not identify an underlying cause and so we proceeded to a water load test. This demonstrated a marked inability to excrete a free water load (just 15% of a 20 mL/kg oral load by 240 mins post-ingestion), with the onset of hyponatraemia (Na^+ 125 mmol/L, urine

osmolality 808 mOsm/kg). However, AVP levels were low throughout the test (0.4-0.9 pmol/L), consistent with a diagnosis of NSIAD. *AVPR2* sequencing revealed a previously described hemizygous activating mutation (p.Arg137Cys). Through structural modelling of AVPR2, we suggest the disruption of a hydrogen bond between residues Thr269 and Arg137 may promote stabilisation of the receptor in its active conformation. Since diagnosis, the patient has adhered to modest fluid restriction and remained well, with no further episodes of hyponatraemia.

Conclusion

NSIAD should be considered in young patients with unexplained hyponatraemia. A water load test with AVP measurement is a potentially informative investigation, while *AVPR2* sequencing provides a definitive molecular genetic diagnosis and a rationale for long-term fluid restriction.

Introduction

Hyponatraemia is relatively common, affecting 15-20% of hospital inpatients, and is associated with increased morbidity and mortality[1-5]. The aetiology is frequently multifactorial[2]. Once pseudo-hyponatraemia, factitious causes and hyperosmolar/dilutional hyponatraemia have been excluded, there remains the broad category of hypoosmolar hyponatraemia, which may be sub-classified by volume status[6]. Hypervolaemic and hypovolaemic hyponatraemia complicate excessive water retention or water and sodium loss respectively. Euvolaemic hyponatraemia is associated with diminished free water excretion; the accompanying expansion in circulating volume is partially counter-acted by suppression of plasma renin and aldosterone, which in turn exacerbates the hyponatraemia. The most common cause is the syndrome of inappropriate antidiuresis (SIAD) due to inappropriate release of arginine vasopressin (AVP) – i.e. the syndrome of inappropriate antidiuretic hormone (SIADH). Recent European Society of Endocrinology guidelines conclude that a diagnosis of SIAD in the context of hyponatraemia requires: i) effective serum osmolality <275 mOsm/kg, ii) urine osmolality >100 mOsm/kg at some level of decreased effective osmolality, iii) clinical euvolaemia, iv) urine sodium concentration >30 mmol/L with normal dietary salt and water intake, v) absence of adrenal, thyroid, pituitary or renal insufficiency and vi) no recent use of diuretic drugs[7]. SIADH may arise in the context of neoplasia, neurological disorders, lung disease, infections, treatment with a variety of drugs and other miscellaneous conditions including acute intermittent porphyria and ectopic AVP production. This leaves a proportion of cases labelled as 'idiopathic'. The incidence of such cases increases with age[8] and SIADH *per se* is very uncommon in children[9].

Recently, Feldman and colleagues described two paediatric patients with hyponatraemia, both of whom met the criteria for SIAD but in the absence of a classical cause[10]. Furthermore, AVP was undetectable and subsequent molecular genetic studies identified mutations at codon 137 (p.Arg137Cys and p.Arg137Leu) that render the arginine vasopressin receptor type 2 (AVPR2) constitutively active, thereby mediating downstream actions of AVP, including

95 water reabsorption, in the absence of ligand binding. This monogenic 'nephrogenic'
96 syndrome of inappropriate antidiuresis (NSIAD) has since been described in a small number
97 of kindreds, including adult cases, with a markedly heterogeneous phenotype.
98
99 Here, we report a case of intermittent hyponatraemia in a 38-year-old man with clinical and
100 biochemical findings consistent with SIADH. Given the relative rarity of this diagnosis in his
101 age group and the lack of an obvious precipitant, we performed a water load test with AVP
102 measurement, which suggested a diagnosis of NSIAD. This was subsequently confirmed by
103 sequencing of *AVPR2* and demonstration of the p.Arg137Cys mutation, with structural
104 modelling offering novel insights into the molecular basis for constitutive activation by the
105 mutant receptor. We further discuss the implications for management of the patient in the
106 context of a diagnosis of NSIAD.
107

Materials and Methods

All studies were performed in accordance with local standard clinical practice.

