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REVIEW

VGF: A BRIEF INTRODUCTION TO IT'S STRUCTURE, TISSUE DISTRIBUTION AND FUNCTIONS

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ABSTRACT

VGF (a non-acronymic name) a ~68 kDa neurosecretory protein, belongs to the extended granin family of proteins, initially identified as a nerve growth factor (NGF) inducible gene product, is selectively synthesised mostly in neuronal and neuroendocrine cells. Due to the presence of paired basic amino acid residues (R – Arginine, and K – Lysine), the VGF sequence undergoes endoproteolytic cleavage to produce several smaller peptides, released upon stimulation via the regulated secretory pathway both in vitro and in vivo. There are data suggesting that the VGF-derived peptides are the biologically active, stored in dense core vesicles and secreted in order to play a role in inter cellular communication, and responsible for the diverse range of biological functions associated with VGF. Several of these VGF-derived peptides have been characterised and are involved in energy balance, reproductive behaviour, pain modulation, mood order, etc. In this review the most important findings regarding the VGF derived peptides, structure, tissue distribution and functions of VGF have been summarized, mentioning its future perspective.

Keywords: *VGF, peptide, regulation, pain, psychiatric disease*

INTRODUCTION

VGF gene was originally identified as a nerve growth factor (NGF) responsive gene and should not be confused with VEGF (vascular endothelial growth factor). NGF33.1, a nervous system-specific mRNA was cloned by treatment of PC12 cells with NGF. After elucidating the nucleic acid as well as amino acid sequences of the NGF33.1 cDNA clone, **Levi *et al*.** (**1985**) designated this clone corresponding to the NGF-inducible mRNA as VGF. The term 'VGF' derived from the selection of this clone from plate **V** of the nerve **G**rowth **F**actor induced PC12 cell cDNA library (**Levi *et al*., 1985; Possenti *et al*., 1989**). Indeed, in response to NGF treatment, VGF was independently identified by several groups using differential hybridization techniques (**Cho *et al*., 1989; Levi *et al*., 1985; Salton *et al*., 1991**). In this review, structure, tissue distribution and functions of VGF will be discussed.

STRUCTURE OF VGF

The human and mouse VGF genes are located on chromosome 7q22.1 and 5 (**Hahm *et al*., 1999; Canu *et al*., 1997a; Canu *et al*., 1997b**) and encode a 615 amino acid (human) and 617 amino acid (rat), respectively (**Levi *et al*, 1985**). VGF is a single copy gene (**Canu *et al*., 1997a**) and is simply organized as depicted in Figure 1 and 2. It is composed of 3 exons but the complete VGF protein is encoded by exon 3 only while two introns interrupt the region encoding the 5' untranslated sequence of VGF (**Hahm *et al*., 1999**). C-terminal peptide regions encode a number of bioactive peptides. As depicted in Table 1, VGF is a proline, and glycine rich and acidic polypeptide. Although there are evolutionary conservation of other invertebrate granins (a family of proteins comprising chromogranins, secretogranins and some other related proteins (**Bartolomucci *et al*., 2011**) found in the secretory granules of endocrine, neuroendocrine cells and neurons (**Deftos *et al*., 1986; Fischer-Colbrie *et al*., 1987**), that function to control the supply of neurotransmitters, hormones, growth factors and peptides (**Bartolomucci *et al*., 2011**); no invertebrate VGF proteins have been identified so far. And several regions of high sequence conservation were found in both lower and higher vertebrate VGF proteins (Figure 3).

Table 1: Introductory informations on VGF, at a glance. *(Number of amino acids (AA) and calculated molecular mass (MM) of the preprotein, number of amino acids and calculated molecular mass of the mature protein, observed molecular mass of the mature protein, number of dibasic sites, number/content of proline, number/content of glutamate, calculated (calc) and observed (obs) pI, and secondary structure (percent α-helix) are shown for human (h), ND, not determined. From* ***Bartolomucci et al., 2011****).*

	Preprotein		Mature protein							
	AA[h]	Calculated MM[h] (kDa)	AA[h]	Calculated MM[h] (kDa)	Observed MM (kDa)	Dibasic sites[h]	AA/% proline[h]	AA/% glutamate[h]	pI calc[h]/obs	% α-Helix[h]
VGF	615	67	593	65	90[h]	10	77/12.5	97/15.8	4.5/ND	39

