Neuropeptide Y as a risk factor for cardiorenal disease and cognitive dysfunction in CKD: translational opportunities and challenges

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ABSTRACT

Neuropeptide Y (NPY) is a 36-amino-acid peptide member of a family also including peptide YY and pancreatic polypeptide which are all ligands to Gi/Go coupled receptors. NPY regulates several fundamental biologic functions including appetite/satiety, sex and reproduction, learning and memory, cardiovascular and renal function and the immune function. The mesenteric circulation is a major source of NPY in the blood in man and this peptide is considered a key regulator of the gut-brain cross-talk.

A progressive rise in circulating NPY accompanies the progression of CKD toward kidney failure and NPY robustly predicts cardiovascular events in this population. Furthermore, NPY is suspected as a possible player in the accelerated cognitive function decline and dementia in patients with CKD and in dialysis patients.

In theory, Interfering with the NPY system has relevant potential for the treatment of diverse diseases from cardiovascular and renal diseases to diseases of the central nervous system. Pharmaceutical formulations for effective drug delivery and cost as well as the complexity of diseases potentially addressable by NPY/NPY antagonists have been a problem until now. This in part explains the slow progress of knowledge about the NPY system in the clinical arena. There is now a renewed research interest on the NPY system in psycho pharmacology and in pharmacology in general and new studies and a new breed of clinical trials may eventually bring the expected benefits in human health by drugs interfering with this system.

Keywords: cardiovascular, CKD, dialysis, hypertension, renin-angiotensin system

INTRODUCTION

Neuropeptide Y (NPY) is a 36-amino-acid peptide that derives from a 98-amino-acid protein (preproNPY) coded on chromosome 7p15.1. This compound is a member of a family of peptides including peptide Y (PYY) and pancreatic polypeptide (PP) which are all ligands to Gi/Go coupled receptors (Y receptors). PreproNPY generates the 69- amino-acid prohormone proNPY which by enzymatic cleavage eventually results in the NPY molecule. The dibasic pair of amino acids, Lys38-Arg39, of the prohormone is cleaved by two convertases, PC1/3 and PC2, to generate NPY (1). The N-terminus of NPY and PYY is readily cleaved by aminopeptidase P and dipeptidyl peptidase 4 (DP4, CD26), giving rise to the fragments NPY2-36, NPY3-36 and PYY3-36 with distinct pharmacological properties.

NPY regulates several fundamental biologic processes including appetite/satiety, sex and reproduction, learning and memory (1)(Figure 1). Furthermore, it has anxiolytic and anti-depressant properties (1) and potently inhibits bone turnover in experimental models (3). This peptide is highly represented in the brain and in the central and peripheral nervous system and is ubiquitous in the cardiovascular system (4). In the arterial system NPY regulates vascular tone by interacting with the sympathetic system, the renin-angiotensin-aldosterone system (5), the nitric oxide system (6)and key vasodilators like atrial natriuretic peptide (7) and vasorelaxant prostaglandins(8). Notably, NPY is an established regulator of several hormone systems (9) and is involved in immune-regulation (10) (11).

The mesenteric circulation is a major source of circulating NPY in man (12) and this peptide is considered as a key regulator of the gut-brain cross-talk (Figure 1). This cross-talk rests on three major pathways, namely neural input transmitted by vagal and spinal afferent neurons, immune signals triggered by cytokines, and endocrine signals by gut hormones(13). The gut microbiota and the gastrointestinal immune system interact with each other at the level of the gastrointestinal mucosa this interaction originates disparate signals for the brain, including cytokines, lipopolysaccharide (LPS) and peptidoglycans (14) that directly activate the central nervous system (CNS). These signals contribute to the regulation of diverse functions from digestion to immunity, metabolic homeostasis and brain function, including the emotional and cognitive dimension. Remarkably, the gut produces over twenty hormones (15) and the reach of gut hormones extends from digestion, hunger and satiety and energy homeostasis to mood and emotion. The three pathways discussed above are closely interrelated. Indeed cytokines and

gastrointestinal hormones act on afferent vagal neurons sending messages to the brain. Deciphering how the gut–brain axis works has implications well beyond gastroenterology extending to neurology and psychiatry.

In this review, we summarize knowledge on NPY, from target receptors to possible clinical implications of this neuropeptide in human diseases at large and in CKD in particular. Especially we have focused our attention on studies focusing on cognitive dysfunction and dementia, which is an emerging problem in the CKD population.