Biochemical analyses

All analytes were measured by a United Kingdom Accreditation Service (UKAS, Middlesex, UK) accredited laboratory with relevant internal and external quality assurance as recommended by the UKAS. Thyrotropin (TSH) was measured by chemiluminescent immunometric assay using a Siemens Centaur® immuno-analyser (Siemens Healthcare, Surrey, UK) with protocols and reagents provided by the manufacturer. Serum free thyroxine was measured using a one-step chemiluminescent analogue method on the Centaur® using reagents provided by the same manufacturer. Cortisol was measured by competitive chemiluminescent immunoassay also using the Centaur®. Serum Na^+, K^+, Ca^{2+}, glucose and urea, and urine Cr and Na^+ were measured using a Siemens ADVIA 2400 using protocols and reagents provided by the manufacturer. Whole blood sodium was also measured using a B221 blood gas analyser using equipment, protocols and reagents provided by Roche Diagnostics (Sussex, UK). Blood glucose was also measured using a Nova Biomedical StatStrip™ blood glucose monitoring system (Nova Biomedical Cheshire, UK). Serum and urine osmolalities were measured using an Advanced micro-osmometer 3300 (Advanced instruments inc. Mass, US). Plasma aldosterone was measured using liquid chromatography tandem mass spectrometry, adapted from a previously published protocol[11] using an AB SCIEX 5500 (AB Sciex UK Ltd, Cheshire, UK) and validated to UKAS standards. Plasma renin concentration was measured using an immunometric assay on a Liason XL® analyser with reagents and protocols provided by Diasorin (Kent, UK). Plasma AVP was measured by the Newcastle (UK) SAS laboratory with an in-house radio-immunoassay method using C18 resin-extracted plasma and a ^{125}I-labelled AVP tracer. Urine PBG was measured using a PBG by Column Test provided by Biorad (Herts UK).

Water load test

A dynamic water load test was adapted from a published protocol[12]. Briefly, this comprises a body mass adjusted oral water load (20 mL/kg) ingested over 15 minutes, followed by a period of observation during which time urine output and biochemical responses are measured. The patient is fasted overnight and should abstain from caffeine and smoking, but is allowed moderate free fluid intake. Following cannulation and weight measurement, the patient fully voids their bladder before resting for 30 minutes. Baseline bloods are drawn for measurement of Na^+, K^+, urea, serum osmolality and AVP, and point of care venous blood gas analysis for Na^+ and glucose. A urine sample is collected for osmolality measurement. The point of care Na^+ analysis allows for real time assessment of natraemic status; the water load should not proceed if there is significant hyponatraemia at baseline. The patient then drinks the oral free water load. Further samples are taken at 60, 120, 180 and 240 minutes, along with a paired urine output volume record. Further measurements are taken over the predicted 'recovery' phase at 300 and 360 minutes. Persistent hyponatraemia at this stage mandates a period of inpatient observation. It has been reported that normal subjects excrete 78-82% of the ingested water load by 240 minutes, which can be reduced to 30-40% in patients with syndrome of inappropriate antidiuresis[12]. In AVP-mediated SIAD, serum AVP levels fail to suppress at serum osmolalities below the standard normal threshold for AVP release (275-285 mOsm/kg)[12]. To exclude urinary retention, an ultrasound scan of the bladder was performed at the bedside at the conclusion of the test, to determine residual volume.

Genetic sequencing

Genomic DNA was extracted from peripheral blood lymphocytes. The coding region of *AVPR2* was then amplified by PCR of genomic DNA and sequenced by conventional Sanger sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Paisley, UK) and analysed on an ABI 3730 automated sequencer (Applied Biosystems, Paisley, UK). Primer sequences are available on request.

Structural modelling

The AVPR2 structural models for each of the wild-type and mutant receptors were generated using the Phyre2 (**P**rotein **H**omology/analog**Y** **R**ecognition **E**ngine 2) web portal, which predicts and analyses protein structures for a submitted amino acid sequence based on homology/analogy recognition to solved protein crystal structures, and MacPymol, a molecular graphics program for visualisation and analysis of 3D structures[13].