VGF DERIVED PEPTIDES

In human, rat, mouse and chimpanzee (**Hahm *et al.*, 1999; Canu *et al.*, 1997a; Possenti *et al.*, 1989**), VGF protein possesses ten (**Levi *et al.*, 2004**) paired basic amino acid residues (as illustrated in the Figure 3) having potential cleavage sites for the best characterized mammalian prohormone processing enzymes, prohormone convertases (PCs), members of the family of subtilisin/kexin-like serine proteinases (**Seidah and Chretien, 1999; Steiner, 1998**). For example, TLQP-62 is produced by PC1/3, while NAPP-129 has been produced by the action of either PC1/3 or PC2 (**Trani *et al.*, 2002**).

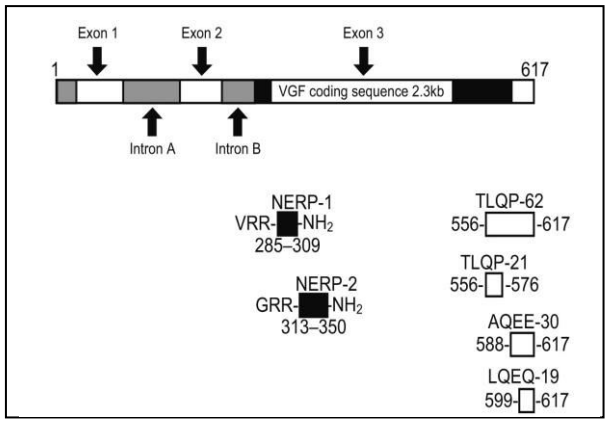

Figure 1: Schematic diagram of the VGF gene and its derived peptides (mouse). *The numbers correspond to the positions of amino acid residues in mouse VGF protein. The closed black boxes represent neuroendocrine regulatory peptides (NERPs): NERP-1 and NERP-2 and the rest of the boxes represent other peptides: TLQP-62, TLQP-21, AQEE-30 and LQEQ-19. (From **Toshinai and Nakazato, 2009**).*

N-terminal VGF peptides (human) are not compatible with known motifs required for PCs. So some VGF peptides must be generated by other yet unidentified endoproteases. As mentioned earlier, in rat, mouse and human VGF protein comprises at least 10 basic amino acid residues susceptible to potential PC cleavage. Hence the number of known VGF derived peptidesis likely to increasefurther. To understand the contributions of 'such a variety of VGF peptides' individually as well as their mode of action has now become a big challenge.

VGF in PC12 cells appears as a doublet of 80–90 kDa when analysed by Western blot though its calculated molecular weight is 68 kDa. The lower band was confirmed to be generated from the upper, probably due to limited proteolysis, providing that no post-translational modification occurred (**Levi *et al.*, 1985**). Finally it was concluded that the very high proline content accounts for the lower than expected electrophoretic mobility of the protein. In addition to a doublet of 80–90 kDa, several smaller peptides were identified in rat brain homogenates, neuronal, endocrine and pancreatic beta cell lines, in extracts of primary cultures of cerebellar granule cells in response to the antibodies raised against the C-terminal nonapeptide of rat VGF protein (**Possenti *et al.*, 1999; Trani *et al.*, 1995**). Several VGF peptides were revealed out in rat brain homogenates by mass spectroscopy followed by affinity purification with C-terminal antiserum, in addition to more lower molecular-weight peptides detected by Western Blot Analysis (**Trani *et al.*, 2002**).