NPY receptors

To date seven Y receptor subtypes (Y1R-Y8R) have been described in vertebrates. The three endogenous ligands -NPY, PYY and PP- exhibit varying degrees of affinity and specificity for four human YRs (Figure 2). NPY and PYY bind with relatively high affinity to Y1R, Y2R and Y5R. In contrast, PP binds predominantly to Y4R and, with lower affinity, to Y5R. The phylogeny of the human YRs results in three receptor subfamilies and defined preference towards a specific receptor subtype: the Y1R subfamily (Y1R, Y4R, y6R and Y8R), the Y2R subfamily (Y2R and Y7R) and the single gene Y5R subfamily (16)(Figure 3). The Y1R subfamily is equally distant from the Y2R and Y5R subfamilies (17), which are also equally distant to one another. The original chromosome containing the receptor genes duplicated twice in vertebrates. Thus, the genes coding for Y1R, Y2R and Y5R arose by a local duplication of a common receptor ancestor and are present on the same chromosome in humans. Subsequently, a second duplication led to the Y1R-like genes Y4R and y6R (18). Only five receptor subtypes are present in mammals (Y1R, Y2R, Y4R, Y5R and y6R). While Y1R, Y2R, Y4R and Y5R are functional in all mammals, the y6R is non-functional in several mammals and in man and Y7R and Y8R were lost in the lineage leading to mammals (16). The receptor which was originally identified as the Y3R, based on pharmacological studies, has now been characterized as CXC chemokine receptor type 4 and is therefore included into the chemokine receptor family (19). Compared to other GPCR families, the human YRs exhibit relatively low levels of sequence identity. The Y1R shares its closest amino acid identity with the Y4R (42%) and the non-active form of y6R (51%) (Figure 3), but lower homology to Y2R (31%) and Y5R (35%) . In addition to distinct amino acid sequences, each of the YRs is characterized by a unique pharmacological profile and distinct tissue localization.

Y1 Receptor

The Y1R subtype is predominantly expressed in the central nervous system and the brain, including regions as the cerebral cortex, hypothalamus, thalamus and amygdala (20) and in a variety of tissues like heart, kidney, lung, colon, muscle cells, gastrointestinal tract and blood vessels (2). The most important Y1R-mediated effects of NPY are vasoconstriction, anxiolysis (2) and the stimulation of feeding, together with Y5R . The Y1R displays a highly conserved structure with overall identities of 94% to its orthologs and the human gene has been localized to chromosome 4q31.3–32 (21) (22). The Y1R exhibits almost equally high affinity for NPY and PYY, but a very low affinity for PP (Figure 2). All N-terminally truncated versions of NPY such as NPY2-36, NPY3-36 or NPY13-36 show intermediate or no affinity for the Y1R (22).

Y2 Receptor

The Y2R is predominantly expressed in the CNS including regions such as the brain cortex and hippocampus (23) but also located in the intestine and blood vessels. The presynaptically-expressed receptor suppresses the neurotransmitter release (24). The human Y2R gene is localized on chromosome 4q31–32 in proximity to the Y1R and the Y5R gene cluster(25). The Y2R shares very low degree of identity to the Y1R (31%) but is highly conserved in mammals showing 90% identity. Like Y1R, the receptor has high affinity for NPY and PYY, but not for PP (Figure 2). The Y2R is pharmacologically characterized by its ability to retain high affinity for N-terminally truncated peptide fragments, for instance, NPY3-36, NPY13-36, PYY3-36 and PYY13-36 (2). This receptor modulates fat mass and fundamental metabolic functions including the control of serum glucose, insulin and serum cholesterol.

Y4 Receptor

The Y4R is predominantly expressed in peripheral tissues such as heart, intestine, colon, pancreas, testis, prostate, lung and skeletal muscle (26) and, to a weak extent, in the hypothalamus, amygdala and thalamus(27). Y4R activation inhibits pancreatic secretion and gall bladder contraction (28). The Y4R has one of the least conserved sequences of the NPY hormone family, making it the fastest evolving receptor subtype. The receptor is most closely related to the Y1R (42%) and the truncated y6R (38%) since they evolved from a common ancestral gene(2). The

conservation between species is much less (75%) than that exhibited by Y1R and Y2R. In contrast to Y1R and Y2R, this subtype exhibits also a high affinity for PP and PYY, particularly for PP(29).