Results

Case history and baseline biochemistry

A 38-year-old man presented to his local Emergency Department with confusion and a brief loss of consciousness. He denied any intake of alcohol and reported that he had only drunk a moderate amount of fluid in the preceding 24 hours. There was no history of intercurrent illness or illicit drug use. His past medical history included depression treated with citalopram, and asthma, for which he used salbutamol and budesonide/formeterol inhalers. No collateral history was available with respect to his early childhood. Systemic enquiry did not reveal any other issues and there was no relevant family history. Physical examination was otherwise unremarkable. His serum sodium at presentation was 117 mmol/L [reference range (RR) 135-145]. Initial management in the Emergency Department was with 0.9% sodium chloride intravenously, which failed to resolve his hyponatraemia; 24 h later his sodium was 115 mmol/L. He was discharged with oral 'slow sodium' and his citalopram was discontinued. He was admitted on several other occasions over the next six months with stereotyped episodes of malaise, nausea and confusion, with serum sodium often below 120 mmol/L. Between admissions, his sodium was reported to be within normal limits. There did not appear to be a specific trigger for the periods of illness and he reported a broadly matched fluid intake and urine output with 'average' thirst.

At his first Endocrine Clinic review following tertiary referral, he was lucid, felt well and his biochemistry was unremarkable [serum Na^+ 134 mmol/L (RR 135-145), serum K^+ 4.4 mmol/L (RR 3.4-5.0), plasma glucose 5.0 mmol/L, serum urea 5.3 mmol/L (RR 0-7.5), serum creatinine 87 µmol/L (RR 35-125), serum osmolality 282 mOsm/kg (RR 280-300), urine osmolality 799 mOsm/kg and urine Na^+ 189.6 mmol/L]. In marked contrast, at his second appointment six weeks later, he appeared mildly confused and was nauseated. He was clinically euvolaemic and normotensive with no postural drop in blood pressure (lying 130/80 mmHg, standing 135/85 mmHg). Investigation confirmed hyponatraemia with a clinical and

biochemical picture consistent with syndrome of inappropriate antidiuresis [serum Na^+ 124 mmol/L (RR 135-145), serum K^+ 4.5 mmol/L (RR 3.4-5.0), plasma glucose 6.0 mmol/L, serum urea 4.7 mmol/L (RR 0-7.5), serum creatinine 76 µmol/L (RR 35-125), serum osmolality 268 mOsm/kg (RR 280-300), urine osmolality 652 mOsm/kg and urine Na^+ 153.6 mmol/L].

Further biochemistry

The patient declined admission but agreed to further investigation. Thyroid and adrenocortical function were normal [serum free T4 14.4 pmol/L (RR 10-19.8), serum TSH 1.1 mU/L (RR 0.35-5.5), 9 am serum cortisol 501 nmol/L, 30 min post-Synacthen® serum cortisol 714 nmol/L (RR >450), plasma renin 27 mU/L (RR 5.4-60)] and plasma aldosterone 292 pmol/L (RR 100-800)]. Serum corrected Ca^{2+} [2.3 mmol/L (RR 2.1-2.5)] and plasma HbA1c [32 mmol/mol (RR 30-45)] were also within normal limits. A contemporaneous screen for acute intermittent porphyria was normal [urine porphobilinogen 4.2 µmol/L (RR 0-10.2), urine porphobilinogen:creatinine ratio 0.7 mmol/mol (RR 0-1.5)].

At his next review one week later, his symptoms and hyponatraemia had both resolved without intervention [serum Na^+ 135 mmol/L (RR 135-145), serum K^+ 4.0 mmol/L (RR 3.4-5.0), plasma glucose 5.3 mmol/L, serum urea 5.3 mmol/L (RR 0-7.5), serum creatinine 97 µmol/L (RR 35-125), serum osmolality 281 mOsm/kg (RR 280-300), urine osmolality 717 mOsm/kg and urine Na^+ 131 mmol/L].

Imaging

A plain chest radiograph, computed tomography of the chest, abdomen and pelvis, and magnetic resonance imaging of the head were all unremarkable.

Water load test

Given the intermittent nature of his hyponatraemia, which was consistent with relapsing and remitting SIAD, but without an identifiable cause for SIADH, a water load test with AVP measurements was performed (**Table 1**). Baseline biochemistry was unremarkable other than a mild degree of hyponatraemia [venous gas Na^+ 133.9 mmol/L (RR 135-148), serum osmolality 274 mOsm/kg (RR 280-300), urine osmolality 805 mOsm/kg]. Following an oral load of 20mL/kg (1510 mL) water, taken over 15 minutes, hyponatraemia developed with a nadir at 180 mins post-ingestion. His biochemistry at this stage was commensurate with a syndrome of inappropriate antidiuresis [venous gas Na^+ 125.3 mmol/L (RR 135-148), urine osmolality 808 mOsm/kg]. His urine output was consistent with this, demonstrating a failure to clear the water load, with a total urine output of just 15% of the ingested fluid at 240 mins, compared with an expected value of 78-82% in normal subjects[12]. Urinary retention was excluded with a bladder scan, which demonstrated a residual volume of just 77 mL. Serum AVP levels were in the low physiological range throughout the test (0.4–0.9 pmol/L). Urine osmolality (745-840 mOsm/kg) was persistently elevated. Together, these findings were consistent with an AVP-independent (nephrogenic) syndrome of inappropriate antidiuresis.