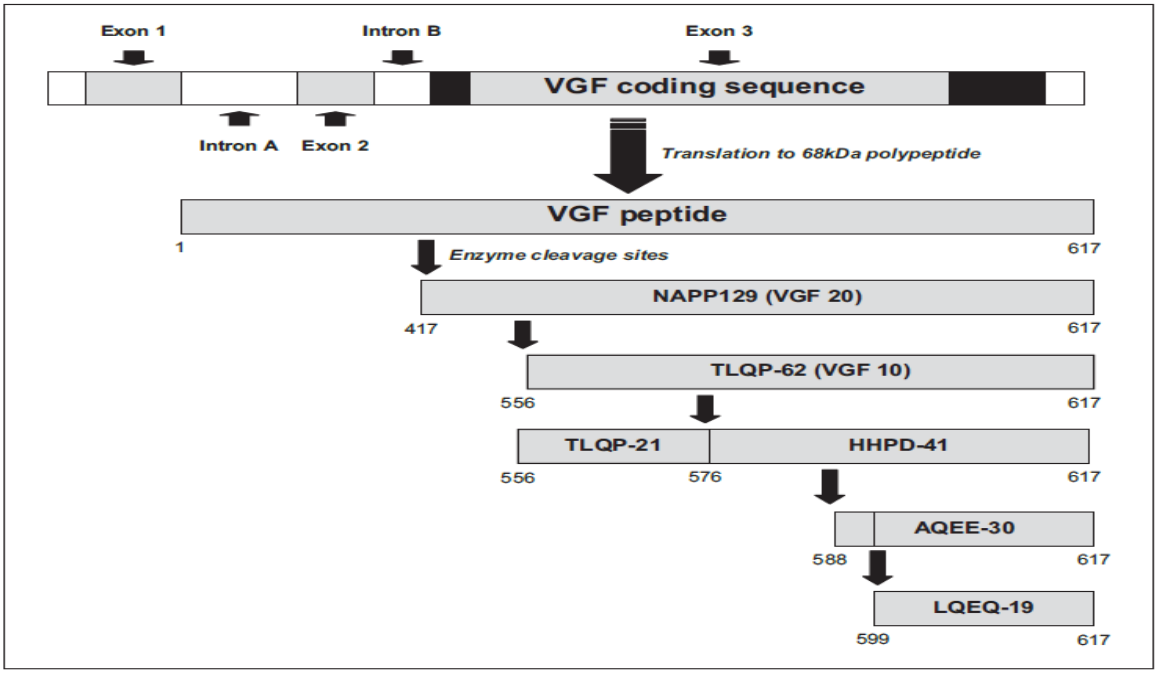

Figure 2: Schematic diagram of the VGF gene and its derived peptides (mouse). *VGF gene with its simple structure encodes a 617-amino acid protein in rats, and a 615-amino acid protein in humans, subjected to cleavage into several peptides. Two introns interrupt the region encoding the 5' untranslated sequence of VGF, and the entire VGF protein is encoded by exon 3. Most of the fragments are capable to show biological activity, indicated in Table 2 (From **Jethwa and Ebling, 2008**).*