Y5 Receptor

The Y5R gene generates two splice variants, that differ in the N-terminal 10-amino acid extension. The long isoform consists of 455 amino acids and the short isoform of 445 amino acids, which show no differences in their pharmacological profile (30). Y5R is mainly expressed in the hippocampus and hypothalamus where it regulates food intake while it is rarely observed in peripheral tissues. However, Y5R mRNA has also been detected in the pancreas, gastrointestinal tract, muscle cells and cardiomyocytes (31). The Y1R and Y5R genes are transcribed in opposite directions from a common promoter region on chromosome 4q31.3–32 (31). The Y5R is almost always localized in neurons that also express Y1R. The Y5R exhibits very low identity to other YRs, with the highest identity (35%) to the Y1R. Some unusual features are the extended intracellular loop (ICL) 3 and short C-terminus. The protein is very well conserved in mammals with 90% overall identity. The Y5R pharmacological profile shares many features with that of the Y1R. The Y5R exhibits almost equally high affinity for NPY, PYY and PP. N-terminally truncated NPY, NPY13-36, shows intermediate affinity for Y5R and the receptor has substantial affinity for Y2R-specific agonists, such as NPY2-36 and NPY3-36 (2). The first Y5R-selective agonist was [Ala31,Aib32]-NPY, where residue 31 and 32 of NPY were substituted by the dipeptide Ala-α-aminoisobutyric acid in order to induce a more flexible, 310-helical turn structure in the C-terminal peptide region (32). Thus, Y5R-selectivity is related to the destabilization of the α-helical conformation at the C-terminal tetrapeptide, as shown for both Y5R-selective analogs [Ala31, Aib32]-NPY and [Ala31, Pro32]-NPY (33). The variants [D-Trp32]-NPY and [D-Trp34]-NPY show particularly improved affinity for Y5R, with significantly reduced potency at Y1R, Y2R, Y4R and y6R (34). In vivo investigation demonstrated that the orexigenic potency of [D-Trp34]-NPY exceeded that of [D-Trp32]-NPY (106).

Extensive knowledge on NPY and NPY receptors in animal models has been gathered over the last 30 years. However, apart from a dose finding study testing NPY administered intranasally in post traumatic depression (35), until now no clinical trial has been performed. The issue of clinical trials targeting YRs is discussed in the conclusive part of this review.

NPY, the cardiovascular system and the kidney

Coherent with its representation in the autonomic system and the co-release with nor-epinephrine, circulating NPY increases in response to physical exercise and orthostatism and in diseases states characterized by high sympathetic tone like heart failure and cardiac ischemia (4). Sympathetic nerve activity (SNA) is augmented early on in CKD (36) and increases progressively with declining renal function (37) which goes along with the severity of hypertension and left ventricular hypertrophy (LVH) (38) (39) in this condition. Similarly, a study based on two CKD cohorts documented a gradual rise in circulating NPY at progressively more severe degrees of renal dysfunction (40). Like nor-epinephrine, this peptide exerts pro-atherogenic effects because it promotes vascular smooth muscle proliferation, stimulates monocyte migration and activation, activates platelets, and stimulates angiogenesis (41). In injured rat carotid arteries, local delivery of NPY intensifies neointimal hyperplasia and this alteration improves by Y1R antagonism (42,43). Independently of hypertension, in CKD patients carotid intima thickness is strongly associated (R2=0.71) with the gene expression levels of NPY(44). However, the molecular NPY fragment 3-36 has favorable, protective, effects for the cardiovascular system because it stimulates Y2R mediated neo-angiogenesis in experimental ischemia and has an anti-fibrotic effect at myocardial level (45).

Observations in patients with kidney failure (stage G5 CKD) associated circulating NPY levels with LVH (46) and incident cardiovascular complications(47) and the link between NPY and incident cardiovascular events was more recently confirmed in pre-dialysis CKD patients (48). The direct link between NPY and left ventricular mass in kidney failure goes along with experimental observations showing that long-term subcutaneous infusion of NPY induces cardiac hypertrophy and dysfunction in rats (49), an effect mediated via calcineurin signaling (50) and the microrna-216b/foxo4 signaling pathway(51). On the other hand, other experimental data indicate that NPY co-released with norepinephrine mitigates the hypertrophic response of adult ventricular cardiomyocytes to norepinephrine(52). Overall, the direct hypertrophic effect of NPY probably prevails on the mitigation of nor-epinephrine induced LVH by the same peptide. NPY is a compound characterized by slow release and persistent actions under physiological conditions. Chronically increased NPY levels in the rat cause a doubling in cardiomyocyte mass (49). Sitagliptin, a dipeptidyl-peptidase 4 (DPP-4) inhibitor that raises NPY levels and potentiates

the vasoconstrictive response to NPY in healthy humans (5), augments the risk for heart failure in diabetic patients on dialysis(53), a population where LVH is robustly associated with NPY levels (46,47).

Renal disease progression is a multifactorial problem and sympathetic overactivity is a well documented risk factor for adverse renal outcomes (36)(54)(55). Together with hypertension, proteinuria is considered as the most important modifiable risk factor for CKD progression. NPY levels correlated with proteinuria and the GFR and predicted a faster progression rate toward kidney failure in the two cohorts study in CKD patients discussed above(40). The rs16139 polymorphism in the NPY gene associates with proteinuria (41) and increased susceptibility to nephropathy in type 1 diabetic patients(42) suggesting that the link between NPY and proteinuria is causal in nature, at least in type 1 diabetic patients . NPY levels reduce after renal denervation(56) and future studies applying this technique in patients with treatment-resistant hypertension may explore whether reduction in NPY is key to renoprotection in these patients. As alluded to before, NPY is an immunomodulatory factor (11). Inflammation is a fundamental risk factor the progression of CKD towards kidney failure (57) and future studies should test whether NPY may contribute to CKD progression by interfering with the inflammatory pathway. NPY is down-regulated in insulin-resistant vs. insulin-sensitive mouse podocytes and in human glomeruli of patients with diabetic kidney disease (58). This contrasts with the increased NPY levels that are commonly observed in CKD patients. However, the NPY-knockout mice, a model of NPY deficiency, exhibits less severe degrees of albuminuria and podocyte injury. Furthermore, NPY signaling in cultured podocytes via the Y2R stimulates phosphoinositide 3-kinase, mitogen-activated protein kinase, and nuclear factor of activated T-cells which are all fundamental factors in the immune response (58).