Sequencing of arginine vasopressin receptor type 2 (AVPR2)

Analysis of the *AVPR2* gene, which is situated on the X chromosome, demonstrated a hemizygous mutation, with a substitution of cytosine to thymine at nucleotide 770 leading to a single amino acid change, p.Arg137Cys, which has been previously reported as an activating mutation causing nephrogenic syndrome of inappropriate antidiuresis[10]. This is a highly conserved residue in AVPR2 across species (**Fig. 1a**) and across G-protein coupled receptors in general[14, 15].

Structural modelling of AVPR2

In silico structural modelling of the AVPR2 receptor in both Arg137 and p.Arg137Cys forms identified a putative hydrogen bond between residues Arg137 and Thr269 in the second intracellular loop of the G-protein coupled receptor (GPCR) (**Fig. 1b, 1c**), which is not

253 present in the mutant form (p.Arg137Cys). We speculate that loss of this interaction may
254 permit adoption of an activated form of the receptor, independent of AVP ligand binding.
255

Discussion

Nephrogenic syndrome of inappropriate antidiuresis (NSIAD) resulting from constitutive activation of AVPR2 was first described by Feldman and colleagues in 2005[10]. Both probands in the original report presented in infancy (<3 months of age), the first with irritability and the second with generalised seizures. In each case, the biochemical picture was consistent with a syndrome of inappropriate antidiuretic hormone release. However, SIADH is a rare occurrence in the paediatric population (Huang and colleagues identified just 13 published cases between 1954 and 2004)[9]. Moreover, Feldman *et al* were unable to identify a classical cause for SIADH in their subjects, and AVP was subsequently found to be undetectable in both cases.

A central step in salt and water homeostasis is the signalling of AVP via its renal G-protein coupled receptor AVPR2. Ligand binding and activation leads to cAMP-mediated recruitment of aquaporin-2 channels to the luminal membrane of the medullary collecting duct, leading to reabsorption of water and urine concentration. Inactivating mutations in AVPR2 are a well recognised cause of nephrogenic diabetes insipidus, with reduced AVPR2-mediated transactivation resulting in diminished cAMP production and thus aquaporin translocation[16]. Point mutations leading to constitutive activation of a GPCR are rarer in biology than inactivating mutations, but there is precedent in GPCRs involved in other endocrine signalling pathways, e.g. TSH receptor, luteinising hormone receptor and calcium sensing receptor[17,18,19]. As such, activating mutations in AVPR2 are therefore an obvious candidate to explain inappropriate antidiuresis occurring independently of AVP. One of the original mutations identified by Feldman and co-workers was the same as that demonstrated in our case, p.Arg137Cys, while the second resulted in a different substitution at the same codon (p.Arg137Leu)[10]. Arginine 137 is highly conserved in AVPR2 across species (**Fig. 1a**) and is situated in the DRY/H domain of the second intracellular loop, at its juncture with the third intra-membrane domain, which is highly conserved across the broader GPCR family. This region appears to play a central role in stabilisation of the receptor in either its active or