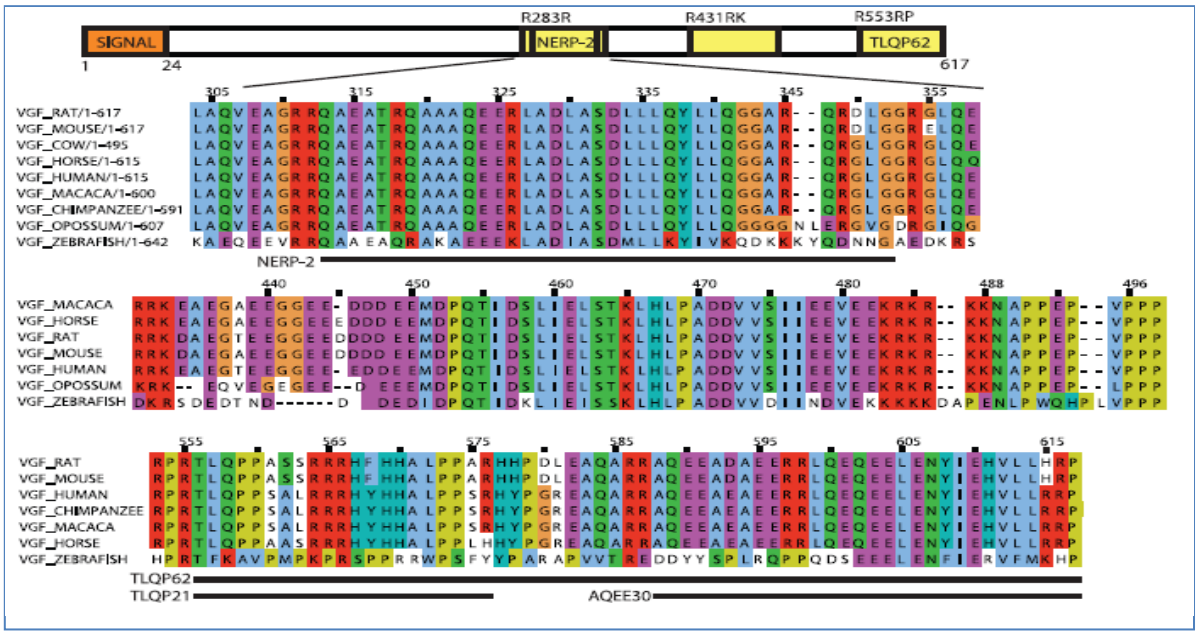

Figure 3: Evolutionary conservation of VGF. *Each of the three highly conserved regions of VGF: NERP-2, TLQP and AQEE peptides, are conserved in both higher and lower vertebrates (From* ***Bartolomucci et al., 2011****).*

Table 2: VGF derived peptides. *Nomenclature of the peptides was simplified by the first four amino acids and by its length, such as: TLQP-21 and TLQP-62 are two peptides with TLQP (Thr-Leu-Gln-Pro, respectively) sequences at N-terminus with total length 21 and 62 amino acids, respectively except the neuroendocrine regulatory peptides, NERPs (an acronym for neuroendocrine regulatory peptides) and VGF 20 (20 kDa, based on the apparent molecular weights observed by Western blot analysis). (Adapted from* ***Jethwa and Ebling, 2008****).*

Peptide names	Amino acid position on VGF precursor	Length	Biological activity known?
APPG-40	23-62	40	No
APPG-37	23-59	37	No
GRPE-37	26-62	37	No
NERP-1	302-309	8	Yes
NERP-2	341-350	10	Yes
NAPP-129 (VGF 20)	417-617	201	No
VGF 18	Unknown	---	No
TLQP-62 (VGF 10)	556-617	62	Yes
HFHH-51 (VGF 6)	567-617	51	No
HHPD-41	576-617	41	No
AQEE-30 (Peptide V)	588-617	30	Yes
LQEQ-19	599-617	19	Yes
TLQP-21	556-576	21	Yes

TISSUE DISTRIBUTION OF VGF

VGF mRNA is expressed in the main and accessory olfactory bulbs, hippocampus, cortex, basal ganglia, thalamus, amygdala, midbrain and the brainstem, withthe highest expression in neurons in the hypothalamus and in the granular layer of the cerebellum (**van den Pol *et al*., 1989; 1994; Snyder *et al*., 1997; 1998**). After birth, rats express VGF throughout brain (in neurons exclusively), andin peripheral endocrine and neuroendocrine tissues (**Snyder *et al*., 1998**). Later on in the adult rat brain, VGF mRNA becomes widely distributed in the spinal cord, olfactory system, cerebral cortex, hypothalamus, and hippocampus, and also in thalamic, septal, amygdaloid, and brain stem nuclei (**Snyder *et al*., 1998; van den Pol *et al*., 1989; 1994**). Before birth, rats express VGF at first on embryonic day 11.5 in the dorsal root and sympathetic ganglia, neural crest cells migrating to enteric ganglia and in primordia of the vagal (X) complex (**Snyder *et al*., 1998**). VGF mRNA continues to be expressed in the ventral spinal cord, cranial nuclei, basal forebrain, adrenal and pituitary in between of embryonic day 13.5 to 15.5. Later in embryogenesis, VGF expression starts to additional CNS sites as well as becomes prevalent in the esophagus, stomach (both in endocrine cells and myenteric plexus), and pancreas in between of embryonic day 17.5 to 19.5). High expression of VGF mRNA was evident in oligodentrocyte precursors though it was found to disappear following differentiation (**Dugas *et al*., 2006; Cheishvili *et al*., 2007**). VGF derived peptides were also found to be expressed in peripheral leukocytes (**Cattaneo *et al*., 2010**) and glial fibrillary acidic (GFA) protein-expressing astrocytes of the spinal cord in transgenic mutant SOD1G93A mice. In the spinal cords of sporadic amyotrophic lateral sclerosis (ALS) patients, VGF expression was found low in comparison to control patients. A small molecule, SUN N8075 was capable to slow down disease progression in SOD1 mouse and rat models of ALS, resisting the lowering of VGF level in the spinal cords of ALS mice (**Shimazawa *et al*., 2010**). However, further investigation regarding the unexpected localization of VGF may point out towards unexpected functional roles. To summarize, VGF is synthesized by neurons in the CNS and PNS, as well as in the adult pituitary, adrenal medulla,

endocrine cells of the stomach and pancreatic beta cells, moreover, VGF mRNA is obvious at embryonic day 11·5 in the rat dorsal root and sympathetic ganglia (**Snyder *et al*., 2003**).