NPY, and the central and peripheral nervous system

NPY is one of the most evolutionarily conserved peptides across mammalian species (59). It is widely represented throughout the CNS including the cortical, limbic, hypothalamic, and brainstem regions, the neocortex, the amygdala, the hippocampus and the basal ganglia, the periaqueductal grey, dorsal raphe nucleus, and the A1-3 and A6 noradrenergic cell groups in the brainstem (60)(Figure 4). In the peripheral nervous system NPY is mainly expressed in sympathetic ganglia and the Y1R is densely represented in the colonic nerve plexuses.

NPY is a key neuro-mediator of appetite and has a relevant impact on nutrition and metabolism (61). In in the rat intracerebroventricular or intrahypothalamic administration of NPY triggers hyperphagia, body weight gain and increased adiposity, hyperinsulinemia, high leptin and high cortisol levels, and reduced thermogenesis in brown adipose tissue (62) (63). Furthermore, conditional knockdown of Y2 receptors at an adult stage prevents diet-induced obesity(64).

Importantly, NPY modulates stress-related emotions, anxiety and depression and is considered key for stress resilience. Plasma NPY concentration is raised in situations of extreme stress like in military survival training(65). However, when prolonged over time, stress eventually produces NPY depletion and a decline in plasma levels (ibidem). NPY levels are indeed reduced in patients with post-traumatic stress disorder (66). At least in experimental models in rodents, in most brain areas NPY levels and NPY-expressing cell counts are lower in females than in males, a phenomenon that could explain a higher susceptibility of stress related disorders in females (67).

NPY is involved in various domains of cognitive function .The highest concentrations of neuropeptide Y receptors are located in the hippocampus and high levels of NPY expression occurs in brain regions important for learning and memory (68). These effects of NPY are very diverse and complex. Indeed this peptide can inhibit or promote memory depending on the memory type or phase (i.e. acquisition, consolidation, retention, or retrieval), NPY dose applied, receptor type, and brain region (69). NPY enhances retention, a critical memory phase, when infused into the rostral hippocampus and septum, but inhibits this process when infused into the amygdala and caudal hippocampus and is ineffective when infused into the thalamus, caudate, or cortical regions above the rostral hippocampus and septum (70). Furthermore, NPY is more effective in enhancing memory consolidation, retention, and retrieval than memory acquisition. Recently, studies on social and non-social cognition have been performed in mice (71). Social memory is the ability of mice to discriminate between a previously encountered mice and a novel mice (social discrimination test) while non-social memory is the ability to discriminate between a previously encountered and a novel object (object discrimination test). Intracerebroventricular infusion of NPY prolonged retention of non-social memory, but not social memory and the Y1R antagonist BIBO3304 trifluoroacetate blocked the effect of NPY (71).

The effects of NPY on cognitive processes have been little investigated in man. Assessing the role of NPY in these process is fundamental in the clinical perspective of dementia. Studies in the nineties (72) observed subnormal NPY levels in the cerebrospinal fluid of patients with

dementia of Alzheimer type, a condition characterized by neuronal degeneration of temporo-parietal and temporo-limbic structures. This phenomenon was specific to Alzheimer dementia because no such alteration was registered in patients with frontotemporal degeneration of non-Alzheimer type. Of note, cerebrospinal-fluid levels of NPY correlated with clinical symptoms, such as restlessness, anxiety, irritability and depression (73). In contrast, other studies failed to confirm these observations (74). Data in experimental models have coherently shown that NPY is a neuroprotective peptide because it facilitates neurogenesis, has trophic effects on the nervous system and inhibits neuroinflammation (ibidem). However, no clinical trial has until now tested the hypothesis that these effects may translate into real clinical benefits in dementia. A randomized dose-ranging study of NPY in 26 patients with posttraumatic stress disorder has shown that higher doses of NPY administered by nasal route are associated with a greater treatment effect, favoring NPY over placebo on the Beck Anxiety Inventory score(35). In 2019 a clinical trial was registered for testing intranasal NPY in level 2 trauma patients with post traumatic stress disorder (75) but apparently recruitment of patients has yet to be started. About a quarter of hemodialysis patients in New Orleans developed post traumatic stress disorder after hurricane Katrina (76). Several other examples of exposure of the hemodialysis population to environmental disasters exist (77). These disasters provide an interesting opportunity for studying the association between NPY levels and this disorder. Indeed NPY in hemodialysis patients is about 6 times higher than in healthy individuals (46)(47) and at least in theory accumulation of NPY may protect hemodialysis patients from the same disorder. Cognitive dysfunction is common in the CKD population, particularly so among ESKD patients (78). At present we have no key to understand whether increased NPY levels in CKD and hemodialysis patients are protective, neutral or deleterious for cognitive dysfunction and dementia in these populations.