284 inactive form[14,15]. The importance of this particular residue in AVPR2 signalling is confirmed
285 by the demonstration that an alternative substitution at the same residue, p.Arg137His,
286 produces an inactivating mutation with resultant nephrogenic diabetes insipidus.
287 Interestingly, in the basal state the inactivating mutation induces similar basal cAMP
288 production to its wild type counterpart, which contrasts markedly with the 4–7.5-fold higher
289 level of transcriptional activity seen with the constitutive activating mutations, p.Arg137Cys
290 and p.Arg137Leu[10]. Rochdi *et al* have also shown that AVP binding to the p.Arg137Cys and
291 p.Arg137Leu mutant receptors does not produce a further increase in cellular cAMP or CRE-
292 luciferase reporter assay activity over their already elevated basal levels, nor do inverse
293 agonists have any effect[20], findings which have been replicated by others[21]. Together, these
294 observations suggest that the GPCR complex is 'locked' in an active form in the presence of
295 such mutations, and a change in the interaction between the GPCR $G\alpha_s$ subunit and β-
296 arrestin2 has been postulated to underlie this[20]. Carpentier and coworkers have recently used
297 structural modelling to demonstrate that the relative positioning of the AVPR2
298 transmembrane helices is altered in another constitutively active form (p.Phe229Val) of the
299 receptor[22]. Extending these findings, we have now identified a putative hydrogen bond
300 between Arg137 and Thr269 in the second intracellular loop of the wild-type AVPR2 that is
301 not present in the p.Arg137Cys mutant (**Fig. 1b, 1c**), and speculate that disruption of this
302 interaction contributes to the structural change(s) required to allow constitutive activation in
303 the absence of AVP. However, additional molecular studies would be required to formally
304 test this hypothesis.
305
306 To date, fewer than 30 cases of NSIAD due to activating AVPR2 mutations have been
307 reported[10, 23-32]. Whilst the majority of affected individuals harbour mutations at codon 137,
308 activating mutations affecting other amino acids (p.Phe299Val and p.Ile130Asn) have also
309 been described[22, 33]. It is interesting to note the marked heterogeneity with respect to age at
310 diagnosis and severity of the disorder between subjects. For example, several probands
311 presented with neonatal seizures in the context of hyponatraemia but, on screening family

312 members, causative mutations and abnormal water load test dynamics were also observed in
313 otherwise apparently unaffected individuals. Accordingly, it seems that a subgroup of those
314 with activating AVPR2 mutations only come to attention in adult life after an unusually
315 severe water load or in the context of exogenous salt loss[27], suggesting there may be a
316 protective element of chronic voluntary fluid restriction in these subjects[30]. Importantly,
317 female subjects harbouring heterozygous activating AVPR2 mutations have also been
318 reported to exhibit reduced water excretion in response to an oral load, and may thus be
319 similarly affected to some male hemizygotes[25, 30, 32].
320
321 Fluid restriction is the mainstay of treatment for NSIAD and can be effective in attenuating
322 the risk of hyponatraemia, as in our patient. Oral urea, which reduces natiuresis while
323 maintaining aquaresis, is an alternative, especially in paediatric cases where strict fluid
324 restriction may be hazardous or difficult to achieve[9,10,23,24,25,27,28,30,31]. Oral sodium
325 supplementation has been used in some patients, while furosemide has been suggested as an
326 alternative for refractory cases[25]. Inverse agonists (e.g. the vaptan class of drugs) have been
327 shown to be ineffective *in vitro* against the p.Arg137Cys and p.Arg137Leu variants[20,21], and
328 indeed one individual harbouring the p.Arg137Cys mutation was identified through a failure
329 to respond to tolvaptan and satavaptan in the phase III trials of these oral inhibitors[25]. Of note
330 however, the more recently described p.Phe299Val and p.Ile130Asn mutant forms of AVPR2
331 appear to behave differently. Phe299 is located at the base of the 5th GPCR transmembrane
332 domain and *in vitro* studies demonstrated a marked (30-fold) increase in basal cAMP
333 production when compared to the wild-type receptor[22]. Unlike the Arg137 variants however,
334 constitutive signalling is abrogated with vaptan treatment, suggesting that the p.Phe299Val
335 mutation does not permanently lock the receptor in an active configuration. It has been
336 postulated that this may be related to the lack of constitutive β-arrestin recruitment to the
337 receptor, which is present with the Arg137 mutants[22]. Similar *in vitro* findings and response
338 to vaptan treatment have been described for the p.Ile130Asn activating mutation. Together,

these findings raise the possibility of targeted therapy for a subset of NSIAD patients and highlight the value of confirming a molecular genetic diagnosis.