All these informations regarding tissue specific expression and distribution of VGF suggest that VGF has different roles in the concerned tissues.

FUNCTIONS OF VGF

Regulation of energy homeostasis

Studies on VGF knockout mice demonstrated that they were hyperactive, hypermetabolic, lean, and small with less abdominal fat (**Hahm *et al*., 1999**). VGF derived peptide, TLQP-21 was found to decrease food intake and to rise energy expenditure in Siberian hamsters (**Jethwa *et al*., 2007**). Also in mice fed high-fat diet (HFD) for 14 days, TLQP-21 decreased body weight and white adipose tissue (WAT) blocking hormonal changes associated with HFD and provoking the autonomic activation of the adrenal medulla and adipose tissue. Furthermore, intracerebroventricular (icv) administration of TLQP-21 reduced early phase diet induced obesity (**Bartolomucci, *et al*., 2006**). Thus, VGF derived peptide TLQP-21 shows catabolic activity (**Jethwa *et al*., 2007; Bartolomucci *et al*., 2006**) whereas VGF knockout mice demonstrated the opposite trend (**Hahm *et al*., 1999**). The hyphothesis to explain this inconsistency could be: one or more VGF derived peptides possess opposite biological roles with respect to TLQP-21. There are evidences that upkeep the hyphothesis: administration of TLQP-62, HHPD-41 increased food intake in fasting mice (**Bartolomucci *et al*., 2007**). While, NERP-2 icv administration to rats and mice increased food intake, body temperature, O_2 consumption, locomotor activity promptly (**Yamaguchi *et al*., 2007; Toshinai and Nakazato, 2009; Toshinai *et al*., 2010**).

Regulation of gastrointestinal function

There are evidences suggestive of the central role of TLQP-21 in intermediating gastroenteric functions by the initiation of prostaglandin (PG) synthesis. Icv, but not intraperitoneal (ip), or intravenous (iv) injection of TLQP-21 inhibited gastric emptying almost ~40% in a time (**Severini *et al*., 2009**) as well as dose dependent manner and reduced ethanol induced gastric lesions in rats through nitric oxide synthase and PG E_2. It also reduced gastric acid secretion through somatostatin and PG (**Sibilia *et al*., 2010a; 2010b**). The peptide also induced contraction of gastric fundic strips ex vivo by PG mucosal release (**Severini, *et al*., 2009**).

Regulation of hormone, neurotrophin, and/or neurotransmitter release

TLQP-62, in hippocampal slices, provoked electrical potentiation that was found to be blocked by the brain-derived neurotrophic factor (BDNF) scavenger TrkB-Fc, Trk tyrosine kinase inhibitor K252a, and tissue plasminogen activator STOP (**Bozdagi *et al*., 2008**), pointing out the probable role of TLQP-62 in inducing release of BDNF in the hippocampus and other regions of CNS. The BDNF-receptor, TrkB, is progressively activated in vitro by TLQP-62 to initiate the TLQP-62 induced proliferation of neural progenitor cells (NPCs) (**Thakker-Varia *et al*., 2014**) in consistent with the upregulation of VGF protein by BDNF treatment (**Alder *et al*., 2003**). Accordingly, AQEE-30 and LQEQ-19 upregulate phosphorylation of MAPK p^{38} in microglia (**Riedl *et al*., 2009**) in nociceptive signaling with the probable induction of BDNF (**Coull *et al*., 2005**). Another VGF derived peptide, neuroendocrine regulatory peptide (NERP) increased food intake in mice through the activation of an orexin dependent mechanism indicating that NERP induces the release of neuropeptide orexin in hypothalamus (**Toshinai K *et al*., 2010**).

