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Angiopoietin-like proteins in multiple sclerosis

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ABSTRACT

Angiopoietin-like proteins (ANGPTLs) are a group of proteins with functions in lipid metabolism, angiogenesis, and inflammation. Here, we investigated their involvement in multiple sclerosis (MS) progression and response to treatment in 100 MS patients and 77 healthy controls. ANGPTLs significantly associated with MS progression and response to therapy. High ANGPTL6 levels associated with slow disease progression and good response to fingolimod treatment and low ANGPTL4 associated with poor response to natalizumab treatment. Therefore, we propose high ANGPTL4 and 6 levels as markers for positive response to MS treatments either natalizumab or fingolimod respectively. Further investigations into their role in MS is warranted.

1. Introduction

Multiple Sclerosis (MS) is a progressive demyelinating, autoimmune, neuro-inflammatory complex disorder. In MS, myelin and myelin producing oligodendrocytes are targeted by myelin-reactive immune cells promoting a cascade of inflammatory activators that resolve in MS plaques scarring the central nervous system (CNS) and perturbing the neural network. Many factors have been shown to be involved in MS pathogenesis, however, there is no definite known aetiology for MS (Zetterberg, 2017). Lipid metabolism is thought to be involved in MS pathogenesis, progression, and associated increased risk for the metabolic syndrome (Reale and Sanchez-Ramon, 2017). One of the proteins that regulate plasma level of lipoproteins is lipoprotein lipase. One of the key regulators of lipoprotein lipase activity are members of the angiopoietin-like protein family.

Angiopoietin-like proteins (ANGPTLs) that are structurally similar to angiopoietins and contain an amino-terminal coiled-coil domain as well as a fibrinogen-like domain except for ANGPTL8 which lacks the fibrinogen-like domain. Functional attributes of these proteins encompass lipid metabolism, angiogenesis, and inflammation (Santulli, 2014). The eight members in the ANGPTL protein family display variable and specific tissue expression. The most ubiquitously expressed member is ANGPTL4 (Ortega-Senovilla et al., 2013). Whereas ANGPTL3 and 6 are exclusively expressed in the liver, and ANGPTL5 and 7 are specifically expressed in the heart and eyes; respectively (Santulli, 2014). Due to their functions, ANGPTLs have been investigated for their association with different diseases including diabetes, cancer, cardiovascular diseases and the metabolic syndrome (Kadomatsu et al., 2011; La Paglia et al., 2017). ANGPTL2 expression promotes inflammation, neovascularization and epithelial tissue regeneration (Thorin-Trescases and Thorin, 2014). ANGPTL4 plays a major role in lipoprotein B metabolism, angiogenesis, and has multisystem effects compared to other ANGPTLs due to its wider tissue distribution (Zhu et al., 2012). ANGPTL6 functional attributes are deduced from several genetic variants in its encoding gene associating with the metabolic syndrome (Legry et al., 2009). Like other ANGPTLs ANGPTL6 is involved largely in regulating lipid and glucose homeostasis (Namkung et al., 2011). Despite ANGPTLs investigations in many inflammatory disorders, none of these proteins have been investigated in demyelinating, neurodegenerative disorders for which disturbances in vascular, metabolic, and immune pathways are reported (Hamid and Mirshafiey, 2016; Jorissen et al., 2017). ANGPTLs' function in lipid metabolism might be involved in promoting myelin synthesis by regulating cholesterol synthesis in MS (Lichtenstein et al., 2007). In addition, the angiogenic functions of ANGPTLs could play a role in scar healing and regeneration in the MS brain. However, it is plausible that ANGPTLs have different functions in the brain than in other tissues, but these functions are currently unknown (Vienberg et al., 2015). Here, we investigated ANGPTL2, 3, 4, and 6 for their involvement in MS pathogenesis, progression, and response to treatment.