Exploring the potential implication of NPY in cardio-renal diseases and cognitive dysfunction and dementia by the mendelian randomization approach

Mendelian randomization is an established approach to explore cause-effect relationships minimizing or abolishing confounding in observational settings (79). This approach needs a genetic single nucleotide polymorphism (SNP) known to modify a risk factor suspected to cause a given health outcome. In the specific case of NPY, SNPs that reflect the availability and/or activity of NPY or its receptors can be used as unconfounded predictor variables for cognitive function. Indeed, SNPs are inherited randomly and as such are not subject to confounding by

environmental risk factors that my influence the gene product (NPY and NPY receptors in our case). Therefore, any link between the SNP and the health outcome of interest should be interpreted as causative. In other words, if a SNP known to increase NPY is also linked to an outcome, high NPY levels must be considered the cause of the outcome. Because some SNPs explored as genetic markers are found only in certain ethnicities and because it is possible that different populations differ for multiple SNPs of diverse genes, mendelian randomization studies demand homogeneous study populations. Mendelian randomization has been used to study genetically regulated variables as possible causes of dementia and telomere length has been associated with Alzheimer dementia (80).

NPY SNPs

According to the Genome Aggregation database (gnomAD) (81), which comprises three databases (ExAC, gnomAD 2.1 and 3.1) with more than 80,000 genes, there are 198 NPY variants in the population. Most are too uncommon (from 3 in a million to 9 in 1,000) to be used for a Mendelian approach. The main SNPs associated with phenotypes of interest for this review are rs16139, rs16147 and rs3037354 (Table 1 and further details shown in Suppl Table 1). The rs16147 (−399C) variant in the promoter region of the NPY gene and the rs2234759 in the Y2R gene (82) associate with faster iconic memory fading in individuals carrying the (rare) G allele of the rs16147 variant and the rs2234759 variant in the Y2R gene which also associates with increased expression of the Y2R.

rs16139 is a benign missense SNP resulting in substitution of leucine by proline at residue 7 in the signal peptide of pre-pro-NPY. It is considered a gain-of-function SNP that modifies the efficiency of NPY processing, facilitating the accumulation of NPY rather than of pro-NPY in endothelial cells and increasing NPY in response to sympathetic stimulation (83). However, in resting conditions NPY concentration is lower in the L7P subjects than in L7L subjects (84) suggesting that the Leu7Pro polymorphism has different effects on the plasma NPY kinetics at rest and exercise. It is possible that in resting conditions, this polymorphism leads to impaired release and intracellular retention of NPY, followed by an exaggerated release of NPY in high- intensity sympathetic stimulation (84). The L7P variant has a global frequency of 3%, but its distribution is uneven ranging from absent in East Asia to 3 to 4% in South Asia and Europe and 7% in Finland (85). Thus, studies in East Asia will likely be uninformative and inclusion of East Asians in global studies may introduce biases. Information on disease associations of L7P originated in Finland, where more

than 20 years ago it was associated with traits of the metabolic syndrome such as weight gain, hyperlipidemia, impaired glucose tolerance, insulin resistance, earlier onset of T2DM and increased risk of vascular disease (86)(87)(88)(89)(90). It has since been also associated with hypertension (91) , coronary heart disease (92), diabetic kidney disease (93), diabetic retinopathy (94) and alcohol dependence (95).

rs16147 (−399C) is a 5' UTR SNP also reported to influence NPY levels that may potentially interact with L7P. Located in YNP promoter, it accounted for a 30% decrease in basal gene expression and was a key component of haplotypes associated with lower NPY gene expression(96). It was associated with higher emotion-induced activation of the amygdala, and diminished resiliency as assessed by pain/stress-induced activations of endogenous opioid neurotransmission in various brain regions (ibidem). In Finland, five haplotypes were found in 94% of chromosomes. −399C belongs to the frequent H1 haplotype (0.45) and the infrequent H4 haplotype (0.04), both associated with low NPY expression, while the frequency of the H5 haplotype uniquely containing L7P was 0.05 and its association with gene expression could not be determined as it was too infrequent in a US sample. In obese males, rs164147 was associated with an increased risk of metabolic syndrome and its related phenotypes, such as central obesity and hyperglycemia (97). It was also associated with ischemic stroke (98) and early onset coronary artery disease(99). By contrast, rs3037354 (∇-880Δ) was associated with increased NPY secretion, enhanced BP response to environmental (cold) stress, and higher basal systemic vascular resistance (100) (Suppl Table 1). Finally, rare copy variants and copy number variants have been associated with early onset obesity (101).