Regulation of pain

In the first study highlighting the role of TLQP-21 in pain modulation , the forepaw-injected formalin test confirmed the induction of analgesic effect by TLQP-21, additionally it was established that the inflammatory modulatory effects of TLQP-21 depend on the route of administration : being pronociceptive at the periphery and antinociceptive at the central level (**Rizzi *et al*., 2008**). TLQP-21 has been found to provoke a hyperalgesic response when it was subcutaneously injected into the hind paw of mice. Mechanical hypersensitivity was apparent in rat following inoculation of TLQP-21-stimulated macrophages into rat hind paw and intracellular Ca^{2+} levels in macrophages was lessened via siRNA suggesting that TLQP-21 plays an important role in inducing heperglesia/hepersensitivity/ chronic pain through activation of macrophages (**Chen *et al*., 2013**). Moreover, *in vivo*, long-lasting mechanical and cold behaviouralallodynia (pain produced by stimuli which usually do not provoke pain (**Merskey and Bogduk, 1994**) like temperature, cold or physical stimuli (**Hooshmand and Hooshang, 1993**) was persuadedby TLQP-62 (**Moss *et al*., 2008**). AQEE-30 and LQEQ-19 caused hyperalgesia via microglial p38 MAPK activation (**Riedl *et al*., 2009**). To sum up, both the short and long VGF derived peptides like LQEQ-19 (**Riedl *et al*., 2009**), TLQP-21 (**Fairbanks *et al*., 2014; Chen *et al*, 2013; Rizzi *et al*., 2008**), AQEE-30 (**Riedl *et al*., 2009**), TLQP-62 (**Moss *et al*., 2008**) were found to be responsible for the consistent induction of analgesia/ hyperalgesia/hepersensitivity/chronic pain in diverse models of pain.

Regulation of emotion/psychiatric disease

Probably out of the granin family members, VGF has been the most explored for its role as well as mechanism in emotional behavior and psychiatric disease (**Bartolomucci *et al*., 2011**), while there are very few data suggesting the role of other granin family members like secrotogranin II (SgII)-derived peptides GE19, GAIPIRR and SN (secretoneurin) in emotional behavior (**Wakonigg *et al*., 2002**). Since the time period when VGF mRNA was found in the hippocampal areas, VGF was thought to play a crucial role in this area (**Snyder and Salton, 1998**) which was later confirmed.

VGF has antidepressant-like actions in rodents **(Hunsberger *et al*., 2007, Thakker-Varia *et al*., 2007; 2010**). For the last

two decades, this VGF peptide is getting more importance due to the unveiling of its role as an anti-depressant. VGF $^{+/-}$ mice exhibit deficits in experimental paradigms of depression (**Hunsberger *et al*., 2007**). VGF mRNA extensively co-localizes throughout the central nervous system (CNS) with mRNA encoding brain derived neurotrophic factor (BDNF) and the BDNF receptor, TrkB (**Snyder *et al*., 1997**), suggesting that VGF could potentially mediate some of the behavioral and electrophysiological actions ascribed to BDNF signalling. BDNF is a central player in the "adult neurogenesis theory of depression" (**Gilhooley *et al*., 2010**).

Recently, using a proteomic approach, VGF was found to be one of the proteins whose expression more conspicuously decreased as a result of ShRNA-based DISC1 (Disrupted-in-schizophrenia 1, one of the very few genes directly correlated with psychiatric illness and was co-segregated with schizophrenia, bipolar disorder and major depression in a large Scottish pedigree (**Chubb *et al*., 2008**) silencing in human SH-SY5Y neuroblastoma cells. Subsequently, this result was confirmed by means of Western blot analysis in the same cells and in ShRNA-silenced murine primary neurons (**Ramos *et al*., 2014**). In this context, the finding that silencing of DISC1 causes a marked decrease of expression of VGF, a protein that is so critically involved in depression, bipolar disorder, and schizophrenia, is of particular relevance. Further, a recent study found that NPAS3, a transcription factor that is a replicated genetic risk factor for psychiatric disorders, regulates VGF (**Sha *et al*., 2012**). Therefore, three independent proteins with strong associations with mental disease, DISC1, NPAS3 and BDNF, seem to have VGF as a downstream effector, making VGF, and more specifically, the putative receptor of VGF-derived peptides, a very attractive target of pharmacological intervention to develop new drugs effective in the treatment of mental diseases. Though till to date, VGF derived peptide TLQP-62 (**Lin *et al*., 2014; Bozdagi *et al*., 2008**) has been found to be linked up with mental depresión, etc., not TLQP-21.