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2. Materials and methods

2.1. Sample collection

One hundred Kuwaiti MS patients were recruited for this study at Dasman diabetes institute MS clinic. This study's protocols were approved by Dasman diabetes institute ethical review committee which adheres to the declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. Seventy-seven gender and relatively age (± 3 years) matched healthy control individuals were allocated from the general population. All study protocols and objectives were fully explained to all participants before securing their informed written consent. MS patients' inclusion criteria were as follows; a detailed clinical history (demographics, body mass index (BMI) measurement, age of MS onset, disease duration, expanded disability status scale (EDSS) score, and treatment history), being a Kuwaiti citizen, and an MS disease duration of ≥2 years. Exclusion criteria included; having an EDSS score of 0 and being a pediatric MS case (< 18 years). In addition, criteria used to categorize natalizumab treated MS patients as responsive included; having a stable or reduced EDSS, no occurrence of relapse during natalizumab adherence, and regression or lack of new MS-related CNS lesions on magnetic resonance imaging (MRI). Whereas, natalizumab unresponsive MS patients were identified based on the following; having an increased EDSS despite treatment, occurrence of a relapse during treatment course, and detection of new MS-related lesions in the CNS on MRI compared to pre-treatment MRI results. All MRI and EDSS assessments were conducted by our expert co-author neurologist MS consultant according to standardized accepted criteria (Kurtzke, 1984). Healthy controls' exclusion criteria were; having a family history of MS, and a diagnosis of an autoimmune or neurodegenerative disorder. Collected blood samples were centrifuged at 2500 ×*g* at room temperature for 10 min, and plasma fractions were collected and stored at −80 °C until use.

2.2. ANGPTL2, 3, 4 and 6 multiplexing assay

Plasma levels of ANGPTL2, 3, 4 and 6 were assessed using the multiplexing immunobead array platform according to manufacturer instructions (R&D systems Inc., MN, USA). The samples were analysed using the Bioplex-200 system (BioRad Laboratories, CA, USA). Plasma samples were diluted 1:2 before analysis in sample buffer. Data was processed by the Bioplex Manager software version 6 using five-parametric curve fitting. Assay coefficient of variation for all analytes ranged from 6.0% to 13%.

2.3. Statistical analysis

D'Agostino and Pearson normality tests were performed for all assessed ANGPTLs and none were normally distributed. Log transformed ANGPTLs levels were analysed for their association with study variables in a case-control model and in an intra-MS cohort model. Statistical analyses used included Student *t*-test and linear regression analysis adjusted for sex, age, smoking status, and BMI. A *p*-value of < 0.05 was considered significant for all tests. All statistical analyses were performed using statistical package for the social sciences (SPSS) v. 25 software (IBM corporation, NY, USA).

3. Results

3.1. ANGPTLs' levels in studied cohorts

Demographic and clinical characteristics of the studied cohorts are shown in Table 1. ANGPTLs' levels did not significantly differ between the two cohorts (Table 2). Age and sex did not affect levels of ANGPTLs 2, 3, and 6. However, low ANGPTL4 levels associated with older age (β = −0.004, 95%CI:-0.008–0.00, *p* = .039).

Table 1
Cohorts' demographics, clinical characteristics and ANGPTLs levels.

Criteria	MS patients (*n* = 100)	Healthy Controls (*n* = 77)
Sex [n]		
Female	70	51
Male	30	24
Age [Median (IQR[a])]	32 (26–38)	28 (24–35)
Smoking status		
Smoker	29	14
Non-smoker	71	61
MS type (RR/SP/PP)	88 / 10 / 2	–
EDSS [median (IQR)]	2 (1–3.375)	–
MSSS [median (IQR)]	3.46 (2.16–4.94)	–
Type of treatment [n]		–
Fingolimod	39	
Natalizumab	34	
Other	27	

[a] IQR: Interquartile range.

3.2. ANGPTLs correlation with MS clinical variables

Intra-MS cohort analysis revealed the association of ANGPTL2 levels with BMI (β = 0.008, 95%CI: 0.001_ 0.015, *p* = .03) and smoking status (β = 0.185, 95%CI: 0.068_ 0.3, *p* = .002) when adjusted for sex and age, but not with EDSS or MSSS scores. Similarly, ANGPTL3 levels were influenced by BMI (β = 0.012, 95%CI: 0.003_ 0.02, *p* = .008), as were ANGPTL4 levels (β = 0.01, 95%CI: 0.003_ 0.016, *p* = .004). ANGPTLs 3 and 4 did not associate with MS disability or smoking status. ANGPTL6 levels significantly associated with lower EDSS scores when adjusted for sex, age, BMI and smoking status (β = −0.053, 95%CI: -0.091 _ -0.016, *p* = .006). In addition, ANGPTL6 levels association with MS severity scores persisted when using MSSS to adjust for disease duration (β = −0.045, 95%CI: -0.076_ -0.015, p = .004). Dividing the MS cohort based on treatment administered being fingolimod (*n* = 39) or natalizumab (*n* = 34) we analysed ANGPTLs levels in the two sub-groups to identify any changes in levels specific to treatment administered. MS patients on fingolimod had higher levels of ANGPTL6 (*p* = .001) than those on natalizumab when adjusted for age, sex, BMI and smoking status (Fig. 1). No other associations were found.