SNPs for NPY receptors

NPY1R, NPY2R and NPY5R are expressed in the brain [Allen Brain Atlas (102)] and Human Protein Atlas database (60)]. The variants with greater frequency in the population are synonymous variations or variations in the 5’ UTR, some of which may influence gene expression and have been associated with phenotypes of interest for kidney, cardiovascular or CNS disease (Suppl Table 1).

Mendelian randomization design for NPY SNPs

Because its frequency in the general population (3.6% in non-Finnish Europeans) and its repeatedly reported association with outcomes of interest, L7P appears well suited for a mendelian randomization study to disentangle the role of NPY in cognitive impairment in CKD patients. However, such study should ideally recruit non-Finnish Europeans and/or South Asians to

minimize confounding by other genetic or environmental characteristics found in populations with higher (e.g. Finland) or lower (e.g. East Asia, Africa, American First nations and descendants) frequencies of the allele. Furthermore, the study should be controlled for common genetic variants in NPY or NPY receptors that have been associated with phenotypes. As alternatives, other NPY SNPs reported to have a functional impact may be used, such as rs16147.

UK Biobank collected data from over 500,000 adults. The cognitive assessment in UK Biobank is brief and customized and it is administered without supervision but the test-retest reliability is reasonably good (103). This Biobank provides unparalleled genetic data (104) and represents an unique opportunity for mendelian randomization studies testing the relationship between cognitive function and NPY and YRs genes at general population level and in the CKD population. Furthermore, databases including CKD patients linked with plasma and sera biobanks that collected information on cognitive function exist and these databases may complement genetic databases (105).

Conclusive remarks: why knowledge on the NPY system accumulated so far is still untranslated

In theory, interfering with the NPY system has relevant potential for the treatment of diverse diseases, from CNS diseases to metabolic, cardiovascular and renal diseases. Anxiety, depression, learning and memory and, more in general, cognitive problems are all potentially addressable by interventions on the NPY system. However, in the face of a large series of experimental studies in animal models and in genetically engineered animals, until now no pharmacologic intervention on the NPY system has been tested in adequately powered clinical. The main problem is the complexity of diseases potentially treatable by interfering with the NPY system. The diversity of actions of NPY on organ systems which are in part of opposite sign (e.g. noxious effects in the cardiovascular system and potentially useful effects on the CNS) has probably restrained clinical investigators and the industry to invest on clinical research. Furthermore, because mechanisms underlying energy homeostasis are highly integrated and redundant, interventions on just one component of the system may elicit counter-regulatory responses cancelling out the primary effect of the intervention. Furthermore, pharmaceutical formulations for effective drug delivery and cost have been a problem and this in part explains the slow progress of knowledge about the NPY system in the clinical arena. In experimental models NPY agonists and antagonists have been often administered intracerebrally which hinders the

translation value of findings in these studies to human diseases. Yet Y-receptors are expressed in peripheral organs like the adipose tissue and the pancreas, indicating a direct function of the NPY system in the control of glucose and energy homeostasis . This suggests that antagonism of peripheral receptors may be useful for the treatment of obesity by routes that can be applied in clinical practice. A recent study (106) tested selective antagonism of peripheral Y1R by BIBO3304, an antagonist that does not pass the blood-brain barrier in diet-induced obesity in mice. Remarkably, BIBO3304 reduced energy expenditure and body weight and fat mass following exposure to a high calorie diet. Importantly, the Y1R is also expressed in blood vessels acting as a vasoconstrictor. Therefore, peripheral Y1R antagonism has the potential for reducing BP which has obvious, additional benefits. These observations in mice warrant a renewed interest on the NPY system by pharmacologists and clinical investigators alike and will hopefully generate useful application of Y1R antagonism in metabolic and cardiovascular diseases. Cognitive dysfunction and dementia represent the most complex diseases that medicine has to face. With the exception of the NPY trial in post traumatic depression (35) no human studies have been performed in diseases of the CNS. We are perhaps at a critical juncture in NPY research. New drug formulation and renovated research efforts may propel NPY system research in neurology into a new era. Psychopharmacology developed as a discipline from the mid-20th century. After the discovery of the antidepressants, antipsychotics, anxiolytics and mood stabilizers currently in use today, research slowed down and most major pharmaceutical companies disinvested from psychopharmacology. However, new avenues are now being explored (107). Renewed interest in this area and research on the NPY system may eventually bring the expected benefits on human health by drugs interfering with this system