Regulation of sexual function

In consistent with both male and female infertility observed in VGF knockout mice (**Hahm *et al*., 1999; 2002**), following repeated administration of TLQP-21 on adolescent males with chronic food deprivation the gonadotrophin response of hypothalamic-pituitary-gonadal axis was reduced. However, in case of properly nourishley fed mice, only puberty onset was late (**Pinilla *et al*., 2011**). Other VGF derived peptides namely AQEE-11, LQEQ-19, AQEE-30 and HHPD-41were found to facilitate penile erection capability in rats via the mechanism of nitric oxide production in the paraventricular nucleus (PVN) followed by the activation of PVN oxytocinergic neurons (**Succu *et al*., 2004; 2005**).

Regulation of body fluid homeostasis

With the aid of immunogold electron microscopy, neuroendocrine regulatory peptides (NERPs) colocalization was confirmed with vasopressin in storage granules (**Toshinaiand Nakazato, 2009**). VGF mRNA in rats was found to be upregulated as a consequence of water deprivation, in consistent with the upregulation of the vasopressin mRNA. VGF mRNA was also increased with vasopressin mRNA level following salt inoculation into the brain of rats (**Mahata, *et al*., 1993**). Icv inoculation of hypertonic NaCl stimulated vasopressin release while inoculation with NERP-1 and 2 inhibited the NaCl induced vasopressin release. Moreover, NERP antibodies were found to reduce plasma vasopressin following water loading (**Yamaguchi *et al*., 2007**). All these data including in vivo and immunocytochemical observations suggest the possible roles of VGF derived peptides, NERPs in modulation of the central body fluid balance.

Table 3: VGF derived peptides linked to diseases in humans and animals, at a glance (**Bartolomucci *et al*., 2010**)

Disease	Peptide/fragment aa residue (species)	Effect	Stage	Reference
Neurological disease				
Alzheimer's disease	378–397 (human)	Decreased in patient's CSF	Identified as potential biomarker	Carrette et al., 2003
	Fragment not specified (human)	Decreased in patient's CSF	Identified as potential biomarker	Simonsen et al., 2007
Amyotrophic lateral sclerosis	398–411 (human)	Decreased in patient's CSF	Identified as potential biomarker	Pasinetti et al., 2006
	Entire pro-peptide (human)			
	Entire pro-peptide (mouse)	Decreased in CSF, serum and motor neurons	Pre-clinical	Zhao et al., 2008
Frontotemporal dementia	26–62 (human)	Decreased in patient's CSF	Identified as potential biomarker	Rüetschi et al., 2005
Pain	*TLQP*-21 556–576 (mouse)	Hyperalgesia (peripheral) analgesia (icv)	Pre-clinical	Rizzi et al., 2008
	TLQP-62 556–617 (mouse, rat)	Allodynia	Pre-clinical	Moss et al., 2008
	AQEE-30 588–617 (mouse, rat)	Hyperalgesia	Pre-clinical	Riedl et al., 2009
	LQEQ-19 598–617 (mouse, rat)	Hyperalgesia	Pre-clinical	Riedl et al., 2009
Psychiatric disease				
Schizophrenia/depression	23–62 (human)	Increased in patient's CSF	Identified as potential biomarker	Huang et al., 2006, 2007
Depression	*TLQP*-62 556–617 (mouse, rat)	Antidepressant-like effect	Pre-clinical	Thakker-Varia et al., 2007
Depression/anxiety	*AQEE-30* 588–617 (mouse, rat)	Antidepressant- and anxiolytic-like effects	Pre-clinical	Hunsberger et al., 2007
Other disease				
Obesity/eating disorders	*TLQP-21* 556–576 (mouse, hamster)	Limits diet induced obesity (mice, hamster) anorexia (hamster)	Pre-clinical	Bartolomucci et al., 2006; Jethwa et al., 2007
	NERP2 313–350 (rat)	Enhance food intake in an orexin-dependent manner	Pre-clinical	Toshinai et al., in press
	TLQP-62 and *HHPD-41* 556–617 and 577–617 (mouse)	Enhanced food intake	Pre-clinical	Unpublished, referred to by Bartolomucci et al., 2007