Longitudinal 2.5 years follow-up of patients on natalizumab subdivided natalizumab treated patients into those responsive (*n* = 23) and those non-responsive (*n* = 11) based on EDSS, occurrence of relapse, and detection of new CNS lesions compared to pre-treatment initiation on MRI. We found that ANGPTL4 levels were higher in those responsive to natalizumab than poorly responding MS patients (*p* = .007, Fig. 2) while ANGPTL6 did not differ among the sub-groups. Given ANGPTL4 level associating with age, we performed a linear regression analysis of ANGPTL4 levels among the subgroups corrected for age, sex, and BMI. We found a significant difference between responders and non-responders to natalizumab (*p* = .039). ANGPTL6 levels did not associate with response to natalizumab despite it being significantly lower in these patients than those on fingolimod.

4. Discussion

ANGPTLs are structurally similar to angiopoietins and are characterized by an N-terminal coiled-coil domain, a C-terminal fibrinogen-like domain, and an N-terminus highly hydrophobic signal sequence region for protein secretion. All ANGPTLs have been shown to have a role in lipid metabolism, however only few have an established role in inflammation, mainly ANGPTL2 and 4 (Thorin-Trescases and Thorin, 2014; Zhu et al., 2012). Here, we investigated ANGPTL2, 3, 4, and 6 for their involvement in MS pathogenesis, progression, and response to treatment. We found several associations of ANGPTLs with MS progression and response to treatment. Increased ANGPTL2 levels in MS smokers and those with high BMI represent its pro-inflammatory

Table 2
ANGPTLs levels in cohorts investigated and their associated functions and disease.

ANGPTLs levels Median in ng/mL (IQR)	MS cohort (n = 100)	Healthy controls (n = 77)	Function (Associated disease)
ANGPTL2	1.73 (1.3–2.3)	1.8 (1.5–2.4)	Neovascularization
ANGPTL3	43.1 (32.6–60.2)	39.1 (29.5–54.8)	Angiogenesis, lipid metabolism (Familial hypobetalipoproteinemia 2)
ANGPTL4	93.3 (70.2–117.8)	89.4 (72.5–121.5)	Glucose homeostasis, lipid metabolism, insulin sensitivity (Type 2 diabetes)
ANGPTL6	25.7 (19.8–34.2)	25.3 (17.8–32.1)	Wound healing, neovascularization (Familial cerebral saccular aneurysm)

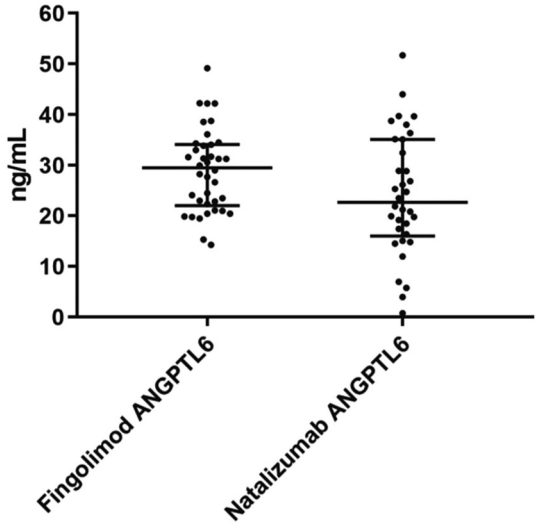

Fig. 1. Levels of ANGPTL6 in MS patients treated with natalizumab were significantly lower than those treated with fingolimod (β = −0.272, 95%CI: −0.43 . −0.115, p = .001). Bars represent median and interquartile range.