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Carmine Zoccali (CZ) designed the review plan and wrote the first version of the review with Francesca Mallamaci (FM), Alberto Ortiz (AO), Inga Arune Blumbyte (IAB), Annette G. Beck-Sickinger (AGBS), Jolanta Malyszko (JM) and Goce Spasowsky (GS). Sarina Rudolf, Sol Carriazo, Davide Viggiano refined the literature search and integrated the writing the first version by CZ, FM, AO,IAB, AGBS JM and GS. Justina Kurganaite, Vaiva Sarkeviciene, Daiva Rastenyte, Andreja Figurek, Merita Rroji, Mayer Christopher, Mustapha Arici, Gianvito Martino, Gioacchino Tedeschi, Annette Bruchfeld, Belinda Spoto, Ivan Rychlich, Andrzej Wiecek, Mark Okusa, Giuseppe Remuzzi critically read the first version of the manuscript and provided suggestions for changes and additions to the manuscript. Carmine Zoccali prepared the final version of the paper which was approved by all authors.

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APPENDIX

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Table 1. Genetic variants of NPY and its receptors and their associated phenotypes. Further details on other gene variants associated with central nervous system and cardiovascular disease are shown in suppl table 1. n.a.=not available

Gene	Total number of variants	Number of variants with a frequency higher than 1:100 and their type	Variants reported in Clinvar and their frequency in the population	NPY levels and Neurological phenotype	Cardiovascular phenotype
NPY	198	7 (three synonymous variants, one 5' UTR variant, two intron variants, and one missense variant)	rs16139 (3%)	↓ plasma NPY ↑ alcohol intake	↑ serum LDL ↑ carotid intima-media thickness
NPY1R	313	2 (5' UTR variants)	rs5578 (0.5%)	n.a.	n.a.
NPY2R	347	2 (synonymous variants)	rs141746382 (0.04%); rs200831948(<<0.001%); rs142187929 (0.03%)	n.a.	n.a.
NPY5R	359	2 (5' UTR variants)	rs188410293 (0.03%); rs77419821 (<<0.001%)	n.a.	n.a.

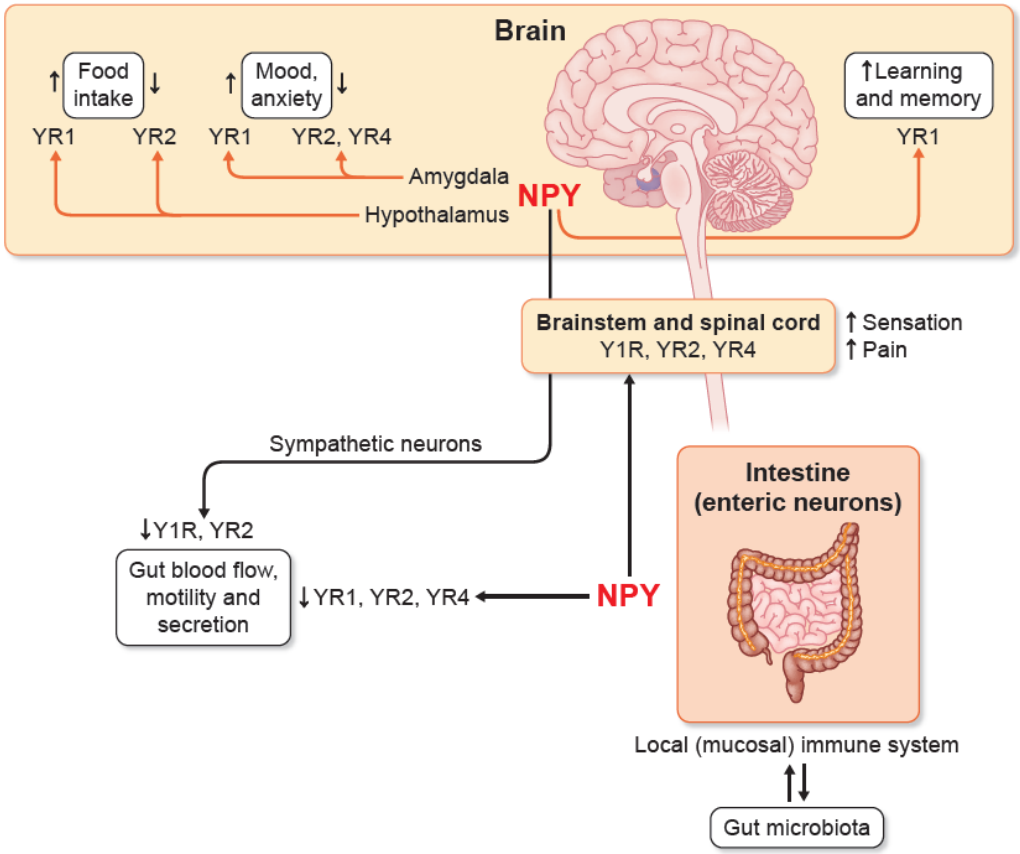

Figure 1 The NPY and the gut–brain axis. The graph shows the Y receptor subtypes which mediate the effects of NPY in the Central Nervous System and at the different levels of the gut–brain axis. (↑) increase, (↓) decrease.