Role of VGF as neuroprotective agent

TLQP-21 was found to protect the cerebellar granule cells (CGCs) dose-dependently in rats which (CGCs) were supposed to go through cell death due the serum and potassium deprivation via the modulation of extracellular signal-regulated kinase1/2, ERK1/2 and by the enhancement of intracellular Ca^{2+}release (**Severini, C. *et al*, 2008**). SUN N8075, an inducer of VGF mRNA, protected cells from ER stress induced cell death. But its protective mechanism was entirely diminished by VGF knockdown with siRNA validating that the protective effect of SUN N8075 is mediated by VGF. VGF level was found to be lower in the spinal cords of sporadic ALS patients. This inducer was also found to slow the disease progress in the mutant SOD1 animal models of familial ALS and thus elongating the survival of the animals (**Shimazawa *et al*., 2010**). From all these findings, the involvement of SUN N8075/ VGF, either unconnectedly or concurrently, in progression of ALS can be speculated, and either or both of them may become a potential therapeutic candidate for treatment of ALS.

VGF derived peptides as diagnostic tools and/or targets in drug discovery

It has already become evident that VGF derived peptides are produced at altered levels in different disease conditions. VGF expression is down-regulated in the brains of rodents subjected to animal depression paradigms (**Thakker-Varia *et al*., 2007**). VGF is also reduced in post-mortem brain of patients with bipolar disorder and contributes to some of the behavioral and molecular effects of lithium, the canonical drug used to treat this disorder (**Thakker-Varia *et al*., 2010**).

Data referring to expression of VGF in schizophrenia are conflicting: one study reported increased VGF expression in prefrontal cortex of four brains from schizophrenic patients, and an increased concentration of in CSF of a 40-amino acid VGF-derived peptide, VGF 23–62, in CSF of first-onset, drug-naive schizophrenia patients and, to a lesser degree, in patients with depression. Interestingly, a VGF 26–62 peptide with an identical sequence to the VGF 23–62 peptide, except for the first three amino amino-terminal amino acids, did not appear to be differentially expressed in CSF from schizophrenia or depression patients compared to healthy volunteers, in the same study (**Huang *et al*. 2006**). On the other hand, results from the Stanley Medical Research Institute Genomics Database showed decreased VGF in prefrontal cortices of schizophrenia and bipolar disorder patients (**Thakker-Varia *et al*., 2010**). More recently, a reduced density of hypothalamic VGF-immunoreactive neurons was detected in post-mortem brains from patients with schizophrenia as compared to control subjects (**Busse *et al*., 2012**). All these strongly suggest that VGF does play an important role in the development of schizophrenia. Like schizophrenia, VGF derived peptides are also related to some other diseases as summarized in Table 3.

The number of diseases for which VGF-derived peptides have already been proved or are being investigated to act as disease biomarkers are increasing day by day. Some of them are namely breast cancer, gastroenteric tumors, insulinoma, lung tumors, medullary thyroid carcinoma, neuroblastoma, ganglioneuroma, parathyroid adenoma, pheochromocytoma, pituitary carcinoma, fronto-temporal dementia (FTD), schizophrenia (SCZ), major depressive disorder (MDD), alzheimer's disease (AD), Amyotrophic lateral sclerosis *(*ALS) (**Bartolomucci *et al*., 2011**). Though the number of experimental proofs are increasing day by day to recommend VGF derived peptides for widespread clinical use, not yet enough data confirmed the clinical utility of the VGF derived peptides as 'surrogate endpoints' for diseases, especially for life threatening, extensively prevalent neurological, psychriatic disorders (**Bartolomucci *et al*., 2010**). It should be noted that it took forty five years for the first granin protein, chromogranin A (CgA) to be used as a biomarker for neuroendocrine tumors (**Harsha *et al*., 2009**).

FUTURE PERSPECTIVE

Biological activities of a number of VGF derived peptides as well as their diverse tissue specific distribution and their corresponding multi-functional roles have already been elucidated with experimental evidences, as described earlier. What is now left and of upmost important in the field of VGF is complete elucidation of physiologically important receptors/binding proteins/partners with further characterization of receptor-dependent and/or receptor-independent signaling pathways.

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