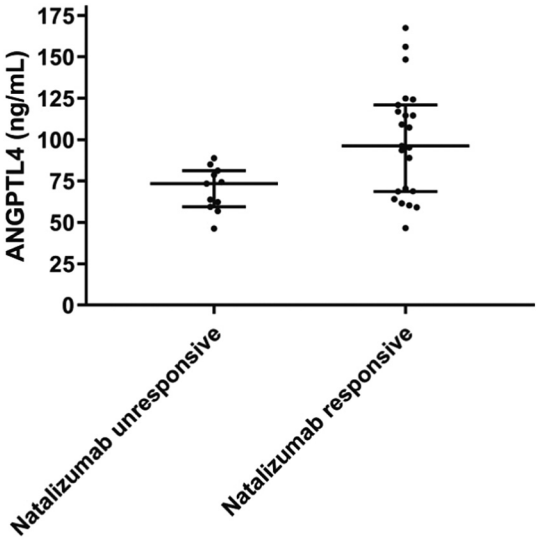

Fig. 2. High ANGPTL4 levels significantly associated with positive response to natalizumab treatment in MS patients (β = 0.13, 95%CI: 0.008 . 0.25, p = .039).

functions. ANGPTL2 has been shown to be increased in many inflammatory pathological conditions such as cancer, diabetes, periodontal, obesity and cardiovascular disease (Toiyama et al., 2015; Ohno et al., 2017; Gellen et al., 2016; Kim et al., 2018). Most notably, ANGPTL2 association with inflammation for which smoking is a risk factor might explain its increased levels in smoking MS patients that already have an inflammation prone physiological environment (Knight et al., 2016; Amadatsu et al., 2016). The association of high ANGPTL6 levels and low MS disability and severity we have shown here may be a result of elevated ANGPTL6 promoting healing, reparative, regenerative and angiogenic events following MS attacks (Zhang et al., 2006; Akhter et al., 2013). The latter might also explain high ANGPTL6 level associating with fingolimod treatment which sequesters lymphocytes to the lymph nodes inhibiting their transmigration to the CNS and re-initiation of neuroinflammation. The effects of fingolimod would possibly give ANGPTL6 protein better conditions to detect CNS damage and promote its healing and repair functions in the CNS than would natalizumab treatment. Natalizumab is a mono-clonal antibody against α4-integrin that blocks the binding of autoreactive immune cells to endothelial expressed α4-integrin at the blood-brain-barrier site (Yednock et al., 1992). The most studied function of ANGPTL4 is its role in systemic angiogenesis and metabolic health through its ubiquitous expression in the body mediating its pleiotropic functions (Santulli, 2014). ANGPTL4 association with response to natalizumab might be explained by its angiogenic and wound healing functions by binding αvβ5-integrins and β1-integrins (Chong et al., 2014; Goh et al., 2010). It is plausible that some cross-reactivity exist between the involved integrins prompting a positive competitive interaction between natalizumab and ANGPTL4 augmenting the levels of the latter. However, this hypothesis is not supported by existent evidence, as integrins while sharing some sequence homology; display committed ligand and pathway specificities (Barczyk et al., 2010). Alternatively, it is possible that the reduction in inflammation by natalizumab allows for ANGPTL4 levels to increase concomitantly to promote its angiogenesis and wound healing and repair activities in an efficient spatio-temporal manner. The latter hypothesis may also explain the associated reduction in MS disability in responsive natalizumab treated MS patients. In addition, it has been shown that ANGPTL4 stabilizes the blood-brain-barrier inhibiting hyper-permeability thus augmenting the inhibitory effects of natalizumab (Zhang et al., 2017). Moreover, we cannot rule out the role of genetic and environmental factors such as nutrition and exercise in altering ANGPTL4 level as it is tightly regulated by these factors (Catoire and Kersten, 2015; Janssen and Kersten, 2017; Cushing et al., 2017), nor can we rule out the possibility of a novel role for ANGPTL4 in the nervous system (Vienberg et al., 2015). Therefore, while we cannot surmise the mechanism by which ANGPTL4 enhances response to natalizumab it is evident that ANGPTL4 is a candidate predictor of response to natalizumab. However, the obvious limitation of this finding is the small sample size of this sub-cohort that needs to be validated in a larger natalizumab treated MS cohort.

In conclusion, the reported data on ANGPTLs functions have focused on lipid metabolism, glucose homeostasis and association with cancer and diabetes. Our findings support their roles in inflammation, neuronal regeneration and wound healing. We conclude, based on the presented evidence here; that ANGPTL4 and 6's metabolic and vascular functions might play complex roles in MS injury reperfusion, response to treatment, and possibly remyelination at the site of MS lesions thus further investigation of their roles in MS is warranted.

Conflict of interest

The authors declare that there is no conflict of interest.

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