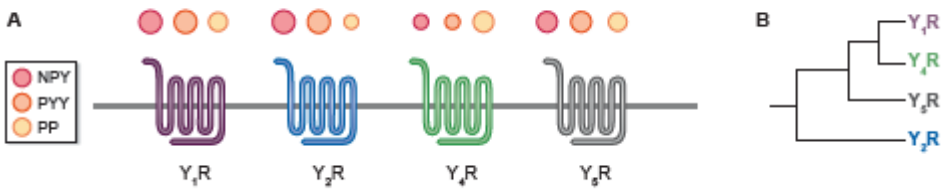

Figure 2 Selectivity within the NPY family. (A) The three endogenous ligands -NPY, PYY and PP- exhibit varying degrees of affinity and specificity for four human YRs. The ligand potency at the respective receptor is reflected by the size of the above sphere. NPY and PYY bind with relatively high affinity to Y1R, Y2R and Y5R. In contrast, PP binds predominantly to Y4R and with lower affinity to Y5R. (B) Phylogeny of the human YRs resulting into three receptor subfamilies and defined preference towards a specific receptor subtype.

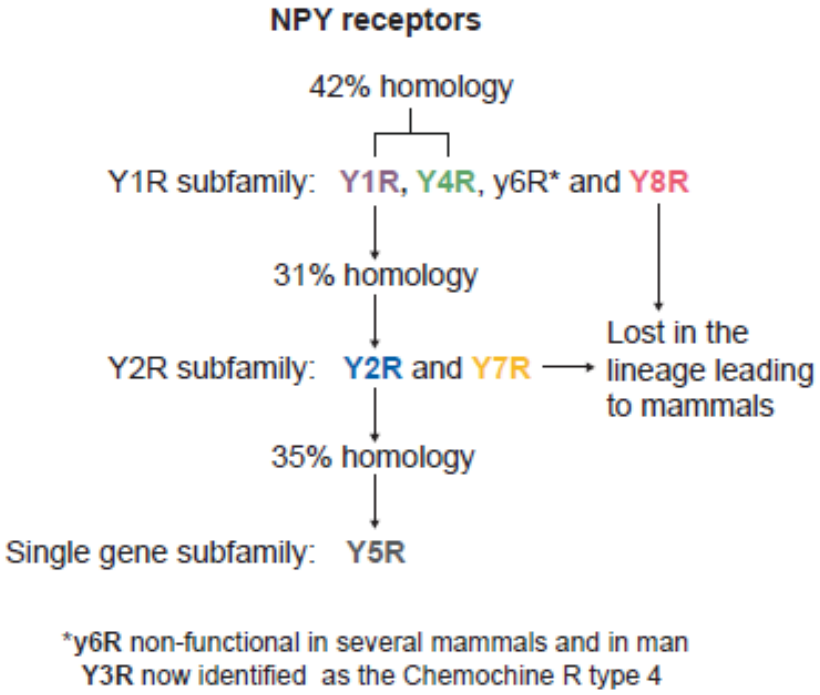

Figure 3 NPY receptors. Y1R, Y2R, Y4R and Y5R are the four active receptors in mammals. yR6 is functionally inactive and YR7 and YR8 got lost in the lineage leading to mammals

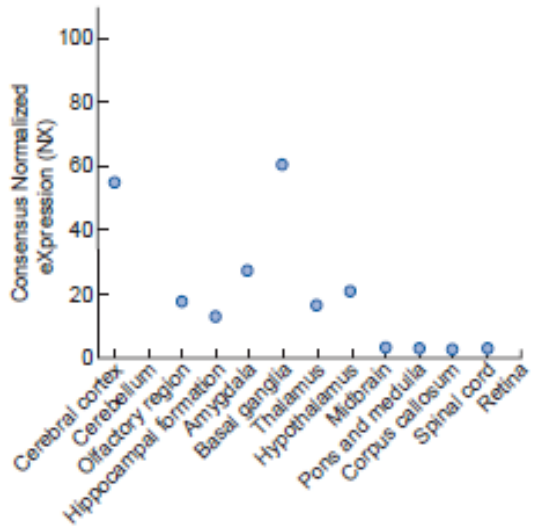

Figure 4 The figures whereupon the graph is based were derived from data reported in the human protein atlas. Consensus Normalized eXpression (NX) levels of NPY for the Central Nervous system created by combining the data from the three transcriptomics datasets using internal normalization criteria (se also main text).