The true prevalence of nephrogenic SIAD is uncertain. One study screened the R137 codon of *AVPR2* in two large cohorts of asymptomatic individuals (i.e. 'normal' populations). A number of those screened had serum sodium levels below the population reference range. Genotyping failed to detect any variants at R137 in these cohorts, leading the investigators to conclude that NSIAD-associated AVPR2 variants are exceedingly uncommon[34]. It is possible though that NSIAD may be more common than is implied by this or by the low number of reported cases to date, and that cases may be more readily identified by screening symptomatic hyponatraemic patients, particularly those in whom no obvious cause for SIADH is identified. Some support for this notion is provided by historical studies which identified a subset of patients (14% of a cohort meeting biochemical criteria for SIADH) who could neither maximally dilute their urine nor excrete a water load normally, in the absence of any detectable abnormality in vasopressin secretion. This has been termed 'hypovasopressinemic antidiureses' or 'type D' SIAD and it has been suggested that the pathology in these cases reflects either an increase in renal sensitivity to low concentrations of AVP, or the action of another antidiuretic factor[12, 35, 36]. While these early studies have their limitations, which do not permit distinction between these possibilities, it is tempting to speculate that nephrogenic SIAD accounts for a proportion of these cases.

The suspicion of nephrogenic SIAD in our patient was raised by his relatively young age, the intermittent nature of the hyponatraemia, and the absence of a classical cause for SIADH or other causes of hyponatraemia despite meeting the diagnostic criteria for SIAD. We would advocate considering the diagnosis of NSIAD in such individuals. Whilst a water load test is seldom required for a diagnosis of SIAD, by including measurement of AVP, we were able show in this case that a markedly reduced water clearance and the development of dilutional hyponatraemia were associated with AVP levels persistently in the low physiological range.

These levels, in the context of a paradoxical markedly elevated urine osmolality (which serves as a bioassay for AVP activity) were consistent with NSIAD due to constitutive AVPR2 activation and led us to perform the confirmatory genetic testing (**Table 1**).

Finally, there are significant benefits for the patient in making the diagnosis of NSIAD. First, as in our case, it provided validation for the patient, who had suffered many years of intermittent symptoms for which no cause had been attributed. Second, it provided a rationale for management. Moderate fluid restriction has markedly improved his quality of life and he has been issued with 'sick day rules', advising close clinical and biochemical monitoring (i.e. fluid input/output charting with periodic measurement of serum/plasma sodium) in the event of intercurrent illness such as fevers, vomiting or diarrhoea; or hospitalisation, where medical staff must be alerted to the potential dangers of fluid overload. The genetic diagnosis also offers the possibility of screening family members and the involvement of a genetics service to discuss potential implications for family planning. Importantly, genotyping may also guide therapeutic choices, e.g. use of a vaptan in the context of a 'responsive mutation'.

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Disclosures

The authors have nothing to declare.

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Figure legends

Fig 1. a Sequence alignment of AVPR2 across several species, demonstrating conservation of the Arg137 residue within the DRY/H domain of the GPCR second intracellular loop**. b** *In silico* crystallographic modelling showing the position of wild type (Arg137 - cyan) and mutant (Cys137 - orange) residues in the second transmembrane domain of AVPR2. **c** Magnified view demonstrating the putative hydrogen bond (dotted line) between Arg137 and Thr269 (green) in the wild-type receptor, which is absent in the presence of the p.Arg137Cys mutation (orange).

Table 1. Water load test in proband

Time (min)	**Urine output** (mL/h)	**VBG sodium** (mmol/L)	**Serum sodium** (mmol/L)	**Serum potassium** (mmol/L)	**Capillary glucose** (mmol/L)	**Serum urea** (mmol/L)	**Serum creatinine** (μmol/L)	**Serum osmolality** (mOsm/kg)	**Urine osmolality** (mOsm/kg)	**Plasma AVP** (pmol/L)
0	-	133.9	129	3.9	5.4	4.2	88	274	805	0.9
60	100	130.9	131	4.4	5.8	4.8	92	272	836	0.5
120	50	130.2	129	3.9	6.0	4.8	-	266	745	0.4
180*	40	125.3	-	-	6.0	-	-	-	808	0.8
240	40	129.4	127	3.8	5.6	4.0	82	266	833	0.8
300	30	128.9	129	4.1	-	4.2	90	-	845	0.6
360	20	129.7	128	3.8	-	4.8	93	266	840	-

Key: Following baseline measurements (0 min), 20 mL/kg of water (=1510 mL) was ingested over 15 min; AVP, arginine vasopressin; VBG, venous blood gas sample; * denotes serum sample haemolysed at this time point.

a

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H.sapiens              QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGSGAHWNR
P.troglodytes          QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGSGAHWNR
M.mulatta              QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGGGAHWNR
C.lupus                QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGGGARWNR
B.taurus               QMVGMYASSYMILAMTLD  R  HRAICRPMLAHRHGGGTHWNR
M.musculus             QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGGGARWNR
R.norvegicus           QMVGMYASSYMILAMTLD  R  HRAICRPMLAYRHGGGARWNR
D.rerio                QIVGMFASSYMIVAMTVD  R  HYAICCPLQAYRGGATSRWNT
X.tropicalis           QVVGMYASSYMIVAMTFD  R  HQAICRPMMTFKKGS-ARWNI
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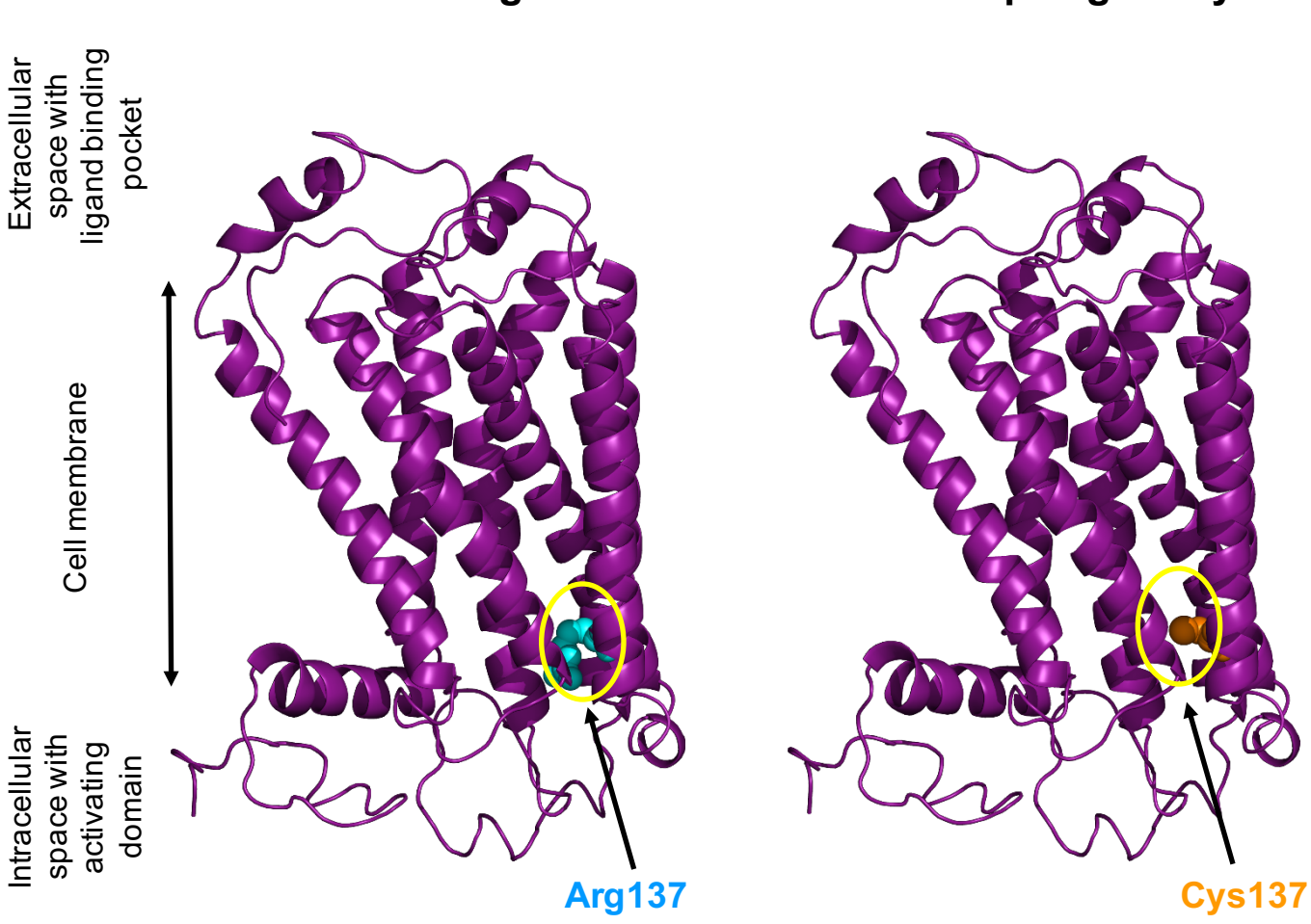
b



c